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Blood Profile of Tilapia (*Oreochromis niloticus*) Given Feed Mixed with Soursop Leaf Extract (*Annona muricata* L.)

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ABSTRACT

Efforts to combat disease in farmed fish are typically done using antibiotics. However, long-term use of antibiotics can lead to disease resistance and environmental pollution. One herbal extract with potential as an immunostimulant is the soursop plant (*Annona muricata* L.). This study aims to understand the hematology of tilapia (*Oreochromis niloticus*) that were fed a diet mixed with soursop leaf extract (*Annona muricata* L.). The study design included four treatments and three repetitions, comprising treatment A (control), treatment B (2.5 g/kg feed), treatment C (5 g/kg feed), and treatment D (7.5 g/kg feed). The results showed that feeding a diet mixed with soursop leaf extract at a concentration of 7.5 g/kg feed (treatment D) could influence the increase in hematocrit levels by 26.11%, hemoglobin levels by 7.11 g/dl, and total leukocytes by 4.41 x 10^4 cells/mm^3, but did not affect the total erythrocytes of tilapia.

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is an introduced fish from Africa that entered Indonesia since the 1970s (Khairuman & Amri, 2005). Riauwaty & Syawal (2016) said that tilapia is one of the fish species that is very suitable to be developed in the aquaculture sector. The main reasons are good growth ability, delicious meat flavor, and high resistance to environmental changes.

An obstacle for tilapia farmers is the presence of disease outbreaks that can attack cultured fish at any time. Disease management is generally done with the use of antibiotics. However, antibiotics that are used excessively in the long term can cause resistance to disease, and can pollute the environment (Noga & Edward, 2010). Therefore, there is a need for a better alternative to other treatments for fish disease management. One alternative that can be used is the use of herbal plants that have anti-fungal, antibacterial and anti-viral properties.

One of the herbal plants found in Indonesia and can be used as an alternative treatment is the soursop plant (*Annona muricata* L). Rachimi *et al*. (2017) mentioned that soursop leaves contain a lot of phytochemical bioactive compounds, which include antibacterial, antiparasitic, antipasmodic, anticancer and able to remove toxins. Ohashi *et al*. (2003) said that soursop leaf extract has a lot of flavonoids from the flavonol group that act as immunostimulants. Soursop leaf extract is known to improve the immune system of fish, as well as fish health.

As part of fish health assessment, blood picture can be an important parameter to detect pathogenic infections or the effects of feed ingredients. Thus, information about fish blood picture can provide clues about the overall health status of fish. Based on the above description, feeding with soursop leaf extract is expected to increase the immune response of fish by looking at parameters such as blood picture that shows the health status of fish.

METHODS

Time and Place of Research

The research was conducted from January to April 2023. This research activity was carried out in the laboratory of the Department of Fisheries, Faculty of Agriculture, Palangka Raya University, then for hematological examination was carried out at the Fish Quarantine Center, Quality Control and Safety of Fishery Products Palangka Raya.

Tools and Materials

The tools used in this research are centrifuge, syringe, haemometer, hemocytometer, microscope, and DO meter. The materials used include tilapia size 10-12, commercial pellets, soursop leaf extract, EDTA 10%, Turk's solution, Hayem HCL 0.1N solution, and distilled water.

Research Design

This study used a completely randomized design (CRD) experimental method with four treatments, namely treatment A (control), B (2.5g/kg feed), C (5g/kg feed) and D (7.5g/kg feed) with three replications. The reference basis for determining the treatment dose in this study is based on the results of preliminary experiments that have been conducted to identify a safe and effective dose range before selecting the final treatment level. With the treatment using soursop leaves (*Annona muricata* L.) and the fish used in the research conducted were tilapia (*Oreochromis niloticus*) with a size of 10-12 cm and weighing 18-25 grams. This size range corresponds to the juvenile stage of tilapia, which is a critical period for rapid and uniform growth so that variations in physiological and immunological responses can be minimized (Bittencourt *et al*., 2003).

Research Procedures

- **Soursop Leaf Preparation**

Soursop leaves used were obtained from soursop trees owned by residents in Palangka Raya, Central Kalimantan. The soursop leaves taken are leaves that are in the 3rd to 5th order from the tip of the leaf stem and picked at 5-6 am (Zuhud, 2011). The leaves collected are leaves that are neither too young nor too old, namely the 3rd to 7th leaves from the top (Sulastrianah *et al*., 2014).

- **Preparation of Soursop Leaf Extract**

Soursop leaves (*Annona muricata* L.) were first washed using water and then cut into small pieces. After cutting the soursop leaves into small pieces, they were dried at room temperature until dry. The dried leaves were crushed until they were powdery. After that, the dose of soursop leaf extract was dissolved with 100 mL of heated distilled water and homogenized for 40 minutes. Then the solution is filtered with filter paper and separates the filtrate and particulates. The filtrate will be the soursop leaf extract and mixed into the feed by spraying. This procedure is based on the research of Dotulong *et al*. (2020) which has been modified.

- **Feeding Process**

The feed given during the study amounted to 3% of the total weight of the fish kept (Amalia *et al*., 2017) per aquarium per day. Soursop leaves that have been extracted into liquid

are mixed into the feed by spraying on the feed with a dose according to the treatment. Feed that has previously been sprayed is air dried for ± 1 day so that the feed is dry before storage. Feed is stored in a jar container, then placed at room temperature (Arianti *et al*., 2022). Feeding is done twice a day at 08:00 and 16:00, for 10 days. For the rest of the feed in the maintenance media, watering and water changes are carried out 3 days.

- **Calculation of Hematocrit Level**

Calculation of hematocrit levels is done by filling the microcapillary tube with fish blood until it fills ¾ of the tube. Then the end was closed using a lid and placed into a centrifuge machine and rotated at 5000 rpm for 5 minutes. Then, the tube was removed and the hematocrit value was determined by measuring it with a ruler and expressed as % (Samsisko *et al*., 2013).

- **Hemoglobin Level Calculation**

Hemoglobin levels are calculated using the Wedemeyer & Yasutake method (1977), by filling the sahli tube using 0.1 N HCl solution until it touches the number 10 (the bottom scale line on the sahlinometer tube). Then the tube is placed in the middle between 2 other tubes that have a standard color. After that, fish blood is taken from the affendorf tube by sucking it using a sahli pipette as much as 0.02 ml then clean the tip of the sahli pipette with a tissue and insert the blood sucked into the sahli tube, then let stand for 3 minutes. Distilled water was added with a dropper pipette slowly and continuously while stirring the stirring glass until the color on the tube was the same as the color standard of the other two tubes. Hemoglobin levels are expressed in g/dl.

- **Total Erythrocyte Calculation**

Calculation of total erythrocytes is done by sucking blood with an erythrocyte pipette to the limit of 1.0 blood then mixed with Hayem's solution to the limit of 101 and obtained a dilution factor of 100x. If the erythrocytes obtained are still too dense, the process is repeated by sucking blood with an erythrocyte pipette to the limit of 0.5, then adding Hayem's solution to the limit 101, the dilution factor obtained is 200x. The contents of the pipette were stirred by making a circular motion like a figure 8 to mix. The stirred liquid was put into the counting chamber and counted under the microscope. The counting chamber that has a striped field is placed at the bottom of the objective, while the focus of the microscope is directed at the lines of the counting chamber so that the erythrocytes will be seen. This counting procedure is in accordance with Blaxhall & Daisley (1973) with the formula:

$$N = \frac{n}{V} \; x \; P$$

Description:

N : Number of erythrocytes counted (cells/mm3)
n : Number of erythrocytes sampled
V : Volume of the observed field
P : Dilution factor (100x/200x)

Calculation of Total Leukocytes

Calculation of total leukocytes is done by sucking blood with a pipette until the limit of 1.0 blood then mixed with Hayem solution until the limit of 11 and obtained a dilution factor of 10x. If the leukocytes obtained are still too dense, the process is repeated by sucking blood with a leukocyte pipette to the limit of 0.5, then adding Hayem's solution to the limit of 11, the dilution factor obtained is 20x. The contents of the pipette are stirred with a movement like number 8 until mixed. The stirred liquid was put into the counting chamber and counted under the microscope. The counting chamber with a striped field is placed on the lower part of the objective. This counting procedure refers to Blaxhall & Daisley (1973) with the formula:

$$N = \frac{n}{V} \; x \; P$$

Description:
N : Calculated leukocyte count (cells/mm3)
n : Sample leukocyte count
V : Volume of the observed field
P : Dilution factor (10x/20x)

Data Analysis

Blood features observed were hematocrit levels (%), hemoglobin levels (g/dl), total erythrocytes (10^6 cells/mm^3), total leukocytes (10^4 cells/mm^3), and water quality temperature (°C), and DO (mg/l). The data obtained is the percentage of the number of blood cells in fish which is then obtained in the form of tables and graphs using the Microsoft Word program, then the data obtained is analyzed and analyzed using the SPSS version 25 program with analysis of variance (ANOVA) and then conducted further tests with the Duncan test.

RESULTS

Blood picture is an important parameter to detect pathogen infection or the effect of feed ingredients given. In this case, the results of soursop leaf extract administration can be observed based on hematocrit levels, hemoglobin levels, total erythrocytes and total leukocytes.

Hematocrit Level

Hematocrit is the percentage of solids to blood fluid in the blood. Hematocrit is a parameter that affects the measurement of erythrocyte volume. The results of this study obtained that the hematocrit percentage of tilapia (*Oreochromis niloticus*) during the study given feed with a mixture of soursop leaf extract (*Annona muricata* L.) can be seen in Figure 1.

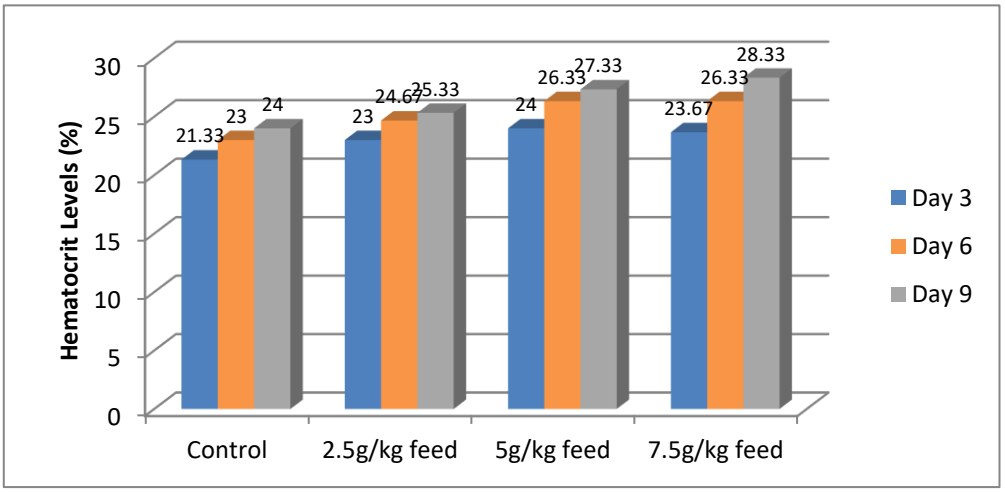

Figure 1. Hematocrit Levels During 10 Days of Research

Hemoglobin Level

Hemoglobin is an important protein in the blood consisting of globin protein which is clear in color and heme pigment made from erythrocytes. The results of this study showed that there was an effect of feeding with a mixture of soursop leaf (*Annona muricata* L.) extract

on the blood picture of tilapia (*Oreochromis niloticus*). The graph of total erythrocyte data can be seen in Figure 2.

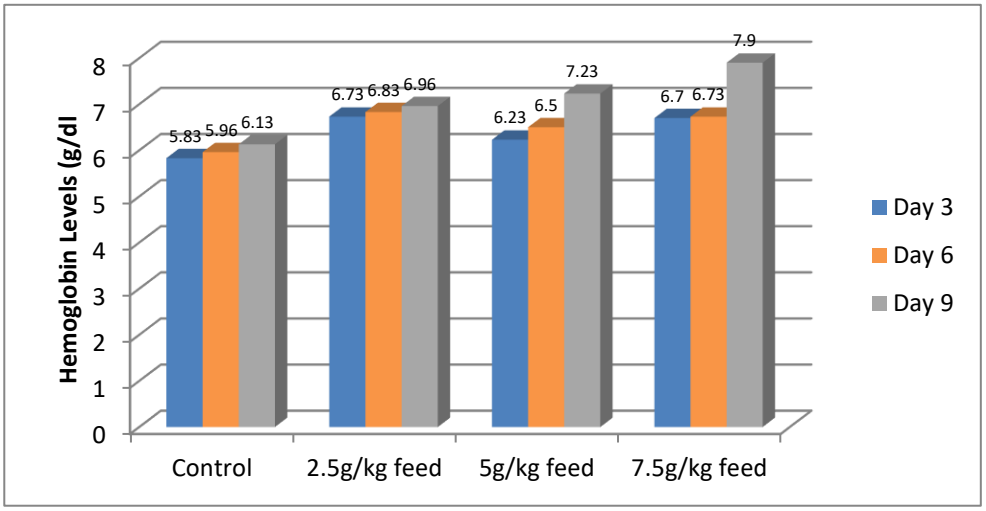

Figure 2. Hemoglobin Level During 10 Days of Research

Total Erythrocytes

Erythrocytes or red blood cells are the most cells found in the blood. The main function of erythrocytes as gas transportation (Kokasih & Kosasih, 2008) Based on the results of the research conducted, the graph of total erythrocyte data can be seen in Figure 3.

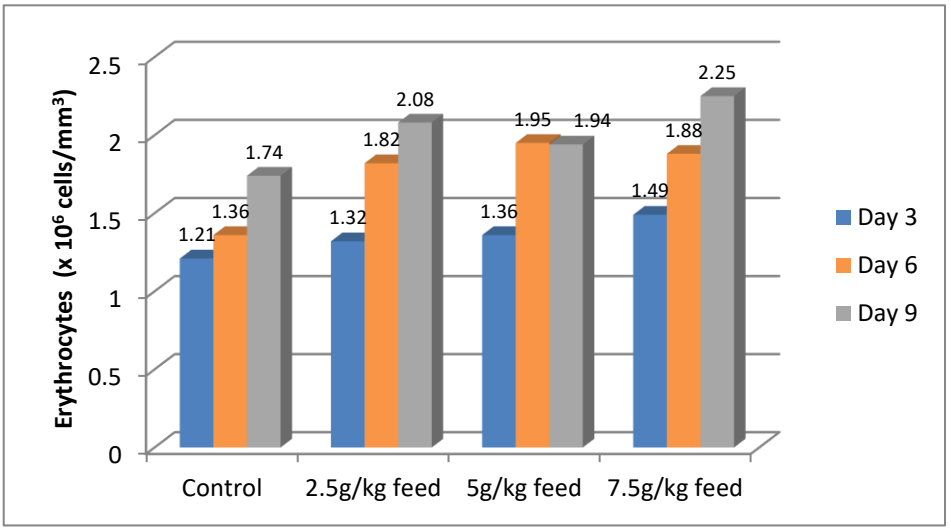

Figure 3. Total Erythrocytes During 10 Days of Research

Total Leukocytes

Leukocytes are part of an active body defense response that is mostly produced in the bone marrow and partly in bone tissue. The results of the research conducted showed an increase in tilapia leukocytes (*Oreochromis niloticus*) given feed with a mixture of soursop leaf extract (*Annona muricata* L.) can be seen in Figure 4. as follows.

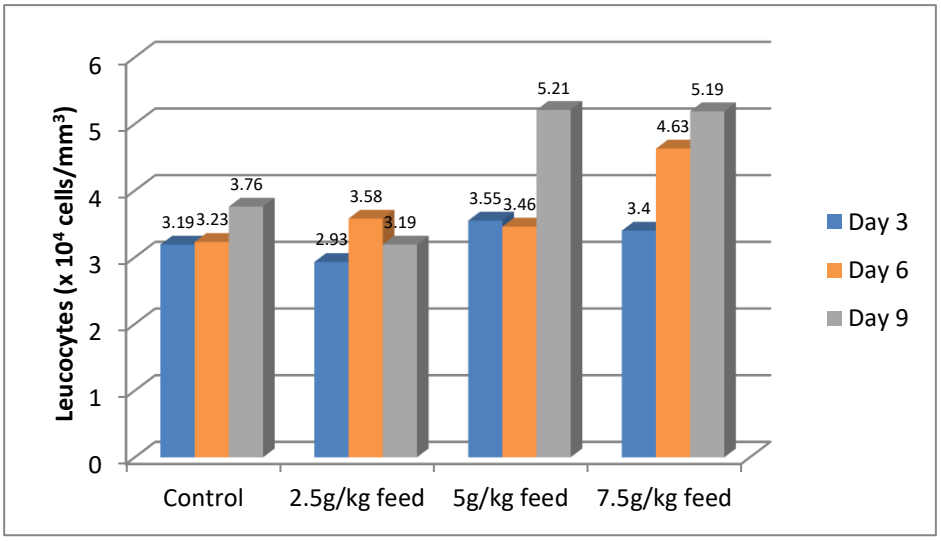

Figure 4. Total Leukocytes During 10 Days of Research

Water Quality

The water quality of the cultivation media temperature ranged from 29°C - 29.2°C and DO of 6.3 mg/l - 6.7 mg/l. The temperature and DO values in the study were in the normal range.

DISCUSSION

Hematocrit Level

Data from the processing of tilapia hematocrit levels (%) during the study showed an increase during the 10 days of the study. The results of hematocrit levels showed that the average hematocrit levels given feed with a mixture of soursop leaf extract at different doses ranged from 21.33%-28.33%. Royan *et al*. (2014) said that the increase in hematocrit levels of tilapia itself is thought to be due to the fish starting to acclimatize to the environment and the feed given, so that the fish can start eating and moving normally and get a supply of nutrients in its body. Fauzan *et al*. (2017) stated that hematocrit levels and hemoglobin levels reflect the condition of oxygen in the blood.

From the observation, it is known that treatment D has the best results with a hematocrit level of 26.11%. Hematocrit levels in this study are still normal values for tilapia hematocrit, this is also supported by the opinion of Royan *et al*. (2014), that normal fish hematocrit ranges from 21%-22.67%, as well as Putra's statement (2015) the range of normal fish hematocrit values between 20-30%. The increase in hematocrit is thought to be due to the flavonoid content in soursop leaves as according to Syawal *et al*. (2022), flavonoid content is known to increase the ability of blood-producing organs (lymphomyeloid) so that blood production can increase. Zulaikhah (2017) said that flavonoids donate their electrons to compounds that are antioxidants, which in turn inhibit the activity of oxidant compounds and protect the erythrocyte lipid membrane from exposure to free radicals. Hidayat (2018) said the amount of hematocrit value can be caused by the value of erythrocytes, the high hematocrit indicates that the number of erythrocytes in the fish is high, causing blood viscosity to increase as well. The hematocrit value can also indicate that the fish is anemic or stressed if the value is below the normal range.

Hemoglobin Level

The average hemoglobin level of tilapia during the 10 days of research by feeding with a mixture of soursop leaf extract ranged from 5.83 to 7.9 g/dl. Matofani *et al*. (2013) argue that hemoglobin is closely related to erythrocytes, the less hemoglobin levels, the possibility that fish will experience anemia. Azhari *et al*. (2020) said that hemoglobin concentration is determined based on the color or density of red blood cells. The increase in hemoglobin levels in tilapia is thought to be caused by flavonoid activity obtained by feed mixed with soursop leaf extract. This is supported by the statement of Setyarini *et al*. (2023), flavonoids can bind to metals that play a role in oxygen metabolism such as iron ions, so as to increase the number of erythrocytes and hemoglobin levels.

Treatment D is known to have the best results with a hemoglobin level of 7.11 g/dl. Hemoglobin levels during this study were in the normal range according to the statement of Azhari *et al*. (2020), that the average hemoglobin is generally normal fish in the range of 6-11.01 g/dl. Increased hemoglobin levels are influenced by total erythrocytes in the fish body, while low hemoglobin is known to cause anemia in fish. According to Prasetio *et al*. (2017), low hemoglobin levels can affect the decrease in metabolic rate in fish, so that the energy produced by the body becomes low and causes the fish to become weak, have no appetite, and will look still at the bottom or float on the surface of the water. Hemoglobin has a link to the resilience of the fish body in terms of oxygen binding power with blood (Nirmala *et al*., 2012). The ability of oxygen to bind to blood depends on the amount of hemoglobin levels present in red blood cells.

Total Erythrocytes

The average results of erythrocytes of tilapia (*Oreochromis niloticus*) given feed with a mixture of soursop leaf (*Annona muricata* L.) extract for 10 days of research at the beginning of the study ranged from 1.87 x 10^6 -1.21 x 10^6 cells/mm^3. This is because tilapia experience stress. The stress experienced by tilapia is thought to be due to adapting to the feed treatment and the place of maintenance.

The increase in the number of erythrocyte cells in tilapia itself is thought to be because the fish began to stabilize, and the level of stress experienced decreased. Treatment D with the addition of soursop leaf extract at 7.5 g/kg feed has the highest total erythrocyte value. The results showed that the total erythrocytes obtained were lower than the research of Kurniawan *et al*. (2013) with total erythrocytes in sangkuriang catfish (*Clarias gariepinus* Var. Sangkuriang) from the first to the seventh day in the range of 1.8x106-2.5x106. The addition of soursop leaf extract in feed is also known to have no effect on erythrocyte levels, but there is no adverse effect on fish because the total erythrocytes of fish are in the normal average range. Hartika *et al*. (2014) said the number of fish erythrocytes in general ranges from 20,000-3,000,00 cells/mm^3. This opinion is then supported by Royan *et al*. (2014), teleostei fish, has a normal range of 1.05 x 10^6-3.0 x 10^6 cells/mm^3.

According to Yanto *et al*. (2015), that the factors that influence the total erythrocytes of fish include age, sex, environment, nutrition and oxygen deprivation conditions. Fajrianti *et al*. (2017) also stated that environmental factors also affect the total erythrocytes of fish. In situations where the temperature increases, the absorption of oxygen by erythrocytes is known to increase. The fish body will then compensate for the lack of oxygen it experiences by increasing its total erythrocytes (Hidayat, 2018). The number of erythrocytes is influenced by the health condition and appetite of the fish. Fish that are infected with disease or have no appetite, the hematocrit value becomes abnormal, and the erythrocytes will be low (Fauzan *et al*., 2017).

Total Leukocytes

Based on the results of feed research with a mixture of soursop leaf extract, it is known to increase total leukocytes in tilapia. Treatment D is the treatment that has the highest total leukocytes of 4.41 x 10^4 cells/mm^3. The results showed that the total erythrocytes obtained were higher than the research of Kurniawan *et al*. (2013), with total leukocytes in sangkuriang catfish (*Clarias gariepinus* Var. Sangkuriang) from the first to the seventh day in the range of 3.5x10^4-2.0x10^4 cells/mm^3. The value in all treatments is in the normal range, this is in line with the statement of Hartika *et al*. (2015), that the range of total fish leukocytes is between 20,000 cells/mm^3-150,000 cells/mm^3. The increase in leukocyte count is thought to be influenced by the presence of tannin, alkaloids and flavonoids in soursop leaves compared to treatment A (control). Flavonoids have immunostimulatory activity by increasing lymphocyte proliferation and macrophage activation (Maleki *et al*., 2019). Luhurningtys & Amaliasari (2023), also said tannin can increase the phagocytic activity of macrophages to destroy microbes, while alkaloid compounds are cytokine compounds that function to regulate immune responses, increase the proliferation and function of T cells, B cells, and Natural Killer Cell (NK Cell). This is also supported by the opinion of Arindita & Prayitno (2014), that the increase in total leukocytes in fish can be influenced by the presence of medicinal plants mixed into feed as immunostimulants for the fish body.

The average hemoglobin level of tilapia during the 10 days of research by feeding with a mixture of soursop leaf extract ranged from 5.83 to 7.9 g/dl. Matofani *et al*. (2013) argue that hemoglobin is closely related to erythrocytes, the less hemoglobin levels, the possibility that fish will experience anemia. Azhari *et al*. (2020) said that hemoglobin concentration is determined based on the color or density of red blood cells. The increase in hemoglobin levels in tilapia is thought to be caused by flavonoid activity obtained by feed mixed with soursop leaf extract. This is supported by the statement of Setyarini *et al*. (2023), flavonoids can bind to metals that play a role in oxygen metabolism such as iron ions, so as to increase the number of erythrocytes and hemoglobin levels.

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Water Quality

The results showed that the water quality in the parameters observed was within the normal range. This is in accordance with the research of Mulyani *et al*. (2014), which shows that the appropriate temperature for tilapia rearing is 27-29^oC. According to Pramleonita *et al*. (2018), the optimal DO levels for tilapia aquaculture activities range from 6.1 mg/l - 14.5 mg/l.

CONCLUSION

The results of this study showed that soursop leaf extract has an effect on the blood picture of tilapia. The mixture of soursop leaf extract in treatment D feed at a dose of 7.5 g/kg feed is known to have the best effect in increasing hematocrit levels by 26.11%, hemoglobin levels by 7.11 g/dl, and total leukocytes by 4.41 x 10^4 cells/mm^3, but has no effect on total erythrocytes. Water quality values obtained during the study were also within the normal range that can be tolerated by fish life and growth

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