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HISTOPATHOLOGICAL FEATURE OF GILLS OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) EXPOSED TO LEAD NITRATE

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ABSTRACT

This study aims to determine changes in gill damage of African catfish (*Clarias gariepinus*) exposed to lead nitrate. This study used 24 African catfish with an average weight of 20-25 g, size 10-12 cm, age ± two months. This study uses a completely randomized design (CRD). All African catfish populations were divided into four groups, each consisting of six replications, namely P1, P2, P3, and P0 as negative controls, each P1 was given a lead nitrate concentration of 7.26 mg/L, P2 was given 14.53 mg/L, and P3 given 29.06 mg/L. Based on macroscopic observations, it shows that the gills become swollen and pale. Histopathological picture of the gills was examined under a light microscope at a magnification of 100 times. The scoring method uses the Semiquantitative Histopathological Scoring Method to check for edema, hyperplasia and lamella fusion. Then, the Crucial Wallis test was carried out followed by the Mann Whitney test. Analysis of the average edema P0 (0.30), P1 (1.90), P2 (1.60), and P3 (0.50). The average number of hyperplasia P0 (0.30), P1 (2.30), P2 (2.20) and P3 (1.00). The average number of lamella fusion P0 (0.10), P1 (0.70), P2 (1.60) and P3 (3.40). From these results it can be concluded that the most severe lamella edema damage occurs at a concentration of 7.26 mg/L, and the worst lamella hyperplasia occurs at a concentration of 7.26 mg/L and 14.53 mg/L, and the worst lamella fusion occurs at a concentration of 29.06 mg/L.

KEY WORDS: *Clarias gariepinus*, Lead nitrate (Pb (NO_3) 2), Gills.

INTRODUCTION

The need for fish consumption has increased in line with the increase in population and public awareness of nutrition. According to the Ministry of Maritime Affairs and Fisheries statistical data that the national fish consumption figure in 2015 was 41.11 kg/cap / year, and an increase in 2016 reached 43.94 kg / cap / year. African catfish (*Clarias gariepinus*) are one of the freshwater fish that are in great demand and are cultivated by the people of Indonesia. Statistics of the Ministry of Maritime Affairs and Fisheries states that the fish most cultivated by the people of Indonesia are tilapia by 29% and catfish by 20%. This figure shows that African catfish is one of the fish that is much in demand by Indonesian people, besides that African catfish have several advantages including fast growth and good ability to adapt to the environment (Purnamasari, 2012).

Increased fish consumption is not matched by an increase in the quality of water that is the fish ecosystem. Based on the Decree of the Minister of Environment No. 115 of 2003 concerning the Guidelines for Determination of Water Quality Status states that there are 23 provinces in Indonesia which have a river water quality index that is less than the target. Decrease in river water quality can be caused by several factors, one of which is water pollution.

One of the dangerous water pollutants is lead. Lead is a dangerous heavy metal which is mostly available in industrial environments. Lead found in water can originate from human activities such as industry, shipping and so on (Bengen, 2001).

Heavy metal pollution in Indonesian waters tends to increase in line with the increasing industrial processes that produce pollutants as waste (Siregar *et al.,* 2012). The presence of heavy metals in waters can cause heavy metals to accumulate in the waters (Alifia, 2013). In accordance with the statement of Fadhlan (2016) that lead is a heavy metal that can not be destroyed (non-degradable) so that it can accumulate into the environment, settles at the bottom of the water and form complex compounds. Lead is a metal that can accumulate into organism tissues such as fish and the lead content in the tissue will continue to increase in line with the increase in lead concentration in water and the length of time the organism is in lead polluted water. Lead toxicity (Pb) to aquatic organisms can cause organism tissue damage especially in sensitive organs such as gills and intestines then the liver and kidneys as the place where the metal accumulates (Siregar *et al*., 2012).

Fish is an aquatic biota that is most often used as an indicator of water environment pollution, one of which is catfish. Organs that can be used as indicators of observation when lead accumulation occurs in the waters, one of which is gills.

Damage to the gills can be in the form of thickening in the lamela, this can affect the respiratory system of fish, causing fish to experience difficulty in breathing and even death. Toxic entry into fish tissue will cause gills to experience damage such as edema, hyperplasia, and lamella fusion. This happens because toxic compounds cause cells to be unable to metabolize so that energy is not formed for survival (Tandjung, 1995).

MATERIALS AND METHODS

Experimental animals and histological protocol

The material used in this research was 24 African catfish (*Clarias gariepinus*) measuring 10-12 cm with a body weight of about 20-25 grams, with a range of two months of age. Preparations (Pb $(NO_3)2$), PDAM water, catfish feed in the form of MS Prima brand PF 1000 R, and clove oil. Materials for the preparation of gill histopathology preparations include 10% formalin solution for fixation, alcohol (70%, 80%, 85%, and PA alcohol) for dehydration, xylol for clearin, paraffin, glisserin, and Hematoxylin Eosin (HE).

Research Procedure

Containers and tools such as aquariums, aerators, filters and hoses used are washed and then dried. A total of four buckets filled with water and allowed to stand for 24 hours, then the water at the bottom of the bucket is discarded to remove the possibility of heavy metal that settles to the bottom of the bucket. African catfish were adapted in a bucket for two days before the treatment period. The African catfish are fed with pellets during the adaptation period and are kept in water circulation so they can adapt quickly.

Trial groups

African catfish (*Clarias gariepinus*) are treated as follows:P0: six African catfish (*Clarias gariepinus*) without exposure (Pb (N03) 2); P1: six African catfish (*Clarias gariepinus*) exposed (Pb (N03) 2) 7.26 mg/L; P2: six African catfish (*Clarias gariepinus*) exposed (Pb (N03) 2) 14.53 mg / L; P3: six African catfish (*Clarias gariepinus*) exposed (Pb (N03) 2) 29.06 mg/L; In the P0 group used as a control group, six African catfish (*Clarias gariepinus*) were put into the aquarium without being exposed (Pb (N03) 2). In the treatment group (P1, P2, P3) each aquarium was given lead nitrate according to a predetermined concentration and stirred evenly. Then the African catfish (Clarias gariepinus) of 18 fish were put into three aquariums, each as many as six fish. Each treatment group and control group were given 50 grams of feed every morning and evening.

The treatment was carried out for 30 days. On the 31st day the catfish was taken using a fish filter, then euthanasia was done using clove oil. Clove oil is dissolved by dropping 50 drops of clove oil into a bucket of four liters of water, then stir it evenly. Catfish are euthanasia by putting it in clove oil that has been dissolved until the fish lie in the bottom of the aquarium for about 10-15 minutes. Catfish are surgically removed after euthanasia, gill organs that have been removed from the body of the fish are carried out macroscopic pathological examination and gill histopathology preparations are made.

Making histopathology preparations

Gill organs were taken in the entire group. The gill organs that have been removed from the fish's body are macroscopically examined and the gill histopathology preparation is made. The method used to make histopathological preparations is a routine method or a paraffin method consisting of stages of tissue fixation, dehydration, clearing, embedding, sectioning, staining and mounting.

Macroscopic and Microscopic Observations

Macroscopic examination of the gills of African catfish (*Clarias gariepinus*) gills was carried out by observing the visible pathological changes in the physical organs of the gills. Meanwhile, microscopic examination of the gills of African catfish (*Clarias gariepinus*) was carried out by making histopathological preparations and observed under a 100 × magnification microscope as much as five fields of view. After that scoring was done by the scoring method conducted by Mitchell *et al.* (2012). The scoring values of histopathological changes in the gills of African catfish can be seen in Table 1.

Data Analysis

The data obtained were analyzed using the Krusskal Wallis test and if there were significant differences then proceed with the Mann Whitney test.

RESULTS AND DISCUSSION

Macroscopic observations of African catfish (*Clarias gariepinus*) gills showed changes such as thickening of the lamella, gills appear moreshiny, organs appear enlarged and gills appear paler than normal gills. Arborescent organs which are additional respiratory organs in catfish have also undergone a change in the arborescent organ paler. Contact between lead nitrate with gills will cause irritation and release mucus as a protective gill against toxic, but the mucus will cover the surface of the lamella, resulting in oxygen is not bound and the color of the gills will fade and subsequently will die. Pale colored organ Arborescent caused by lead nitrate which enters the gills continuously will make it difficult for the fish to breathe and cause low oxygen content so that Hb is difficult to bind oxygen.

Analysis of lamella edema damage showed significantly different results. The results of gill lamella edema analysis are listed in Table 2.

The analysis showed that the lamella gill edema was most severe in the P1 group and the mildest in the P0 group. Groups P0 and P1 showed significantly different results ($P < 0.05$) while groups P1 and P2 showed significantly different results ($P < 0.05$) and groups P2 and P3 showed significantly different results ($P < 0.05$).

Table 2. Average number and standard deviations of gill damages in African catfish damage

Treatment	Means ± SD
P0	$0.43^{a} \pm 0.29$
P1	$2.13^{d} \pm 0.64$
P2	$1.50^{c} \pm 0.24$
P3	$0.60^{b} \pm 0.35$

Note: Different superscripts show significant differences between treatments ($p < 0.05$); P0: negative control without lead nitrate exposure, P1: positive control exposed ($Pb (NO_3) 2$) at 7.26 mg/L, P2: positive control exposed ($Pb (NO_3) 2$) 14.53 mg/L, P3: the positive control was exposed ($Pb (NO_3) 2$) at 29.06 mg / L.

This occurs because the entry ($Pb (NO_3) 2$) into the gill lamella will cause the lesion so that the endothelial wall becomes more permiable and the fluid easily exits into the intersitial tissue. Fluid that comes out of the interstitial tissue interferes with the normal mechanism of re-absorption in the tissue, which will cause edema because the lymphatic ducts are unable to reabsorb the fluid. Edema causes swelling in the inflamed tissue due to accumulation of the fluid. Histopathological changes in gill lamella edema can be seen in Figure 1.

Analysis of lamella hyperplasia damage showed significantly different results. The results of gill lamella edema analysis are listed in Table 3.

The analysis showed that lamella hyperplasia of gills was most severe in the P1 and P2 groups, while the mildest was in the P0 group. Groups P0 and P1 showed significantly different results ($P<0.05$) while groups P1 and P2 were not significantly different ($P<0.05$) and groups P2 and P3 showed significantly different results ($P<0.05$).

Hyperplasia is an increase in the number of cells in tissue and hyperplasia is a form of the body's adaptive response to lead exposure ($Pb (NO_3) 2$). The body's adaptive response occurs at low concentrations. Hyperplasia occurs in lamellar gill

Table 1. Histopathological scoring of gill damage

The shape of the lesion	Score	Information
	0	There was no damage
	1	If damage occurs between 1-20% of one field of view
	2	If damage occurs between 21-40% of one field of view
	3	If damage occurs between 41-60% of one field of view
	4	If damage occurs between 61-80% of one field of view
	5	If damage occurs between 81-100% of one field of view

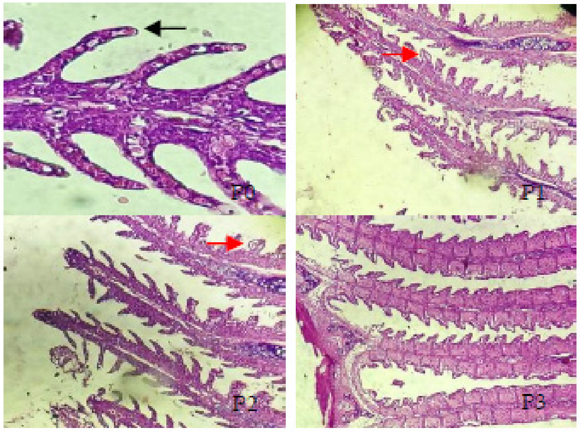

Fig. 1. Histopathological features of gills lamella edema of African catfish; red arrows = lamella edema.

Table 3. Average number and standard deviations of lamella hyperplasia damage in African catfish gills

Treatment	Means ± SD
P0	$0.33^{a} \pm 0.16$
P1	$2.36^{c} \pm 0.38$
P2	$2.23^{c} \pm 0.36$
P3	$0.90^{b} \pm 0.32$

Note: Different superscripts show significant differences between treatments ($p < 0.05$); P0: negative control without lead nitrate exposure, P1: positive control exposed (Pb (NO3) 2) at 7.26 mg / L, P2: positive control exposed (Pb (NO3) 2) 14.53 mg / L, P3: the positive control was exposed (Pb (NO3) 2) at 29.06 mg / L.

epithelial cells of African catfish where epithelial cells are unstable cells. Unstable cells are cells that routinely undergo proliferation and are prone to hyperplasia.

Hyperplasia in this study is caused by the entry (Pb (NO_3) 2) into the gills will cause disruption of the balance of lamella cells, so that the cells become irritated accompanied by an increase in the number of mucous cells at the base of the lamella and protect by releasing mucus. The mucus cells function as a protection against lead nitrate, but these mucus cells will actually cover the surface of the gill lamela and the exchange of O_2 and CO_2 becomes inhibited, so that the binding of O_2 by blood hemoglobin does not occur. This incident caused transportation of O_2 throughout the body to be disrupted so that the fish lacked oxygen and experienced hypoxia as a result of secondary lamella damage. Exposure to lead nitrate will trigger excessive division of gill epithelial cells because many cells are damaged by the lead nitrate. However, an increase in the number of cells causes partial closure of the secondary lamella so that the oxygen exchange process in the gills will be disrupted. Hyperplasia itself is characterized by tissue thickening in the gill lamellae. Hyperplasia occurs at a lower level of irritation and is accompanied by an increase in the amount of mucus at the base of the lamella and results in lamella fusion. Histopathological changes in lamellar hyperplasia of the gills can be seen in Figure 2.

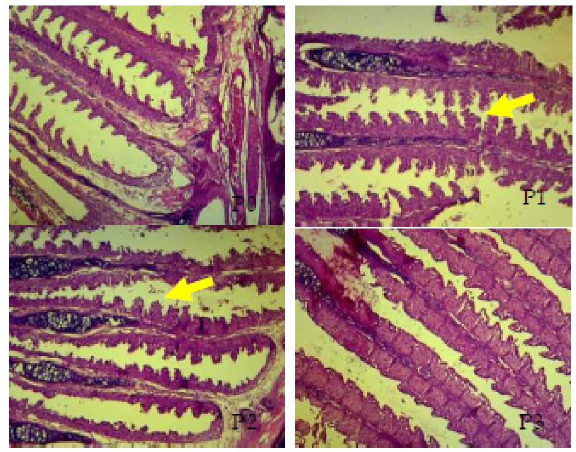

Fig. 2. Histopathological features of lamella hyperplasia of gills of African catfish; yellow arrows = lamella hyperplasia.

Analysis of lamella fusion damage showed significantly different results. The results of the analysis of lamella edema gills are listed in Table 4.

The analysis showed that lamella hyperplasia of gills was most severe in the P3 group and the mildest was in the P0 group. P0 and P1 groups showed significantly different results ($P < 0.05$). P1 and P2 groups showed significant differences ($P<0.05$) and P2 and P3 groups also showed significantly different results ($P<0.05$).

The high concentration of lead and the amount of time the catfish was in water contaminated with lead can cause death in the catfish. This is due to the fusion of the secondary lamella. Lamella fusion is the end result of lamella hyperplasia which causes a reduction in the surface area of the gills. The lamella fusion in this study can occur because of the continuous increase in pathology of hyperplasia and will cause secondary intermellal space to be filled by new cells, triggering the attachment of both sides of the lamella. Besides lamella fusion is also caused by the presence of excess mucus cells in the gills so that

Table 4. Average number and standard deviations in the damage of lamella fusion gills of African catfish

Treatment	Means ± SD
P0	$0.13^{a} \pm 0.16$
P1	$0.70^{b} \pm 0.41$
P2	$1.63^{c} \pm 0.48$
P3	$3.53^{d} \pm 0.98$

Note: Different superscripts show significant differences between treatments ($p<0.05$); P0: negative control without lead nitrate exposure, P1: positive control exposed (Pb (NO_3) 2) at 7.26 mg/L, P2: positive control exposed (Pb (NO_3) 2) 14.53 mg/L, P3: the positive control was exposed (Pb (NO_3) 2) at 29.06 mg / L.

it will close the secondary lamella. This event resulted in the inhibition of the process of respiration in the body of catfish. Fusion is a level of severe damage, because lamella fusion is an advanced stage of hyperplasia damage (Mitchell *et al.,* 2012). Histopathological changes in gill lamella fusion can be seen in Figure 3.

CONCLUSION

Based on the results of research conducted there are differences in the level of gill damage of exposed African catfish (Pb (NO_3) 2) namely the highest gill

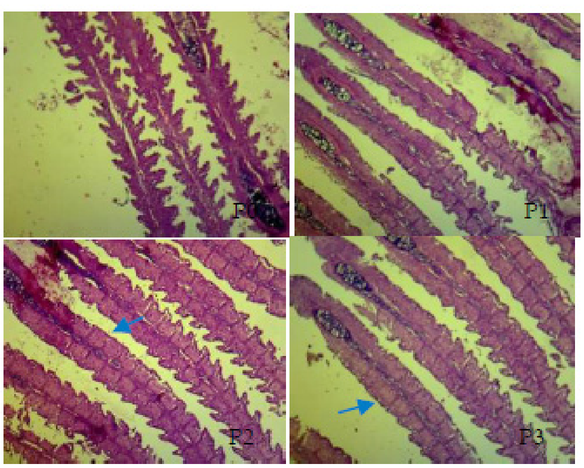

Fig. 3. Histopathological features of lamella fusion gills of African catfish; Blue arrows = lamela fusion.

lamella edema occurs at a concentration of 7.26 mg / L, the highest gill lamplain hyperplasia occurs at a concentration of 7.26 mg/L, and the highest lamella fusion occurred at a concentration of 29.06 mg/L. This shows that the higher the concentration of lead nitrate given to African catfish, the higher the level of damage produced.

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