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Effectiveness of kepok banana stem extract (*Musa Acuminata*) on the immune response of catfish (*Clarias gariepinus*) infected with *Aeromonas hydrophila*

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ABSTRACT

Catfish have high economic value, fast growth, and are easy to cultivate. This is the reason why catfish cultivation is increasing. However, there are still obstacles in the cultivation process, namely the threat of disease, one of which is the Motile Aeromonas Septicaemia (MAS) disease caused by *Aeromonas hydrophila* bacteria. The countermeasure can be done by using active compounds found in plants, including banana stems. This study aimed to determine the effectiveness of giving banana stem extract to carp culture media and treating *A. hydrophila* disease. This research used an experiment with a completely randomized design. The treatments tested were the addition of banana stem extract at a dose of 5% (treatment A), 10% (treatment B), 15% (treatment C), and antibiotics (treatment K-), and without extract treatment (treatment K+). Each treatment was repeated three times. The results showed that the administration of banana stem extract significantly affected the fish's erythrocytes, leukocytes, and blood glucose. Leukocyte differential observation showed that kepok banana stem extract had a significant effect on eosinophils, lymphocytes, and neutrophils but no significant effect on monocytes. In analyzing fish blood, the optimal values were 7.64±0.190a cells/mm3 for leukocytes, 3.60±0.116d cells/mm3 for erythrocytes, 65±6.028b mg/dl for glucose, 74.67±0.577d% for lymphocytes, 5.67±0.577a% for neutrophils, and 0.33±0% for eosinophils. The results indicate that treatment B with 10% kepok banana stem extract was the most effective in administering the extract to catfish infected with *A. hydrophila* bacteria.

Introduction

The freshwater fish farming business is increasingly lucrative and promising. According to a report by the UN Food Agency, in 2021, the quantity of fish consumed per capita by the world's population will reach 19.6 kg per year. Although fish consumption is currently supplied more by marine fish, 2018 freshwater fish production can rival capture fisheries. This is because capture fisheries will gradually decline due to overfishing. To meet the fish consumption needs of people in the world, it is necessary to increase the production of freshwater fish farming as a substitute for marine fish (Ministry of Marine Affairs and Fisheries, 2017).

One type of freshwater fish that is very popular in Indonesia is catfish (*Clarias gariepinus*). The fish is widely cultivated because it has high economic value, has high environmental adaptability, has fast growth, and is easy to grow. So, no wonder many people are interested in cultivating dumbo catfish. The development of dumbo catfish cultivation has obstacles that are often faced, namely the presence of diseases that attack the farmed dumbo catfish. According to Simatupang and Anggraini (2013), cultivation problems hamper efforts to increase production, including failures due to pathogenic fish outbreaks from the bacterial group. One disease that often attacks catfish is caused by *Aeromonas hydrophila* (Laith and Najiah, 2013).

Aeromonas hydrophila is an aquatic microorganism that resides in marine and fresh waters. The bacteria become pathogenic and are an opportunistic pathogen in fish hemorrhagic septicemia (red spot disease) under stress conditions (Yogananth *et al.*,

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Aeromonas hydrophila is an aquatic microorganism that resides in marine and fresh waters. The bacteria become pathogenic and are an opportunistic pathogen in fish hemorrhagic septicemia (red spot disease) under stress conditions (Yogananth *et al.*,

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2009; Mellisa *et al.*, 2024). Clinical symptoms in fish infected with *A. hydrophila* bacteria are slow movement, fish tend to stay at the bottom of the aquarium, sores on the infected area (Rahmaningsih, 2012), bleeding at the base of the tail fin and dorsal fin, and the lower abdomen looks distended and swollen (Agustina, 2007). This condition occurs after *A. hydrophila* infects two days

One of the efforts to treat catfish attacked by *A. hydrophila* bacteria is using phytopharmaceuticals. Phytopharmaceuticals can be used to prevent or treat fish diseases. Some phytopharmaceuticals that can be used in fish disease prevention efforts are curcuma (Sari *et al.*, 2012), turmeric (Muchtaromah, 2010), aloe vera extract (Kamaludin, 2011), papaya leaves (Sumiati, 2014), inai leaves (Karina *et al.*, 2015), and kepok banana stems (*Musa acuminata*). Kepok banana stems are often considered agricultural waste, making them readily available and inexpensive than those phytopharmaceuticals, it also lowers cost due to the abundance and the fact that the stems are typically discarded as a byproduct of banana cultivation (Hasanah *et al.*, 2023).

According to research by Ningsih *et al.* (2013), ethanol extract from the kepok banana stem has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Banana stems contain several secondary metabolite compounds, including alkaloids, tannins, flavonoids, and steroids. Flavonoid compounds have antibacterial activity by disrupting the metabolic functions of these microorganisms, damaging cell walls, and denaturing microorganism cell proteases (Ogofure and Emoghene, 2016).

This study aimed to treat *A. hydrophila* bacteria with kepok banana stem extract immersion. It knows the immune response in catfish after being given kepok banana stem extract. In addition, to determine the optimal concentration of kepok banana stem extract to improve the immune system in catfish.

Materials and Methods

Location and time of research

This research was implemented from March to April at the Fisheries Laboratory, Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang. The tools used include aerators, aeration hoses, 15 aquariums (40x30x30 cm3), measuring cups, pH meters, thermometers, DO meters, haemocytometer sets, siphon hoses, siphons, glucose test kits, syringes, microscopes, and analytical scales.

The materials used included catfish, pelleted feed, banana stem extract, *A. hydrophila* bacteria, 10% EDTA, truck, Giemsa, gloves, masks, and distilled water. The research design was completely randomized (CRD), with five treatments and three replications. The treatment details are as follows refers to Ningsih *et al,* (2013):

A: 5% (50 ml/L) kepok banana stem extract
B: 10% (100 ml/L) kepok banana stem extract
C: 15% (150 ml/L) kepok banana stem extract
K-: A. hydrophilla bacteria with antibiotic-soaking treatment
K+: A. hydrophilla bacteria without banana stem extract.

The research stages to be carried out consist of three steps, including (1) preparation; (2) making banana stem extract; and (3) testing banana stem extract on catfish.

The Research Procedure

The containers used for this study were 15 aquariums sized 40 x 30 x 30 cm. Before using the aquarium, the tanks were cleaned with soap after soaking in chlorine for half an hour to kill microorganisms that may stick, then rinsed and dried for one day. Next, the aquariums were filled with 30 liters of water with a density of 10 dumbo catfish sized 12 - 15 cm (Agustina, 2007). After that, aeration was installed to supply oxygen to the water. After that, the aquarium was labeled with the treatment. After that, the water in the aquarium remained aerated for several days before the test fish were put into the aquarium. Dumbo catfish were acclimatized to the aquarium for approximately seven days. During the acclimatization period, catfish were fed regularly every morning at 08.00 WIB and in the afternoon at 16.00 WIB.

The preparation of banana stem extract, referred to by Fitriahani (2017), was done by washing the banana stem first to remove the dirt that sticks to it and then slicing or chopping to facilitate the mashing process. Banana stem pulverization was done by blending. Once smooth, separate the pulp from the filtrate using a sieve. The obtained filtrate was then macerated with 70% ethanol (1:1 ratio) for 24 hours using a magnetic stirrer. The obtained maceration results were then filtered using a vacuum pump and evaporated with an evaporator to remove the remaining ethanol. The banana stem extract that had been obtained was stored in the refrigerator (at 4 °C) until it was ready for use. The five kilograms of kepok banana stem material produced as much as 1.5 liters of extract.

Testing banana stem extract on catfish was intended to determine the effect of kepok banana stem extract on the immune response of catfish

infected with *A. hydrophila* bacteria. The research implementation can be done as follows:

- Entering banana stem extract according to the treatment concentration. Observing the test parameters at the end of fish rearing.
- Analyzing and collecting research parameters, including data on total erythrocytes, total leukocytes, differential leukocytes, and glucose levels in fish blood.

Erythrocyte Total

The calculation of total erythrocytes was that the blood sample was sucked with a pipette containing a red stirring grain to a scale of 0.5, and then Hayem's solution was added to a scale of 101. The blood in the pipette is stirred by shaking the pipette in a figure-eight shape for 3-5 minutes so that the blood is evenly mixed. The first two drops of blood solution in the pipette were discarded, and then the blood solution was dripped on a hemocytometer with a glass cover (Svobodova *et al.*, 1991). The number of red blood cells could be counted under a microscope with 400x magnification. Calculations were made on five large boxes of hemocytometers, and the number was calculated by the formula (Nabib and Pasaribu, 1989):

Total Leukosit

The total leukocyte count was that the sample blood was sucked with a pipette containing a white stirring bulb to a scale of 0.5, and then Turk's solution was added to a scale of 11 (Amlacher, 1970). The blood in the pipette was stirred by shaking the pipette in a figure eight shape for 3-5 minutes so that the blood was evenly mixed. The first two drops of blood solution in the pipette were discarded, then the blood solution was dripped on a hemocytometer with a glass cover. The number of red blood cells could be counted under a microscope with 400x magnification. Calculations were made on five large boxes of hemocytometer, and the number was calculated by the formula Svobodova *et al.* (1991).

Diferensial Leukosit

The leukocyte (white blood cell) differential was measured to determine the percentage of each type of leukocyte present in the blood. The counting was done by observing the blood review preparations. A drop of blood was placed on a clean (methanol-soaked) glass slide, and the tip of the second glass slide was placed on top of the first glass slide to form a 30° angle. The second object glass was slid towards the back, touching the blood drop until it spread. Then the second object glass was shifted in the opposite direction until a thin layer of blood was formed, left to dry. The preparations were fixed using absolute methanol for 5 minutes, removed, and air-dried. The trials were stored for 10 minutes in a staining container with Giemsa solution, then removed, rinsed with running water, and allowed to air dry. Observation of the preparations was carried out under a microscope using immersion and observed at 400 times magnification. Leukocyte differentials were calculated based on their type (monocytes, lymphocytes, and neutrophils) using the following formula (Hartika *et al.*, 2014) :

$$Monocyte\ percentage\ (\%) = \frac{the\ number\ of\ monocytes}{100\ leukocyte\ cell} \times 100$$

$$Lymphocyte\ percentage\ (\%) = \frac{the\ number\ of\ lymphocytes}{100\ leukocyte\ cell} \times 100$$

$$Neutrophil\ percentage\ (\%) = \frac{the\ number\ of\ neutrophils}{100\ leukocyte\ cell} \times 100$$

$$Eosinophil\ percentage\ (\%) = \frac{the\ number\ of\ eosinophils}{100\ leukocyte\ cell} \times 100$$

Glucose

Glucose observation parameters use enzymatic methods that generally use enzyme work, one of which is the strip test. The principle of this method was that blood was dripped on the reaction zone of the strip test. There was the enzyme glucose dehydrogenase and the coenzyme (PQQ). The glucose catalyst is reduced in the blood. The intensity of the electrons formed in the strip is equivalent to the glucose concentration in the blood. After being given a blood sample, the gold metal on the strip will react with the electrodes on the gold strip and glucose oxidase, which produces harmless DC electricity, so that the check tool can interpret blood sugar (Susanto *et al.*, 2014).

Data analysis

Data analysis was obtained from quantitative data and analyzed by descriptive analysis and ANOVA (analysis of variance) diversity analysis using a completely randomized design (RAL). If there was a difference in the effect on the treatment, the BNT (Smallest Real Difference) further test was carried out with a real level of 5%.

Results

According to the results of the observation of total erythrocytes in catfish infected with *A. hydrophila* bacteria and then given kepok banana stem extract, the highest erythrocyte value was found in treatment C (15% extract), reaching 3.60 ± 0.116d cells/mm^3, and the lowest results were found in treatment K- (giving antibiotics), namely 1.92 ± 0.025a cells/mm3. Based on the results of the ANOVA, it was found

that kepok banana stem extract had a significant effect (P<0.05) on the total erythrocyte count. The administration of kepok banana stem extract in treatment A (5% extract) was not significantly different from treatment K- (antibiotics). In comparison, treatments B (10% extract), C (15% extract), and K+ (no extract treatment) were significantly different from other treatments. These results indicated that administering kepok banana stem extract in treatment C (15% extract) could increase the number of erythrocytes in the blood of catfish infected with *A. hydrophila*. The graph of the erythrocytes can be seen in Figure 1.

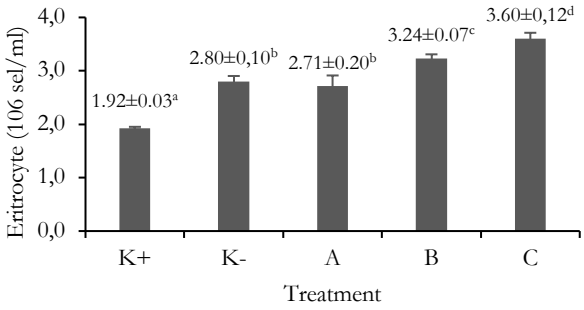

Figure 1. Total eritrocytes

The results of the observation of total leukocytes in dumbo catfish infected with *A. hydrophila* bacteria and then given the extract of kepok banana stems (Fig. 2), the highest leukocyte value was found in the K+ treatment (without extract treatment), contributing 10.1 ± 0.204c cells/mm3, and the lowest results were found in treatment B (10% extract), namely 7.64 ± 0.190a cells/mm3. The ANOVA results showed that the kepok banana stem extract significantly affected the total leukocyte count (P<0.05). The administration of kepok banana stem extract in treatments A (5% extract), C (15% extract), and K- (antibiotic treatment) was not significantly different. In comparison, treatments B (10% extract) and K+ (no extract treatment) significantly differed from other treatments.

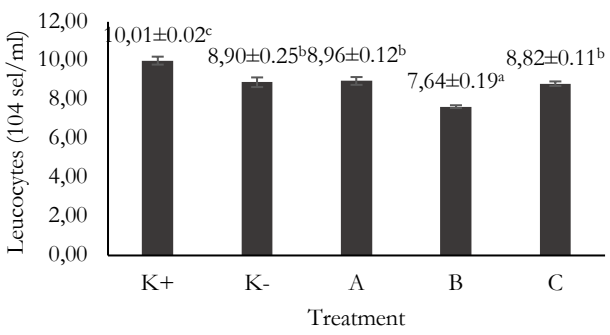

Figure 2. Total Leucocytes

This shows that administering kepok banana stem extract in treatment B (10% extract) could reduce the number of leukocytes in fish, reducing infection caused by *A. hydrophila* bacteria (Figure 2).

Based on the observation of the percentage of lymphocytes in the blood of catfish infected with *A. hydrophila* bacteria and then given a kepok banana stem extract, the highest lymphocyte value was found in treatment B (10% extract), which was 74.67 ± 0.577%, and the lowest result was found in treatment K- (antibiotic administration), which was 63.66 ± 0.577%. The ANOVA results show that kepok banana stem extract significantly affects lymphocyte numbers (P <0.05). The administration of kepok banana stem extract in treatments A (5% extract) and K+ (no extract treatment) was not significantly different. Meanwhile, treatments B (10% extract), C (15% extract), and K- (antibiotic treatment) were very different from other treatments. These results indicate that administering kepok banana stem extract in treatment B (10% extract) could increase the number of fish lymphocytes in fish, reducing infection in fish caused by *A. hydrophila* bacteria. The graph of the results of the value of lymphocytes can be seen in Figure 3.

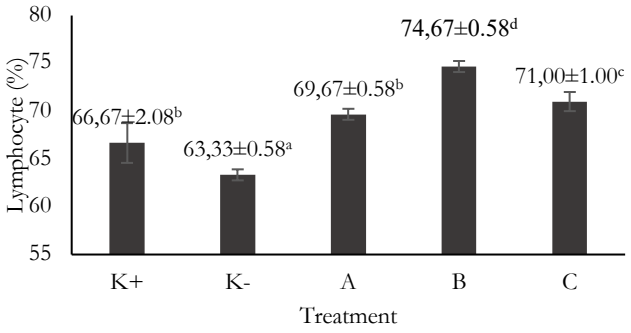

Figure 3. Total Lymphocyte

Based on the observation of monocytes of blood catfish dumbo catfish infected with *A. hydrophila* bacteria and then given kepok banana stem extract, the highest monocyte value was found in treatment B (10% extract), which was 19.67 ± 0.577%, and the lowest result was found in treatment K- (giving antibiotics), sharing 13.33 ± 0.577%. The ANOVA test results show that kepok banana stem extract did not significantly affect catfish monocytes (P>0.05). The administration of kepok banana stem extract in treatments A (5% extract), K+ (without extract treatment), B (10% extract), C (15% extract), and K- (antibiotic treatment) were not significantly different. Graphs of monocyte percentage results can be seen in Figure 4. (Add notations that show real differences between treatments)

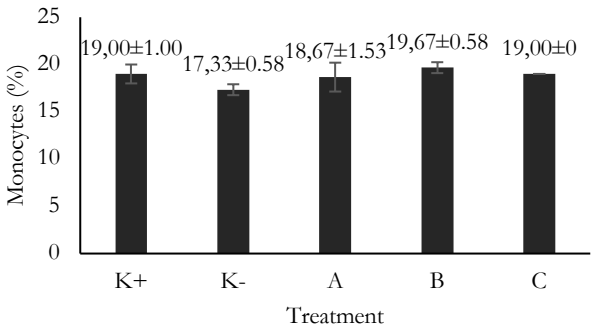

Figure 4. Monocyte Percentage

The results of the observation of neutrophil percentage in dumbo catfish infected with *A. hydrophila* and then given kepok banana stem extract, the highest neutrophil value was found in treatment K- (antibiotic administration), reaching 14.33 ± 0.557%, and the lowest result was found in treatment B (10% extract), namely 5.67 ± 0.577%. Based on the results of the ANOVA test, it was found that kepok banana stem extract had a significant effect (P <0.05) on the total percentage of neutrophils. The administration of kepok banana stem extract in treatments A (5% extract), C (15% extract), and K+ (no extract treatment) was not significantly different. In comparison, treatments B (10% extract) and K- (antibiotic administration) significantly differed from other treatments. This shows that administering kepok banana stem extract in treatment B (10% extract) could reduce the percentage of neutrophils in fish, reducing infection caused by *A. hydrophila* bacteria. The graph of neutrophil percentage results can be seen in Figure 5.

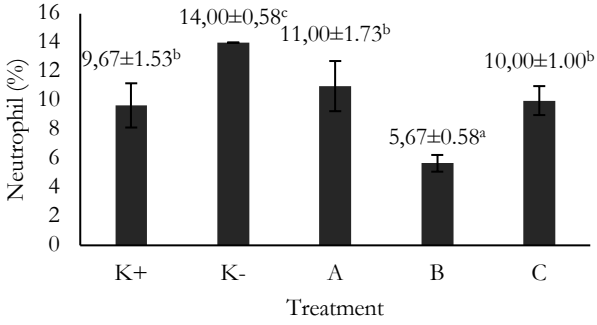

Figure 5. Neutrophil Percentage

The percentage value of eosinophils in the observation of catfish infected with *A. hydrophila* and then given kepok banana stem extract, the highest eosinophil value was found in treatment K- (antibiotic administration), having 5 ± 0%, and the lowest results were in treatment B (10% extract), namely 0 ± 0%, and treatment C (15% extract), namely 0 ± 0%. Based on the results of the ANOVA test, it was shown that kepok banana stem extract had a significant effect (P < 0.05) on the total percentage of eosinophils. The administration of kepok banana stem extract in treatment A (5% extract) was significantly different from other treatments, while treatments B (10% extract) and C (15% extract) were not significantly different. Treatment K+ (without extract treatment) and K- (antibiotic treatment) were not quite different. This shows that administering kepok banana stem extract in treatments B (10% extract) and C (15% extract) can reduce the percentage of eosinophils in fish, which means it can reduce infection in fish caused by A. hydrophilla bacteria. The graph of the rate of eosinophil results can be seen in Figure 6.

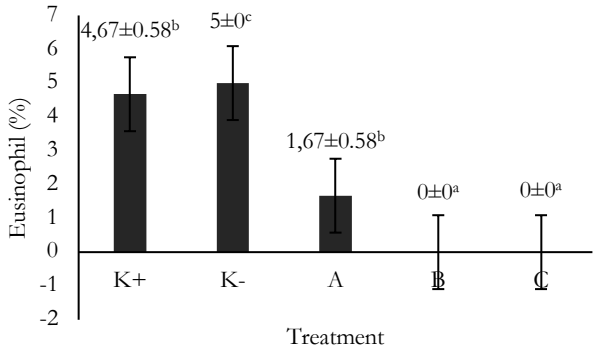

Figure 6. Eusinophil Percentage

The results of glucose observations in dumbo catfish infected with *A. hydrophila* bacteria and then given kepok banana stem extract got the highest glucose value in treatment K + (without extract treatment), which was 100 ± 9,504 mg/dl. The lowest result was in treatment B (10% extract), 65 ± 6,028 mg/dl. Based on the results of the ANOVA test, it was shown that kepok banana stem extract had a significant effect (P <0.05) on glucose values. The administration of kepok banana stem extract in treatments B (10% extract), C (15% extract), K+ (without extract treatment), and K- (administration of antibiotics) were not significantly different. Treatment A (5% extract) significantly differed from other treatments. These results indicate that administering kepok banana stem extract in treatment B (10% extract) can reduce glucose levels in the catfish's blood, reducing infection in fish caused by A. hydrophilla bacteria. A graph of the value of glucose levels can be seen in Figure 7. Results in Figure 7 show that the values of K+, K-, treatment of B and C are not significantly different, but the value of glucose levels is quite far away. (Please review the data and analysis results.)

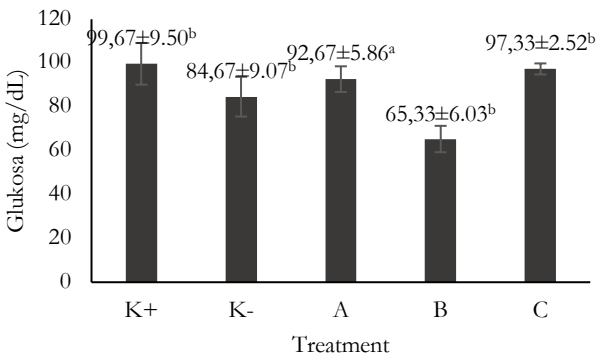

Figure 7. Glucose Value

Discussion

The low value of erythrocytes in fish blood can cause fish difficulty taking oxygen in the water, even though the availability is abundant. As a result, fish will lack oxygen, which can cause death. According to Rahma *et al.* (2015), fish's anoxia (lack of oxygen) can be caused by a lack of erythrocyte levels in their blood. Low erythrocyte values indicate anemia, while high erythrocyte values indicate that fish are experiencing stress (Purwanto, 2006). Erythrocytes in the blood are hemoglobin carriers that carry oxygen to all body tissues. Based on these results, it can be seen that treatment C (15% extract) was the treatment that had the best total erythrocyte results from other treatments, it is indicating that the extract helps in maintaining a healthier erythrocyte level, thus ensuring better oxygen transport and reducing the risk of anemia and anoxia (Kasem *et al.*, 2012)). Some plant extracts possess antioxidant compounds that can protect erythrocytes from oxidative damage, enhancing their lifespan and function (Goldefroy *et al.*, 2023).

High leukocyte counts in fish blood may indicate that the fish is diseased. Leukocytes, or white blood, are the part of the blood that produces antibodies. Infected fish will tend to have more leukocytes than healthy fish. An increase in leukocyte cells can occur due to an infection that triggers leukocyte cell division (Hardi *et al.*, 2011). Arry (2007) stated that the increase in leukocytes occurs due to the response of the fish body to poor environmental conditions, stress factors, and disease infection. The number of leukocytes in fish infected with pathogens will increase as a defense effort (Martins *et al.*, 2008). Based on these data, it can be seen that treatment B (10% extract) was the treatment that had the best total leukocyte results from other treatments.

The normal range of fish lymphocytes is between 74-86% (Preager *et al.*, 2016). Based on these results, it can be seen that treatment B had better lymphocyte values than other treatments. The decrease in the number of lymphocytes is because lymphocytes are the body's leading defense against infection (Alamanda *et al.*, 2006). Lymphocytes are not phagocytic but play an essential role in antibody formation (Baratawidjaja 2012). The immune system of catfish in treatment B with a dose of 10% proved that administering kepok banana stem extract could increase the number of lymphocytes and reduce infection in catfish attacked by *A. Hydrophila*.

In fish blood, the value of monocytes is deficient in leukocytes, which is 0.1-3% (Andayani *et al.*, 2010). The increasing number of monocytes in fish blood is thought to be a fish phagocytosis process against pathogen attacks on the body. Suhermanto *et al.* (2013) stated that monocytes function in phagocytosis against harmful pathogen attacks and to remove dead or damaged cells from the blood. The number of monocytes in fish will increase quickly if the fish is infected (Robert, 2012).

The number of neutrophils in normal fish blood ranges from 6-8% (Robert, 2012). The high percentage of neutrophils in fish blood can be caused by fish stress (Listiyanti, 2011), which states that a high percentage of neutrophils is likely due to stress. The increase in neutrophil rate results from the immune mechanism that works in response to infection in the fish body (Utami *et al.*, 2013).

According to Gunanti *et al.* (2011), the average percentage of eosinophils in healthy fish is 1.4%. The increasing number of eosinophils in the blood indicates that parasites or diseases attack the fish. This is because eosinophils in leukocytes have a function or relationship with parasitic infections, so the high percentage of eosinophils in fish leukocytes indicates the extract helps manage eosinophil levels, indicating a better ability to combat parasitic infections and maintain a healthy immune response (Gunanti *et al.*, 2011).

A good or standard fish glucose value ranges from 40-90 mg/dl (Rahardjo *et al.*, 2011). In these results, it can be seen that treatment B (10% extract) had an average glucose value, and the results in treatment K- (without extract treatment) showed that the glucose value was above the standard value. High glucose levels can indicate that fish are stressed (Sulmartiwi *et al.*, 2013). Based on the results above, it can be concluded that treatment B (10% extract) had the best glucose level results from other treatments.

Conclusion

The administration of banana stem extract can enhance the immune response of catfish (Clarias gariepinus) infected with Aeromonas hydrophila and

54 significantly affect the erythrocytes, leukocytes, and blood glucose of fish.

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