Analysis of EM4 Probiotics Added in The Water on The Hematological Profile of Tilapia (*Oreochromis niloticus*)

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ABSTRACT

This journal reviews the effect of giving EM4 probiotics on the hematological profile of tilapia. This study aims to determine the effects that will arise after administering EM4 probiotics for several days on the blood of tilapia fish. This research uses experimental methods so that the results used are the results of experiments carried out by the author. The results of this study explain the blood profile of tilapia after administration of EM4 probiotics including levels of erythrocytes, leukocytes, hematocrit, hemoglobin and differential counting. There was a decrease in erythrocyte, hemoglobin, hematocrit levels, and an insignificant increase in leukocyte and hematocrit levels.

Keywords: hematology, tilapia, EM4 probiotics

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INTRODUCTION

Tilapia is a type of freshwater fish with a fairly high consumption rate, causing demand for tilapia to increase (Arlanda *et al*, 2018). In its cultivation, tilapia has several advantages compared to other fish, namely that it is easy to maintain in various maintenance media, easy to breed, reproduces every month, has high resistance to extreme environments and has high nutritional and economic value (Maulina and Sri Herlina, 2022) .

In tilapia cultivation there are many disturbances, one of which is disease attacks from pathogenic organisms due to an unfavorable environment for tilapia. Physiological deviations in fish will cause blood components to also change. Changes in blood picture and blood chemistry, both qualitatively and quantitatively, can determine health conditions. Fish that are attacked by the disease will experience changes in hematocrit values, hemoglobin levels, number of red blood cells and number of white blood cells. Blood tests can be used as an indicator of the severity of a disease (Fahmi *et al*, 2014).

Probiotics are an alternative in overcoming water quality problems in aquaculture. The function of probiotics is to help degrade organic materials thereby producing substances that are beneficial for plankton growth. This organic material can be used directly by phytoplankton in the water and becomes food for zooplankton so that it is abundant. In this way, the availability of natural food for fish is maintained (Sri Hartini *et al*, 2013). Based on the description above, this study aims to determine changes in the hematology of tilapia given EM4 probiotics in their water to evaluate the physiological response of tilapia.

METHODS

This research used laboratory experimental methods followed by

hematological examination of tilapia fish carried out in the laboratory using the Sahli Adams method, the improved Neubauer chamber calculation method, and the microhematocrit method.

The samples for this research were 6 tilapia fish (*Oreochromis niloticus*) aged 4 months. Fish were divided into control groups and treatment groups, each group consisting of 3 fish. Other ingredients used in this research include EM4 probiotics, tilapia fish feed, clean water, and dacies solution.

The research was carried out using experimental methods by looking directly at the treated tilapia fish. In this study, 6 samples of tilapia fish were used, with 3 treated tilapia fish and 3 control tilapia fish. Before conducting the research, 6 tilapia fish underwent hematology tests. then the 6 tilapia fish will be divided into 2 groups, namely the treatment group and the control group. All tilapia fish will be treated for 5 days for hematological tests to be carried out again by comparing the results of the control group and the treatment group.

The samples in this study used 6 4 month old black tilapia (*Oreochromis niloticus*) fish obtained from the Veterinary Clinical Pathology Lab, Faculty of Veterinary Medicine, Airlangga University.

Data is presented and analyzed quantitatively. Data obtained from measuring erythrocyte levels through fish blood samples taken using three techniques, namely the caudal vessel puncturing technique, cardiac puncture technique, and dorsal aortic puncture technique. The normal level of tilapia erythrocytes varies depending on the fish species and the sex of the fish. The number of erythrocytes in male tilapia was 60.84 ± 24.31 x 10^6/mm^3 while the erythrocytes in female tilapia were 43.38 ± 6.79 x 10^6/mm^3 (Yanto *et al.*, 2015). The number of erythrocyte counts in the treatment group given EM4 probiotics will be compared with the control group that was not given EM4 probiotics. The results of this research data will show the effect of the EM4 (Effective Microorganism) probiotic on the erythrocyte levels of the test tilapia fish.

RESULT AND DISCUSSION

Erythrocytes

There were differences in the number of erythrocytes in the control group and the treatment group. Fish in the control group had an erythrocyte count of 2.6 x 10^6 cells/mm^3, while fish treated with probiotics added to the water had an erythrocyte count of 2.14 x 10^6 cells/mm^3. It can be seen that the tilapia fish in the treatment group have a lower number of erythrocytes than the tilapia fish in the control group. Normally, the erythrocyte content of tilapia fish is 0.02-3x10^6 cells/mm^3 (Hartika., *et al*, 2004). The decrease in the number of erythrocytes is thought to have occurred due to poor water quality when the research was conducted and the fish's failure to adapt.

Hemoglobin levels

According to Salasia *et al.* (2001) normal hemoglobin levels in tilapia range from 5.05 to 8.33 g%. Tilapia hemoglobin levels in the control group were 3 g%. Meanwhile, the hemoglobin level of tilapia treated using probiotics in water was 7 g%. The results showed that the hemoglobin levels of tilapia treated with probiotics in water were greater than those in the control group.

Hematocrit Test

According to Hardi *et al.* (2011), normal tilapia hematocrit levels range from 27.3% - 37.8%. In this study, the results of the hematocrit test between the control group and the treatment group did not have a significant difference, namely the control group was 15.4% and the treatment group was

16%, namely a difference of 0.6%. According to Maulina and Herlina (2022), giving probiotics has no effect on hematocrit values. Meanwhile, Alamanda *et al.* (2007) in Sukenda *et al.* (2016), believes that low hematocrit levels can be an indicator that the fish is suspected of having anemia.

Table 1. Hematological Profile of Control Group and Treatment Group

Test	K group	T group
Erythrocytes	2,6 x10^6 cell/mm^3	2,14 x 10^6 cell/mm^3
Hemoglobin	3 g%	7 g%
Hematocrit	15,4 %	16%
Leukocytes	27.500 cell/mm3	52.500 cell/mm3
Differential Counting Leukocytes	Ba/Eo/St/Sg/Ly/Mo 15/6/13/16/35/15	Ba/Eo/St/Sg/Ly/Mo 7/7/17/10/34/25

Leukocyte Count

The normal range for the number of white blood cells in normal tilapia is generally around 20,000 - 150,000 cells/mm^3 (Rastogi, 1977 in Hartika *et al.*, 2014). The results of the study showed that the number of leukocytes obtained during observations with control treatment was 27,500 cells/mm^3 and the number of leukocytes increased after administration of EM4 probiotics with a total of 52,500 cells/mm^3. Giving EM4 probiotics has been proven to increase the number of leukocytes in tilapia.

Differential Counting Leukocytes

According to Sayed and Moneb (2015), the average value of the number of lymphocytes in tilapia is 90.98%, monocytes are 2%, neutrophils are 5.67%, and eosinophils are 1.44%. A decrease in the number of neutrophil cells indicates that the fish is in healthy condition. The results showed that the number of lymphocytes was 34 cells, the number of monocytes was 25 cells, the number of basophils was 7 cells, the number of segment neutrophils was 10 cells, the number of stable neutrophils was 17 cells, and the number of eosinophils was 7 cells. In the control group, the number of lymphocytes was 35 cells, the number of monocytes was 15 cells, the number of basophils was 15 cells, the number of segmental neutrophils was 16 cells, the number of stable neutrophils was 13 cells, and the number of eosinophils was 6 cells.

The results of a low neutrophil percentage indicate that there is no attack by microorganisms, so the body does not produce significant neutrophil cells during the acclimatization process. In accordance with the opinion of Hartika *et al.*, (2014), a low percentage of neutrophils indicates that there is no attack by microorganisms so that not many neutrophils are produced by the fish's body. An increase in the number of lymphocyte cells indicates an improvement in the immune system of tilapia, in accordance with the findings of Hartika *et al.* (2014) who stated that increasing lymphocytes plays a significant role in increasing the immune response and body resistance of tilapia to disease and infection. This

research reveals that lymphocyte cells have the highest percentage compared to other cells, consistent with their main function as producers of immune substances, as explained by Rustikawati (2012) who stated that the number of lymphocyte cells in fish is greater than neutrophils or monocytes. Setiawan *et al*. (2012) also emphasized that the high proportion of lymphocyte cells is due to their role in providing immune defense, where lymphocyte cells will enlarge, proliferate and form specific antibodies according to the antigen that stimulates them.

CONCLUSION

Giving EM4 probiotics to tilapia did not increase the number of erythrocytes in tilapia. In the treatment group given EM4 probiotics, a decrease in the number of erythrocytes was found. The decrease in erythrocyte levels can be due to poor water quality and low levels of activators and protein in the fish's body. Meanwhile, hemoglobin and hematocrit levels in the treatment group did not increase significantly.

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