Asian Journal of Aquatic Sciences, December 2022. Vol 5, Issue (3) 421-428 e-issn: 2716-4608
p-issn: 2655-366X

KIDNEY AND LIVER HISTOPATHOLOGY OF STRIPED CATFISH (*Pangasianodon hypophthalmus*) INFECTED WITH *Edwardsiella tarda*

Melin Siburian[1*], Henni Syawal[1], Morina Riauwaty Siregar[1]
[1]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Riau
Kampus Bina Widya KM. 12,5, Simpang Baru, Kec. Tampan, Kota Pekanbaru, Riau 28293
*melin.siburian2822@student.unri.ac.id

ABSTRACT

This research was conducted in April – June 2021in the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine, Universitas Riau. Histological preparations were carried out at the Bukittinggi Veterinary Center (BVet). The aim of this study was to analyze the kidney structure of striped catfish (*P.hypophthalmus*) infected with *Edwardsiella tarda* with different bacterial densities. The benefit of this research is to provide information about changes in the structure of kidney tissue due to infection with *E.tarda*, so that later treatment solutions can be sought. The method used was an experimental method with a completely randomized design (CRD) consisting of 4 treatment levels and 3 replications. The treatments applied were P0 (fish not infected with *E.tarda*), P1 (fish infected with *E.tarda* at a density of 10^6 CFU/mL), P2 (10^7 CFU/mL), and P3 (10^8 CFU/mL). Striped catfish (*P.hypophthalmus*) used are 8-12 cm in size with a density of 1 fish/3 L. Infection is done by injection in the intraperitoneal as much as 0.1 mL/fish. The results of the study found that clinical symptoms caused by dull and protruding eyes, dull body surface color, flaky fins, body surface wounds, and bleeding. In histopathological observation, the kidneys with different bacterial densities experienced the highest damage at P3 (108 CFU/mL), namely necrosis, hemorrhage, and hypertrophy.

Keywords: Histopathology, *Pangasianodon hypophthalmus, Edwarsiella tarda*, Kidney

I. INTRODUCTION

Striped catfish (*Pangasianodon hypophthalmus*) is a freshwater consumption fish commodity that has important economic value because of its several advantages such as fast growth, easy to cultivate and can be maintained in waters with low oxygen content. Various efforts have been made to increase the production of striped catfish, one of which is by cultivating intensification. However, improper management in intensive cultivation systems can cause disease. One of the diseases that often attack striped catfish farming is *Edwardsiellosis. Edwardsiella tarda* is a pathogenic bacterium that causes *Edwardsiellosis*, *Emphisematous Putrefactive Disease of Catfish* (EPDC) and *Red Pest*. *E.tarda* has been found in Indonesia (Java) in catfish. This bacterium attacks the host's defense mechanisms, therefore the process of proliferating these bacteria is very fast in the host and causes death [1-2]. In some cases, mortality from this bacterial attack is very low, which is less than 5%, but in some cases, it shows obvious disease symptoms and causes up to 50% death. One of the factors causing *E.tarda* attacks is that fish experience stress, especially due to overcrowding before harvest, poor water quality conditions and high organic matter [3]. Mortality due to this bacterial attack is very low, which is less than 5%, but in

Received : 19 October 2022
Accepted : 21 November 2022

some cases, it shows obvious disease symptoms and causes death of up to 50%. One of the factors causing *E.tarda* attacks is that fish experience stress, especially due to overcrowding before harvest, poor water quality conditions and high organic matter [3].

Clinical symptoms of fish infected with *E.tarda* experience, such as changes in behavior and morphology of the fish's body, because of *E.tarda* infection, it is characterized by loss of body color, formation of gas-filled cavities with muscles and swelling of some of the internal organs and changes in behavior occur in decreased feeding response, slow swimming and fish swimming closer to aeration [2]. The internal organs that are often attacked are the liver, spleen and kidneys, which are characterized by the presence of abscesses or swelling of the intestines and the presence of gas pale liver and kidneys.

Histopathological examination of fish can provide an overview of tissue changes infected with pathogens and to detect the presence of pathogenic components that are infective through micro-observation of abnormal changes at the tissue level. Tissues that can be used as indicators of observation are the kidney and liver. Kidneys are excretory organs with the function of filtering metabolic waste to be excreted in the form of urine. *E.tarda* bacteria that enter the blood easily reach important organs in fish such as the kidney sinusoids. Furthermore, bacteria will use the kidneys as a place to multiply themselves, and take the nutrients around them for metabolic processes. The liver is the metabolic center of the body, the liver produces bile as a fat emulsifier, which plays an important role in the process of digesting food. If bacteria attack the kidneys and liver, it will disrupt the function of the kidneys and liver, so that these organs cannot work according to their respective functions and will disrupt metabolic processes and cause death. This research aims to analyzing the structure of the kidney tissue of striped catfish due to infection *E.tarda* with different bacterial densities.

2. RESEARCH METHOD

Time and Place

The research was carried out from April to June 2021 at the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine, Universitas Riau. Histology preparations were carried out at the Bukittinggi Veterinary Center (bVet).

Method

The method used in this study was an experimental method by applying a Completely Randomized Design (CRD) consisting of four treatments and 3 replication, so that 12 experimental units were needed. The treatment used namely:

P0 : Fish is not infected *E.tarda*
P1 : The fish is infected *E.tarda* density of 10^6 CFU/mL
P2 : 10^7 CFU/mL
P3 : 10^8 CFU/mL

Research Procedure

Fish Adaptation

Striped catfish was acclimatized for 7 days. During maintenance, striped catfish were fed three times a day, namely at 08.00, 12.00 and 16.00 WIB adlibitum. The maintenance container is cleaned 2 times a day by siphoning.

Pathogenicity Test

Pathogenicity test was conducted to determine the density of bacteria that can kill 50% of striped catfish infected with *E.tarda* for 72 hours using the LD50 test). The pathogenicity test used four treatments, namely control, 10^6CFU/mL, 10^7CFU/mL, 10^8CFU/mL with three repetitions. Fish were reared for 7 days in a 30 L container with commercial feed ff-999 (35% protein) given ad libitum three times a day at 08.00,

12.00 and 16.00 WIB. Prior to infection, the striped catfish was anesthetized by immersing it in water that had been given clove oil 1/4 mL/L water until it fainted. Next, the injection is carried out using a 1 mL syringe of 0.1 mL/fish in the lower abdomen (intraperitoneal) with a density of 10^6, 10^7, and 10^8 CFU/mL. After infection, clinical symptoms were observed and recorded for 72 hours.

Preparation of Fish Histology

According to [4], the body organs of the striped catfish will be fixed with 10% formalin for 24-48 hours and transferred to 4% formalin. After that, the dehydration process was carried out, namely the fixed sample was transferred into a series of graded alcohol starting from 70%, 80%, 90%, 96% and absolute alcohol for 1 hour each.

Data analysis

The data that were analyzed were the kidneys and liver of striped catfish. Data on kidney and liver abnormalities in striped catfish were analyzed and discussed descriptively. The level of damage and abnormalities that occur in the kidney tissue of striped catfish is calculated and categorized based on the Histological Alteration Index (HAI) assessment.

3. RESULT AND DISCUSSION

Clinical Symptoms of Striped Catfish

The clinical symptoms of striped catfish during the study showed a change between fish that were not infected with *E.tarda* and those that were infected with *E.tarda*. Clinical symptoms in the control treatment (P0) looked healthy such as active body movement, bright body surface, normal mucus production, normal fish appetite, intact fins without damage. This happened because the control treatment (P0) was not infected with bacteria so the fish in the control treatment were healthy fish. Presented in Table 1.

Table 1. Shows that there are changes in the fins such as bleeding on the caudal fin, the body surface is dull, and there are wounds and excess mucus production. This is due to the bacteria *E. tarda* producing a toxin. According to [5] *E.tarda* bacteria produce two exotoxins dermatonecrosis that causes the lesion, both types of toxins produced are antigenic. Toxins that have entered the animal's body will cause necrosis and gas production. In addition, this toxin causes cells to come out of the blood vessels and causes red spots on the skin [6]. Excessive mucus production indicates that the fish is fighting an attack by the *E.tarda* bacteria.

Table 1. Clinical symptoms of striped catfish (*P.hypophthalmus*) for 72 hours

Clinical Symptoms	Treatment
	P0
Movement	Active
body surface	Bright
Slime Production	Normal mucus production
Appetite	Normal fish appetite
Fin	Intact without damage
Eye	Bright black in color and concave in shape

Striped Catfish (*P.hypophthalmus*) Kidney Tissue Structure

The structure of normal fish kidney tissue is indicated by the presence of a glomerulus that is still clearly visible, not completely round but in the shape of a number six and Bowmen's capsule looks neatly wrapped around the glomerulus. [7], states that the structure of normal fish kidney tissue is characterized by the presence of cells that make up the glomerulus which are still clearly visible, not completely round but in the shape of a six and Bowmen's capsule looks neatly wrapped around the glomerulus. For more details can be seen in Figure 1 and Table 1.

In P1, the level of damage is categorized as 2 and is categorized as a normal group. P2 with a level of damage of

3 and P3 with a level of damage of 4 are categorized as damaged kidneys. This is in accordance with [4] that a damage level of 0-2 is categorized as a normal group while a damage level of 3-6 is categorized as a damaged group.

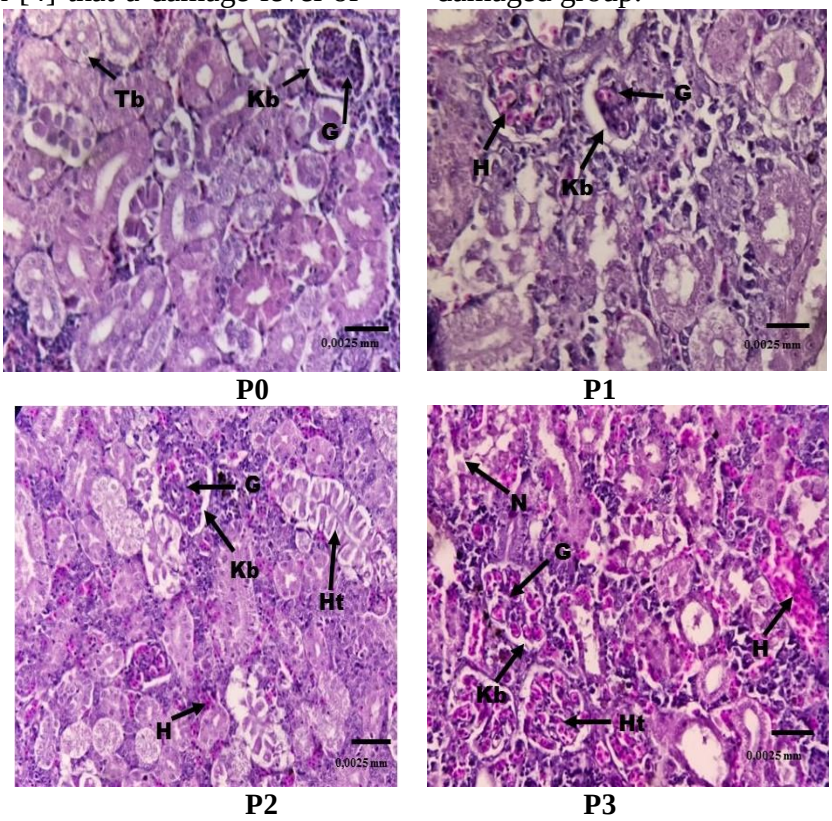


Figure 1. Photomicrograph of Striped catfish Kidney (P. hypophthalmus) HE staining (Magnification 400X)
Description: I: Cell nucleus, S: Sinusoid, Tb: Tubulus, G: Glomerulus, N: Necrosis, H: Hemorrhage, Ht: Hypertrophy

Table 2. Kidney Damage of Striped catfish based on index.

Treatment	Kidney Damage			Damage Rate	Category Level of Damage
	H	ht	N		
P0	-	-	-	0	Normal
P1	+	-	-	2	Normal
P2	+	+	+	3	Damaged
P3	+	+	+	4	Damaged

Based on the value of the level of damage to the fish kidney tissue structure in Table 2, it is known at P0 the fish kidney structure was marked by the presence of bowmen's capsule, glomerulus, and tubules. This is in accordance with the statement of [8] the structure of normal fish kidney tissue is characterized by the presence of cells that make up the glomerulus which are still clearly visible, not completely round but in the shape of a six and Bowmen's capsule looks neatly wrapped around the glomerulus.

Damage to kidney tissue at P1 (10^6 CFU/mL) was found to be hemorrhagic in the glomerulus belonging to the normal category. Meanwhile, at P2 (10^7 CFU/mL) there was increased hemorrhagic damage and hypertrophy in the tubules, and included in the damaged category. At P3 (10^8 CFU/mL) found necrotic damage characterized by loss of epithelial cell

nuclei in the tubules and glomerulus, increased hemorrhage in the tubules and glomerulus and hypertrophy of the tubules cells which are categorized as damaged due to attack from *E.tarda.* It is suspected that infection with *E.tarda* in striped catfish is characterized by the presence of bacteria that have very high virulence and *E.tarda* bacteria contain a toxin hemolysin that can cause swelling of cells [9]. The most severe damage occurs at P3 (10^8CFU/mL) is caused by the high pathogenicity of *E.tarda* which damages the structure of kidney tissue.

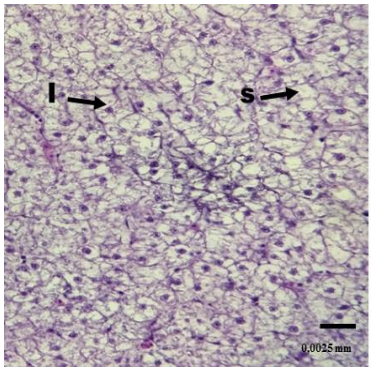
Hemorrhage is bleeding due to damaged blood vessels. This is in accordance with the statement of [10] that hemorrhage is bleeding in cells caused by rupture of blood vessels, causing blood to flow in inappropriate places both outside the body and into body tissues. Hemorrhage on histopathology observed was visible in the presence of dark red or black. This is in accordance with [11] that hemorrhage is characterized by the presence of blood spots in the blood vessels of the body's tissues due to congestion in the liver that is so severe that it causes the blood vessels to become damaged. Furthermore, [12] stated that when there is damage to blood vessels due to exotoxin, then the blood will come out of the blood vessels and there was hemorrhage on the surface of the body. The continued effect of exotoxin will cause more and more cells in the tissue to die, so that clinical symptoms will appear in the form of necrosis on the surface of the body.

Hypertrophy is tissue damage that is characterized by an increase in the size of the organ due to the increase in cell size, so that one cell separates from another. Hypertrophy occurs due to blockage of toxic compounds, even though the concentration is low, but is contaminated for a long time in the fish's body.

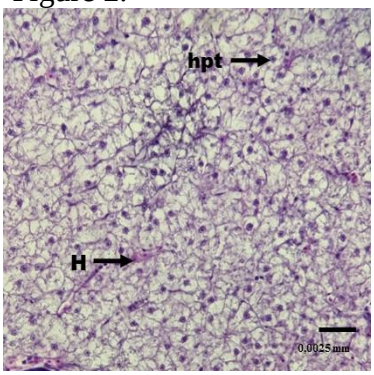
Necrosis causes an inflammatory response in the living tissue around the area where necrosis occurs. According to [7] states that kidney tissue abnormalities due to necrosis are irreversible or irreversible which describes a condition where there is a decrease in tissue activity, which in a short time will experience the death of the structure of the kidney organs, as well as abnormalities in the form of hemorrhage.

Liver Tissue Structure of Striped Catfish (*P.hypophthalmus*)

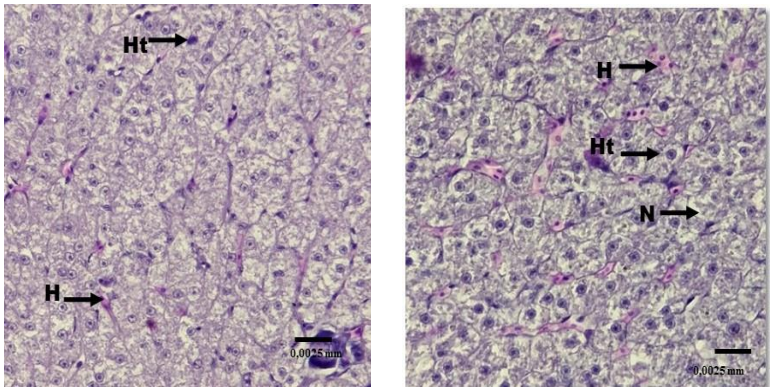
The structure of the striped catfish liver tissue looks normal which is characterized by clearly visible hepatocyte nuclei or round nuclei, central position and sinusoids are clearly visible. Besides that, you can see the central vein as the center of the lobe looks round and empty. According to Lubis (2014) that hepatocyte cells are clearly visible, the round nucleus is located in the middle and the sinusoids are clearly visible, and the central vein as the center of the lobule is seen to be round. To see more details of liver tissue damage can be seen in Figure 2.



P0



P1



P2 **P3**

Figure 2. Photomicrograph of striped catfish liver HE staining (Magnification 400X)
Description: H (Hemorrhage); Ht (Hypertropy); N (Necrosis); hpt (hepatocyte).

Table 3. Striped catfish liver damage based on index (Windarti et al., 2017).

Treatment	Liver Damage			Damage Rate	Category Level of Damage
	H	ht	N		
P0	-	-	-	0	Normal
P1	+	-	-	2	Normal
P2	+	+	+	3	Damaged
P3	+	+	+	4	Damaged

Based on the level of damage to the structure of fish liver tissue in Table 3, it can be seen that the damage found in the liver includes hemorrhage, necrosis, and hypertrophy caused by infection with *E.tarda*. Treatments P1 (10^6 CFU/mL) and P2 (10^7 CFU/mL) had damage levels of 2 and 3 so they were categorized as normal. Whereas in the P3 treatment (10^8 CFU/mL) it had a damage level of four, so it was categorized as a damaged liver. [4] States that a damage level of 0-2 is categorized as a normal group, while a damage, level of 3-6 is categorized as a damaged group. Where in the P1 treatment (10^6 CFU/mL) one type of damage was found, namely low-intensity hemorrhage, which was classified in the normal category. Followed by P2 treatment (10^7 CFU/mL) two types of damage were found, namely hemorrhage and hypertrophy which were quite severe in intensity and included in the damaged category.

The most and highest damage was in the P3 treatment (10^8 CFU/mL). There were three types of damage ranging from hemorrhage, hypertrophy and necrosis, which were included in the damaged category. So it can be stated that the level of damage to the liver has a relationship with the density of bacteria where the higher the density of bacteria, the higher the damage to the liver.

The changes seen in P1 are hemorrhagic damage. Hemorrhage was found in all treated livers. Hemorrhage is bleeding due to damaged blood vessels. This is in accordance with [13] stating that hemorrhage is bleeding in cells caused by rupture of blood vessels, causing blood to flow where it should not, both outside the body and into body tissues.

Changes in P2 show two types of damage, namely hemorrhage and necrosis. Necrosis is tissue damage, namely cell death that is irreversible or irreversible. In the structure of liver tissue, necrosis is characterized by loss of cell nuclei in hepatocytes, cell membranes rupture due to dilated sinusoids. This is in accordance with [7], necrosis is characterized by loss of tissue structure. Cell death usually occurs

with rupture of the plasma membrane and no structural changes to the membrane can be detected prior to rupture. Cells that experience necrosis can no longer return to their original state, at the end of necrosis the cells will die.

Hemorrhage (bleeding) is a condition characterized by discharge of blood from the blood vessels, both out of the body and into the body's tissues, it appears that there are spots of hemorrhage in the mucous lining of the body's organs. This is in accordance with [14], the presence of hemorrhage can be caused by damage to the capillary endothelium due to infectious agents circulating in the blood vessels. When there is damage to the blood vessels due to exotoxin, blood will come out of the blood vessels and hemorrhage will occur on the surface of the body. The continued effect of exotoxin will cause more and more cells in the tissue to die, so that clinical symptoms will appear in the form of necrosis on the surface of the body. This is in accordance with [15], *E.tarda* has a toxicity mechanism through the production of two dermatonecrosis exotoxins. Both types of toxins produced are antigenic and are not found in the animal's immune system. Toxins that have entered the animal's body can cause necrosis and gas production in the stomach (dropsy).

This is presumably due to the multiplication activity of bacteria that develop due to toxins produced by gram-negative bacteria in the form of endotoxins or exotoxins and the result of the multiplication of hydrophilic bacteria that cause the bacteria to damage tissues [16].

Histopathological changes in the P3 treatment included hemorrhagic damage, hypertrophy and necrosis. Hypertrophy was found characterized by the presence of enlarged hepatocytes. [10] Stated, hypertrophy is a cell that swells due to increasing size causing cells to separate from one another. [11], stating that hypertrophy is tissue damage characterized by an increase in organ size due to increased cell size so that one cell separates from another and is an early symptom of necrosis.

4. CONCLUSION

Based on histopathological observations of the kidneys and liver of striped catfish (*P. hypophthalmus*) infected with *E.tarda* with different bacterial densities, it was found that tissue damage was in the form of necrosis, hemorrhage and hypertrophy.

REFERENCES

1. Sari, D.R., Prayitno, S.B., Sarjito. (2014). Pengaruh Perendaman Ekstrak Bawang Putih (*Allium sativum*) terhadap Kelulushidupan dan Histologi Ginjal Ikan Lele *(Clarias gariepinus)* yang Diinfeksi Bakteri *Edwardsiella tarda. Journal of Aquaculture Management and Technology.* 3(4) : 126-133
2. Prastiti, L.A., Sarjito., Slamet, B.P. (2015). Pengaruh Penambahan Ekstrak Jahe Merah (*Zingiber officinale* var. Rubrum) pada Media Pemeliharaan Terhadap Kelulushidupan dan Pertumbuhan Ikan Gurami (*Osphronemus gouramy*) yang Diinfeksi Bakteri *Edwardsiella tarda. Journal of Aquaculture Management and Technology.* 4(3): 31-37.
3. Diniarti, E., Triyanto., Murwantoko. (2019). Isolasi, Indentifikasi dan Uji patogenitas *Edwardsiella tarda* Penyebab Penyakit pada Ikan Air Tawar di Yogyakarta. *Jurnal Perikanan*. 21(1): 41-44
4. Windarti, Simarmata, A.H., Eddiwan. (2017). *Buku Ajar Histologi*. Penerbit Unri Press. Pekanbaru. 105 hlm.
5. Maghrabi, O. (2017). Histopathological Effects of Some Helminthic Parasities in The Liver of Serranid Fish. Research & Review : *Journal of Zoological Science*. 5(1): 36-43

6. Pardamean, E.S., Henni, S., Siregar, M.R. (2021). Identifikasi Bakteri Patogen pada Ikan Mas (*Cyprinus carpio*) yang Dipelihara dalam Keramba Jaring Apung. *Jurnal Perikanan dan Kelautan*, 26(1): 26 – 32.
7. Hutabarat, M.V. 2018. Histopatologi Hati dan Ginjal Ikan Jambal Siam (*Pangasius hypophthalmus*) yang Direndam Dengan Larutan Daun Inai (*Lawsonia inermis L.*). *Jurnal Perikanan dan Kelautan*. 16 hlm
8. Safratilofa. (2017). Histopatologi Hati dan Ginjal Ikan Patin (*Pangasionodon hypophthalmus*) yang diinjeksi Bakteri *Aeromonas hydrophila*. *Jurnal Akuakultur Sungai dan Danau*. 2 (2) : 83-88.
9. Wahyuni, S. (2017). *Studi Komparatif Struktur Jaringan Insang dan Ginjal Ikan Gabus (Channa striata) dari Sungai Sibam dan Sungai Kulim Provinsi Riau. Skripsi.* Fakultas Perikanan dan Kelautan. Universitas Riau. Pekanbaru. 73 hlm
10. Jamin., Erlangga. (2016). Pengaruh Insektisida Golongan Organofosfat Terhadap Benih Ikan Nila Gift (*Oreochromis niloticus*): Analisis Histologi Hati dan Insang. *Jurnal Acta Akuatik*a, 3(2): 46-53.
11. Andayani, S., Henny, S., Galih, D.A. (2017). Pengaruh Pemberian Bakteri *Lactobacillus plantarum* terhadap Histopatologi dan Hematologxi Ikan Patin Jambal (*Pangasius djambal*) yang Diinfeksi Bakteri *Edwarsiella tarda*. *Jurnal Perikanan dan Kelautan*. 1(1): 31 – 32
12. Arsal., Hasanuddin, A., Rizal, A. (2016). Patogenitas *Edwardsiella tarda* pada Ikan Sidat (*Anguilla marmorata*) Selama Penyimpanan Beku -25^{0}C. Universitas Tadulako. 6(2): 111-118.
13. Alif, A., Syawal, H., Riauwaty, M. (2021). Histopatologi Hati dan Usus Ikan Jambal Siam (*Pangasianodon hypophthalmus*) yang Diberi Pakan Mengandung Ekstrak Daun *Rhizophora apiculata*. *Jurnal Ilmu Perairan (Aquatic Science)*. 9 (2): 152-161
14. Nopilitia, E. (2017). *Histopatologi Hati dan Ginjal Ikan Nila (Oreochromis niloticus) yang Diinfeksi Bakteri Streptococcus iniae. Skripsi*. Fakultas Perikanan dan Kelautan. Universitas Riau. Riau
15. Yanti, M. (2018). *Histopatologi Ginjal dan Hati Benih Ikan Kakap Putih (Lates calcarifer Bloch) yang Diinfeksi dengan Lima Isolat Bakteri yang Berbeda. Skripsi*. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor. Bogor.
16. Ogueji, E.O., Stanley, C.I., Christian, C.M. (2020). Effect of Partial and Complete Replacement of Soybean with Discarded Cashew Nut (*Anacardium occidentale L*) on Liver and Stomach Histology of *Clarias gariepinus* (Burchell, 1822). *Journal Aquaculture and Fisheries*. 5: 86 – 91.