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The Effect of Exposure to Microplastic Polystyrene (PS) in Feed on the Haematology of Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Indonesia is one of the countries that has the largest archipelago area in the world. Indonesia's vast territorial waters also present major challenges in terms of environmental management. One problem that is getting worse is plastic waste. Over time, plastic waste scattered in the environment will degrade into small particles which are usually called microplastics. Tilapia (*Oreochromis niloticus*) is one of the economically and ecologically important freshwater fish species. This fish is not only an important source of protein for humans, but also plays a role in the balance of the marine ecosystem. Exposure to microplastics in fish can occur in various ways, including through contaminated feed. The aim of this study was to determine the effect of exposure to polystyrene (PS) microplastics in feed on the blood profile of tilapia (*Oreochromis niloticus*). The treatments given were P1 without exposure to microplastics, P2 with 0.01 mg/0.75 g, P3 with 0.1 mg/0.75 g, and P4 with 1 mg/0.75 g. The parameters measured in the study were blood profiles, including hematocrit, hemoglobin, erythrocytes, leukocytes, leukocyte differential and survival rate. The results obtained in this study were that hematocrit levels and survival rates had an effect on fish growth, while erythrocyte, leukocyte and leukocyte differential values had no significant effect on the blood profile of Nile fish exposed to polystyrene microplastics.

INTRODUCTION

Indonesia is one of the countries with the largest archipelago area in the world. The water area is very large, covering more than 3.25 million square kilometers of ocean and 2.01 million square kilometers of the Exclusive Economic Zone (EEZ). With a coastline of more than 95,000 kilometers. According to Setiawan (2022) states that Indonesia is one of the countries with the highest marine biodiversity in the world. Indonesia's seas and waters are not only

vital natural resources, but also important transportation routes and the economic base for millions of people who depend on fisheries and marine tourism.

Indonesia's vast territorial waters also present major challenges in terms of environmental management. One problem that is increasingly worrying is plastic waste. Over time, plastic waste scattered in the environment will degrade into small particles which are usually called microplastics. According to Azizah *et al*. (2020) stated that microplastics have a size of less than 5 mm. With its small size, it often spreads to various elements, one of which is tilapia farming activities.

Tilapia (*Oreochromis niloticus*) is one of the economically and ecologically important freshwater fish species. This fish is not only an important source of protein for humans, but also plays a role in the balance of aquatic ecosystems. This has caused many people in Indonesia to carry out tilapia cultivation activities because it is very easy to cultivate and is in great demand by the public. However, with increasing microplastic pollution, there is growing concern about its impact on the health of tilapia and other farmed organisms.

Exposure to microplastics in fish can occur in various ways, including through contaminated feed. Microplastics that enter the fish's body can cause various negative effects, both physical and chemical (Aulia *et al*., 2023). One way to assess the impact of microplastics is by analyzing fish blood profiles. Blood profiles can provide insight into the health condition of fish, including their physiological function and immune response to environmental stressors. The aim of this study was to determine the effect of exposure to polystyrene (PS) microplastics in feed on the blood profile of tilapia (*Oreochromis niloticus*).

METHODS

This research was carried out for 45 days starting on January 16, 2024 - February 29, 2024 at the Fish Production and Reproduction Laboratory and blood checks of tilapia fish were carried out at the Fish Health Laboratory and Environmental Laboratory, Aquaculture Study Program, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram. The tools used in this research are airator, DO meter, bucket, elenmeyer, haemocytometer, container, microscope, tray, pH meter, tweezers, tweezers pipe, knife, sieve, scoop, ammonia test kit, thermometer, analytical balance, digital scale, cover glass, beacker glass, avendov, sahli pipette, dropper pipette, blander and preparation glass. Meanwhile, the materials used in this research were tilapia fish, feed, microplastic polystyrene, 5% H_20_2 solution, turk solution, hayem solution, 0.1 N HCL, turks solution, methanol solution, and distilled water.

The design used in this research was a completely randomized design (CRD) consisting of 4 treatments and 3 replications. The treatment in this research refers to previous research of Suwartiningsih *et al.* (2023) The treatments given were P1 without exposure to microplastics, P2 with 0.01 mg/0.75 g, P3 with 0.1 mg/0.75 g, and P4 with 1 mg/0.75 g. The parameters measured in the study were blood profiles, including hematocrit, hemoglobin, erythrocytes, leukocytes, leukocyte differential and survival rate.

The feed used in this research is H-Pro-vite 781 - 2 feed which will then be mixed with microplastics. The microplastic that will be used in this research is polystyrene, commonly known as styrofoam. Before styrofoam is given to test animals, it needs to be crushed first using a blender to micro size. After that, it is dried and filtered using a flour sieve. The filtered microplastics are stored in zip plastic. The sifted polystyrene microplastics are mixed into the feed according to the dose to be used for each treatment.

In this study, tilapia with a size of 10 - 12 cm and a weight of 16.8 - 48.1 g were used. The test animals used were obtained from the Lingsar Fish Seed Center, Lingsar District, West Lombok Regency. Before stocking, acclimatization is carried out first so that the tilapia used do not experience stress in the new environment so they can survive. The tilapia fish used in containers is 1 fish per 2 L of water so that the water used in 1 container is 30 liters with 15 tilapia fish. During activities, tilapia fish are fed 3 times a day with a feeding rate of 5%.

Hematocrit

The hematocrit value is read using the microhematocrit scale, which is expressed in percent (%) (Hartika *et al*., 2014). Calculation of hematocrit can be done by preparing a blood sample, then the first step is carried out, namely sucking the tilapia blood sample using a hematocrit capillary in ¾ of the tube and covering it with wax, then centrifuging at a speed of 3500 rpm for 15 minutes. The hematocrit value is determined by comparing the length of the volume of red blood cells that settle divided by the length of the total volume of blood in the tube.

Hemoglobin

Hemoglobin can be measured using a tool in the form of a sahli haemometer. The Sahli haemometer has a pipette called a sahli pipette which is used to take fish blood samples on a scale of 20 mm. After that, the blood sample that has been taken is transferred to a sahli tube containing 0.1 N HCL until it reaches the number 10. Next, the tube is placed in the middle between the 2 tubes which are the standard color and a little distilled water is added until the color is the same as the color in the standard tube after that. it was found that the results would be expressed in units of g% (Oktafa *et al*., 2017).

Erythrocytes

The first thing that needs to be done is that the sample or fish blood that is already in the tube is sucked using a toma pipette up to 0.5. Then mixed with hayem solution to a level of 101 and homogenized. If it is felt that it is homogeneous, then discard the first 3 drops because they are assumed to be not homogeneous, then drop them into a hemocytometer, cover with a cover glass and observe using a microscope (Alipin & Sari, 2020). After observing, calculations are carried out using the following formula:

$$\text{Erythrocytes} = \text{number of erythrocytes counted} \times 10^4 \text{ cells/mm}^3$$

Leukocytes

The blood sample to be tested is sucked using a toma pipette with a scale of 0.5 then Turk's solution is added up to a scale of 11, then homogenized so that it is mixed evenly. The first 3 drops were discarded, then the blood sample was dropped into the hemocytometer and covered using a cover glass. After that, observations were made using a microscope and the total leukocyte count was taken by taking 5 boxes with the following formula:

$$\text{Leukocytes} = \text{number of leukocyte cells counted} \times 50 \text{ cells/mm}^3$$

Differential Leukocyte

Leukocyte differential is done when you want to know the percentage of leukocyte types in the blood. The first step that must be taken is to make a thin blood film where the blood sample is placed 1 drop on a glass object, then spread evenly using the smear method. Next, it is fixed using 5-6 drops of methanol, then left for 5 minutes, then rinsed using distilled water and dried for 2 minutes. Then the final step is observation under a microscope with 100x magnification (Hartika *et al*., 2014).

Survival Rate (SR)

Survival rate is defined as the comparison between the number of living biota and the total number of biota stocked at the start of the study. The survival rate value can be expressed using the formula according to Setyono *et al*. (2023) as follows:

$$SR = \frac{Nt}{No} \; x \; 100\%$$

Information:
SR : Survival Rate
Nt : Number of fish at the end
No : Number of fish at the beginning

RESULTS

Hematocrit

The results of the calculation of tilapia hematocrit levels obtained during the research can be seen in Figure 1.

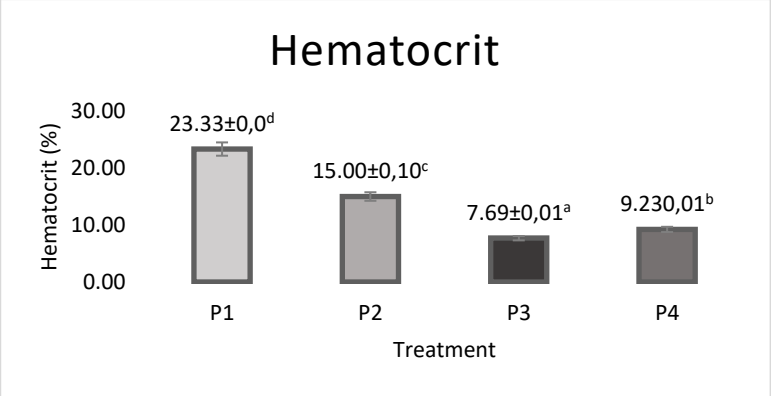

Figure 1. Hematocrit levels of Tilapia (*Oreochromis niloticus*)

Based on the results of the hematocrit test, the hematocrit value obtained ranged from 7.96%-23.33%. The highest hematocrit value was found in P1, namely 23.33%, followed by P2 with a hematocrit value of 15.00%, then P4 with a hematocrit level of 9.23% and the lowest hematocrit level in P3, namely 7.96%. The hematocrit levels produced based on the ANOVA test show that exposure to microplastics has a significant effect (<0.05) on the hematocrit value of tilapia fish.

Hemoglobin

The results of calculating the hemoglobin levels of tilapia obtained during the research can be seen in Figure 2.

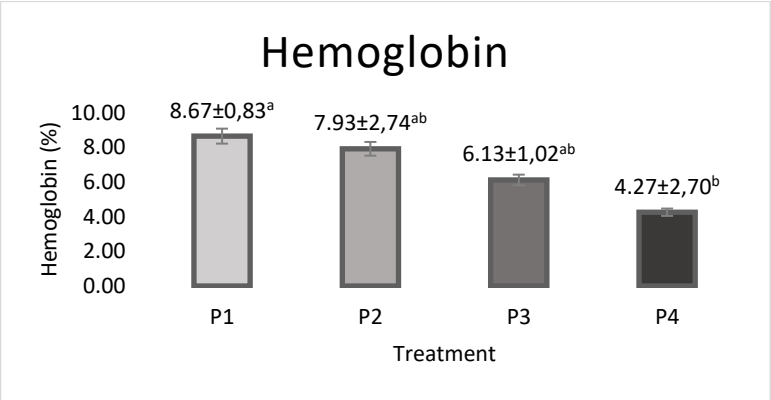

Figure 2. Hemoglobin levels of Tilapia (*Oreochromis niloticus*)

The results of calculating hemoglobin levels obtained from this study, namely P1, are not significantly different from P2 and P3, but are significantly different from P4. The highest hemoglobin level was found in P1 with a hemoglobin level of 8.76%, followed by P2 with a hemoglobin level of 7.93%, then P3 with a hemoglobin level of 6.13% and the lowest hemoglobin level in P4 was 4.27% and was significantly different (<0.05) P1.

Erythrocyte

The results of calculating tilapia erythrocyte levels obtained during the research can be seen in Figure 3.

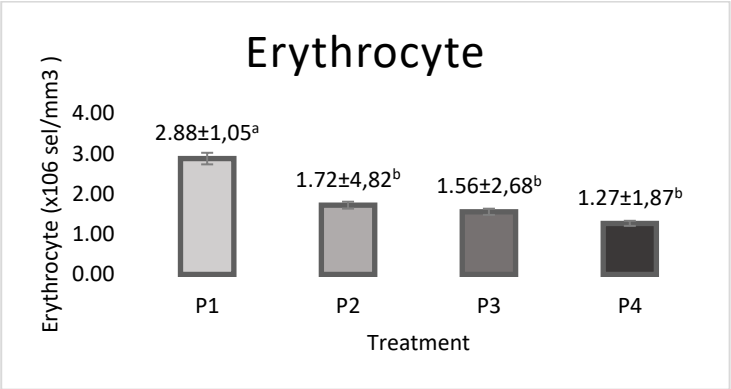

Figure 3. Erythrocyte levels of Tilapia (*Oreochromis niloticus*)

The erythrocyte levels obtained in this study ranged from $1.27x10^6$ cells/mm^3 – $2.88x10^6$ cells/mm^3. The highest total erythrocytes were in treatment P1 (Control), namely $2.88x10^6$ cells/mm^3 and the lowest total erythrocytes were in treatment P4, namely $1.27\ x10^6$ cells/mm^3. The effect of microplastic exposure in P1 feed was not significantly different from P2 but was significantly different from P3 and P4. The P1 treatment had higher erythrocyte levels compared to P1.

Leukocyte

The calculation results of tilapia leukocyte levels obtained during the research can be seen in Figure 4.

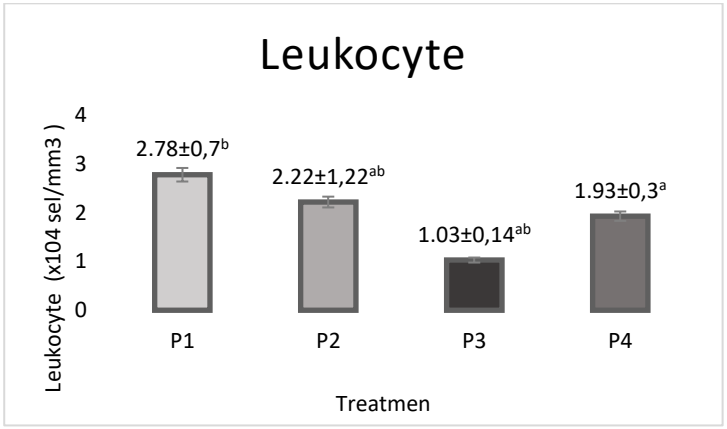

Figure 4. Leukocyte levels of Tilapia (*Oreochromis niloticus*)

The results of calculating leukocyte levels obtained from this study ranged from 1.03 x50 cells/mm^3 to 2.78 cells/mm^3. The highest leukocyte level was in P1, namely 2.78 cells/mm3, the lowest erythrocyte level was in P3, which was 1.03 cells/mm3. The leukocyte levels obtained had no real effect on treatment (>0.05). P1 is not significantly different from treatments P2 and P4 but P1 is significantly different (<0.05) from P3.

Differencial Leukocyte

The results of calculating the differential leukocyte levels of tilapia fish obtained during the research can be seen in Figure 5.

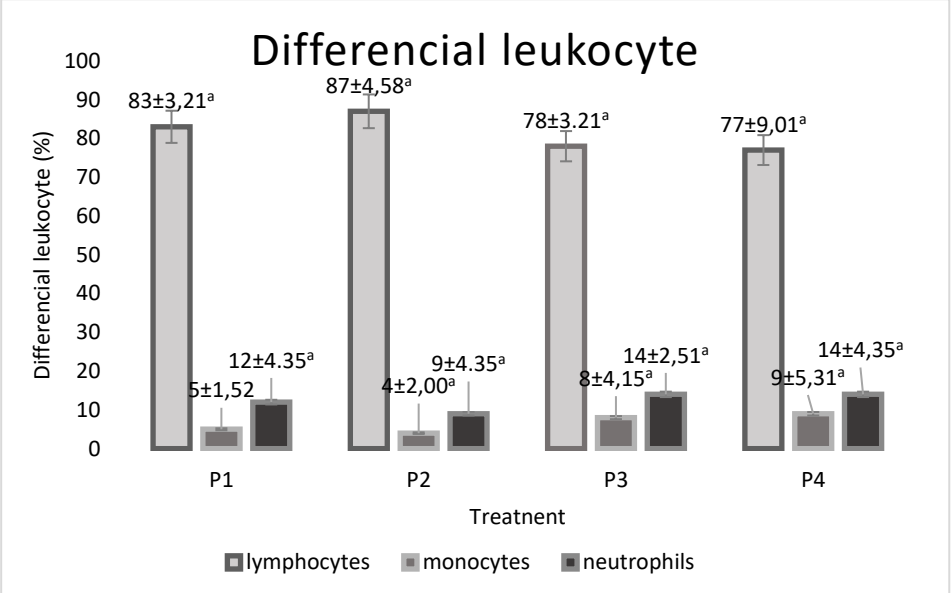

Figure 5. Differential Leukocyte Levels of Tilapia (*Oreochromis niloticus*)

The differential percentage of leukocytes observed consisted of 3 types, namely lymphocytes, monocytes and neutrophils. Exposure to microplastics at P3 and P4 had a significant effect on the differential percentage of tilapia leukocytes. Meanwhile, P1 and P2 did not show a significantly different effect from the effect of exposure to microplastics. The

difference in percentages obtained in each treatment was caused by microplastic levels so that it affected the differential leukocyte levels of tilapia fish.

Survival Rate (SR)

The results of calculating the hematocrit levels of tilapia obtained during the research can be seen in Figure 6.

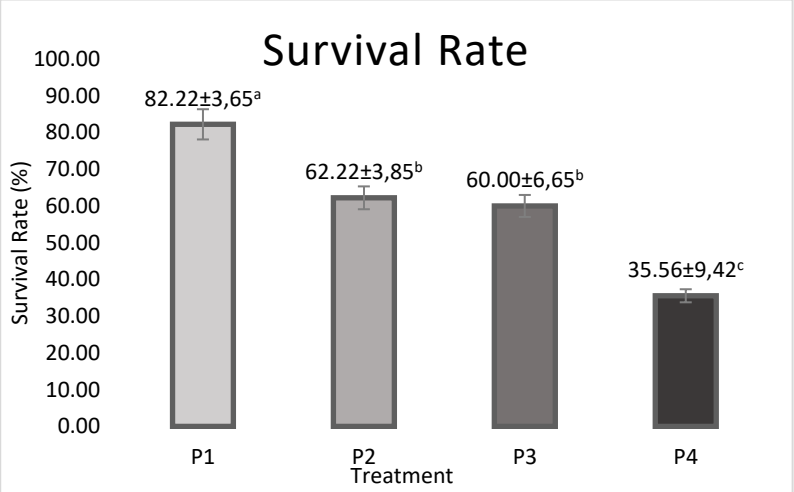

Figure 6. Survival Rate of Tilapia (*Oreochromis niloticus*)

The average SR yield of tilapia fish obtained from rearing ranges from 35.56% - 82.22%. The highest SR value is found on P1 with a value of 82.22%, the SR value on P2 obtained is 62.22%, on P3 the SR value obtained is 60.00% and the lowest SR value on P4 is 35.56%. Based on the results of the ANOVA test that has been carried out, it shows that exposure to polystyrene microplastics is significantly different (<0.05) to the SR of tilapia fish. From the results of the Duncan test, the highest SR value is found in P1, which is significantly different from P4, but not significantly different from P2 and P3.

DISCUSSION

Hematocrit

Hematocrit levels obtained during the maintenance period ranged from 7.96% -23.22%. According to Royan *et al*. (2014) the optimal hematocrit level for tilapia is around 20-30%. The hematocrit levels obtained by each treatment were different. The highest hematocrit level was found in P1 with a hematocrit value of 23.22%, while in treatments P2, P3 and P4 it was lower than P1. Hematocrit levels can be influenced by the presence of foreign objects entering the fish's body so that when treated with exposure to microplastics, the hematocrit levels appear to be lower. This happens because microplastics enter the fish's body, causing the fish to experience stress. Dosmi *et al*. (2022) low hematocrit levels can be caused by toxic materials entering the fish's body. The introduction of toxic materials will disrupt the body's physiology so that the fish will lose their appetite.

A fish's appetite can affect its stress level, the more stressed a fish is, the more its appetite will decrease. If the fish loses its appetite, the fish will experience stress very easily. According to Nainggolan *et al*. (2021) environmental contamination or bacterial infection, as well as stress are factors that cause fluctuations in the hematocrit percentage to be low.

Hemoglobin

The percentage of hemoglobin levels obtained from this study was 4.27%-8.76%. According to Hidayat *et al*. (2023) Tilapia hemoglobin levels decrease because tilapia fish experience stress due to exposure to foreign objects in the form of microplastics. If microplastics are consumed by living creatures, they will cause toxicity due to the chemicals they contain. This is in line with the statement from Rahman (2022) that microplastics contain chemical ingredients that are dangerous for living creatures, one of which is if exposed to tilapia fish.

Exposure to polystyrene microplastics can disrupt the binding process in fish which is obtained through the gills so that the more contaminated the fish rearing environment is by microplastics, the lower the oxygen obtained so that the hemoglobin level in the fish has a low value. This is in line with the statement from Yanto *et al.* (2015) That hemoglobin functions in the process of transporting O_2 from gills, transporting nutrients, removing metabolic waste. Low hemoglobin levels will be able to bind little oxygen so that the fish will experience stress, causing the fish to die.

Erythrocyte

The total erythrocyte test results obtained during the treatment had different values. According to Royan *et al*. (2014) that the normal number of erythrocytes for tilapia ranges from 1.05 x 10^6–3.0 x 10^6 cells/mm^3. So the erythrocyte value obtained is quite high. The number of etyrocytes obtained in tilapia can be influenced by environmental factors. The more contaminated the environment with foreign objects, the worse the rearing environment, so that the fish will experience stress which causes the number of erythrocytes obtained to be low. According to Gusti (2021) The number of erythrocytes can be influenced by the condition of the fish and the temperature of the aquatic environment.

According to Liana *et al.* (2024) optimal erythrocyte levels in tilapia range from 20,000-3000,000 cells/mm^3. The tilapia fish's erythrocyte levels are not optimal due to stress and the number of erythrocytes reflects the condition of the fish's body. Low erythrocytes indicate that the fish is anemic. This is in line with the statement from Lomi *et al*. (2023) that a low erythrocyte value indicates that the fish is anemic. According to Tukan *et al*. (2023), anemia that occurs causes the number of erythrocytes to decrease, thereby reducing the supply of food to cells, tissues and organs and causing metabolic processes to decrease.

Leukocyte

The number of leukocyte levels obtained from this study ranged from 1.03 x10^4 cells/mm^3 - 2.78 x 10^4 cells/mm^3. According to Firman *et al*. (2022) the normal range for tilapia leukocytes is 20,000 cells/mm^3-150,000 cells/mm^3. The number of leukocyte levels obtained was relatively low. Low leukocyte levels are caused by unstable environmental conditions, causing fish to experience stress. Apart from that, the number of leukocyte cells can also be caused by foreign objects in the fish's body and leukocytes also help cleanse the body of foreign objects or invading pathogens.

Microplastics found in the aquatic environment of tilapia cultivation contain toxic chemical compounds which cause contamination of the tilapia fish media. Microplastics that are ingested through feed can change the fish's immune system so that it is not optimal so that the health of the tilapia is disrupted because it enters the food network. This is in line with the statement from Wicaksono (2021) that microplastics can enter networks and can also accumulate to each trophic level. Microplastics that enter the fish's body can damage the cells in the fish's body. So the low level of leukocytes found in tilapia cannot increase the fish's immunity which causes the fish's immune system to decrease and even cause death.

Diferential Leukocyte

The differential observations carried out consisted of 3 observations, namely observing lymphocytes, monocytes and neutrophils. Each type of observation carried out by each treatment has different levels. The highest lymphocyte percentage was in P2, namely 87%, then continued with P1 with a lymphocyte level of 83%, after that the lymphocyte level in P3 was 78% and the lowest lymphocyte level was in P4 with a lymphocyte percentage of 77. According to Preanger *et al*. (2016) stated that an unfavorable environment that can cause fish to experience stress can cause hormone secretion which can affect the number of lymphocytes in the blood of tilapia fish. According to Utami *et al*. 2013 optimal lymphocyte levels for tilapia ranged from 60-67%.

Apart from lymphocytes and monocytes, neutrophils are one of the types observed in the leukocyte differential. Neutrophils have a role in destroying foreign objects that enter the body of tilapia fish. The percentage of neutrophils obtained shows that the percentage obtained is different for each treatment. The highest percentage of neutrophils was found in P3 and P4 at 14%, then continued with P1 which was equal and the lowest percentage was obtained at P2 which was 9%. According to Arifin (2016) stated that the optimal number of neutrophil cells in the fish body is around 6 – 8%. The high percentage of neutrophil levels is thought to be caused by stress in the fish so that activities to stimulate incoming foreign objects cannot be carried out optimally.

One part of the tilapia immune system is also called monocytes. The percentage of monocytes obtained at the end of maintenance decreased. The highest percentage of monocytes was found in P4 and P3 with monocyte levels of 9%, then continued with P1 with monocyte levels of 5%, and the lowest monocyte levels were in P2, namely 4%. Through the results obtained, it can be seen that the value of monocytes obtained is influenced by exposure to microplastics given through feed. High levels of monocytes indicate that the fish are experiencing stress so they can phagocytose foreign objects and the tilapia will die due to exposure to microplastics. According to Subryana *et al*. (2020), states that the percentage of normal tilapia monocytes ranges from 3.9-5.9%.

Survival Rate (SR)

Based on the research results, the survival rate (SR) values obtained ranged between 35.56% - 82.22%. P2, P3, and P4 have SR values that are not optimal, this is thought to be due to exposure to microplastics given to the feed. According to *Hendriana et al*. (2023), the optimal sr value of tilapia according to BSN (SNI 6141:2009) is above 75%. Microplastics contain dangerous ingredients because they contain chemicals so that when consumed by fish it will cause damage to the fish's organs so that the fish's body resistance level becomes increasingly reduced. Fish that have been exposed to microplastics can experience stress and cause death, therefore the SR in fish becomes lower.

Feed given exposure to microplastics is thought to cause the fish's digestive tract to not function properly so that the feed given causes damage and increases the potential for fish death. According to Rizkia (2023) states that microplastics found in the aquatic environment can clog and damage organs and even kill organisms. With the large number of deaths occurring in fish, the survival rate of tilapia becomes lower.

CONCLUSION

Based on the research that has been carried out, it can be concluded that the effect of exposure to polystyrene microplastics in feed at doses of 0.01 mg/0.75 g, 0.1 mg/0.75 g, and 1 mg/0.75 g. It has a real influence on hematocrit levels and survival rate, while the values of

erythrocytes, leukocytes and differential leukocytes do not have a real influence on the blood profile of nile fish exposed to polystyrene microplastics.

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