

TIK-29 Hematological and Histological Analysis of Tilapia (Oreochromis Niloticus) Cultured in Floating Net Cages After Disease Outbreak

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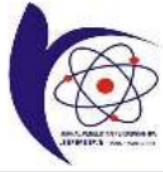
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Hematological and Histological Analysis of Tilapia (*Oreochromis Niloticus*) Cultured in Floating Net Cages After Disease Outbreak

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Abstract: Recently, Tilapia fish cultivated in floating net cages in Karang Intan District experienced a sudden death from June 4 to June 5. At some point, mortality reached 50% of the population in floating net cages. The purpose of this study was to analyze the hematology and histology of tilapia (*Oreochromis niloticus*) after exposure to disease outbreaks that were cultivated in floating net cages. The research location was carried out at the Laboratory of Fish Pests and Diseases, Faculty of Fisheries and Maritime Affairs, University of Lambung Mangkurat. The animal samples used were tilapia showing clinical symptoms of sick fish obtained from floating net cages in Mali-Mali Village, Karang Intan District, which experienced quite high fish mortality cases and 3 fish were taken. Based on the results of hemtological analysis of tilapia (*Oreochromis niloticus*) after being exposed to disease outbreaks which were cultivated in floating net cages, the hemoglobin levels of tilapia obtained in this study ranged from 6.9-7.8 g/dl, the average hematocrit of tilapia was obtained in this study has a range between 37.2 - 58.9%, the average tilapia leukocrit obtained in this study has a range between 2.89 - 6.61% and blood plasma is a liquid component of blood consisting of 99% water and 8-9% protein.

Keywords: Disease outbreaks; Hematology; Histology; Tilapia

Introduction

Tilapia (*Oreochromis niloticus*) is one of the fish that people like to consume. Recently, Tilapia fish cultured in floating net cages in Karang Intan District experienced a sudden death from June 4 to June 5 (Mandia et al., 2013). At some points, mortality has reached 50% of the population in floating net cages. From the results of measurements carried out by the Department of Fisheries and Banjar Regency, it was found that the water quality on June 5, 2023 had low oxygen levels from standard 4. So the cause of death was thought to be due to low oxygen levels and this fish death could also be due to the presence of pathogens that attack fish (Rousdy & Linda, 2018).

Several types of bacteria that most commonly attack freshwater fish such as *Aeromonas* sp. and *Streptococcus* sp. (Ashari et al., 2014). This disease causes a lot of losses due to the high death rate due to bacterial attack, which is up to 30-50% (Eldar et al., 1994; Gardenia et al., 2011). Fish infected by *Streptococcus* sp. experiencing symptoms including decreased appetite, moving irregularly, swimming to the surface, lethargy, blackened body, scales easily detached, wounds, bleeding in the operculum and anus, cloudy and protruding eyes (Eldar et al., 1994). Internal organs that are often attacked are the spleen, liver and brain, then spread to the kidneys, intestines and heart (Austin et al., 2007). The spleen appears enlarged and damaged, the liver looks pale and necrotic in several places. Intestine

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contains fluid and some hemorrhagic. Often found acute meningitis, with the surface of the brain looks yellowish and contains lots of bacteria.

Tilapia (*Oreochromis niloticus*) is one of the fish that people like to consume (Hamada et al., 2018; Kori-Siakpere et al., 2005; Levina et al., 2021). Recently, Tilapia fish cultured in floating net cages in Karang Intan District experienced a sudden death from June 4 to June 5. At some points, mortality has reached 50% of the population in floating net cages. From the results of measurements carried out by the Department of Fisheries and Banjar Regency, it was found that the water quality on June 5, 2023 had low oxygen levels from standard 4. So the cause of death was thought to be due to low oxygen levels and this fish death could also be due to the presence of pathogens that attack fish (Indarti et al., 2012).

Several types of bacteria that most commonly attack freshwater fish such as *Aeromonas* sp. and *Streptococcus* sp. (Ashari et al., 2014). This disease causes a lot of losses due to the high death rate due to bacterial attack, which is up to 30-50%. Fish infected by *Streptococcus* sp. experiencing symptoms including decreased appetite, moving irregularly, swimming to the surface, lethargy, blackened body, scales easily detached, wounds, bleeding in the operculum and anus, cloudy and protruding eyes (Bhat et al., 2023). Internal organs that are often attacked are the spleen, liver and brain, then spread to the kidneys, intestines and heart. The spleen appears enlarged and damaged, the liver looks pale and necrotic in several places. Intestine contains fluid and some hemorrhagic (Nuridin, 2008). Often found acute meningitis, with the surface of the brain looks yellowish and contains lots of bacteria.

Method

The location of the research was carried out at the Laboratory of Fish Pests and Diseases, Faculty of Fisheries and Maritime Affairs, University of Lambung Mangkurat. The animal samples used were tilapia showing clinical symptoms of sick fish obtained from floating net cages in Mali-Mali Village, Karang Intan District which experienced quite high fish mortality cases and 3 fish were taken.

Tilapia Hematological Analysis

The measurement of hematocrit levels refers to the method of (Anderson & Siwicki, 1994; Dianti et al., 2013), in which the blood sample is inserted into the microhematocrit capillary up to approximately 4/5 of the tube, then the end (marked red) is plugged with cretoseal (cover wax). The microhematocrit capillaries were then centrifuged for 15 minutes at 3500 rpm.

Centrifuge speed and centrifugation time have an effect on the results of the hematocrit examination, the higher the speed and the longer the centrifugation is carried out, the faster the precipitation will occur and the results obtained will be more optimal (Saleh et al., 2019). The length of the erythrocyte deposits in the hematocrit capillaries was measured with the hematocrit scale and the volume percentage was calculated. Hematocrit levels are expressed as % solid volume of blood cells.

The procedure for calculating hemoglobin using the Sahli method according to Hartika et al. (2014) begins with taking blood samples from test fish using a Sahli pipette with a scale of 20 mm³ or 0.2 ml. Then the pipette tip was cleaned with a tissue and transferred to the HB-meter tube which had been filled with 0.1 N HCL to a scale of 10 (red). The blood in the tube is stirred with a stir bar for 3-5 minutes (Shen et al., 2019). After that the tube is given Sodium Citrate in a Sahli pipette. Then add distilled water in the tube until the blood color shows the same color as the Sahli solution (the standard in the HB-meter). Hemoglobin levels are expressed in g % (Hidayat et al., 2014).

Measurement of leukocrit levels aims to determine the health condition of fish. It can be calculated using the leukocrit calculation formula according to Pratiwi et al. (2019). Observation of blood plasma color was carried out descriptively. According to Aisiah et al. (2015), blood plasma is calculated based on the difference between the total number of blood with leukocytes and erythrocytes.

Tilapia Histological Analysis

Histopathological observation of organs in the test fish samples was carried out in the Mandiangin BPBAT testing laboratory. Histopathological examination is an examination technique by studying abnormal changes in cells or tissues (Insivitawati et al., 2015).

Result and Discussion

Tilapia Hematology

The results of measuring the blood profile of tilapia when analyzed in the laboratory of fish pests and diseases of the Faculty of Fisheries and Marine Affairs are as follows:

Hemoglobin

The hemoglobin value of tilapia blood obtained from a blood test using a sahlimeter can be seen in Table 1.

Table 1. Tilapia Blood Hemoglobin Levels

Sample (fish)	Rate hemoglobin (g/dl)
A1	6.9
A2	7.8
A3	7.0

Table 1 shows that the amount of tilapia hemoglobin obtained in this study has a range of 6.9-7.8 g/dl. Blood is one of the parameters that can be used to see abnormalities that occur in fish, whether they occur due to disease or due to environmental conditions. Diseased fish will experience changes in hematocrit values, hemoglobin levels and white blood cell counts. Blood tests can be used as an indicator of the severity of a disease. The haematological study is an important criterion for the diagnosis and determination of the health of the fish.

Hemoglobin is the part of the red blood cells that binds oxygen which is called oxyhemoglobin. Hemoglobin is responsible for the transport of oxygen and carbon dioxide in the blood. Transport of oxygen in the blood depends on the iron component of the respiratory pigment, usually hemoglobin. Hemoglobin functions to bind oxygen which will then be used for the catabolic process to produce energy. The ability to bind oxygen in the blood depends on the amount of hemoglobin contained in the red blood cells, low hemoglobin levels cause the metabolic rate to decrease and the energy produced is low (Hartika, 2014).

The highest hemoglobin level was found in the A2 fish sample of 7.8 g/dL, while in the A3 fish sample it was 7.0 g/dL, and the A1 fish sample was 6.9 g/dL which was the lowest hemoglobin level. Samples A2 and A3 had normal hemoglobin levels but in sample A1 it decreased and the response to feed was reduced but did not affect their body resistance.

Rahmaningsih et al. 2019 stated that blood hemoglobin levels greatly affect the hematocrit value, where the lower the number of red blood cells, the lower the hemoglobin content in the blood. Conversely, if the hemoglobin level is high, it indicates that the fish is under stress. According to Lusiastuti et al. (2004) tilapia hemoglobin levels are 6-11.01 (g%). Based on this, the results of this study indicate that tilapia hemoglobin levels are in normal conditions.

Hematocrit

The average hematocrit value of tilapia blood obtained from a blood test can be seen in Table 2.

Table 2. Tilapia Blood Hematocrit Average Value

Sample (fish)	Rate hematocrit (%)
A1	58.9
A2	47.3
A3	37.2

Table 2 shows that the average percentage of tilapia hematocrit obtained in this study ranged from 37.2 to 58.9%. Hematocrit is a term that indicates the total volume of erythrocytes in 100 mm³ of blood and is expressed as a percentage (%) (Yuni et al., 2019). The

hematocrit value or “packed cell volume” is a term that means the percentage derived from the volume of blood, which is composed of red blood cells. Hematocrit serves to measure red blood cells. This measurement is carried out if there is a suspicion of a disease that interferes with red blood cells, either excessive or lacking.

The highest average hematocrit value was found in the A1 fish sample of 58.9%, while in the A2 fish sample it was 47.3%, and the A3 fish sample was 37.2%, the lowest hematocrit level. Fish samples A1 and A2 in this study were classified as stressed. This is consistent with the statement of (Dewantoro et al., 2022) that the hematocrit value of fish under normal circumstances is usually around 25-40% and after being given a shock (stress) the fish hematocrit reaches 40-50% or more. The A3 fish sample in this study had normal hematocrit levels. Hematocrit values in teleost fish range from 20-30%, while hematocrit in tilapia is 23.6-37.4% (Lusiastuti & Esti, 2004).

Leukocrit

The average leukocrit value of tilapia blood obtained from a blood test can be seen in Table 3.

Table 3. Tilapia Blood Leukocrit Average Value

Sample (fish)	Rate leukocrit (%)
A1	5.49
A2	2.89
A3	6.61

Table 3 shows that the average leukocrit percentage of tilapia obtained in this study ranged from 2.89 to 6.61%. Leukocrit is the percentage of leukocyte volume in fish blood and hematocrit is the percent volume of erythrocytes in fish blood. Both provide clues about fish health and help determine the occurrence of abnormalities due to the use of immunostimulants and drugs which are calculated.

The highest average leukocrit value in sample A3 was 6.61%, sample A1 was 5.49% and sample A2 was 2.89%. being the lowest leukocrit value but still relatively normal. The average leukocrit value of tilapia ranged from 2.89 - 6.61%, higher than the leukocrit value of rainbow trout of 1-2% (Titrawani et al., 2014). The leukocrit value of tilapia ranged from 1.75-2.91% (Aisiah, 2012) and the leukocrit value of paweh ranged from 1.72-3.12%.

The difference in leukocrit values is probably due to illness during blood sampling, so that the leukocrit levels in the blood are high. According to Soetrismo (1987) in Aisiah et al. (2015), infected fish will have a large number of leukocytes, because leukocytes protect the body from infection. The body condition of the fish when sick produces a higher number of leukocytes if in

the blood circulation the number of leukocytes is less than the erythrocytes (Pearce, 1989 in Aisiah et al., 2015).

Blood Plasma

The color of blood plasma obtained when taking tilapia blood samples can be seen in Table 4.

Table 4. Blood Plasma Color

Sample (fish)	Plasma color
A1	Clear white
A2	Clear white
A3	Clear white

Blood consists of a liquid component (plasma) and various elements carried in the plasma, namely blood cells. Blood plasma is a liquid component of blood which consists of 99% water and 8-9% protein (Aisiah, 2015).

Based on the research results in table 4 showed that all samples had clear white blood plasma color, indicating that the tilapia was in normal condition. In general, fish blood plasma has a clear white and yellowish color. Blood plasma in healthy fish is clear and slightly yellowish indicating the condition of the fish is normal, while blood plasma which is reddish indicates the condition of blood that has lysis in the erythrocytes caused by the fragility of cells, while the whitish color indicates high fat content, and a reddish color. shows the occurrence of hemolysis (Aisiah et al., 2015).

Tilapia Histology

The results of histopathological tests of tilapia at the time of the study can be seen in figure.

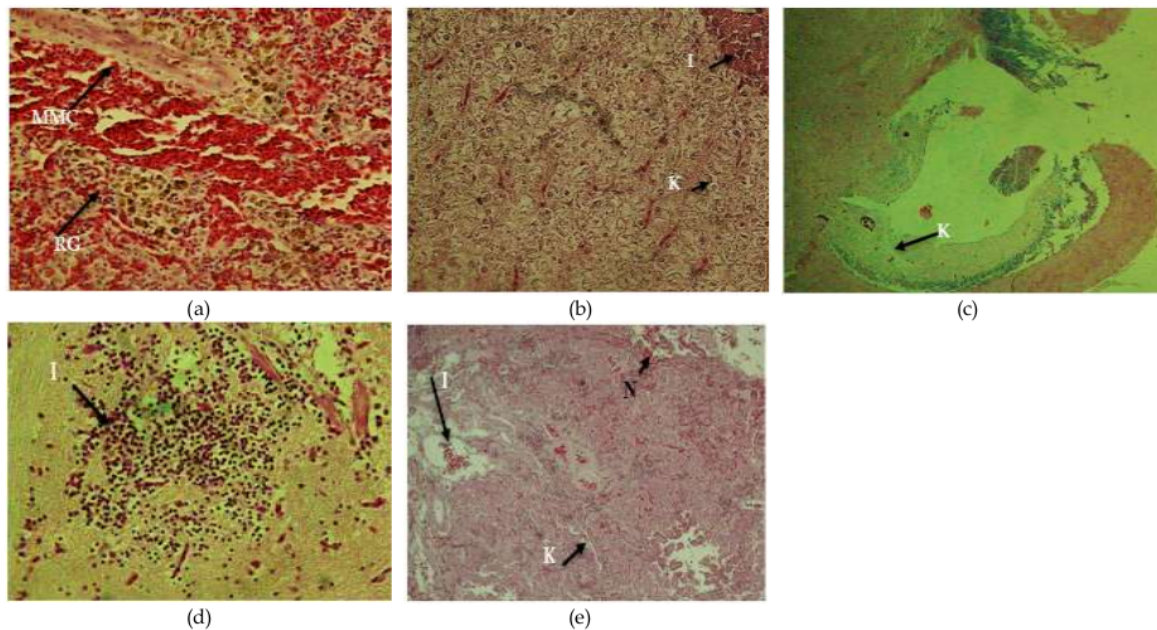


Figure 1. Histological condition: (a) tilapia spleen with HE staining at 400x magnification enhancement *Melano macrophage center* (MMC); (b) tilapia liver with HE staining at 100x magnification there was mild congestion (K) in the blood vessels of the liver and leukocyte infiltration (I); (c) tilapia brain with HE staining at 40x magnification visible mild congestion in the blood vessels of the brain (K); (d) tilapia spleen with HE staining at 400x magnification histological condition of tilapia brain with HE staining at 400x magnification; and (e) tilapia kidneys with HE staining at 100x magnification renal vascular congestion (K), leukocyte infiltration (I) and renal tubular necrosis (N) are seen

Analysis of histopathological testing was carried out after the processing of tissue preparations was completed. The results of the network observations obtained were thoroughly observed and the diagnosis was determined. In the sample test carried out on tilapia samples, the kidneys, liver, spleen and brain were taken as the target organs for observation.

Target organ harvesting is carried out by considering the function of each organ in the fish's body. such as kidney organs in the osmoregulation system, liver organs in the digestive system, lymph organs in the lymphatic system and brain organs in the nervous system. These organs have an important function in the body's metabolism so they can be used as an early diagnosis of health problems in an infectious disease

organism and treatment with antibiotics such as gills, liver, kidneys and so on. The results of histopathological observations of tilapia spleen showed tissue changes such as granulomatous inflammation, lysis of the periper spleen, splenic vascular congestion and an increase in the melano macrophage center in the spleen. The spleen is a very important organ for fish. Blood and foreign substances that pass through the capillaries enter the splenic cavity through a collection of arterial blood vessels (He et al., 2021). This substance is agglomerated by melano macrophage (mm) which is around the lining of the arteries. MMC (melano macrophage center) is formed as a result of the activity of the immune system that is adaptive (Steinel & Bolnick, 2017). The size of the MMC will increase if the fish is under stress due to the environment. Stress conditions can induce changes.

MMC (melano macrophage center) is a stage of an inflammatory reaction (Rofiani et al., 2017). MMC is characterized by the presence of an inflamed part and a collection of macrophages around it (yellow in color), the presence of MMC indicates inflammation, namely the presence of a collection of macrophages as a result of a self-protective response against parasite invasion in the tissue (Riyadi, 2022). The cause of lysis of the periphery of the spleen of tilapia is thought to be caused by the rupture of red blood cells due to a bacterial toxin in the blood called haemolisin. This toxin will lyse and destroy hemoglobin (Jusuf, 2009). Low hemoglobin levels can be an indication that the fish is experiencing an infection by bacteria. Inflammation is a vascular and cellular reaction of living tissue to irritation caused by infectious agents (bacteria, viruses, parasites, fungi). The degree of duration of the inflammatory process consists of acute inflammation, chronic inflammation, and inflammatory reactions that involve immunological processes (Maryadi et al., 2010).

The results of histopathological observations on the tilapia brain showed tissue abnormalities, namely mild congestion in the cerebral blood vessels and leukocyte infiltration in the brain meninges (Alifia & Djawad, 2000). The brain is a very important part of the fish's body, because the brain is the nerve center that controls all fish activities, changes that occur in the fish's body as a whole (stress, disease, changes in environmental conditions) also affect the brain structure, because in the brain there are many there are blood vessels. Changes that are often found in the brain are congestion and hemorrhage (Marwati et al., 2016).

The congestion is the presence of blood clots (erythrocytes) in the organs. Congestion (blockage) in the blood vessels, namely an increase in the amount of blood in the vessels, which is indicated by the dilated blood capillaries filled with erythrocytes in the cranial

vessels. Vacuolization occurs due to cell damage (necrosis). Furthermore, the cell is destroyed so that it is left as an empty space in the brain tissue. The brain tissue of tilapia infected with Streptococcus is damaged and has a negative impact by weakening nerve function, as a result of which the fish swims in circles (whirling) which ultimately causes death (Sukarni et al., 2012).

Histopathological observations of the liver showed tissue damage in the form of periphery hepatocyte lysis, mild congestion and infiltration of leukocytes in the liver. The liver is the largest organ in the fish's body which is located on the side of the abdomen, in the peritoneal cavity and covers the viscera. The liver is capable of synthesizing or storing absorbed nutrients, producing bile, and removing some waste products from the blood. Based on its function, the liver is the organ that accumulates the most toxic substances that enter the body so that it can be easily exposed to toxic effects (Musada, 2022).

Leukocyte infiltration on liver histopathology shows red spots on tissue cells. Infiltration, namely cell infiltration or entry of inflammatory cells from outside the tissue. Microscopically, the entire cell infiltrating tissue is marked by the presence of purplish inflammatory cells (Sugihartini et al., 2016). The congestion in the liver, starting from the central vein which then extends to the sinusoids, is arranged irregularly and contains erythrocytes which are thought to be due to the rupture of the sinusoidal walls. Another sign of congestion is blood spots in blood vessels exposed to chemical agents (Musada, 2022).

Histopathological observations of the fish kidneys showed tissue changes, namely vascular congestion, melano-macrophage centers, leucocyte infiltration and renal tubular necrosis. Renal vascular congestion is thought to be caused by bacteria (Lestari et al., 2017; Nelmi, 2016). Congestion on histopathological kidney looks red swelling. It is suspected that this is caused by a buildup of blood due to blood flow that slows down or even stops. Causes of congestion include obstruction and stenosis. The obstruction in this case can be caused by an inflammatory response (Atibah, 2022). Another abnormality in the kidney is the MMC, in the form of macrophage aggregates which are collections of cells that contain pigment in the tissue. MMC proliferation is an indication of the body's defense reaction in fish because of the presence of infecting bacteria (Susanti et al., 2016).

Infiltration is the diffusion or accumulation (in tissues or cells) of a foreign substance or in an amount that exceeds normal (Fatimah, 2012). Leukocyte infiltration itself is a decrease or buildup of leukocytes or blood cells. Infiltration is the body's response due to harmful stimuli, such as bacterial pathogenic agents that

function as a non-specific defense that localizes pathogens through phagocytosis.

Other damage is also found renal tubular necrosis, necrosis can be caused by trauma, biological agents (viruses, bacteria, fungi, and parasites), chemical agents such as toxic substances or the occurrence of interference with the supply of blood (Khalil, 2013). Satria (2017) stated that necrosis describes a state of decreased tissue activity which is characterized by the loss of several parts of cells one by one from one tissue, which histopathologically is characterized by the appearance of cell boundaries and the cell nucleus is not clear or even disappears.

Conclusion

Based on the results of hemtological analysis of tilapia (*Oreochromis niloticus*) after exposure to disease outbreaks which were cultivated in floating net cages, namely the hemoglobin of tilapia obtained in this study had a range between 6.9-7.8 g/dl, the average hematocrit of tilapia obtained in this study has a range between 37.2 - 58.9%, the average tilapia leukocrit obtained in this study has a range between 2.89 - 6.61% and blood plasma is the liquid component of blood consisting of 99% water and 8-9% protein. The results of histological analysis of tilapia after exposure to the outbreak showed clinical.

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Author Contributions

This article was prepared by four authors, namely R.K.R, S.A, O, and L.N. All authors members worked together at every stage of preparing this article.

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Conflicts of Interest

The authors declare no conflict of interest.

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