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INTESTINE AND LIVER HISTOPATHOLOGY OF STRIPED CATFISH (*Pangasianodon hypophthalmus*) FEEDING CONTAINING PAPAYA LEAF FERMENTATION

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ABSTRACT

Aeromonas hydrophila bacteria often attack internal organs such as the liver and intestines. This research was conducted in June – August 2021 at the Laboratory of Fish Parasites and Diseases, Faculty of Fisheries and Marine, Universitas Riau. Histology preparations were made at the Bukittinggi Veterinary Center. This research aimed to obtain the best dose addition of papaya leaf fermented solution which could reduce intestinal and liver tissue damage of striped catfish (*P.hypophthalmus*) which was challenged with *A.hydrophila*. The method used is an experimental method with a completely randomized design (CRD) with 4 treatment levels and 3 replications; Kp (Feed not given papaya leaf fermented solution + infected with *A.hydrophila*), P1 (16 ml/kg feed of papaya leaf fermented solution + infected with *A.hydrophila*), P2 (16 ml/kg feed of papaya leaf fermented solution) + infected with *A.hydrophila*), P3 (16 ml/kg of papaya leaf fermented solution feed + infected with *A. hydrophila*). The fish used was 8-10 cm with a density of 1 fish/3 L totaling 10 fish with a container measuring 40x30x30 cm. Infection by injection in the intramuscular section as much as 0.1 mL/head with a bacterial density of 10^8 CFU/mL. The parameters observed were clinical symptoms for 72 hours and histopathology of the intestine and liver of striped catfish. The results showed that the intestine saw the presence of hemorrhage, necrosis, and edema. While the liver shows abnormalities, such as hemorrhage, necrosis, hypertrophy, and fatty degeneration.

Keywords: *Aeromonas hydrophila*, Histopathology, Intestine, Liver

1. INTRODUCTION

The striped catfish (*Pangasianodon hypophthalmus*) is a fish that is much loved by people, especially in the Riau area. Market demand for striped catfish consumption per capita tends to increase every year, reaching 21.9% from 2014 to 2017 with a preference for fresh fish products of 76%[1]. Based on this demand, cultivation activities need to be increased intensively. According to Azhari et al.[2] intensive fish farming can reduce water quality which affects physiological processes, including the growth and survival of farmed fish as a result of the accumulation of leftover feed waste and metabolic results.

Intensive aquaculture also has a greater risk of disease, so it is necessary to improve fish health. If it is not handled properly, disease-causing microorganisms such as bacteria will grow in the waters, one of which is the *Aeromonas hydrophila* bacterium which can cause *Motile Aeromonas Septicemia* (MAS). Attack of *A.hydrophila* bacteria can kill fish fry with a mortality rate reaching 80-100% within 1-2 weeks[3]. These bacteria can generally attack the internal organs of the body such as the intestines and liver of fish.

The use of chemicals in feed mixtures is no longer recommended in aquaculture activities, especially if they are added to feed because they have adverse side effects.

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The use of synthetic chemicals and antibiotics that are not appropriate for the long term is not recommended because of the risk of antibiotic resistance (AMR), environmental pollution, and accumulation of toxic residues in fish[4]. Efforts to improve fish health can be done by providing supplements from extracts of natural ingredients added to feed. One of the natural ingredients is papaya leaves (*Carica papaya* L).

According to Afrianda et al[5,] papaya leaf is a medicinal plant because it contains alkaloid compounds and proteolytic enzymes, papain, chymopapain, and lysozyme which are useful in the digestive process and make it easier for the intestines to work. The papain enzyme in papaya leaves has antimicrobial activity. Papaya leaves have compounds that cause a bitter taste, anti-nutrients, and high crude fiber, to get rid of it need to be fermented. Papaya leaves without going through the fermentation process have several weaknesses, namely, they are not durable, and contain high crude fiber which is difficult for fish to digest. The benefits of fermentation can also add to the aroma and flavor of the ingredients so that the fermented ingredients are preferred and easily digested by fish[6].

Histopathological examination is a support for a diagnosis and can be the main diagnostic examination of a disease by finding changes in cells or tissues that are specific to a particular disease. Based on the problem information above, the researcher is interested in researching the histopathology of the intestine and liver of striped catfish (*P.hypophthalmus*) which is fed feed containing fermented papaya leaf solution which is expected to act as an alternative to antibiotics that are safer for the environment and can improve the structure of intestinal and liver tissue of striped catfish post-infection with *A.hydrophila.*

2. RESEARCH METHOD

Time and Place

This research was carried out from June to August 2021 at the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine, Universitas Riau. Histological preparation was carried out at the Bukittinggi Veterinary Center (bVet).

Methods

The research method used in this study was an experimental method using a completely randomized design (CRD), one factor, namely the dose of adding fermented papaya leaf solution to the feed with 4 treatment levels. To reduce the error rate, each treatment was repeated 3 times so that 12 experimental units were needed. The treatment carried out in this study refers to Lase[7], to calculate the dose in the form of a solution:

P0 : The feed was not given fermented papaya leaf solution and was challenged with *A. hydrophila*

P1 : The feed was given a solution of fermented papaya leaves at a dose of 16 ml/kg of feed and was challenged with *A. hydrophila*

P2 : The feed was given a solution of fermented papaya leaves at a dose of 18 ml/kg of feed and was challenged with *A. hydrophila*

P3 : The feed was given a solution of fermented papaya leaves at a dose of 20 ml/kg of feed and was challenged with *A. hydrophila*

Procedure

Container Preparation

The maintenance containers used were 12 units of aquariums measuring 40x30x30 cm. Before use, the aquarium must first be washed clean and filled with water until it is full, then given a solution of Potassium permanganate (KMnO4) and left for 24 hours so that the aquarium is free from pathogenic microorganisms. After that, the aquarium is rinsed with water until clean and then dried for 24 hours. Once clean, each aquarium is filled with water as

high as 25 cm from a drilled well which is precipitated in a tank and aerated. Then striped catfish with a size of 8-10 cm and an average initial weight of 94 g of fish were put into each aquarium with a stocking density of 10 fish/30L (1 fish/3L).

Preparation of Fermented Papaya Leaf Solution

After conducting a preliminary test, the ingredients and dosages to be used are obtained. The materials needed in the manufacture of this fermented solution are 50 g of papaya leaves (the papaya leaves used are taken from the Universitas Riau's land provided the leaves are light green and the petioles are small, only the leaves are taken), 1000 ml of boiled water (cooled to 45-50°C), 1.4 g of tape yeast, 1 bottle of the probiotic solution containing 65 ml of *Lactobacillus casei* bacteria, 50 g of bran, and 10 g of brown sugar. Papaya leaves are cut into small pieces, then blended with boiled water, and blend until smooth (juice). Once smooth, papaya leaves are filtered and put into bottles that have been cleaned. Then the other ingredients are added, namely 50 g of bran, 10 g of sugar (dissolved first), 1 bottle of Probiotic Solution containing 65 ml of *Lactobacillus casei* bacteria, 1.4 g of tape yeast that has been mashed, and the rest of the water. Then the bottle is closed and stirred until evenly mixed, after which it is stored in a safe and dark place in a cupboard so that it is not exposed to sunlight. After 4 days stir again to keep it homogeneous. On the 7th day, the fermented papaya leaf solution is ready to use with the characteristics of successful fermentation, namely, it smells like tape[8].

Adaptation and Maintenance of test fish

The test fish used were striped catfish measuring 8-10 cm as many as 120 individuals. The test fish seeds were acclimatized for 15 minutes in a fiber tub and adapted for 7 days. During the adaptation period, the test fish were given commercial F-999 feed three times a day, at 07:30, 12:30, and 17:30 WIB ad satiation. The test fish-rearing container is cleaned every day by siphoning. On the 6th day of adaptation, the fish fasted for 1 day to empty the fish's stomach so that the response to the test feed given to the fish during rearing was immediately consumed.

The maintenance of the fish was carried out for 44 days and during the maintenance, the test fish were given feed that had been added with fermented papaya leaf solution. Feeding as much as 10% of the fish's body weight and given three times a day. Every 10 days fish body weight is measured to determine the body weight development and determine the amount of feed given.

Challenge Test

After being reared for 30 days, before the fish were challenged, 3 fish/treatment samples were first taken to see the morphology and histology of the fish after being fed with fermented papaya leaf solution. Then the fish were challenged with *A.hydrophila* at a density of 108 CFU/mL as much as 0.1 mL/head utilizing intramuscular injection using a 1 ml syringe on the 31st day. The aim is to see the effectiveness of the fermented papaya leaf solution added to the feed. Before being infected, the fish were first anesthetized using clove oil as much as 0.1 ml/L of water to reduce stress on the fish. Then 14 days post-infection another 3 fish samples were taken/treated to see the morphology and histology of the organs because usually on the 14th day after infection the fish experience healing. Especially for P0 if you experience total death before the 14th-day post-infection, then it must be taken immediately to be used as a histological sample.

Preparation of Histology

Preparation of histological preparations for the intestinal and liver tissues of striped catfish was carried out 2 times, namely on the 30th day (after being fed before being infected) and then on the

44th day (14th day after infection). Histological preparations for gills and liver were made according to Windarti et al[9]. Preparations were made by taking 3 test fish/treated for dissecting from the anal upward to the lateral line and then towards the operculum so that a total of 24 preparations were made. Furthermore, the fish that had been dissected was put in its entirety into a container and fixed in 10% formalin for 48 hours, and transferred to 4% formalin so that when it was cut the organs were not destroyed. Then take the intestines and liver of the fish by cutting it to a thickness of ±0.5 cm. This is done by cutting the tissue thinly so that it can be put into a tissue cassette for the dehydration process.

Dehydration begins by putting the sample into a bottle containing alcohol, starting from 70%, 80%, 90%, 96%, and absolute alcohol (100%), each for 1 hour which aims to remove the water content from the organ sample and replaced it with alcohol. Specifically, immersion in absolute alcohol was carried out 2 times, 1 hour each. Then a process (clearing) was carried out where the sample was put back into alcohol: xylol (1:1 ratio) for 1 hour and put again into pure xylol 2 times for 1 hour each.

Paraffin infiltration or embedding of the sample (embedding), in which the sample is placed in a xylol-paraffin solution (1:1 ratio) for 1 hour and then placed again in paraffin 2 times each for 1 hour. The infiltration process is carried out in an Oil Bath at a temperature of 57-60°C. Samples were embedded in paraffin blocks and left to cool/freeze. Then attach it to the holder/wood. Before cutting the paraffin block is placed on an ice pad so that it freezes quickly and is solid and does not break when cutting.

The sample was cut with a 5-7 micron thick microtome to obtain paraffin bands and placed in a 40°C water bath and then affixed to a glass object. The glass object that has been affixed with paraffin tape is dried in an oven (45° C) for at least 24 hours so that the sample dries and sticks perfectly, then the sample is ready to be colored.

Sample staining using Hematoxylin-Eosin. This coloring is a water-soluble material, therefore the sample was first dissolved in xylol 1 and xylol 2 for 5 minutes each and then put into an alcohol solution (absolute alcohol I and II, 96%, 80%, 70%, respectively each for 2 minutes). Then the samples were immersed in Hematoxylin solution for 7 minutes and washed with running water. The samples were immersed in Eosin solution for 5 minutes, after which the samples were put into a series of rising alcohol solutions (70%, 80%, 96%, absolute alcohol I and II) for 2 minutes and xylol I and II solutions for 5 minutes. Next is closing (mounting).

Mounting is done utilizing preparations that have been stained and then covered with a cover glass. The preparation is dripped with entellan neu then covered with a cover glass and kept to prevent bubbles from arising. Samples were observed with a binocular microscope with 400x or 1000x magnification with a drop of immersion oil and then documented using a digital camera.

Water Quality Measurement

Water quality parameters measured were temperature, pH, DO, and NH_3. The tools used are a thermometer, pH meter, DO meter and spectrophotometer. Measurements were carried out twice, namely at the beginning of maintenance and at the end of maintenance.

Data Analysis

Parameter data for observing clinical symptoms of fish and water quality were tabulated in tabular form, then analyzed descriptively. While the research data which included reading histological preparations regarding changes in the histopathological structure of the intestine and liver were analyzed using damage scoring data. The level of damage and abnormalities that occur in the liver tissue

of striped catfish is calculated and categorized based on the assessment of the liver tissue damage index with conditions categorized into normal and damaged and then analyzed descriptively.

3. RESULT AND DISCUSSION

Clinical Symptoms

The clinical symptoms of striped catfish during the study showed differences between fish that were not infected with fish infected with *A. hydrophila*, an example of the form of these clinical symptoms can be seen in Figure 1.

Based on Figure 1 and observations during the study, during pre-infection (before infection) there were not many differences in clinical symptoms in each treatment. At P0 no injuries were found, all fins were intact, eyes were clear and bright, mucus production was normal, and movement was active and in groups, but the body color of the fish looked paler than the fish in the treatment. The difference between P0 and P3, P2 and P1, the clinical symptoms that appear are the same as those of normal fish, such as the absence of ulcers, normal mucus production, intact fins, bright and clean eye color, and active flocking movements

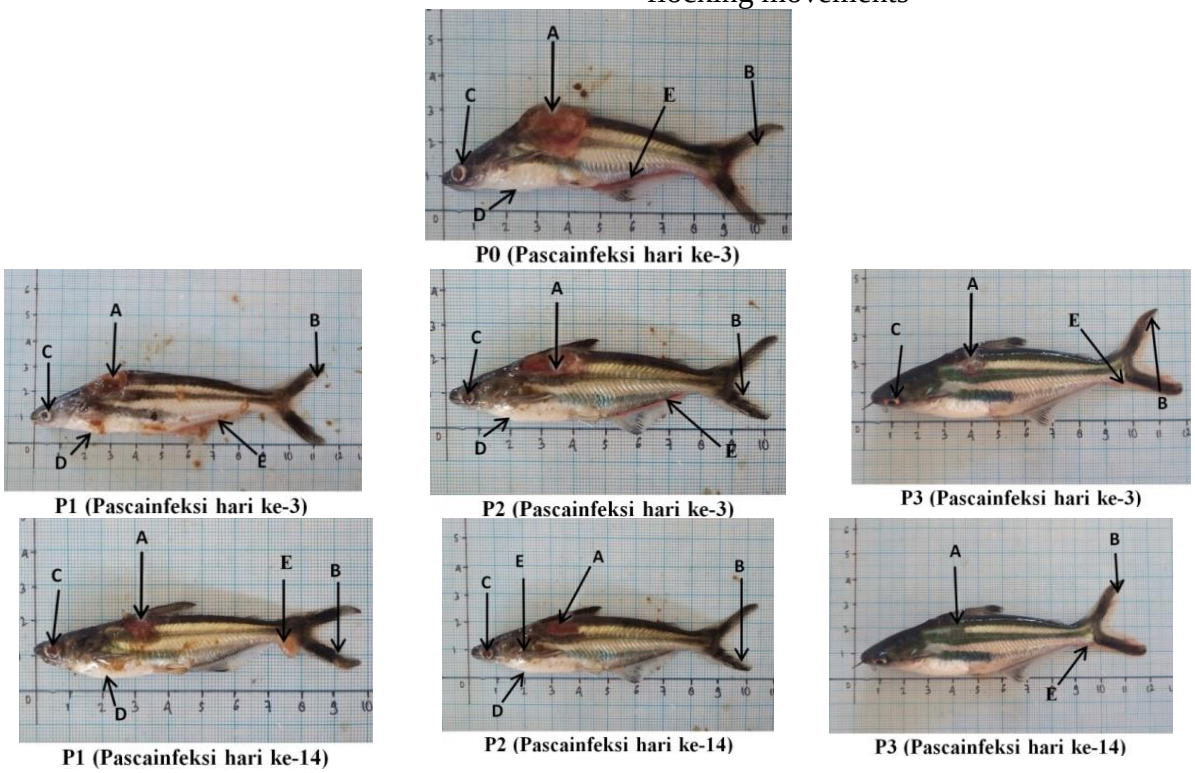


Figure 1. Clinical symptoms of striped catfish (*P. hypophthalmus*)

Information: P0 : (A = ulcers/wounds, B = drizzle, C = red eyes, D = stomach bloating, E = bleeding); P1 : (A = ulcers/wounds, B = drizzle, C = red eyes, D = stomach bloating, E =bleeding); P2 : (A = ulcers/wounds, B = drizzle, C = red eyes, D = stomach bloating, E = bleeding); P3 : (A = ulcers/wounds, B = drizzle, E = bleeding)

It can be seen that on the 3rd day after infection in the P0 treatment, the fish began to experience major changes, including very large ulcers, bleeding at the base of the fins, skin and operculum, sunken and wrinkled red eyes, excessive mucus production and passive movement. Following Pardamean et al[10], fish that have scars (ulcers) on the body parts of the fish and parts of the fins that are damaged (chipped) and have a pale body color are classified as unhealthy or attacked by disease. Fish that are attacked by the disease is usually characterized by clinical symptoms of changes in the body's organs in the fish, namely changes in the color of

the fish's body to red, and over time it will become a large wound, there is excessive mucus and loss of the fish's fins occurs. Meanwhile, before reaching the 14th day post-infection, the study fish at P0 experienced total death on the 4th-day post-infection, so they had to be immediately dissected to make histology.

Any changes in the clinical symptoms of the fish in the P1, P2, and P3 treatments until the 14th-day post-infection which led to healing such as smaller ulcers, reduced bleeding and the growth of new flesh and fins were caused by the addition of papaya leaf fermented solution which was given and consumed by the fish during the maintenance period. P3 was the treatment with the most added doses of papaya leaf fermented solution, in this treatment, the clinical symptoms of the fish experienced the most healing. There are lots of useful ingredients in papaya leaf extract, including as a wound healing medicine because it contains several substances such as saponins.

Saponins are one of the compounds that stimulate the formation of collagen in the wound healing process, apart from saponins papaya leaves also contain vitamins C, E, beta-carotene, and papain enzymes, where vitamins C, E, and beta-carotene function as antioxidants which can neutralize free radicals resulting from neutrophil phagocytosis of debris and bacteria in the healing process of a wound, while the papain enzyme plays a role in helping to speed up the work of macrophages by increasing the production of interleukins which function as wound healing processes and inhibiting widespread infection[11].

Striped Catfish Intestinal Tissue Structure

The intestinal tissue structure of normal fish is different from the intestinal tissue structure of fish infected with bacteria. The structure of fish intestinal tissue consists of several parts including the mucosa, submucosa, muscular, and serous membranes. In the mucous layer, some protrusions or villi form like a wasp's nest. The most common type of cell found in the intestinal epithelium is the enterocyte. Enterocyte cells are cells whose upper surface leads to the intestinal cavity. This cell is the most dominant cell, whose number will increase towards the back of the intestine. Enterocyte cells have small protrusions or small microvilli that act for food absorption. The structure of the intestinal tissue of striped catfish before infection and after infection can be seen in Figure 2.

On the 30th day of P0 treatment, there was only slight congestion in the intestinal tissue structure of the fish, presumably due to the influence of microorganisms or toxins in the rearing medium water. In treatment P0 4 days post-infection, it was seen that the structure of the intestinal tissue of striped catfish was severely damaged with a lot of hemorrhages, necrosis, and edema found, but quite a lot of goblet cells were seen. In addition, the villi/microvilli are not visible, the lamina propria is messy and separated from the lamina epithelia, and the submucosa has a lot of hemorrhages.

Organs for intestinal tissue structures specifically for fish P0 4 days post-infection were taken from dead fish, because before the 14th day post-infection P0 fish experienced total death on day 4. This was due to toxic substances such as the hemolysin enzyme produced by bacteria *A. hydrophila* which can lyse blood flowing in the organs of the body, for example in the intestine, and the maintenance of fish is not given feed with the addition of fermented papaya leaf solution.

The structure of the intestinal tissue of striped catfish on the 30th day of P1 treatment found quite a lot of hemorrhage and few goblet cells. At P1 14th days post-infection, even though he had been fed with the addition of fermented papaya leaf solution at a dose of 16 ml/kg of feed, the structure of the intestinal tissue was still damaged such as considerable hemorrhage

and necrosis, not many goblet cells were seen. Goblet cells that appear or appear in intestinal tissue indicate a good and normal intestinal digestive system, the shape of goblet cells is round. This is to the statement of Sariati et al[12], that goblet cells function to secrete mucus to lubricate and protect the intestinal surface, synthesize and secrete mucus glycoprotein in the form of a gel to protect intestinal epithelial cells.

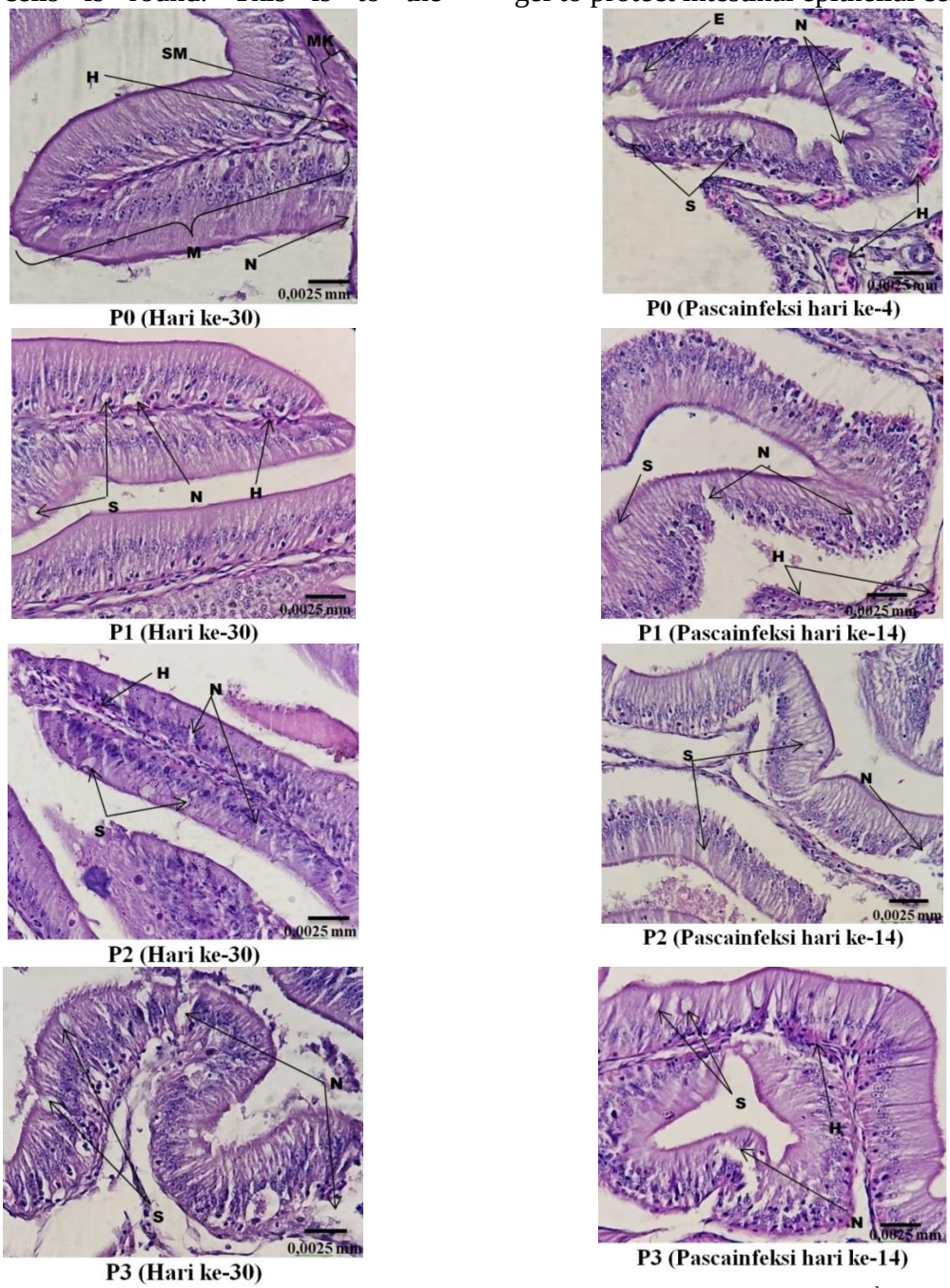


Figure 2. Photomicrograph of intestinal tissue structure of striped catfish 30th day and 14th day post-infection HE staining (Magnification 400X)
Information: Mucosa (M), Sub mucosa (SM), Muscularis (MK), Goblet cells (S), Haemoragi (H), Necrosis (N), Edema (E).

Intestinal histology on the 30th day of P2 treatment showed damage such as slight necrosis and hemorrhage, while the goblet cells had started to be quite numerous. Hemorrhage or bleeding that occurs in the intestine can be caused by several agents, such as materials or foreign bodies that enter the digestive tract with food, causing

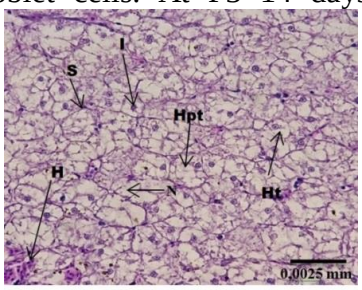
intestinal lesions and the occurrence of hemorrhage. The following statement of Asih[13], hemorrhage can be caused by trauma such as physical damage that damages the tissue vacuole system in the impact area, infection with infectious agents especially causing septicemia, toxic substances that damage capillary endothelium, and other factors that cause weak vascular walls so that blood vessels are prone to leaking.

Figure P0 on the 4th day post-infection also shows the occurrence of edema. Edema is a condition in which the amount of fluid in the intercellular compartment increases. Necrosis of the intestine also occurred in each treatment either on the 30th or 14th day post-infection with different intensities, most commonly occurring in the P2 treatment. Necrosis of the intestinal tissue is characterized by the destruction or loss of part of the normal intestinal structure. In the intestinal mucosa, there are empty spaces and fractures in the lamina epithelia, there are also broken villi that separate from the lamina propria and missing microvilli.

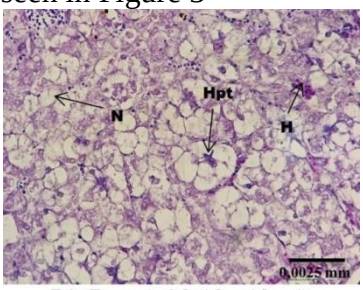
The structure of the intestinal tissue on the 30th day of P3 treatment had damage such as hemorrhage and there were also many goblet cells. At P3 14 days post-infection after being observed it seems to show better results. Where lamina epithelial and villi in the tunica mucosa of the intestine are seen more clearly and firmly. However, in the P3 treatment 14 days post-infection, tissue damage such as necrosis was still found, but the number was very small, while there were many goblet cells. It is suspected that in this study the dose of adding 20 ml/kg of fermented papaya leaf solution in the P3 treatment was the best, but not the most optimal dose in preventing *A. hydrophila* bacterial infection.

Striped Catfish Liver Tissue Structure

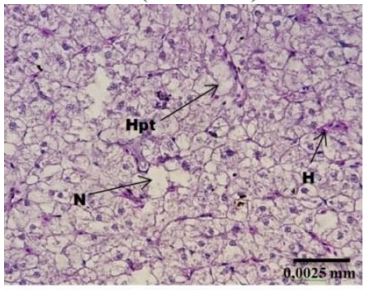
The structure of the normal striped catfish liver tissue is marked or consists of hepatocytes which have a round shape, cell nuclei, and clear sinusoids. The main structure of the liver is liver cells or hepatocytes. Hepatocytes (liver parenchyma cells) are responsible for the liver's central role in metabolism. Normal hepatocytes have the characteristics of cells arranged in a raider, round, oval cell shape and there are hepatocyte plates. Cells appear to have one nucleus and more than one nucleus (binucleate) located in the center of the cell. Striped catfish liver tissue structure before infection and post-infection can be seen in Figure 3



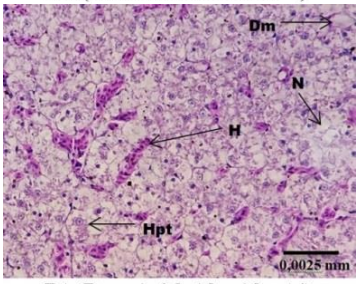
P0 (Hari ke-30)



P0 (Pascainfeksi hari ke-4)



P1 (Hari ke-30)



P1 (Pascainfeksi hari ke-14)

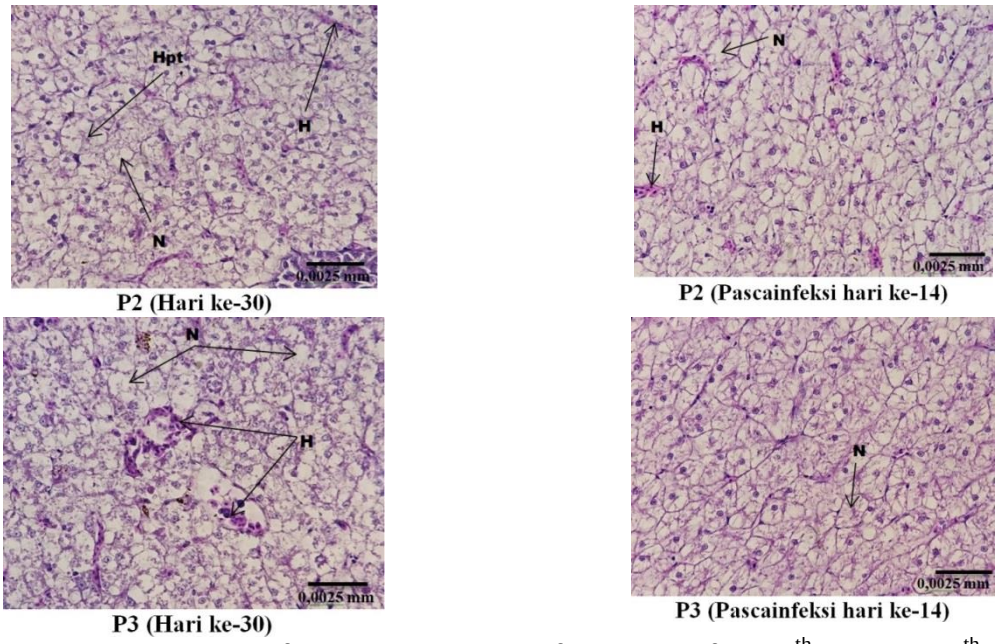


Figure 3. Photomicrograph of the liver structure of striped catfish 30th day and 14th day post-infection, HE staining (Magnification 400x)

Information: Cell nuclei (I), Hepatocytes (Ht), Sinusoids (S), Haemorrhagic (H), Necrosis (N), Hypertrophy (Hpt), Fatty Degeneration (Dm)

The condition of the striped catfish liver tissue on the 30th day of P0 treatment was found to be hemorrhagic, hypertrophied, and necrotic. At P0 4 days post-infection, a lot of damage was found, including hemorrhage, hypertrophy, and necrosis with severe intensity when viewed on all histological preparations. Organs for liver tissue structure specifically for fish P0 4 days post-infection were also taken from fish that experienced death, because before the 14th day post-infection P0 fish experienced total death on day 4.

In treatment P1 on day 30 the liver tissue structure experienced hemorrhage and necrosis, as well as hypertrophy. At P1 14 days post-infection visible liver tissue damage was the most of all treatments and had severe intensity including hemorrhage, hypertrophy, necrosis, and fatty degeneration was found. Furthermore, on the 30th day of P2 treatment, the liver tissue had a hemorrhage, hypertrophy, and some necrosis. In the P2 treatment 14 days after infection there was tissue damage, namely hemorrhage, and necrosis.

Hemorrhage is one of the clinical symptoms of tissue damage in living organisms where there is heavy bleeding or congestion with a high degree of severity. This is due to the increased production of red blood cells and the rupture of blood vessel linings caused by *A.hydrophila* infection. Hypertrophy is tissue damage characterized by cell size much larger than normal cells resulting in an increase in organ size, accumulation of fat and toxic substances from the blood circulation and continuously trapped in hepatocytes so that these cells are unable to synthesize blood cells. becomes blocked and eventually, the hepatocytes enlarge. This is supported by Kahfi et al[14] 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.

Based on the observation results for the 30th day of P3 treatment, the structure of the liver tissue experienced hemorrhage and necrosis, where the sinusoids that form and protect the hepatocytes were not visible or not visible. At P3 14th days post-infection, just like the clinical symptoms that experienced healing, the structure of the

liver tissue also experienced a lot of healing, namely there was only one type of tissue damage in the liver, namely necrosis. However, visible necrotic damage is minimal. The sinusoids have begun to form clearly and the hepatocytes have begun to fill clearly and are visible. This is thought to occur due to the influence of fermented papaya leaf solution which has many compounds/enzymes that are antibacterial. Compounds such as saponins and tannins in papaya leaves can inhibit the growth of microbes or bacteria *A. hydrophila* so that there is not much damage to the striped catfish liver.

The treatment of feeding that added fermented papaya leaf solution at a dose of 20 ml/kg of feed (P3) was the treatment that experienced the most tissue structure improvements. It was seen that there were improvements in the liver tissue of striped catfish. Papain compounds in papaya leaves can produce new proteins and supporting compounds for the addition of new cells to replace damaged cells in fish body tissues. Saponins in papaya leaves have antimicrobial activity through the mechanism of leakage of proteins and enzymes from bacterial cells so that bacterial infection activity is reduced, and organs can focus on regenerating damaged cells/tissues. According to Ramadhian et al[11], the compounds found in papaya leaves (*Carica papaya*) include tannins, alkaloids, flavonoids, terpenoids, and saponins which are anti-bacterial.

Histological structure of the liver tissue of striped catfish which was fed with fermented papaya leaf solution and infected with *A. hydrophila* bacteria found several types of tissue damage including hemorrhage, hypertrophy, fatty degeneration, and necrosis. Data from observations of the level of liver damage were assessed by assessing the liver tissue damage index based on Windarti et al.[9] and shown in Table 1.

Based on the observational data in Table 1, the categories of damage can be seen from how many types of damage to the liver tissue are in each treatment. The most damage (severe category) was found in treatment P1, namely, there were 4 types of damage ranging from hemorrhage, hypertrophy, fatty degeneration, and necrosis. Followed by treatment P0 had 3 types of liver damage (severe category), namely hemorrhage, hypertrophy, and necrosis. In treatment P2 there were 2 types of damage (mild category), namely hemorrhage and necrosis, and finally, in treatment P3 there was only 1 type of damage, namely necrosis.

Table 1. Level of liver tissue damage of striped catfish (*P.hypophthalmus*) 14 days post-challenge

Treatment	Liver Damage				Damage Level Value	Group
	H	Ht	N	Dm		
P0	+	+	+	-	4	Damaged
P1	+	+	+	+	5	Damaged
P2	+	-	+	-	3	Damaged
P3	-	-	-	-	0	Normal

Table 2. Water quality during research maintenance

Parameter	Parameter Range		Quality Standards (PP No.22 of 2021)
	Early	End	
Temperature (^{0}C)	26 - 30	28 - 30	25 - 30
DO (ppm)	3,8 - 4,9	3,4 - 4,2	>4
pH	7,4 - 7,7	7 – 7,5	6 – 9
Ammonia (mg/L)	0,016 – 0,018	0,022 - 0,024	<0,2

Water quality

Measurements were made 2 times during the study, namely at the beginning and end of the study. The range of water quality parameter data during the study can be seen in Table 2.

4. CONCLUSION

be concluded that giving papaya leaf fermented solution can affect changes in the intestinal and liver tissue structure of striped catfish, the gut and liver tissue structure of fish fed with fermented papaya leaf solution, and infected *A. hydrophila* bacteria showed damage. The structure of the intestinal tissue of striped catfish shows damage such as hemorrhage, necrosis, edema, and vacuolar degeneration. Meanwhile, in the structure of the liver tissue of striped catfish, damage such as hemorrhage, hypertrophy, and necrosis can be seen. The best results in this study were the treatments reared by feeding containing fermented papaya leaf solution at a dose of 20 ml/kg of feed and infected with *A.hydrophila*.

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