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# Effect of polyvinyl chloride microplastic on haematological of tilapia (*Oreochromis niloticus*)

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**ABSTRACT**. Plastic is the material most widely used by humans in life and commercial activities. The plastic waste used will ultimately be disposed of into the aquatic environment. Floating particles of plastic waste accumulate in pelagic habitats and form large waste patches. Meanwhile, non-floating debris degrades in the water column and in sediment, forming microplastics. Microplastics are small plastic waste measuring <5 mm. One type of microplastic that has an adverse impact on the life of organisms in waters is polyvinyl chloride (PVC). The aim of this research is to determine the effect of polyvinyl chloride microplastic exposed on haematological of tilapia, so it is hoped that a solution will emerge that can deal with this microplastic problem. This study was conducted in a completely randomized design (CRD) consisting of four treatments with three replications. The treatments were as follows: MP0 = No addition of microplastics (control); MP1 = Addition of 5 mg/L microplastics; MP2 = Addition of 15 mg/L microplastics; MP3 = Addition of 20 mg/L microplastics. Data were analyzed using SPSS version 25, with results expressed as mean ± standard error, and differences between control and treatments assessed by one-way ANOVA followed by Duncan's multiple range test at a significance level of $P<0.05$. The conclusion is exposure to polyvinyl chloride microplastics on the haematological of tilapia through water made a real difference to erythrocytes, leukocytes, lymphocytes, monocytes, neutrophils, hematocrit, haemoglobin, and glucose.

**Keywords**: aquatic pollution; microplastic; polyvinyl chloride; tilapia; haematology

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## INTRODUCTION

Nowadays, plastic is the material most widely used by humans in life and commercial activities. The plastic waste used will ultimately be disposed of into the aquatic environment. Floating particles of plastic waste accumulate in pelagic habitats and form large waste patches. Meanwhile, non-floating debris degrades in the water column and in sediment, forming microplastics (Margaretha *et al*., 2022). Microplastics are small plastic waste measuring <5 mm, most of which come from the decomposition of large plastics (Purnama *et al*., 2021).

Microplastics have quite a large impact on organisms that live in the sea due to their very small size and ability to survive in the sea for quite a long time. This happens because during the foraging process, microplastics can be ingested and enter the bodies of biota, either intentionally or unintentionally (Arisanti *et al*., 2023). The entry of microplastics into the bodies of biota can damage the digestive tract, reduce growth rates, reduce steroid hormone levels, inhibit enzyme production, affect reproduction and cause greater exposure to plastic additives that are toxic (Labibah & Triajie, 2020).

One type of microplastic that has an adverse impact on the life of organisms in waters is PVC (Nosova & Uspenskaya, 2023). This was proven by research by Iheanacho & Odo (2020b) which revealed that PVC microplastics induced changes in enzyme activity and liver damage which were observed histologically in exposed *Clarias gariepinus*. In a study by Darabi *et al*. (2022), it was found that *Cyprinus carpio* exposed to PVC experienced intestinal damage, including epithelial detachment, thinning of the intestinal wall, villous lesions, liver and gill damage, and behavioral changes. Furthermore, research on the effect of PVC exposure on *Oreochromis niloticus* was carried out by

Jawdhari *et al*. (2023), the results of which were that PVC exposure induced oxidative stress and liver histopathological changes.

The negative impact that microplastic have on aquatic organisms has begun to be realized. Currently, only a few studies have focused on the impact of microplastic in freshwater areas, most of which focus only on marine areas without paying attention to research for freshwater areas, so research on microplastic on freshwater aquatic organisms is very necessary, especially on tilapia (*Oreochromis niloticus*).

Tilapia in Indonesia is one of the freshwater fish that has high economic value and is an important commodity in the world freshwater fish business (Istiqomah *et al*., 2018). Tilapia is a source of animal protein that many Indonesians like to use as food (Wibowo *et al*., 2021). In Indonesia, tilapia is included in the 10 priority commodities for cultivation. Tilapia fish production continues to increase every year, the average increase in the number of tilapia fish production reached 31% in the 2013-2017 period. In 2017, tilapia production reached 1.15 million tons, an increase of 3.6% from 2016 which reached 1.14 million tons and is in second place in aquaculture production according to main commodities after biofloc catfish (Prajayati *et al*., 2020). In the cultivation process, tilapia requires good water quality, not too turbid and not contaminated with toxic chemicals, especially those originating from microplastic which have huge consequences for both the survival of the tilapia and the humans who consume the fish.

Based on the impact and potential dangers of microplastic on aquatic organisms, especially tilapia, it is necessary to conduct research on the effect of PVC microplastic exposed on tilapia. The aim of this research is to determine the effect of PVC microplastic exposed on the haematological of tilapia, so it is hoped that a solution will emerge that can deal with this microplastic problem.

## MATERIALS AND METHODS

**Location and time of research**. This research was carried out for 30 days from June to July 2023, which took place at the Fish Production and Reproduction Laboratory. Observations on the abundance of microplastic in fish gut were carried out at the Fish Health Laboratory, Aquaculture Study Program, Faculty of Agriculture, Universitas Mataram.

**Fish collection and maintenance.** Twelve containers are prepared with a size of 45 liters. Twelve containers were used with 4 treatments and 3 replications. The container is washed using soap and then dried. Next, the container is filled with 30 liters of water. A total of one hundred eighty tilapia with an average weight of 0.9 grams were purchased from the Lingsar Fish Seed Center, West Lombok Regency. Before rearing, the tilapia are acclimatized for 2 weeks by keeping them in a tarpaulin pond which has new environmental conditions. Adaptation is carried out until the fish no longer die in a row.

**Preparation of microplastic.** The type of microplastic used comes from PVC pipes (Wavin brand ®) which are crushed using a grinder to micro size. Then sifted using a flour sieve (Vamial Chem brand ®) with mesh 80. The sifted microplastic were stored in a container.

**Experimental design.** This study was conducted in a completely randomized design (CRD) consisting of four treatments with three replications. Exposure of microplastic is carried out before the seeds are put in containers. After that, the seeds were put in at a stocking density of 1 fish per 2 liters in containers with a capacity of 30 liters/container, so that 15 fish were kept in each experimental unit, then the microplastics were spread according to the predetermined concentration. Determination of the dose of microplastics in tilapia refers to same study by Setyono *et al*. (2024). The method of providing exposure to microplastics is through water. The treatments were as follows: (1) $MP_0$ = No addition of microplastics (control); (2) $MP_1$ = Addition of 5 mg/L microplastics; (3) $MP_2$ = Addition of 15 mg/L microplastics; and (4) $MP_3$ = Addition of 20 mg/L microplastics.

If the container looks dirty, the water will be replaced to maintain good growth of fish seeds. After each siphon, they are given another exposure of microplastics at the same concentration

according to the concentration of each experiment. The commercial diet (CP Prima brand ®) was used in this study. It gave twice a day with the ad satiation method in the morning and evening.

**Blood collection.** Tilapia were caught using a fish net, then placed in a bucket filled with water and aerated, then transported to the laboratory. Next, the fish was anesthetized with clove oil (0.1 mL/liter of water). After the fish is limp, the fish's blood is taken by inserting a syringe moistened with 10% EDTA in the caudalis vein (between the fish's scales near the tail). The blood that has been taken is put into a tube that has been moistened with 10% EDTA and analyzed further (Riauwaty & Syawal, 2016).

**Erythrocytes.** Erythrocytes are part of the blood that play a role in carrying and distributing $O_2$ throughout the body's tissues (Aridya *et al*., 2023). The formula for calculating erythrocytes as follows (Blaxhall & Daisley, 1973; Dosim *et al*., 2022), where the measurement of total erythrocytes is that the blood sample is sucked with a 0.5 scale pipette followed by sucking Hayem's solution up to 101 scale, then homogenized by shaking it to form a figure eight. The first drop is discarded and the next drop is put into the hemocytometer and covered with a cover glass. Counting was carried out on 5 small haemocytometer boxes and the amount was calculated using the formula:

$$\Sigma\text{Erythrocytes} = \frac{\Sigma\text{cells}}{\text{large box volume}} \times \text{dilution factor}$$

**Leukocytes.** Leukocytes are part of the blood that function in the body's defense system against infection (Prasthio *et al*., 2022). The formula for calculating leukocytes as follows (Blaxhall & Daisley, 1973; Putranto *et al*., 2019), where the blood sample is sucked with a pipette containing white stirring beads up to a scale of 0.5 then Turk's solution is added up to a scale of 11. Stirring is done in the pipette by swinging the hand holding the pipette in a figure 8 shape for 3-5 minutes until the blood is evenly mixed. The first drop of blood solution in the pipette is discarded, then the blood sample is dropped into the hemocytometer and then covered with a cover glass. The liquid will fill the counting chamber with the help of a microscope with a magnification of 400x. The total number of leukocytes is counted in 4 small boxes and the number is calculated using the formula:

$$\Sigma\text{Leukocytes} = \frac{\Sigma\text{cells}}{\text{large box volume}} \times \text{dilution factor}$$

**Leukocyte differential.** The procedure for calculating differential leukocytes according to (Amlacher, 1970; Hartika *et al*., 2014), first hold the object glass with your index finger and thumb. Drop a little blood on the clean glass slide on the right. Then, place another object glass to the left of the blood drop at an angle of 30°. Pull the object glass to the right until it touches the blood. After the blood has spread along the edge of the second object glass, push the second object glass to the left while still forming an angle of 30° to obtain a blood preparation that is thin enough to be easily observed. After that the review was aired. To make it easier to observe, blood can be stained with Giemsa dye. Blood staining procedure with Giemsa. First, the blood that has just been examined on the object glass is air-dried (air fixation), then fixed in methanol solution for 5 minutes. After that, soak the test preparation in diluted Giemsa solution (1:20) for 15 minutes. Then, rinse with distilled water and air dry. The finished preparations were then placed under a microscope and observed at 400 times magnification. The percentage of leukocyte cells was calculated by observing 10 fields of view and each differential type of leukocyte counted was grouped according to its type (lymphocytes,

monocytes and neutrophils). The calculation of the number of lymphocytes, monocytes and neutrophils is as follows:

$$\%\text{Lymphocytes} = \frac{\text{L}}{100} \times 100\%$$

$$\%\text{Monocytes} = \frac{\text{M}}{100} \times 100\%$$

$$\%\text{Neutrophils} = \frac{\text{N}}{100} \times 100\%$$

Notes:
L = number of lymphocytes cells
M = number of monocytes cells
N = number of neutrophils cells

**Hematocrit.** Hematocrit is the percentage of the entire volume of erythrocytes separated from plasma by rotating them in a special tube at a certain time and speed where the value is expressed in percent (%) (Chairani *et al*., 2022). Hematocrit measurements are carried out by placing a blood sample in a micro hematocrit tube until approximately 4/5 of the tube. The end marked red was plugged with cretoseal then centrifuged for 15 minutes at a speed of 3500 rpm. After that, the percentage of the hematocrit value is measured. Hematocrit levels are expressed as % volume of blood cell solids. The formula calculation is determined by calculation (Royan *et al*., 2014; Maulinia & Herlina, 2022).

$$\text{Hematocrit} = \frac{\text{Length of the volume of red blood cells deposited}}{\text{Length of the total volume of blood in the tube}} \times \mathbf{100}\%$$

**Haemoglobin.** Haemoglobin is a protein in red blood cells which functions to transport oxygen from the lungs throughout the body (Tutik & Ningsih, 2019). According to (Wedemeyer & Yasutake, 1977; Hutasoit *et al*., 2017) states that haemoglobin levels are measured by filling the sahlinometer tube with 0.1 N HCl solution until the number 10 (the bottom scale line on the sahlinometer tube), then the tube is placed between 2 tubes with standard colors, then fish blood was taken from the micro tube with a sahli pipette in the amount of 0.02 mL and put into the sahli tube and left for 3 minutes, beforehand the tip of the pipette was cleaned first then distilled water was added with the pipette little by little while stirring with mixing glass until the color is exactly the same as the standard color. Haemoglobin levels are expressed in g/dL.

**Glucose.** Glucose in the blood is a monosaccharide sugar, the most important carbohydrate used as the main source of energy in the body (Fahmi *et al*., 2020). Fish blood glucose was measured using a glucometer, based on Syamsiyah *et al*. (2022), a strip was inserted into the glucometer. If an indicator appears stating the command to drip blood, the blood is dropped into the sensor box on the glucometer strip. Then wait for the glucometer screen to display a digital number (expressed in mg/dL) which shows the blood glucose level. The use of strips must be different because each strip can only be used in one use.

**Data analysis.** Data analysis was performed using SPSS software (SPSS version 25 for Windows). Results are presented as mean and standard error. The difference between the control and treatments were analyzed using a one-way analysis of variance (ANOVA) followed by the Duncan multiple range test. The significant differences were considered when $P<0.05$.

## RESULTS AND DISCUSSION

**Erythrocytes.** Tilapia erythrocytes can be determined by counting the number on 5 small haemocytometer boxes. The erythrocytes results obtained on average ranged from 1.29 x $10^6$ cells/$mm^3$-3.64 x $10^6$ cells/$mm^3$ can be seen in Fig. 1.

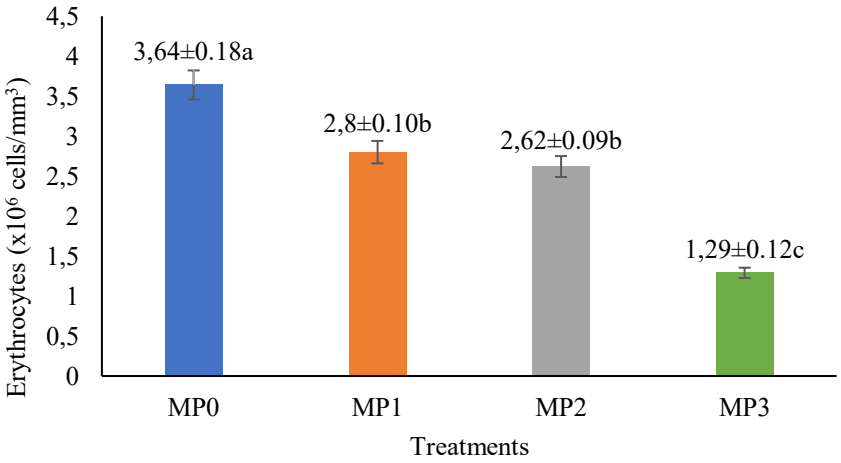


**Fig. 1.** Blood levels of erythrocyte in tilapia

The results of the ANOVA test at the 0.05 level showed that the erythrocytes of tilapia at different microplastic doses had a significant effect ($P<0.05$). The highest erythrocytes were in treatment $MP_0$ with 3.64 x $10^6$ cells/mm$^3$, while the lowest erythrocytes were in treatment $MP_3$ with 1.29 x $10^6$ cells/mm$^3$. Based on the results of further analysis (Duncan's test), the $MP_0$ treatment was very significantly different from $MP_1$, $MP_2$ and $MP_3$, while $MP_1$ and $MP_2$ were not significantly different, but were significantly different from $MP_3$.

The health status of fish can be determined through blood quality checks. Changes that occur in blood quality can be caused by disease or environmental conditions. Changes in hematocrit values, haemoglobin levels, red blood cell counts and white blood cell counts are indicators of fish health (Alipin & Sari, 2020). Erythrocytes (red blood cells) are the most abundant blood cells compared to other cells. Under normal conditions, the number of erythrocytes reaches almost half of the blood volume. The normal number of erythrocytes in tilapia is between 20,000-3,000,000 cells/mm$^3$ (Maulinia & Herlina, 2022). In this study, it was discovered that the number of erythrocytes showed a significant decrease along with the increase in the dose of PVC microplastic. In a study by Hamed *et al*. (2019) found a decrease in the value of red blood cells in *O. niloticus* which was observed after exposure to microplastics. Another study on *Clarias gariepinus* conducted by Iheanacho & Odo (2020a) reported a decrease in the number of red blood cells after exposure to PVC microplastics. Changes in erythrocyte values reflect the fish's defense mechanism against stress due to exposure to environmental toxicity. Microplastics that accumulate in the digestive and circulatory systems after exposure to microplastics can cause toxic effects and cause a decrease in fish hematological parameters such as red blood cells.

It is estimated that 14% of MP particles absorbed in the fish intestine enter the bloodstream and micro- or nano-sized plastics in the bloodstream can cause local inflammation or tissue allergic reactions (Hwang *et al*., 2020; Alberghini *et al*., 2023). The anemia observed in this study may be caused by lysis of erythrocytes (red blood cells) or suppression of erythropoiesis due to damage to hematopoietic tissue (Detzner *et al*., 2020; Afriansyah *et al*., 2021). Current research also shows changes in erythrocytes thus supporting this hypothesis.

**Leukocytes.** Tilapia leukocytes can be determined by counting the number on 4 small haemocytometer boxes. The leukocytes results obtained on average ranged from 14.33 x $10^4$ cells/mm$^3$-32.00 x $10^4$ cells/mm$^3$ can be seen in Fig. 2.

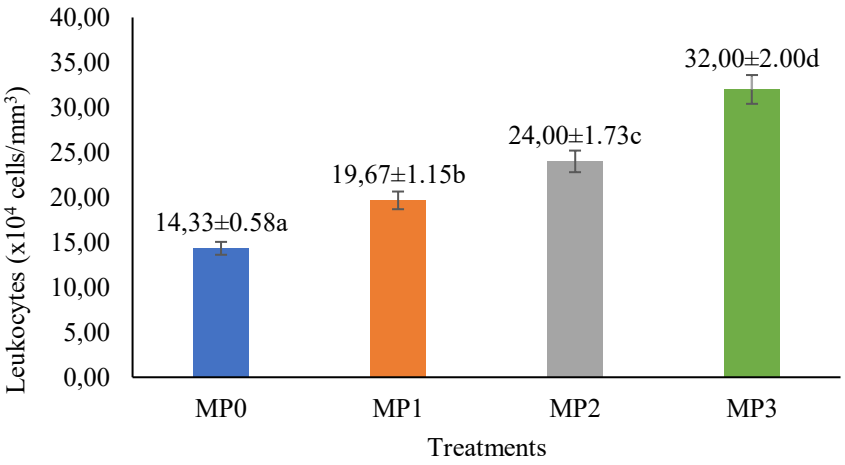


**Fig. 2.** Blood levels of leukocyte in tilapia

The results of the ANOVA test at the 0.05 level showed that the leukocytes of tilapia at different microplastic doses had a significant effect ($P<0.05$). The highest leukocytes were in treatment $MP_3$ with 32.00 x $10^4$ cells/mm$^3$, while the lowest leukocytes were in treatment $MP_0$ with 14.33 x $10^4$ cells/mm$^3$. Based on the results of further analysis (Duncan's test), the $MP_0$ treatment was very significantly different from $MP_1$, $MP_2$ and $MP_3$.

The health status of fish can be determined through blood quality checks. Changes that occur in blood quality can be caused by disease or environmental conditions. Changes in hematocrit values, haemoglobin levels, red blood cell counts and white blood cell counts are indicators of fish health (Alipin & Sari, 2020). The normal range for the number of white blood cells in normal tilapia is generally around 20,000-150,000 cells/mm$^3$ (Maulinia & Herlina, 2022). In this study, it was discovered that the number of leukocytes showed a significant increase along with the increase in the dose of PVC microplastic. Raza *et al*. (2023) stated that exposure to hazardous materials such as microplastics in fish resulted in changes in hematological parameters, such as a significant increase in white blood cells, a significant decrease in red blood cells, haemoglobin and platelet counts. A study on *Clarias gariepinus* conducted by Iheanacho & Odo (2020a) reported an increase in the number of white blood cells as the dose of exposure to PVC microplastics increased. Changes in leukocyte values reflect the mechanism of fish immunity to stress due to exposure to environmental toxicity. Microplastics that accumulate in the digestive and circulatory systems after exposure to microplastics can cause toxic effects and cause an increase in fish hematological parameters such as white blood cells.

The fish immune system consists of innate and adaptive immune systems. The innate immune system is the first line of defense against harmful and detrimental microbes. Innate immunity is also called the non-specific immune system, because this system responds the same to all foreign organisms. Phagocytes, especially white blood cells (leukocytes), are innate immune cells. These cells engulf and digest antigens, the information of which is transferred to the cell surface of macrophages and then recognized by the adaptive immune system (Yang *et al*., 2022).

**Leukocyte differential.** Tilapia leukocyte differential are divided into 3, namely lymphocytes, monocytes and neutrophils. The lymphocytes results obtained on average ranged from 68%-91%. The monocytes results obtained on average ranged from 6%-18%. The neutrophils results obtained on average ranged from 3%-14%. All data are presented in Fig. 3.

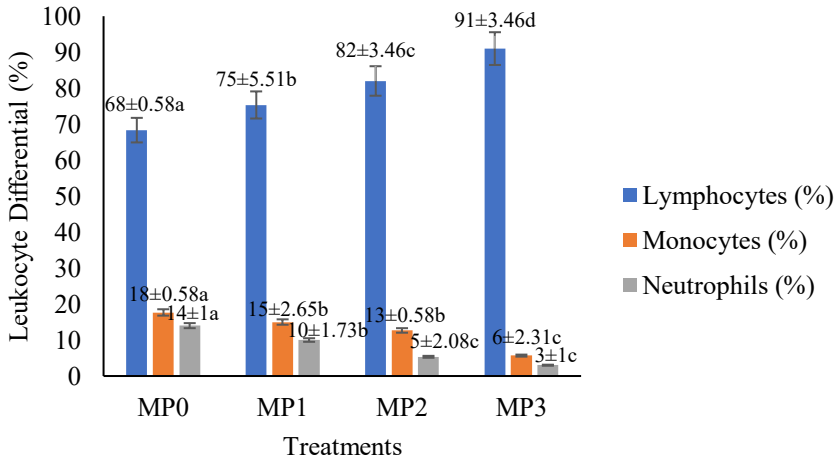


**Fig. 3.** Blood levels of leukocyte differential in tilapia

The results of the ANOVA test at the 0.05 level showed that the leukocyte differential of tilapia at different microplastic doses had a significant effect ($P<0.05$). The highest lymphocytes were in treatment $MP_3$ with 91%, while the lowest lymphocytes were in treatment $MP_0$ with 68%. Based on the results of further analysis (Duncan's test), the $MP_0$ treatment was very significantly different from $MP_1$, $MP_2$ and $MP_3$. The highest monocytes were in treatment $MP_0$ with 18%, while the lowest monocytes were in treatment $MP_3$ with 6%. Based on the results of further analysis (Duncan's test), the $MP_0$ treatment was very significantly different from $MP_1$, $MP_2$ and $MP_3$, while $MP_1$ and $MP_2$ were not significantly different, but were significantly different from $MP_3$. The highest neutrophils were in treatment $MP_0$ with 14%, while the lowest neutrophils were in treatment $MP_3$ with 3%. Based on the results of further analysis (Duncan's test), the $MP_0$ treatment was very significantly different from $MP_1$, $MP_2$ and $MP_3$, while $MP_2$ and $MP_3$ were not significantly different, but were significantly different from $MP_1$.

The health status of fish can be determined through blood quality checks. Changes that occur in blood quality can be caused by disease or environmental conditions. Changes in hematocrit values, haemoglobin levels, red blood cell counts and white blood cell counts are indicators of fish health (Alipin & Sari, 2020). In this study, it was discovered that the number of leukocytes showed a significant increase along with the increase in the dose of PVC microplastic. Raza *et al*. (2023) stated that exposure to hazardous materials such as microplastics in fish resulted in changes in hematological parameters, such as a significant increase in white blood cells, a significant decrease in red blood cells, haemoglobin and platelet counts. A study on *Clarias gariepinus* conducted by Iheanacho & Odo (2020a) reported an increase in the number of white blood cells as the dose of exposure to PVC microplastics increased. Changes in leukocyte values reflect the mechanism of fish immunity to stress due to exposure to environmental toxicity. Microplastics that accumulate in the digestive and circulatory systems after exposure to microplastics can cause toxic effects and cause an increase in fish hematological parameters such as white blood cells.

Leukocyte differential is a sensitive biomarker of environmental stress that serves as the first line of defense against foreign invasion into an organism's system (Iheanacho & Odo, 2020a). There are two types of leukocytes, namely granulocytes (neutrophils, eosinophils and basophils) and agranulocytes (lymphocytes and monocytes). In this study lymphocyte values were higher than monocytes and neutrophils as reported by Hamed *et al*. (2022) on *Cyprinus carpio* and Soliman *et al*. (2023) on *Clarias gariepinus*. Lymphocytes play an important role in the body's immune response to fight viral infections and bacterial infections by forming antibodies to increase the body's immunity against infection. The function of monocytes is as the second layer of the body's defense which can phagocytose and is included in the macrophage group. The main function of neutrophils is to fight

bacterial infections and inflammatory disorders (Indarwati & Prasdini, 2017; Giyartika & Keman, 2020).

**Hematocrit.** Tilapia hematocrit levels are expressed as % volume of blood cell solids. The hematocrit results obtained on average ranged from 23.67%-36.67% can be seen in Fig. 4.

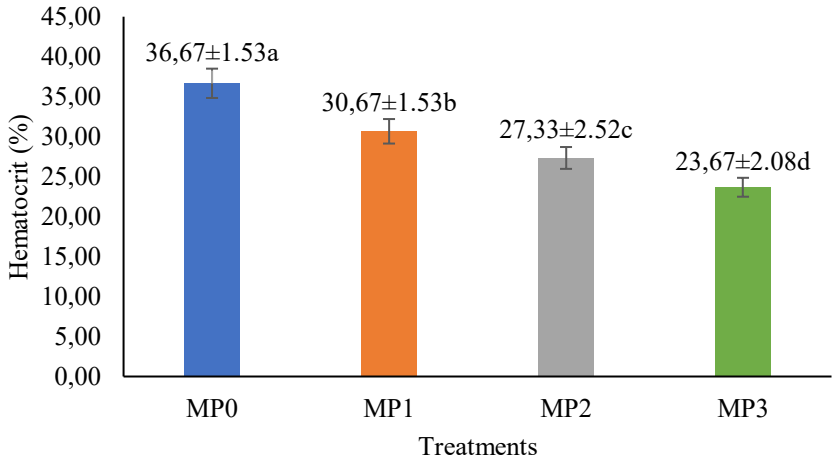


**Fig. 4.** Blood levels of hematocrit in tilapia

The results of the ANOVA test at the 0.05 level showed that the hematocrit of tilapia at different microplastic doses had a significant effect ($P<0.05$). The highest hematocrit were in treatment $MP_0$ with 36.67%, while the lowest hematocrit were in treatment $MP_3$ with 23.67%. Based on the results of further analysis (Duncan's test), the $MP_0$ treatment was very significantly different from $MP_1$, $MP_2$ and $MP_3$.

The health status of fish can be determined through blood quality checks. Changes that occur in blood quality can be caused by disease or environmental conditions. Changes in hematocrit values, haemoglobin levels, red blood cell counts and white blood cell counts are indicators of fish health (Alipin & Sari, 2020). Tilapia hematocrit levels range from 27.3%-37.8% (Maulinia & Herlina, 2022). In this study, it was discovered that the hematocrit value showed a significant decrease along with the increase in the dose of PVC microplastic. In a study by Hamed *et al*. (2019) found that a decrease in hematocrit values in *O. niloticus* was observed after exposure to microplastics. Another study on *Cyprinus carpio* conducted by Hamed *et al*. (2022) reported a decrease in hematocrit values after exposure to microplastics. Changes in hematocrit values reflect the fish's defense mechanism against stress due to exposure to environmental toxicity. Microplastics that accumulate in the digestive and circulatory systems after exposure to microplastics can cause toxic effects and cause a decrease in fish hematological parameters such as hematocrit.

Hematocrit is the percentage of the volume of red blood cells in the fish's body (Putranto *et al*., 2019). The mechanism for changes in hematocrit percentage during stress begins with the receipt of information on the causes of stress factors by receptor organs. Next, this information is conveyed to the hypothalamus part of the brain via the nervous system. The hypothalamus instructs chromaffin cells to secrete catecholamines via sympathetic nerve fibers. The presence of catecholamines will activate lipopolysaccharides which attack blood components whose function is to reduce hematocrit in fish (Madyowati & Muhajir, 2018).

**Haemoglobin.** Haemoglobin is a protein in red blood cells which functions to transport oxygen from the lungs throughout the body. Haemoglobin levels are expressed in g/dL. The haemoglobin results obtained on average ranged from 4.67 g/dL-8.67 g/dL can be seen in Fig. 5.

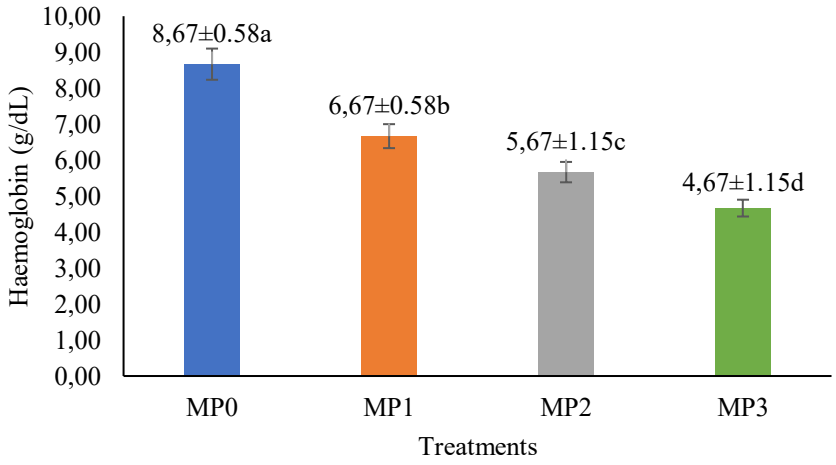


**Fig. 5.** Blood levels of haemoglobin in tilapia

The results of the ANOVA test at the 0.05 level showed that the haemoglobin of tilapia at different microplastic doses had a significant effect ($P<0.05$). The highest haemoglobin were in treatment $MP_0$ with 8.67 g/dL, while the lowest haemoglobin were in treatment $MP_3$ with 4.67 g/dL. Based on the results of further analysis (Duncan's test), the $MP_0$ treatment was very significantly different from $MP_1$, $MP_2$ and $MP_3$.

The health status of fish can be determined through blood quality checks. Changes that occur in blood quality can be caused by disease or environmental conditions. Changes in hematocrit values, haemoglobin levels, red blood cell counts and white blood cell counts are indicators of fish health (Alipin & Sari, 2020). Normal haemoglobin levels in tilapia blood are between 6-11.01 g/dL (Maulinia & Herlina, 2022). In this study, it was found that the amount of haemoglobin showed a significant decrease along with the increase in the dose of PVC microplastic. In a study by Hamed *et al*. (2019) found that a decrease in haemoglobin values in *O. niloticus* was observed after exposure to microplastics. Another study on *Clarias gariepinus* conducted by Iheanacho & Odo (2020a) reported a decrease in haemoglobin values after exposure to PVC microplastics. Changes in haemoglobin values reflect the fish's defense mechanism against stress due to exposure to environmental toxicity. Microplastics that accumulate in the digestive and circulatory systems after exposure to microplastics can cause toxic effects and cause a decrease in fish hematological parameters such as haemoglobin.

Haemoglobin is part of blood plasma cells which has a very important function in the circulatory system in fish. Haemoglobin is a protein in erythrocytes which is composed of colorless globin protein and heme pigment produced in erythrocytes, and the ability of blood to transport oxygen depends on haemoglobin in the blood (Putranto *et al*., 2019). Haemoglobin levels are similar with the number of erythrocytes, the lower the haemoglobin level, the lower the number of erythrocytes (Idzni *et al*., 2018). Low haemoglobin causes anemia in fish and results in a low amount of oxygen in the blood. A decrease in haemoglobin values indicates an abnormality in fish health (Maulinia & Herlina, 2022). The anemia observed in this study may be caused by lysis of erythrocytes (red blood cells) or suppression of erythropoiesis due to damage to hematopoietic tissue (Detzner *et al*., 2020; Afriansyah *et al*., 2021).

**Glucose.** Glucose in the blood is a monosaccharide sugar, the most important carbohydrate used as the main source of energy in the body. Glucose levels are expressed in mg/dL. The glucose results obtained on average ranged from 70.00 mg/dL-101.33 mg/dL can be seen in Fig. 6.

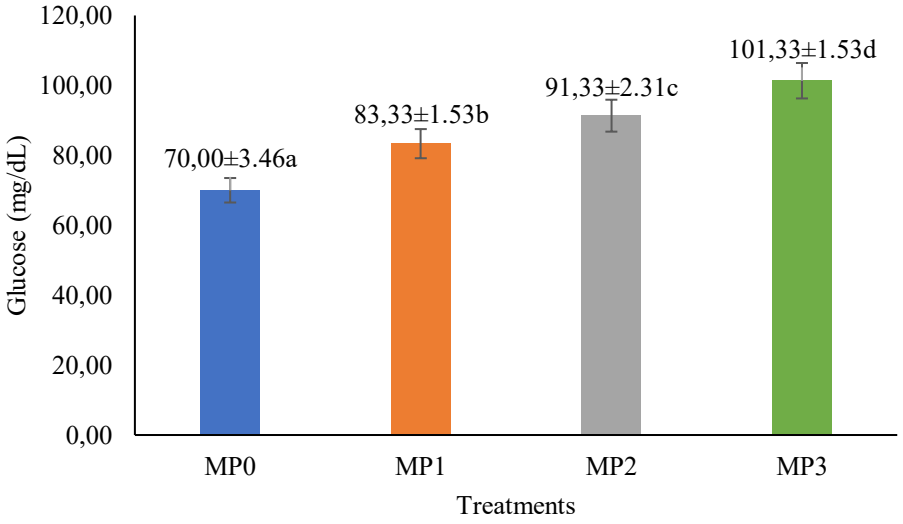


**Fig. 6.** Blood levels of glucose in tilapia

The results of the ANOVA test at the 0.05 level showed that the glucose of tilapia at different microplastic doses had a significant effect ($P<0.05$). The highest glucose were in treatment $MP_3$ with 101.33 mg/dL, while the lowest glucose were in treatment $MP_0$ with 70.00 mg/dL. Based on the results of further analysis (Duncan's test), the $MP_0$ treatment was very significantly different from $MP_1$, $MP_2$ and $MP_3$.

The health status of fish can be determined through blood quality checks. Changes that occur in blood quality can be caused by disease or environmental conditions. Changes in hematocrit and glucose values are indicators of fish health (Hastuti & Subandiyono, 2015). Changes in fish glucose can also be an indication of stress in fish (Hertika *et al*., 2021). Tilapia blood glucose levels ranging from 40-90 mg/dL (Yunus & Yushra, 2023). In this study, it was discovered that the amount of glucose showed a significant increase along with the increase in the dose of PVC microplastic. In a study by Hamed *et al*. (2019); Hamed *et al*. (2022) found that an increase in glucose values in *O. niloticus* was observed after exposure to microplastics. Another study on *Clarias gariepinus* conducted by Iheanacho & Odo (2020b); Iheanacho *et al*. (2020) reported an increase in glucose values after exposure to PVC microplastics. Changes in glucose values reflect the fish's defense mechanism against stress due to exposure to environmental toxicity. Microplastics that accumulate in the digestive and circulatory systems after exposure to microplastics can cause toxic effects and cause an increase in fish hematological parameters such as glucose values.

An increase in glucose levels indicates the disintegration of glycogen in liver tissue or a decrease in glucose absorption. Hyperglycemia can occur in fish due to exposure to adverse conditions, such as exposure to UV A light, heavy metals, and other pollutants such as microplastics (Hamed *et al*., 2019; Hamed *et al*., 2022). In general, glucose is an important biomarker associated with secondary responses that help fish survive and recover from stress. The increase in glucose levels in the treatment given PVC shows an increase in glucose utilization to meet the same metabolic energy needs as stress, which is caused by PVC. Stress conditions are caused by increased corticosteroids which cause hyperglycemia in fish (Iheanacho & Odo, 2020b; Iheanacho *et al*., 2020).

## CONCLUSION

Exposure to PVC microplastics on the haematological of tilapia through water made a real difference to erythrocytes, leukocytes, lymphocytes, monocytes, neutrophils, hematocrit, haemoglobin, and glucose. However, further research is needed with different doses and fish to identify the effect of PVC microplastic exposure on fish haematological parameters. Another

suggestion that can be given is exploring the long-term effects of microplastic exposure or testing different microplastic types.

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