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Growth of African Catfish (*Clarias gariepinus*) Cultured Using the Biofloc System at Different Stocking Densities

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ABSTRACT

The cultivation of African catfish (*Clarias gariepinus*) using a biofloc system is an aquaculture technology that integrates waste management and the production of natural feed in situ, thereby enhancing productivity efficiently. This research aimed to evaluate the effect of EM4 probiotic application in a biofloc system on the growth and survival rate of catfish under different stocking densities. The experiment was conducted using a Completely Randomized Design (CRD) with four stocking density treatments: P1 (30 fish/30 L), P2 (40 fish/30 L), P3 (50 fish/30 L), and P4 (60 fish/30 L), each with three replications. Observed parameters included absolute weight and length growth, floc content, hematocrit, hemoglobin, erythrocytes, leukocytes, differential leukocytes and water quality parameters. The treatment P4 showed the most notable effects, with a final absolute weight gain of 11.66 grams and an absolute length increase of 8.11 cm. Furthermore, the probiotic addition had a significant impact on erythrocyte levels and differential leukocyte counts. In contrast, the use of probiotics in the biofloc system did not result in significant differences in hematocrit levels, total leukocyte counts, or biofloc volume.

INTRODUCTION

Traditional and intensive aquaculture practices for economically valuable freshwater fish species have been widely implemented, particularly for African catfish. This species exhibits rapid growth and strong adaptability to various environmental conditions, which are among its primary advantages. The African catfish (*Clarias gariepinus*), commonly known as "lele dumbo," possesses several favorable traits that make it highly popular among fish farmers. Fundamentally, lele dumbo cultivation does not require large areas of land, demands relatively low capital investment, uses minimal water, is easy to manage, and grows quickly (Wathon, 2018).

Biofloc technology offers several advantages over other aquaculture methods, as it combines waste management to maintain water quality with the in-situ production of natural fish feed. This system is considered effective because it significantly increases productivity even in limited space by allowing higher fish stocking densities, reducing production costs, and shortening the cultivation period compared to conventional systems (Sumardani *et al.,* 2018). The biofloc system is implemented by introducing beneficial bacteria or microbes into the aquaculture medium to enhance and stabilize water quality, prevent the accumulation of toxic residues such as ammonia, and inhibit the growth of harmful (pathogenic) bacteria, enabling fish to grow and develop properly.

Research on the application of biofloc technology in aquaculture remains relatively limited, for example in Nile tilapia. The L1k bacteria used in this research are heterotrophic bacteria known to produce extracellular enzymes such as protease, amylase, and cellulase (Artha, 2018). The addition of these heterotrophic bacteria to the culture media is expected to enhance the microbial composition of the biofloc, which in turn determines the nutritional content of the biofloc. Moreover, the application of L1k bacteria through feed is also expected to improve feed digestibility in catfish, as the heterotrophic microbes in the digestive tract contribute to the production of enzymes that aid in digestion. This research aims to analyze the effect of adding heterotrophic bacterial cells to the culture media and feed in order to improve the production performance of African catfish (*Clarias gariepinus*) in a biofloc based aquaculture system.

Probiotics are live microorganisms that can provide benefits to the host by regulating the balance of microbes in the digestive tract, improving feed efficiency and utilization, enhancing immune responses, and improving environmental quality. Probiotics have the ability to stimulate the immune system to fight disease, increase nutrient absorption in the intestines, and suppress pathogenic populations (Anis & Hariani, 2019). The application of probiotics in aquaculture plays a crucial role in determining the success of cultivation. When probiotics are consumed by fish in sufficient quantities, they provide health benefits to the fish.

METHODS

Time and Place

This research was conducted over 45 days, on January 2024. The research occurred at the Fish Production and Reproduction Laboratory and the Fish Health Laboratory, Department of Fisheries and Marine Science, University of Mataram.

Tools and Materials

In this research, the equipment used included an aerator, stationery, basins, DO meter, BOD meter, measuring cups, camera, ruler, pH meter, measuring pipette, thermometer, water temperature thermometer, digital scale, and jars.

The materials used were 2 weeks old African catfish (*Clarias gariepinus*), EM4 (Effective Microorganisms), water, and FF-999 crumble feed.

Research Design

This research employed an experimental method using a Completely Randomized Design (CRD). The treatment in this research was the variation in stocking density, consisting of four treatments with three replications each. The stocking densities used ranged from 400 to 2400 fish/m³ (Suprapto & Samtafsir, 2017). The specific stocking densities applied in this research were as follows in each treatment container:

Treatment A contained 30 fish in 30 liters of water
Treatment B contained 40 fish in 30 liters of water
Treatment C contained 50 fish in 30 liters of water
Treatment D contained 60 fish in 30 liters of water

Research Procedures

- **Preparation of Container or Pond**

The containers used in this research consisted of 12 units, each with a capacity of 45 liters. Prior to use, all containers were thoroughly cleaned using dishwashing soap to remove dirt and eliminate any bacteria attached to the container surfaces.

- **Water Filling**

Each container was filled with clean water to a volume of 30 liters. Every container was labeled according to its treatment group and supplied with continuous aeration throughout the maintenance period.

- **Preparation of Water Mixed with Probiotics**

Water was first poured into a basin. Probiotics were then added into a container based on the required dosage and diluted with enough water to avoid excessive concentration. The probiotic solution was poured into the water and stirred evenly, then left to sit for approximately 10–15 minutes to ensure thorough mixing. Once the solution was well mixed, it was transferred into labeled plastic bags and administered to the fish three times a day morning, afternoon, and evening. The probiotic solution was given through the feed at a rate of 5% of the fish's body weight, and any leftover feed was weighed.

- **Probiotic Feeding Preparation**

In this research, African catfish were fed three times daily in each rearing aquarium: in the morning at 07:00 WITA, at noon at 12:00 WITA, and in the evening at 17:00 WITA.

- **Maintenance Stage**

The maintenance of African catfish was carried out by feeding them with the same dosage of probiotic mixture. Catfish measuring 5–6 cm in length and weighing approximately 23 grams were placed into 12 containers, with each container stocked after an acclimatization process.

Only catfish with uniform size (5–6 cm), healthy morphology, active movement, and no physical deformities were selected and introduced into the experimental media.

Research Parameters

- **Absolute Weight**

The absolute weight gain of the fish was calculated using the following formula from Rihi (2019):

$$W = W_T - W_0$$

Where:
W = Mean absolute weight (g)
W_T = Mean weight (g) of fish on day t
W_0 = Mean weight (g) of fish on day 0

- **Absolute Length**

The absolute length was calculated using the formula from Zonneveld *et al.* (1991):

$$L = L_T - L_0$$

Where:
L = Mean length growth (cm)
L_0 = Mean length (cm) of fish on day 0
L_T = Mean length (cm) of fish on day t

- **Floc Content**

The floc content was calculated using the following formula from Salamah & Zulpikar (2020):

$$\textbf{Floc Volume} = \frac{(ml)}{(L)}$$

Where:
ml = Settled flocs
L = Collected water sample

- **Hematocrit**

The method for calculating hematocrit levels in the test animals refers to Anderson *et al.* (1993). Blood samples were drawn into hematocrit capillary tubes up to 3/4 of the tube's length, then sealed with wax. The tubes were centrifuged at 3,500 rpm for 15 minutes. The hematocrit value was determined by comparing the length of the packed red blood cells to the total length of the blood column in the tube. The hematocrit result was read using a microhematocrit reader and expressed as a percentage (%).

- **Hemoglobin**

Hemoglobin concentration was measured using a Sahli hemometer. Fish blood samples were drawn using a Sahli pipette up to the 20 mm^3 mark. The blood was then transferred into a Sahli tube containing 0.1 N HCl up to the 10 marks. The tube was placed in the middle between two standard color tubes. Distilled water was then added drop by drop until the color matched the standard. The result was expressed as a percentage (%).

- **Erytrochytes**

The first step involved drawing blood from the Eppendorf tube using a Thoma pipette up to the 0.5 mark. The blood was then mixed with Hayem's solution up to the 101 marks, and the pipette was gently shaken to ensure homogenization. After homogenization, the first three drops were discarded as they were assumed to be non-homogeneous. The next drop was placed onto a hemocytometer, covered with a cover glass, and observed under a microscope. The final step was counting the erythrocytes using the following formula from Cerlina *et al.* (2021):

$$\text{Number of erythrocytes} = \text{Counted erythrocytes} \times 10^4 \text{ cells/mm}^3$$

- **Leukocytes**

The first step involved drawing the blood sample into a Thoma pipette up to the 0.5 mark, followed by the addition of Turk's solution up to the 11 marks. The mixture was then gently shaken to ensure homogenization. The first three drops from the pipette were discarded, and the next drop was placed on a hemocytometer and covered with a cover glass. The total number of leukocytes was counted in five squares using the following formula from Ginting et al. (2021):

$$\text{Number of leukocytes} = \text{Counted leukocytes} \times 50 \text{ cells/mm}^3$$

- **Differential Leukocytes**

The differential leukocyte count was performed to determine the percentage of leukocyte types in the blood. The procedure began by preparing a thin blood smear, where one drop of blood was placed on a glass slide and evenly spread using the smear technique. The smear was then fixed with 5–6 drops of methanol and left for 5 minutes, followed by rinsing with distilled water. Next, the smear was stained with 1–2 drops of Giemsa stain and left for 1–2 minutes, then rinsed again with distilled water and air-dried for 2 minutes. Finally, the slide was observed under a microscope at 400x magnification (Kurniawan *et al.,* 2013).

- **Water Quality**

Water quality is one of the key factors supporting the growth and development of aquatic organisms. These organisms require an optimal environment for proper growth, and thus, water quality significantly affects cultured species. The data collected includes several important parameters, such as dissolved oxygen (DO), pH, and temperature. The data collected includes several important parameters, such as dissolved oxygen (DO), pH, and temperature. The water quality parameters are presented in Table 1.

Table 1. Water Quality Parameters

No.	Parameters	Unit	Equipment Test
1	Temperature	°C	Termometer
2	pH	-	pH meter
3	DO	mg/l	DO meter
4	Ammonia	mg/l	Spektrometer

Data Analysis

The data obtained from the research were statistically analyzed using Analysis of Variance (ANOVA). The experimental design used was a Completely Randomized Design (CRD). ANOVA was performed with a 95% confidence interval to determine whether there were significant differences among the treatments. If a significant difference was found, the analysis was followed by Duncan's Multiple Range Test (DMRT) for further comparison.

RESULTS

Absolute Weight

The results of absolute weight gain in catfish were obtained by weighing the fish on day 0 and at the end of the research. The average absolute weight gain ranged from 10.25-11.66 g, as shown in Figure 1.

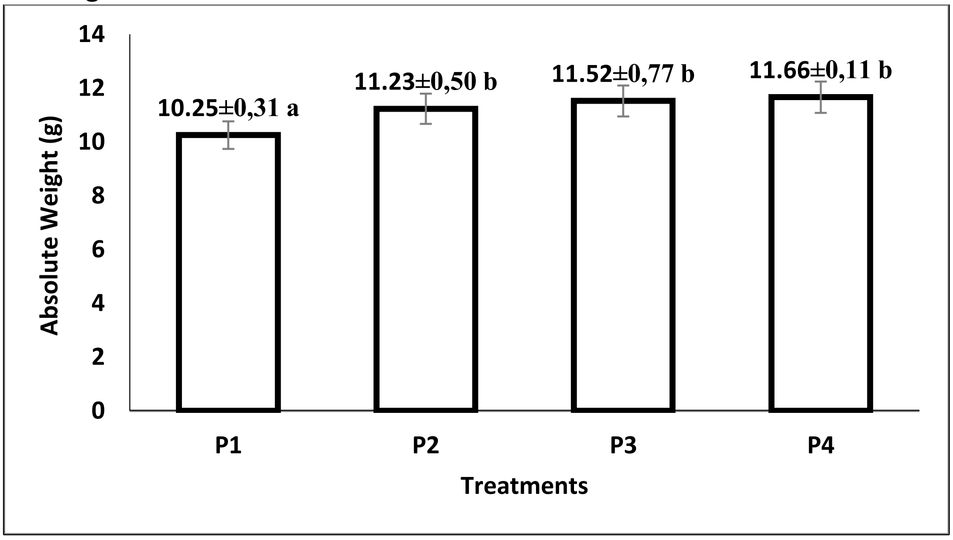

Figure 1. Absolute Weight of Catfish

Absolute Length

The absolute length growth of catfish was determined by measuring the fish on day 0 and at the end of the research. The average absolute length growth ranged from 6.60-8.11 cm, as shown in Figure 2.

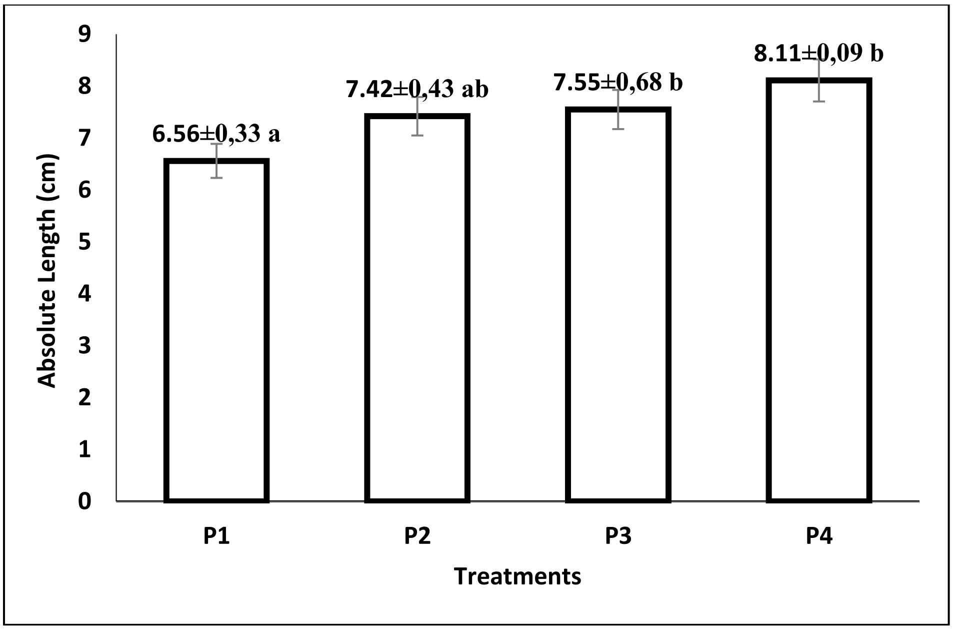

Figure 2. Absolute Length of Catfish

Floc Content

The average floc volume during the research ranged from 0.051-0.080 mL/L. The use of a biofloc system with different stocking densities in catfish culture had no effect on the floc volume, as shown in Figure 3.

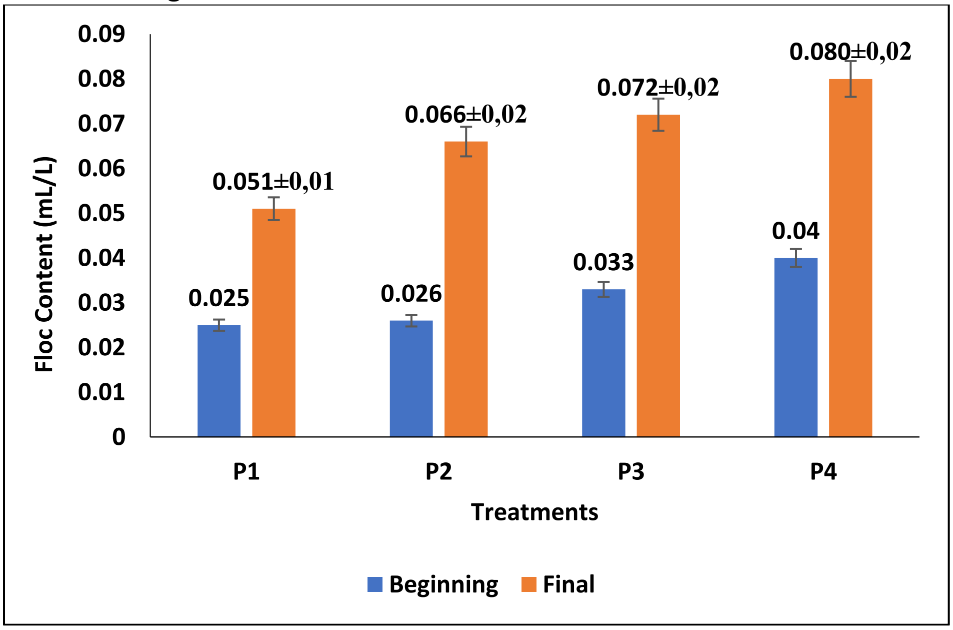

Figure 3. Floc Content

Hematocrit

The average hematocrit values during the research ranged from 15.12-24.29%. The use of a biofloc system with different stocking densities in catfish culture had no effect on hematocrit levels, as shown in Figure 4.

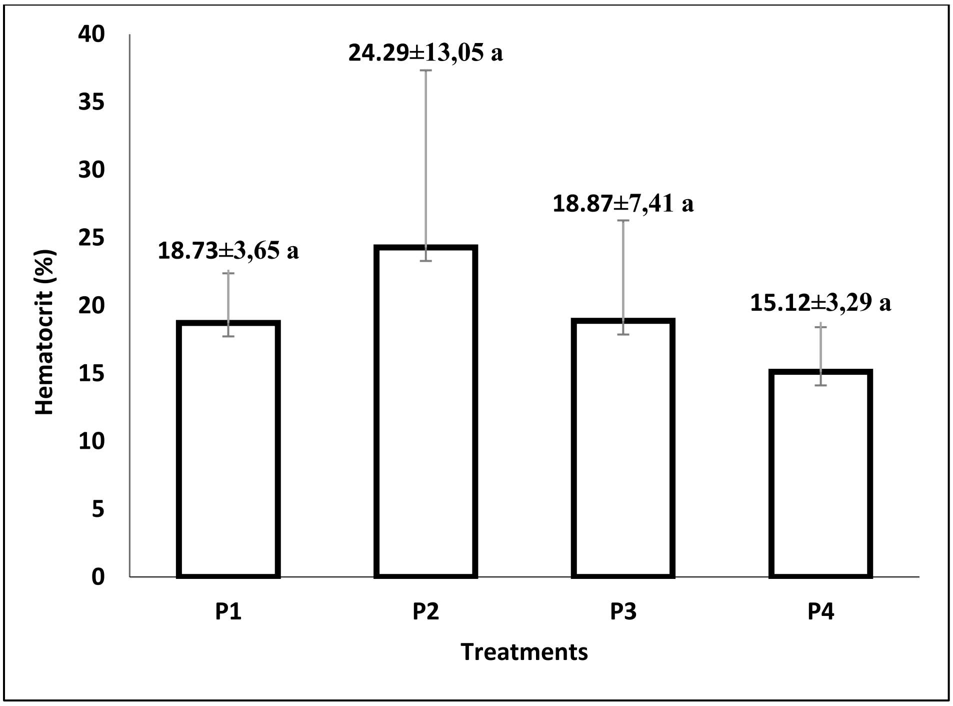

Figure 4. Hematocrit of Catfish

Hemoglobin

The average hemoglobin values during the research ranged from 6.2-7.56 g/dL. The use of a biofloc system with different stocking densities in catfish culture had no effect on hemoglobin levels, as shown in Figure 5.

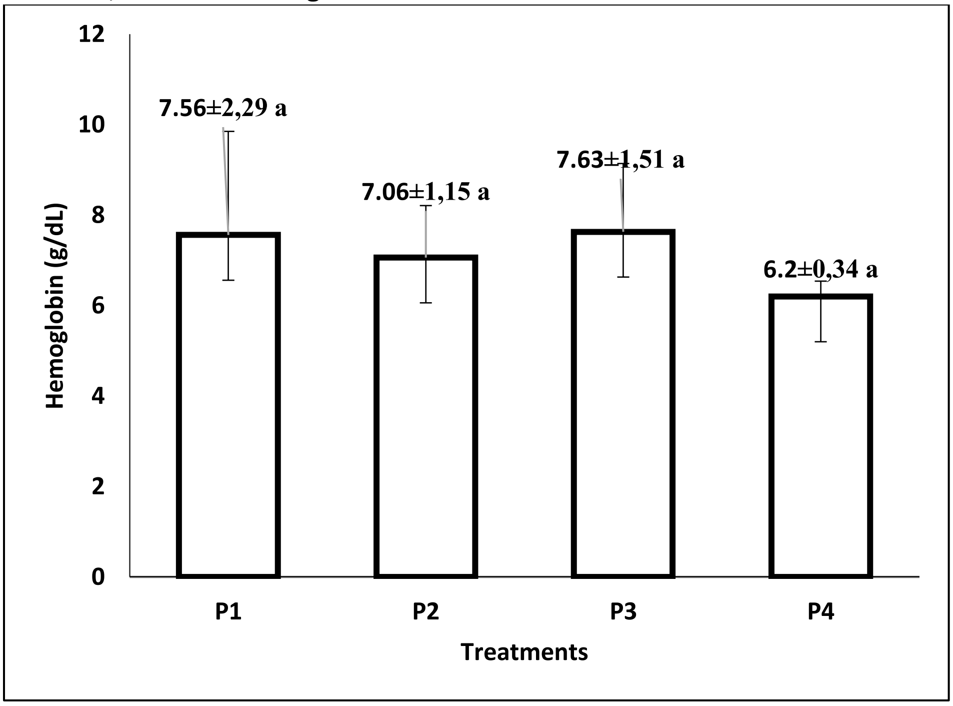

Figure 5. Hemoglobin of Catfish

Erythrocytes

The erythrocyte values during the research ranged from 2.29-4.64 x 10^4 cells/mm^3. The use of a biofloc system with different stocking densities in catfish culture had a significant effect on erythrocyte levels, as shown in Figure 6.

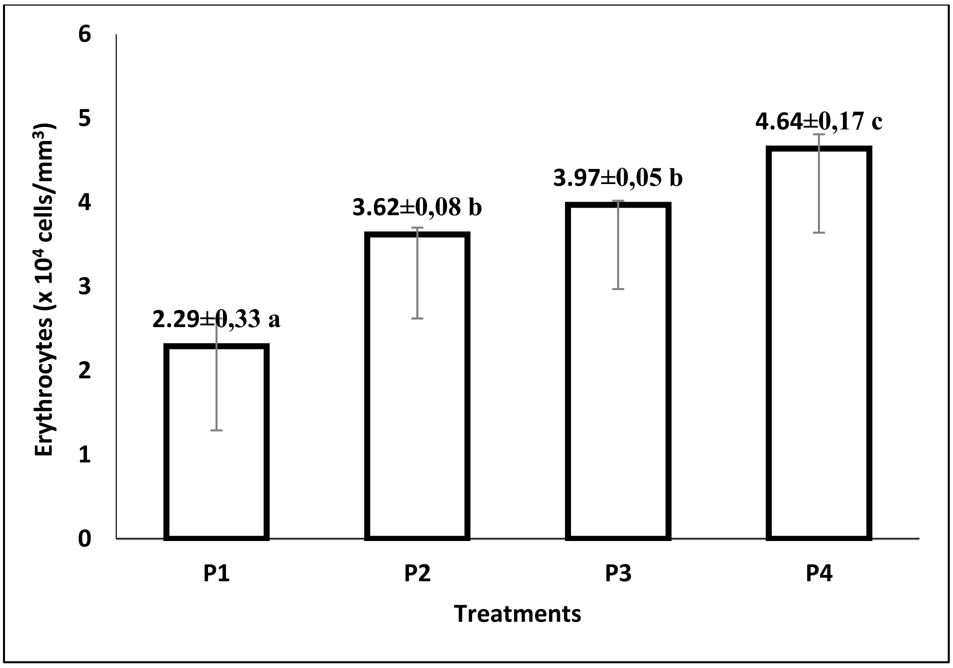

Figure 6. Erythrocytes of Catfish

Leukoytes

The average leukocyte values during the research ranged from 2.25-2.54 x 10^3 cells/mm^3. The use of a biofloc system with different stocking densities in catfish culture had a significant effect on leukocyte levels, as shown in Figure 7.

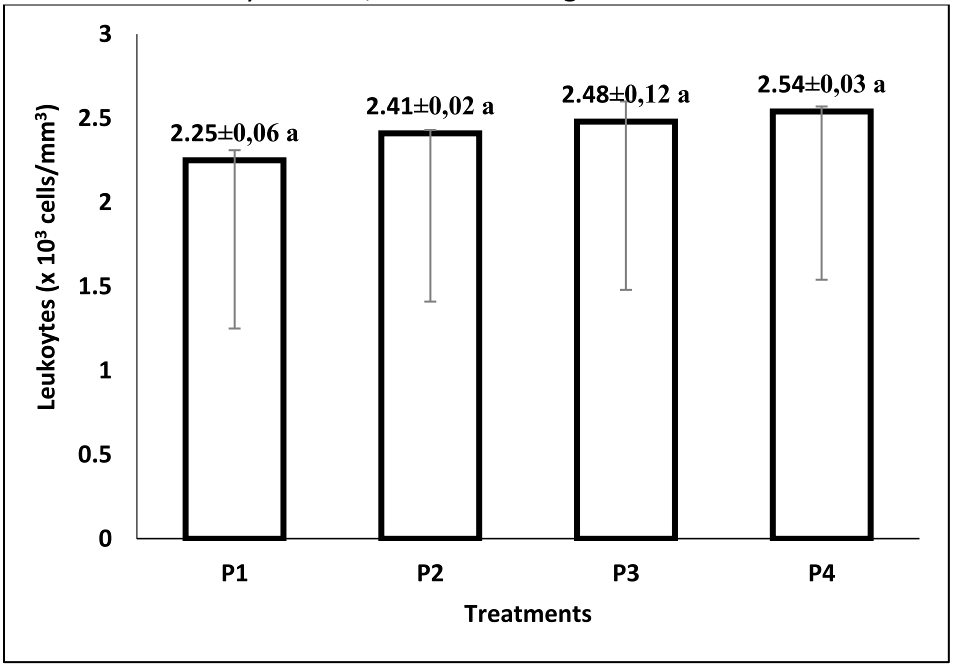

Figure 7. Leukocytes of Catfish

Differential Leukocytes

The average differential leukocyte values during the research ranged from 7.66-11.66% for monocytes, 67.33-90.33% for lymphocytes, 7.33-10% for neutrophils, and 8.33-10.66 cells/mm^3 for thrombocytes. The use of a biofloc system with different stocking densities in catfish culture had a significant effect on the differential leukocyte counts, as shown in Figure 8.

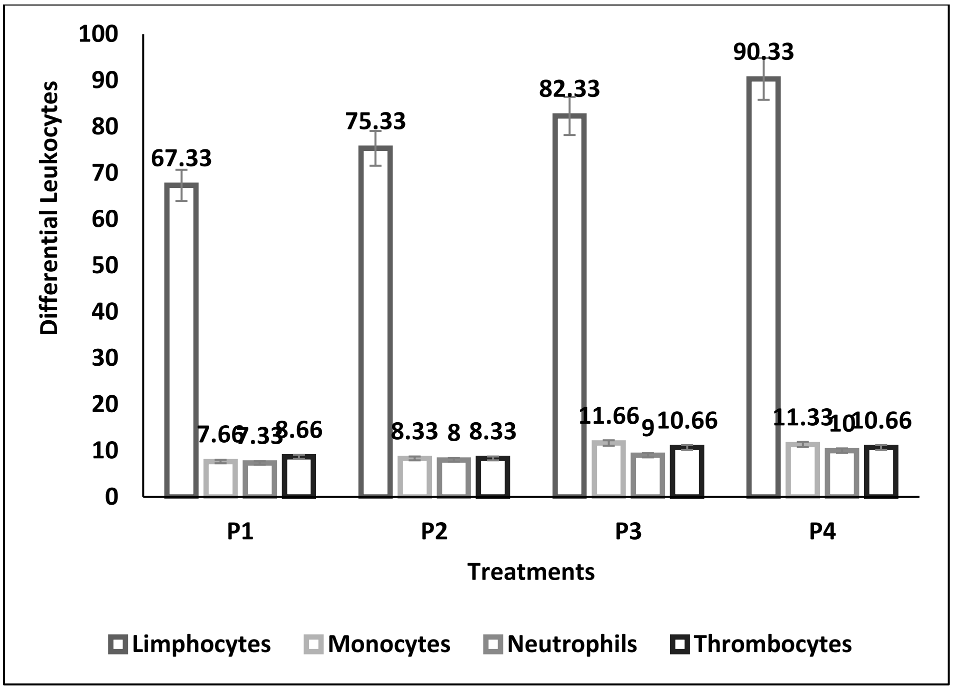

Figure 8. Differential Leukocytes of Catfish

Water Quality

The water quality parameters of catfish during the rearing period are presented in Table 1.

Table 1. Water Quality

Parameters	Observed Range	Optimal Range	Reference
Temperature (°C)	28,4 – 29,6	25 – 30	Wulansari *et al.* (2022)
pH	7 – 7,9	6,5 – 8	Asruti *et al.* (2019)
DO (ppm)	6,1 – 7,9	>3 ppm	Manik *et al.* (2022)
Ammonia (ppm)	0,08 – 0,99	<1 mg/l	Sintiya (2021)

DISCUSSION

Absolute Weight

Absolute weight gain is one of the primary growth performance indicators used to evaluate the effectiveness of rearing conditions during the culture period. It is calculated by measuring the difference between the final and initial body weight of fish. In this research, the results demonstrated that the highest absolute weight gain was recorded in treatment P4, which had a stocking density of 60 fish/m³, whereas the lowest was observed in treatment P1 with a stocking density of 30 fish/m³. The superior growth observed in treatment P4 suggests that a higher stocking density, when combined with the biofloc system, positively influenced the growth performance of catfish (*Clarias gariepinus*), leading to a significantly greater absolute weight gain compared to the control group.

One of the key contributing factors to this improved growth is the synergistic effect of the biofloc system and probiotic supplementation. The biofloc environment enhances water quality by reducing toxic nitrogen compounds and simultaneously serves as a source of natural protein-rich feed. When probiotics are introduced into this system, they further improve nutrient absorption, digestive enzyme activity, and immune response, creating optimal conditions for fish growth. These findings are consistent with the research conducted by Indriyanti *et al.* (2023), which revealed that biofloc technology facilitates high-density catfish farming while significantly enhancing growth performance and reducing feed conversion ratios. Additionally, Liana *et al.* (2024) confirmed that the use of probiotics in aquaculture systems significantly improves growth parameters compared to systems that do not include probiotic supplementation.

Similarly, Rahmawati *et al.* (2022) reported that probiotic-enriched biofloc systems result in improved fish health, increased survival rates, and better feed efficiency. Nuryadi *et al.* (2021) also observed that catfish reared under a biofloc system with probiotic addition exhibited significantly higher absolute and specific growth rates than those in conventional systems. Jaila *et al.* (2021) added that the environmental conditions of fish, in relation to their ability to adapt to their surroundings, are suspected to be one of the factors causing differences in absolute weight growth.

Absolute Length

Absolute length gain is one of the key parameters used to assess the linear growth of fish, measured as the difference between the initial and final body length during the rearing period. In this research, the highest absolute length gain was observed in treatment P4, reaching 8.11 cm, while the lowest was found in treatment P1 at 6.56 cm. Based on the results of a post-hoc ANOVA analysis, the addition of probiotics within the biofloc system under varying stocking densities had a significant effect on the absolute length growth of catfish (*Clarias gariepinus*).

The enhanced length growth in treatment P4 can be attributed to the synergistic effect of the biofloc environment and probiotic supplementation. Probiotics play a critical role in improving the microbial balance in the rearing water, enhancing nutrient absorption, and stimulating digestive enzyme production. These benefits contribute to better growth performance, particularly in terms of linear body development.

These findings align with the results reported by Maspeke *et al.* (2024), who concluded that the application of probiotics in high-density rearing systems significantly promotes fish length growth compared to systems without probiotics. Furthermore, Khotimah *et al.* (2016) emphasized that probiotic supplementation not only enhances water quality by reducing harmful microbial populations and organic waste but also supports fish development by improving physiological conditions and feed utilization.

Additionally, Syahrul *et al.* (2020) found that fish reared in probiotic-enriched environments exhibited improved length and weight growth, as well as higher survival rates. Sukenda *et al*. (2018) also demonstrated that probiotic addition in a biofloc system could significantly improve water quality and length gain, particularly under intensive stocking conditions. The addition of probiotics to Nile tilapia at varying doses can enhance growth more significantly compared to those without probiotic supplementation (Sahendra *et al.,* 2023). According to Liana *et al.* (2024), the addition of probiotics in the biofloc system can improve the digestive system of fish, thereby enhancing optimal growth rates.

Floc Content

The floc volume observed throughout the research exhibited a clear upward trend, particularly in treatments where probiotics and molasses were added as part of the biofloc management strategy. At the beginning of the research, treatment P1 (control) showed no floc development, with a volume of 0 mL/L. In contrast, treatments P2, P3, and P4 had initial floc volumes of 0.026 mL/L, 0.033 mL/L, and 0.040 mL/L respectively, indicating early microbial and organic matter accumulation. By the end of the rearing period, a significant increase in floc volume was observed: 0.066 mL/L in P2, 0.072 mL/L in P3, and 0.080 mL/L in P4, while P1 remained at 0 mL/L. The highest floc volume was recorded in P4, which also correlated with better growth performance parameters in fish, suggesting a strong association between floc formation and water quality improvement.

According to Erlangga *et al.* (2021), a higher floc volume is an indicator of increased microbial biomass, especially the activity of heterotrophic bacteria capable of aggregating organic particles into stable flocs. These bacteria play a crucial role in recycling nitrogenous waste and enhancing the microbial loop within the system. The consistent addition of probiotics and molasses, applied in the morning prior to feeding, was instrumental in stimulating the development of these beneficial microbial communities. Molasses, serving as a carbon source, eerlanhances the C:N ratio, thereby favoring heterotrophic bacterial proliferation. Meanwhile, probiotics such as *Bacillus* species contribute directly to biofloc structure and stability, improve digestive enzyme secretion, and suppress pathogenic bacteria. The floc system developed in this study functioned not only as a biofilter but also as an additional natural feed source, rich in protein, lipids, and microbial biomass. This dual benefit supports both water quality management and nutritional enrichment for the cultured species. However, for this system to operate effectively, certain physical and environmental parameters must be maintained most notably, aeration.

As emphasized by Riani *et al.* (2012), aeration plays a pivotal role in suspending biofloc particles within the water column, maintaining high dissolved oxygen levels, and ensuring continuous circulation. Inadequate aeration can result in stratified zones with limited oxygen availability, causing floc particles to settle at the bottom, which may lead to localized anaerobic conditions, foul odors, and reduced floc efficacy. Therefore, adequate and evenly distributed aeration is essential not only to preserve the floc's physical structure but also to maintain microbial metabolism and fish respiration needs throughout the production cycle.

Hematocrit

Hematocrit in fish is the percentage of erythrocytes in the blood. It is used to measure the ratio between erythrocytes and plasma, thus reflecting the proportion of red blood cells within the total blood volume of catfish. The hematocrit results at the end of the study showed no significant differences based on Duncan’s post hoc test, indicating that the addition of probiotics in the biofloc system did not significantly affect the hematocrit levels in catfish compared to treatments without probiotic supplementation. Hematocrit levels were recorded at 18.73% in treatment P1, 24.79% in P2, 18.87% in P3, and 15.12% in P4. The highest hematocrit value was observed in P2, followed by P3 and P1, while the lowest was found in P4.

Fajriyani *et al*. (2017) stated that hematocrit levels below 30% may indicate erythrocyte differentiation. According to Rahmaningsih *et al*. (2018), hematocrit is a critical parameter in determining erythrocyte volume, with the normal hematocrit range for catfish being between 30.8% and 45.5%. The abnormal hematocrit values observed in this research are likely due to the influence of probiotics in the biofloc system, as the microbes contained in probiotics may affect the hematocrit levels in the red blood cells of catfish.

Hemoglobin

Hemoglobin is a component of erythrocytes, and its primary function is to transport oxygen and carbon dioxide. Hemoglobin plays a vital role in delivering oxygen through the bloodstream to the tissues of the fish’s body. Measuring hemoglobin levels is important to understand the oxidation process in the blood as it is distributed to the tissues (Susandi *et al.,* 2017). Based on the results of the study, hemoglobin levels were recorded at 7.56 g/dL in treatment P1, 7.06 g/dL in P2, 7.63 g/dL in P3, and 6.20 g/dL in P4. The highest hemoglobin level was found in P3, while the lowest was in P4. Overall, the hemoglobin levels were below the normal range.

Statistical analysis using ANOVA indicated no significant difference ($p>0.05$), suggesting that the addition of probiotics in the culture system did not significantly affect hemoglobin levels in catfish. Wilaksana & Arfianti (2021) reported that the normal hemoglobin concentration in catfish blood ranges from 9–13 g/dL, with healthy catfish typically having hemoglobin levels of 12–14 g/dL. According to Alipih & Sari (2020), hemoglobin concentration is positively correlated with erythrocyte count higher erythrocyte levels lead to higher hemoglobin levels, and vice versa. The findings of this study indicate that catfish cultured with probiotic supplementation exhibited hemoglobin levels below the normal range.

Erytrochytes

Red blood cells, or erythrocytes, are the most prevalent blood cell type in vertebrates and serve a crucial function in oxygen transport throughout the body. In fish, erythrocytes play a vital role in ensuring cellular respiration and overall metabolic activity. Under normal physiological conditions, erythrocytes can constitute up to 50% of total blood volume (Jabar *et al.,* 2023), and their abundance often reflects the health and stress status of the fish.

During the rearing period in this study, the erythrocyte counts observed were as follows: 2.26×10^4 cells/mm³ in treatment P1 (control), 3.62×10^4 cells/mm³ in P2, 3.97×10^4 cells/mm³ in P3, and 4.64×10^4 cells/mm³ in P4. The highest erythrocyte level was recorded in P4, which received the highest dose of probiotics in the biofloc system, while the lowest was in the control group (P1), which was not supplemented with probiotics. Statistical analysis confirmed a significant difference among treatments, indicating that the incorporation of probiotics in the biofloc system had a highly significant effect on red blood cell production in catfish.

According to Yanto *et al.* (2015), the normal erythrocyte count in *Clarias gariepinus* (African catfish) is approximately 3.18×10^4 cells/mm³, suggesting that fish in all probiotic treatments (P2–P4) remained within or exceeded this healthy reference range. The elevated erythrocyte levels, particularly in treatment P4, may indicate enhanced hematopoietic activity, improved oxygen transport capacity, and better physiological adaptation to the biofloc environment.

The increase in erythrocyte count observed in biofloc treatments is likely due to the positive impact of probiotics on fish health. Probiotics can improve gut microflora, nutrient absorption, and immune system stimulation, which in turn supports hematological balance. Furthermore, the biofloc system provides a more stable water quality environment, rich in microbial protein and natural immunostimulants, thereby reducing physiological stress and promoting better blood health in fish.

Leukocytes

The total white blood cell count, or leukocytes, obtained in this research did not differ significantly among treatments. The application of probiotics in the biofloc system did not result in statistically significant differences, as indicated by Duncan's test at a 0.05 significance level. The leukocyte counts recorded were 2.25×10^3 cells/mm³ for P1, 2.41×10^3 cells/mm³ for P2, 2.48×10^3 cells/mm³ for P3, and 2.54×10^3 cells/mm³ for P4. The highest leukocyte count was observed in treatment P4, which included probiotic supplementation, while the lowest was found in the control group (P1), which did not receive probiotics.

According to Nainggolan *et al.* (2021), the normal range of total white blood cells in catfish is approximately 34,500 cells/mm³. If probiotics are incorporated into feed, they can directly influence leukocyte levels, as the probiotics are ingested and enter the fish's system directly. However, the leukocyte counts obtained in this study were not within the normal range for catfish.

Despite the lower absolute values, the relative pattern of leukocyte increase from P1 to P4 may still reflect a positive immune-modulating effect of probiotic application in the biofloc system. Probiotics are known to enhance nonspecific immune responses, including leukocyte activity, macrophage proliferation, and phagocytic function. When administered consistently, either through feed or water, probiotics such as *Bacillus* sp. and *Lactobacillus* sp. can stimulate immune cell production and improve disease resistance.

Differential Leukocytes

The differential leukocyte count in catfish consists of three main types: monocytes, lymphocytes, and neutrophils. Based on the results of Duncan's post-hoc test, the application of probiotics in the biofloc system showed a significant effect on the levels of monocytes, lymphocytes, and neutrophils. The monocyte counts obtained were 7.66% in P1, 8.33% in P2, 11.66% in P3, and 11.33% in P4. Monocytes play a critical role in the fish immune system, particularly in phagocytosis the ability to engulf and digest foreign particles or microorganisms.

According to Preanger *et al.* (2016), the normal monocyte percentage in teleost fish is approximately 0.1% of the total leukocyte population, but it can increase rapidly (within 48 hours) following exposure to foreign substances such as carbon particles. The monocyte values obtained in this study (7.66–11.66%) were above the normal range.

The lymphocyte percentages were 67.33% for P1, 75.33% for P2, 82.33% for P3, and 90.33% for P4. As reported by Preanger *et al.* (2016), the normal range of lymphocytes in catfish is 71.12–82.88%. The lymphocyte values in this study mostly fall within or slightly above the normal range.

For neutrophils, the values recorded were 7.33% in P1, 8% in P2, 9% in P3, and 10% in P4. Preanger *et al.* (2016) noted that the normal range of neutrophils in catfish is between 6–8%. Therefore, the neutrophil counts in treatments P3 and P4 slightly exceeded the normal range, possibly indicating an immune response induced by the probiotic-supplemented biofloc environment.

Water Quality

The water quality observed from the beginning of the research until the end of the rearing period showed that the addition of probiotics in the biofloc system did not affect the water quality for catfish. The water quality throughout the research remained within the normal range for catfish growth. The recorded temperature ranged from 28.4-29.6°C, pH levels ranged from 7.0-7.9, dissolved oxygen (DO) levels ranged from 6.1-7.9 mg/L, and ammonia levels ranged from 0.08-0.99 ppm. These results indicate that water quality remained within the optimal range and was suitable to support the growth and survival of catfish. The addition of EM4 probiotics to the biofloc system in this research did not negatively impact water quality, as probiotic application in a biofloc system actually helps improve water quality compared to non-biofloc systems. This finding is consistent with the research by Sudirman *et al.* (2023), which reported that the biofloc system can improve water quality and enhance fish growth rates compared to non-biofloc systems. According to Partiono (2021), water quality is a limiting factor for the types of aquatic organisms that can be cultured in a given environment. Water quality plays a crucial role in determining the survival and growth success of farmed catfish.

CONCLUSION

Based on the results of the study, it can be concluded that the use of EM4 probiotics in the biofloc culture system significantly influenced the growth performance of catfish (*Clarias sp.*). The treatment P4 showed the most notable effects, with a final absolute weight gain of 11.66 grams and an absolute length increase of 8.11 cm. Furthermore, the probiotic addition had a significant impact on erythrocyte levels and differential leukocyte counts. In contrast, the use of probiotics in the biofloc system did not result in significant differences in hematocrit levels, total leukocyte counts, or biofloc volume.

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