

THE EFFECT OF *Rhizophora mucronata* LEAF EXTRACT ON HAEMATOLOGY OF TILAPIA (*Oreochromis niloticus*) AGAINST *Aeromonas hydrophila*

Pengaruh Ekstrak Daun *Rhizophora mucronata* Terhadap Hematologi Ikan Nila (*Oreochromis niloticus*) yang Diuji Tantang *Aeromonas hydrophila*

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ABSTRACT

Tilapia (*Oreochromis niloticus*) is one of the fishery commodities that is widely cultivated. One of the threats to tilapia cultivation is disease attacks, especially diseases caused by the bacteria *Aeromonas hydrophila*. Therefore, it is necessary to control this disease by using new, environmentally friendly immunostimulants. In the context of immunostimulants, the mangrove *Rhizophora mucronata* is a plant that is reported to contain secondary metabolites that are useful for increasing immunity. The method in this research was experimental with a completely randomized design of 5 treatments and 3 replications. The results of the study showed a significant increase in total erythrocytes, hemoglobin and hematocrit in treatments B, C and D. Meanwhile, in treatment A there was an insignificant increase and in treatment E there was a significant decrease. The average values of total erythrocytes, hemoglobin and erythrocytes on all treatments on days 7 and 14 except treatment E were within the normal range. The conclusion in this study showed >96% increase and the average value of total erythrocytes, hemoglobin and hematocrit was influenced by administering *R. mucronata* leaf extract to tilapia (*O. niloticus*) which was tested against *A. hydrophila* bacteria with an optimal dose of $27 \pm 0.2 \text{ mg.kg}^{-1}$.

Keywords: *Aeromonas hydrophila*, Haematology, Immune Response, *Oreochromis niloticus*, *Rhizophora mucronata*

ABSTRAK

Ikan nila (*Oreochromis niloticus*) merupakan salah satu komoditas perikanan yang banyak dibudidayakan. Salah satu ancaman pada budidaya ikan nila adalah serangan penyakit, khususnya penyakit akibat bakteri *Aeromonas hydrophila*. Oleh karena itu, perlu untuk pengendalian penyakit ini salah satunya dengan imunostimulan baru yang ramah lingkungan. Dalam konteks imunostimulan, mangrove *Rhizophora mucronata* adalah tumbuhan yang dilaporkan memiliki kandungan metabolit sekunder yang bermanfaat untuk peningkatan kekebalan. Metode dalam penelitian ini merupakan eksperimental dengan rancangan acak lengkap 5 perlakuan dan 3 ulangan. Hasil penelitian menunjukkan peningkatan secara signifikan

total eritrosit, hemoglobin dan hematokrit pada perlakuan B, C dan D. Sedangkan pada perlakuan A terjadi peningkatan yang tidak signifikan dan perlakuan E terjadi penurunan yang signifikan. Nilai rata-rata total eritrosit, hemoglobin dan eritrosit pada pada semua perlakuan hari ke-7 dan ke-14 kecuali perlakuan E berada dalam kisaran normal. Kesimpulan dalam penelitian ini menunjukkan >96% peningkatan dan nilai rata-rata dari total eritrosit, hemoglobin dan hematokrit dipengaruhi oleh pemberian ekstrak daun *R. mucronata* terhadap ikan nila (*O. niloticus*) yang diuji tantang bakteri *A. hydrophila* dengan dosis optimal sebesar $27 \pm 0,2 \text{ mg.kg}^{-1}$.

Kata Kunci: *Aeromonas hydrophila*, Hematologi, *Oreochromis niloticus*, Respon Imun, *Rhizophora mucronata*

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the fishery commodities that is widely cultivated. This is because of the people's superiority and passion for this fish (Windarto *et al.*, 2023). One of the advantages of tilapia fish is its euryhaline. This means that tilapia can be cultivated in fresh and brackish waters in coastal and marine areas (Windarto *et al.*, 2023). Apart from that, tilapia has advantages in immunity and physiological adaptability to high salinity ranges (Fuadi *et al.*, 2021). The increase in tilapia production is also driven by food requirements to support human population growth (McMurtrie *et al.*, 2022). So the level of market demand, both domestic and foreign, will continue to increase (Mandasari *et al.*, 2023).

At the same time, the increase is still hampered by several threats that can be detrimental, such as disease attacks, especially due to the bacteria *Aeromonas hydrophila*. This bacteria infects all organs of the fish's body accompanied by hemorrhage (Arwin *et al.*, 2016). *A. hydrophila* infection causes Motile Aeromonas Septicemia (MAS) disease and mass death (Salam *et al.*, 2021). Clinically, this disease has symptoms such as ulceration, bleeding, anemia and abscesses (Diab *et al.*, 2023). Therefore, it is necessary to control this disease by using new, environmentally friendly immunostimulants.

In the context of immunostimulants, the mangrove *Rhizophora mucronata* plant is reported to contain secondary metabolites that are useful for increasing immunity (Mulyani *et al.*, 2020). It has been reported that several ingredients in the *R. mucronata* mangrove, such as flavonoids and alkaloids, can modulate immunity (Fahrurrozi *et al.*, 2021). Modulation related to secondary metabolites is by optimizing the humoral immune response (Habotta *et al.*, 2022). This research is important because tilapia cultivation in coastal areas will be hampered by the threat of disease. It is hoped that the use of plants in coastal areas for coastal cultivation commodities can be a solution for developing sustainable product management and developing environmentally friendly technology. Therefore, this study aims to determine the effect of *Rhizophora mucronata* leaf extract on the hematology of tilapia (*Oreochromis niloticus*) which was tested against *Aeromonas hydrophila* bacteria.

METHODS

Time and Place

This research was carried out in March 2024, at the Unikal Krapyak Lor Fisheries Laboratory, North Pekalongan, Pekalongan City.

Tools and Materials

Tools and materials that will be used in this research include tilapia (*Oreochromis niloticus*), *Rhizophora mucronata* leaf extract powder, *Aeromonas hydrophila* bacteria, test aquarium, aerator set, digital scales, TSA, TSB, 1 mL syringe, microtube, incubator, scapel, anticoagulant, petridish, measuring cup, and Hematology Analyzer.

Research Design

The design in this study used a completely randomized design (CRD), with 5 treatments and 3 replications and the research method was experimental. In this study, there was a scheme in the form of positive control treatment or A (treatment without administration of extract and infection), treatment B (administration of *R. mucronata* extract at a dose of 20 mg.kg⁻¹ with *Aeromonas hydrophila* infection), treatment C (administration of *R. mucronata* extract at a dose 30 mg.kg⁻¹ with *Aeromonas hydrophila* infection), treatment D (administration of *R. mucronata* extract at a dose of 40 mg.kg⁻¹ with *Aeromonas hydrophila* infection) and treatment E (*Aeromonas hydrophila* infection without administration of extract).

Research Procedure

The first stage of the research procedure carried out was taking *Rhizophora mucronata* leaves and extracting them using methanol solvent according to previous research procedures (Fahrurrozi *et al.*, 2021). The same thing was done by referring to previous research on bacterial culture test procedures. *A. hydrophila* bacteria were isolated and cultured until they reached a bacterial density of 1.5 x 10⁶ cfu/ml (Rozi *et al.*, 2022). Then prepare 15 test aquariums with a volume of 15 liters/aquarium. Tilapia fish (*Oreochromis niloticus*) with a size of ± 18 grams/fish were then acclimatized in test media with a density of 1 fish/2 liters (Sari, 2017).

Fish are kept and fed 3 times a day with commercial feed at 3% of the biomass. The research was carried out for 14 days to observe the humoral profile of tilapia fish. *Rhizophora mucronata* leaf extract was injected into each tilapia 2 times, namely on day 1 and day 4. On the 7th day, a challenge test was carried out with *Aeromonas hydrophila* bacterial infection. Checking research parameters in the form of erythrocytes, hemoglobin and hematocrit was carried out twice on day 7 (before the challenge test) and day 14 (after the challenge test).

Test Parameters

Sampling and calculation of test parameters were carried out by taking fish blood using the Caudal Vena Punctie technique using a 1 ml syringe. The procedure for taking blood samples is to make the fish unconscious using anesthesia first in an ice water bath. A syringe containing 0.2 ml of 0.1% EDTA is used to take blood samples below the linea lateralis (midline) on the fish's body. The needle is injected into the muscle until it touches the spine and withdrawn with no bubbles. The blood sample is put into a micropipette with EDTA solution for further laboratory testing. Laboratory testing was carried out to calculate total erythrocytes, hemoglobin (Hb), and hematocrit levels in fish blood using a hematology analyzer (Sysmex XT-2000iV, Japan) (Rozi *et al.*, 2022).

Data Analysis

The data analysis that will be used is adapted measures to see changes from the same sample in two time periods. Then it was tested using one-way ANOVA which was further tested with Tukey to see the effect on the differences and accuracy of the data, as well as linear regression as an analysis tool to see the percentage of effect and optimal dose (peak point) of the treatment on the parameters to be observed.

RESULT

The research results on all previous observation parameters were analyzed using normality and homogeneity tests, as a condition for repeated measures analysis and one-way ANOVA. The results of the normality analysis show a significance value of >0.05 for all parameters, which means that the observation data is normally distributed. Apart from that, for homogeneity analysis the results obtained for all observation parameter data also have a

significance value of >0.05 with the understanding that the parameter data is homogeneous. This shows that the data in this study meets the requirements to continue with further analysis.

Total Erythrocytes

Results of observations of total erythrocytes on day 7 and day 14 for the 5 treatments in this study. The observation results showed that there was a significant increase in total erythrocytes in treatments B, C and D between the time period before and after the challenge test using *A. hydrophila* bacteria. Meanwhile, total erythrocytes in treatments A and E experienced a significant decrease after the challenge test. These results were analyzed using repeated measures which can be seen in the bar diagram with the notation (a, b) as a symbol of the significance of the difference in the increase and decrease in total erythrocytes (Figure 1).

Analysis of total erythrocytes using the one-way ANOVA test which was further tested with Tukey, the results can be seen in the column with the notation (a, b, c, d) as a symbol of the significance of the difference in average values (Figure 1). The different notations for all treatments on day 7 showed that the total erythrocyte values were significantly different with the highest value shown in treatment C at $1.2 \pm 0.1 \times 10^6$ cells/mm³, while the lowest was in treatment A ($1.1 \pm 0.4 \times 10^6$ cells/mm³) and E ($1.1 \pm 0.3 \times 10^6$ cells/mm³). The same results were shown on day 14 for the highest and lowest values, however there were significant differences in treatments B and D after the challenge test.

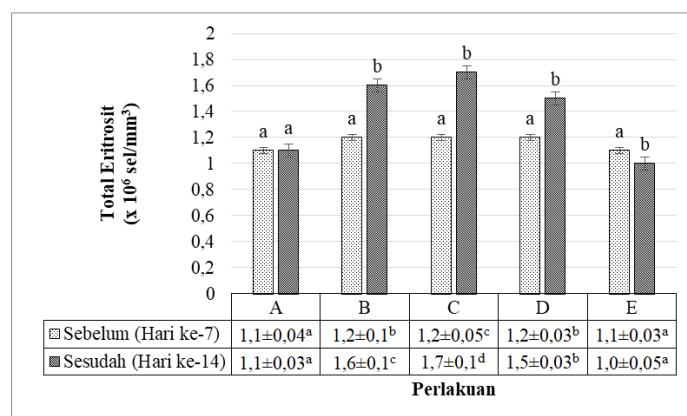


Figure 1. Results of Observations of Total Erythrocytes

The percentage effect was analyzed using a regression test to see the effect of treatment on the observed parameters (Figure 2). Then part of the regression is used to find the peak point using a formula to determine the optimal dose. The results of the analysis showed that the coefficient of determination (R^2) of mangrove leaf treatment on total erythrocytes on day 7 was 0.7975 or it could be interpreted that mangrove leaves could influence the total erythrocytes value with an influence of 80%. Meanwhile, on the 14th day after the challenge test, the coefficient of determination (R^2) was 0.9558 or an influence percentage of 96%. The resulting pattern is quadratic, so the optimal dose can be determined by entering the equation in the peak point formula. The estimated optimal dose was determined at the peak point on day 14 or after the challenge test. The calculation results showed that a value of 27.3 mg.kg^{-1} was the optimal dose with a total erythrocyte production of 1.7×10^6 cells/mm³.

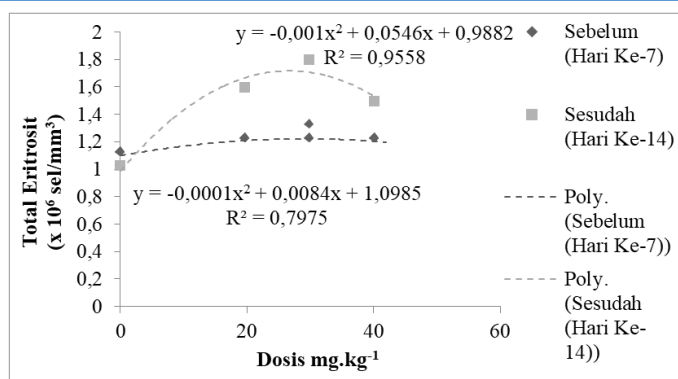


Figure 2. Analysis of The Percentage of Influence and Determination of The Optimal Dose of Total Erythrocytes

Hemoglobin (Hb)

The research results on Hb were obtained from observations on the 7th day and 14th day for the 5 treatments in this study. The observation results showed that there was a significant increase in Hb in treatments B, C and D between the time period before and after the challenge test using *A. hydrophila* bacteria. Meanwhile, Hb in treatment A did not increase significantly and E experienced a significant decrease in Hb after the challenge test. These results were analyzed using repeated measures which can be seen in the bar diagram with the notation (a, b) as a symbol of the significance of the difference in increase and decrease in Hb (Figure 3).

Hb analysis used the one-way ANOVA test which was further tested with Tukey. The results can be seen in the column with the notation (a, b, c, d) as a symbol of the significance of the difference in average values (Figure 2). The different notations for all treatments on the 7th day showed significantly different Hb values with the highest value shown in treatment C at 7.2 ± 0.2 g.dL⁻¹, while the lowest was in treatment A (6.2 ± 0.2 g.dL⁻¹) and E (6.2 ± 0.1 g.dL⁻¹). The same results were shown on day 14 for the highest and lowest values, however there were significant differences in treatments A and E after the challenge test. Where treatment E showed the lowest average value of 4.2 ± 0.1 g.dL⁻¹.

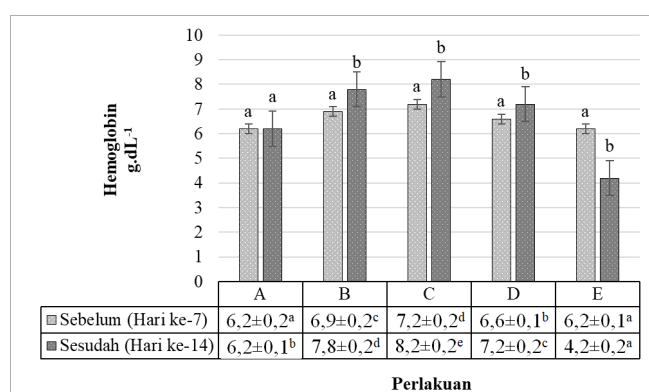


Figure 3. Results of Observations of Hemoglobin (Hb)

The percentage effect was analyzed using a regression test to see the effect of treatment on the observed parameters (Figure 4). Then part of the regression is used to find the peak point using a formula to determine the optimal dose. The results of the analysis show that the coefficient of determination (R^2) of the treatment of mangrove leaves on Hb on the 7th day is 0.8991 or it can be interpreted that mangrove leaves can influence the Hb value with an influence of 89%. Meanwhile, on the 14th day after the challenge test, the coefficient of determination (R^2) was 0.9966 or an influence percentage of 99%. The resulting pattern is

quadratic, so the optimal dose can be determined by entering the equation in the peak point formula. The estimated optimal dose of mangrove leaf extract was determined at the peak point on day 14 or after a challenge test. The calculation results showed that a value of 27.1 mg.kg⁻¹ was the optimal dose with a Hb that could be produced of 8.2 g.dL⁻¹.

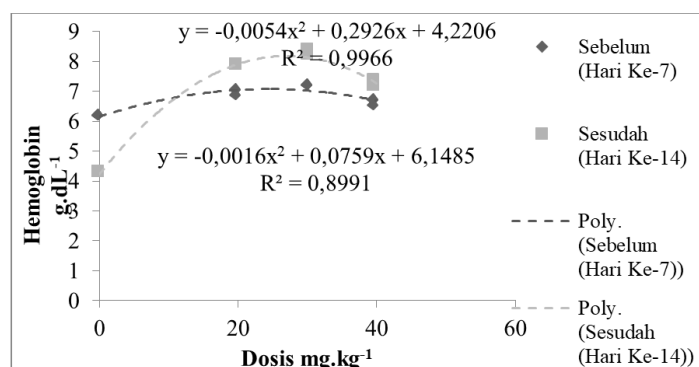


Figure 4. Percentage Analysis of Influence and Determination of Optimal Hemoglobin (Hb) Dose

Hematocrit

The results of research on hematocrit were obtained from observations on the 7th and 14th days for the 5 treatments in this study. The observation results showed a significant increase in hematocrit in treatments B, C and D between the time period before and after the challenge test using *A. hydrophila* bacteria. Meanwhile, the hematocrit in treatment A did not increase significantly and E experienced a significant decrease in hematocrit after the challenge test. These results were analyzed using repeated measures which can be seen in the bar diagram with the notation (a, b) as a symbol of the significance of the difference in increase and decrease in hematocrit (Figure 5).

Hematocrit analysis used the one-way ANOVA test which was further tested with Tukey. The results can be seen in the column with the notation (a, b, c, d) as a symbol of the significance of the difference in average values (Figure 6). The different notations for all treatments on the 7th day showed significantly different hematocrit values with the highest values shown in treatment C at 29.1 ± 2%, B (28.1 ± 1.7%) and E (27 ± 1.9%). Meanwhile, the lowest values were obtained in treatments A (24.1 ± 1.9%) and E (24 ± 1.8%). Different results were shown on day 14 in all treatments where each treatment had a different average value, except for treatments B and D. The highest hematocrit value was obtained in treatment C with a hematocrit value of 30.3 ± 2%. Meanwhile, the lowest hematocrit value was in treatment E or without administration of mangrove leaf extract which was challenged with *A. hydrophila* bacteria with a value of 19.6 ± 2%.

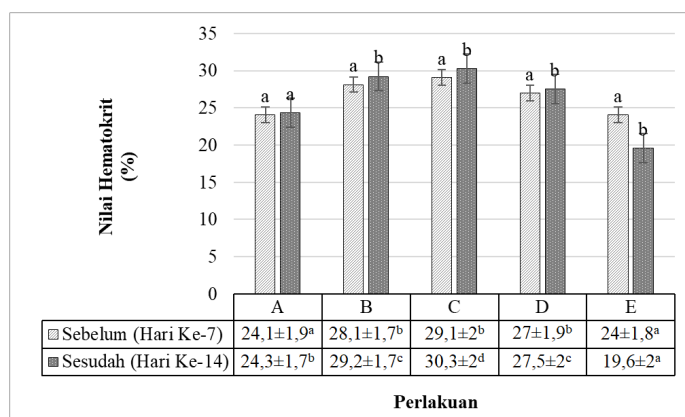


Figure 5. Hematocrit Observation Results

The percentage effect was analyzed using a regression test to see the effect of treatment on the observed parameters (Figure 6). Then part of the regression is used to find the peak point using a formula to determine the optimal dose. The results of the analysis show that the coefficient of determination (R^2) of mangrove leaf treatment on hematocrit on the 7th day is 0.9698 or it can be interpreted that mangrove leaves can influence the hematocrit value with an influence of 97%. Meanwhile, on the 14th day after the challenge test, the coefficient of determination (R^2) was 0.9979 or an influence percentage of 99%. The resulting pattern is quadratic, so that the optimal dose can be determined by entering the equation into a predetermined formula. The estimated optimal dose of mangrove leaf extract was determined at the peak point on day 14 or after a challenge test. The calculation results showed that a value of 27 mg.kg^{-1} was the optimal dose with a hematocrit that could be produced at 30.1%.

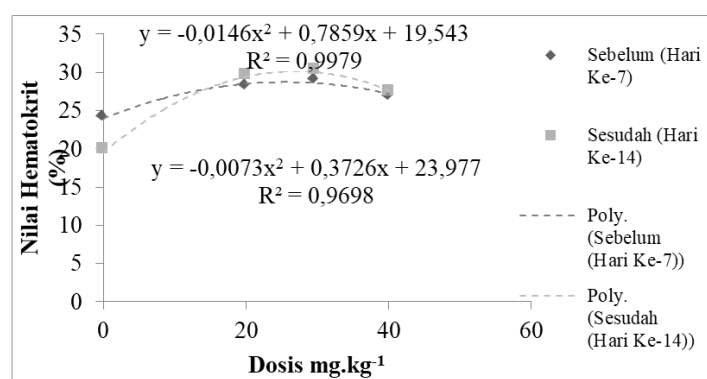


Figure 6. Percentage Analysis of Effect and Determination of Optimal Hematocrit Dose

Water Quality

Water quality measurements using temperature, pH and DO parameters are used as supporting parameters to ensure that the values match the optimal reference for tilapia and that water quality does not affect the observed research parameters. Water quality measurements carried out every day during the research showed that the level of change was relatively small because efforts were made to have an optimal range. Analysis of water quality parameters is carried out by tabulating data and comparing it with predetermined optimal references. The results of observations of water quality parameters can be seen in Table 1.

Table 1. Water Quality Measurement Results

Parameter	Water Quality Range	References
Temperature (°C)	27±0.7 - 30±0.1°C	27-30°C (Fahrurrozi <i>et al.</i> , 2024)
Dissolved Oxygen (DO) (mg.L ⁻¹)	5±0.5 - 6±0.2 mg.L ⁻¹	>3 mg.L ⁻¹ (Scabra <i>et al.</i> , 2022)
pH	8±0.1 - 8±0.6	8-8.7 (Fahrurrozi <i>et al.</i> , 2023)

DISCUSSION

Total Erythrocytes

Observation of total erythrocytes is an important parameter because erythrocytes or red blood cells are the blood cells with the largest quantity compared to the number of other blood cells. Normally, the total number of erythrocytes in the body of tilapia constitutes almost 50% of the total blood volume (Suryadi *et al.*, 2021). In addition, total erythrocytes can reflect the condition of the fish's body in its immune system for defense against pathogenic bacteria (Maryani *et al.*, 2021). Observation of total erythrocytes on day 7 and day 14 showed a significant increase in treatments B, C and D. These results indicate that administration of *R. mucronata* mangrove leaf extract can be an immunomodulator in activating the immune

system, in this case erythrocytes, to fight pathogenic bacteria. This is also supported by the results of the analysis which show that the total erythrocytes in the fish body are 96% influenced by the administration of *R. mucronata* mangrove leaf extract at an optimal dose of 27.3 mg.kg⁻¹.

In this condition, leukocytes or white blood cells do not have enough time to detect pathogens and capture them. Phagocytosis of pathogens by leukocytes occurs in tissues, subepithelium, and the lymph system (Bicker *et al.*, 2008). So in the bloodstream, erythrocytes are charged by the triboelectric effect, which attracts bacteria to the surface where the bacteria will then be killed by the erythrocytes (Minasyan, 2014). Therefore, an increase in total erythrocytes indicates an increased immune response in fighting pathogens through their negatively charged membranes to attract and engulf them (Chan *et al.*, 2022).

The average total erythrocytes of tilapia on the 7th day before the challenge ranged between 1.1-1.2 ($\times 10^6$ cells/mm³). Meanwhile, after the 14th day of challenge with *A. hydrophila* bacteria, the total erythrocytes of tilapia ranged between 1.1-1.7 ($\times 10^6$ cells/mm³) for treatments A, B, C and D, except E (1.0 $\times 10^6$ cells/mm³). This shows that the total erythrocytes in tilapia are still within the normal range, where the normal number of erythrocytes in teleost fish ranges from 1.1-3.0 ($\times 10^6$ cells/mm³) (Maryani *et al.*, 2021). The decrease in treatment E was thought to be due to not giving *R. mucronata* leaf extract so that the immune response in tilapia was not optimal and *A. hydrophila* bacteria could infect and reduce total erythrocytes. During infection, pathogens need iron to maintain their survival, so pathogens attack erythrocytes causing changes in membrane structure and eryptosis (Chan *et al.*, 2022). These changes also result in erythrocyte shrinkage, membrane clumping, and exposure to phosphatidylserine (PS), resulting in degradation and a decrease in the number of erythrocytes (Bissinger *et al.*, 2019).

Hemoglobin (Hb)

Hemoglobin is a protein part of red blood cells or erythrocytes which is composed of colorless globin and heme pigment (Putranto *et al.*, 2019). The presence of Hb is related to the total erythrocytes contained in the fish's blood volume in the body. Apart from that, Hb has a function as an oxygen transporter which circulates it in the bloodstream (Maryani *et al.*, 2021). Observations of Hb on day 7 and day 14 showed a significant increase in treatments B, C and D. These results indicate that administration of *R. mucronata* mangrove leaf extract can be an immunomodulator in activating the immune system, in this case erythrocytes that produce Hb. This is also supported by the results of the analysis which shows that the Hb in the body of tilapia fish is 99% influenced by the administration of *R. mucronata* mangrove leaf extract at an optimal dose of 27.1 mg.kg⁻¹ in relation to resistance to pathogenic bacteria.

The role of Hb in fish health, especially the immune system, occurs when erythrocytes lysed by bacteria release Hb as free radicals, which then degrade the cell walls and membranes of pathogens (Chan *et al.*, 2022). Apart from that, Hb may also in its mechanism induce the activation of macrophages and neutrophils on non-specific immune system receptors in fish (Bozza & Jeney, 2020). Therefore, an increase in Hb can indicate an increase in the immune response against pathogens through induction of the formation of reactive oxygen species (ROS) which break down bacterial cell walls and membranes and activate the immune system (Chan *et al.*, 2022).

The average Hb of tilapia on day 7 ranged from 6.2 to 7.2 g.dL⁻¹. Meanwhile, after the 14th day of challenge with *A. hydrophila* bacteria, the Hb of tilapia fish ranged between 6.2-8.2 g.dL⁻¹ in treatments A, B, C and D, except E (4.2 \pm 0.2 g.dL⁻¹). This shows that the Hb in tilapia fish is still within the normal range, where the normal Hb amount in teleost fish ranges from 6-11.01 g.dL⁻¹ (Thaib *et al.*, 2021). The decrease in treatment E was thought to be due to not administering extracts so that the immune response in tilapia was not optimal and *A.*

hydrophila bacteria could infect and reduce total erythrocytes. During infection, pathogens attack erythrocytes, resulting in a decrease in the number of erythrocytes (Bissinger *et al.*, 2019). This also has an impact on the amount of Hb, because decreasing the number of erythrocytes also has an impact on reducing the amount of Hb (Putranto *et al.*, 2019).

Hematocrit

Hematocrit is the percentage of solid substances in the blood to the liquid, or it can also be explained as a measuring parameter to see the ratio of the total blood volume to the ratio between erythrocytes and plasma which is shown as a percentage (Maryani *et al.*, 2021). The presence of higher hematocrit in the blood has been widely accepted to be associated with health benefits (Esmaeili, 2021). Especially in animals, since ancient times the presence of a higher hematocrit has been closely related to increased production (Lawrence *et al.*, 2024). However, several research results show inconsistencies regarding the usefulness of hematocrit. Therefore, caution is needed when drawing conclusions regarding hematocrit parameters. However, when an increase in hematocrit is within the normal range it can indicate a good sign of optimizing oxygen transport and health (Esmaeili, 2021).

Hematocrit observations on day 7 and day 14 showed a significant increase in treatments B, C and D. These results showed that administration of *R. mucronata* mangrove leaf extract affected the hematocrit value. This is also supported by the results of the analysis which shows that the hematocrit value in the fish body is 99% influenced by the administration of *R. mucronata* mangrove leaf extract at an optimal dose of 27 mg.kg⁻¹. In addition, the increase in hematocrit in this study was still within the normal range for tilapia. Where the normal hematocrit range in tilapia is between 21-30% (Arisa *et al.*, 2024). The average hematocrit of tilapia on the 7th day before the challenge test ranged from 24-29%. Meanwhile, after the 14th day of challenge with *A. hydrophila* bacteria, the hematocrit of tilapia ranged between 24-30% for treatments A, B, C and D, except E (19.6 ± 2%). This is thought to be because *R. mucronata* leaf extract was not given so that the immune response in tilapia was not optimal and *A. hydrophila* bacteria could infect and reduce the hematocrit value. The hematocrit parameter has a dual but opposite effect on systemic oxygen transport or cardiac output and oxygen carrying capacity, so that the relationship between oxygen transport and hematocrit is parabolic (Esmaeili, 2021).

Water Quality

Water quality that is outside the optimal range greatly influences the survival of biota (Fahrurrozi & Linayati, 2022). The water quality in this research is used as a supporting parameter to ensure that there is no influence of water quality on the treatment by optimizing the value of the water quality of the observation media. The research results at temperatures ranged from 27 ± 0.7 - 30 ± 0.1°C, which indicates that the water temperature is in the normal range. The optimal temperature value for tilapia cultivation ranges between 27-30°C (Fahrurrozi *et al.*, 2024).

Poor water quality can cause failure in cultivation efforts due to stress on fish so that they are easily attacked by pathogens and result in death (Fahrurrozi *et al.*, 2023). So, just like temperature, in this study the dissolved oxygen (DO) and pH values were tried to remain in the optimal range with respective values ranging between 5 ± 0.5 - 6 ± 0.2 mg.L⁻¹ and 8 ± 0.1 - 8 ± 0.6. This optimal range is supported by the statement of Scabra *et al.*, (2022), which states that the optimal value of DO is >3 mg.L⁻¹. Meanwhile, the optimal range of pH parameters in fish farming is between 8-8.7 (Fahrurrozi *et al.*, 2023).

CONCLUSION

The conclusion from this study was that >96% increase and average value of total erythrocytes, hemoglobin and hematocrit were influenced by administering *R. mucronata* leaf extract to tilapia (*O. niloticus*) which were tested against *A. hydrophila* bacteria. The optimal dose of *R. mucronata* mangrove leaf extract for the values for all observation parameters (total erythrocytes, hemoglobin and hematocrit) was $27 \pm 0.2 \text{ mg.kg}^{-1}$. This is also supported by water quality values which are within the optimal range.

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