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Effect of Immersion of Striped Catfish (*Pangasionodon hypophthalmus*) in Red Dragon Fruit Peel Solution (*Hylocereus polyrhizus*) on Gill and Kidney Histopathology

Efek Perendaman Ikan Jambal Siam (Pangasionodon hypophtalmus) dalam Larutan Kulit Buah Naga Merah (Hylocereus polyrhizus) terhadap Histopatologi Insang dan Ginjal

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Abstract

Red dragon fruit peel is a natural material with antibacterial potential against fish diseases. This study was conducted from July 2024 to June 2025 at the Laboratory of Fish Parasites and Diseases, Faculty of Fisheries and Marine Sciences, Universitas Riau. The aim was to determine the histopathology of gills and kidneys in striped catfish (*Pangasionodon hypophthalmus*) immersed in red dragon fruit peel extract and challenged with *Aeromonas hydrophila*. The experiment used a Completely Randomized Design (CRD) with five treatments and three replications: Kn (negative control), Kp (positive control, challenged with *A. hydrophila*), P1 (1 mL/L), P2 (1.5 mL/L), and P3 (2 mL/L). Immersion was applied three times at the beginning of rearing, followed by 46 days of culture. A challenge test was carried out on day 32, and gill and kidney samples were collected on days 30 and 14 post-challenge. Results showed that fish challenged with *A. hydrophila* exhibited gill damage, including hypertrophy, lamellar curvature, telangiectasis, and epithelial lifting, as well as kidney damage, including necrosis, hemorrhage, and cell degeneration. The best result was observed in treatment P2 (1.5 mL/L), which effectively reduced tissue damage and improved the histological structure of gills and kidneys in striped catfish.

Keywords: Striped catfish, *Aeromonas hydrophila*, Histopathology

Abstrak

Kulit buah naga merah merupakan bahan alami yang memiliki potensi antibakteri terhadap penyakit pada ikan. Penelitian ini dilaksanakan pada bulan Juli 2024 hingga Juni 2025 di Laboratorium Parasit dan Penyakit Ikan, Fakultas Perikanan dan Kelautan, Universitas Riau. Tujuan penelitian ini adalah untuk mengetahui histopatologi insang dan ginjal ikan jambal siam (*Pangasius hypophthalmus*) yang direndam dalam ekstrak kulit buah naga merah dan diuji tantang dengan *Aeromonas hydrophila*. Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan lima perlakuan dan tiga ulangan: Kn (kontrol negatif), Kp (kontrol positif, diuji tantang *A. hydrophila*), P1 (1 mL/L), P2 (1,5 mL/L), dan P3 (2 mL/L). Perendaman dilakukan tiga kali pada awal pemeliharaan, kemudian ikan dipelihara selama 46 hari. Uji tantang dilakukan pada hari ke-32, dengan pengambilan sampel organ insang dan ginjal pada hari ke-30 dan hari ke-14 pascaujitantang. Hasil penelitian menunjukkan bahwa ikan yang diuji tantang *A. hydrophila* mengalami kerusakan insang berupa hipertrofi, lamela bengkok, telangiektasis, dan pengangkatan epitelium, serta kerusakan ginjal berupa nekrosis, hemoragi, dan degenerasi sel. Perlakuan terbaik diperoleh pada

perendaman dosis 1,5 mL/L (P2) yang paling efektif dalam mengurangi kerusakan jaringan dan memperbaiki struktur insang serta ginjal ikan jambal siam.

Kata kunci: Ikan Jambal Siam, *Aeromonas hydrophila*, Histopatologi

1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*) is a freshwater fishery commodity in high demand among the public, especially in Riau. Its advantages include rapid growth, a good feed response, and the ability to withstand high stocking densities, making it well-suited for intensive production. However, intensive cultivation systems with high stocking densities and excessive feed can lead to a buildup of organic matter, resulting in elevated ammonia levels and triggering disease.

One of the major diseases in striped catfish farming is caused by *Aeromonas hydrophila* bacteria, which causes *Motile Aeromonas Septicaemia* (MAS) or red spot disease. This bacterial infection has been reported to cause heavy losses and even failure in freshwater fish farming (Syaieba et al., 2019). So far, disease management has been carried out using antibiotics, such as oxytetracycline (Mufidah et al., 2022). However, excessive antibiotic use can lead to bacterial resistance and leave residues in fish meat that may be harmful to consumers. Therefore, alternative disease control is needed that is safer, environmentally friendly, and does not cause negative effects, one of which is through the use of natural ingredients. Red dragon fruit skin (*Hylocereus polyrhizus*) is known to contain bioactive compounds such as alkaloids, terpenoids, flavonoids, phenolics, vitamin C, and vitamin E that function as antioxidants (Wahdaningsih, 2022). These compounds have the potential to be antimicrobial agents to suppress *A. hydrophila* infection.

However, studies on the effect of red dragon fruit peel extract on the histopathological condition of target organs, especially the gills and kidneys of striped catfish, are still limited. In fact, histopathological observations are important for determining the extent of tissue damage due to bacterial infection and for assessing tissue repair after treatment. Based on this, research on the histopathology of the gills and kidneys of striped catfish treated with a red dragon fruit peel solution after *an A. hydrophila* challenge is needed.

2. Material and Method

2.1. Time and Place

This research was conducted from July 2024 to June 2025 at the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine Sciences, Universitas Riau. Histology preparations were made at the Bukittinggi Veterinary Center

2.2. Methods

The method used in this research is an experimental method with a one-factor Completely Randomized Design (CRD) with 5 treatment levels. To minimize errors, 3 repetitions were performed for each treatment. The treatments given are:

Kn : Negative control (without soaking and without being tested with *A. hydropila*)
Kp : Positive control (without soaking and tested with *A. hydrophila*)
P1 : Immersion in dragon fruit peel solution at a dose of 1 mL/L water and tested against *A. hydrophila*
P2 : Dose of 1.5 mL/L water and tested against *A. hydrophila*
P3 : Dose of 2 mL/L water and tested against *A. hydrophila*.

2.3. Procedures

2.3.1. Preparation of Dragon Fruit Peel Solution

Dragon fruit peels obtained from the Panam Traditional Market in Pekanbaru City were washed and then cut into small pieces and blended by adding 200 mL of distilled water (1:1 ratio), namely 200 g of dragon fruit peels and 200 mL of distilled water, then heated on a hot plate for ± 5 minutes, this aims to dissolve metabolites from dragon fruit peels in distilled water.

2.3.2 Preparation of Histopathology Preparations

Preparation of histological sections of gills and kidneys of striped catfish is described by Windarti et al. (2017). Organs were fixed in 10% formalin for 24-48 hours, then stored in 4% formalin. Next, multistage dehydration was performed using 70%, 80%, 96%, and absolute alcohol, each for 1 hour, followed by clearing using xylene. Infiltration was performed with xylol-paraffin mixture, then embedding in liquid paraffin using a mold. Paraffin blocks were cut with a microtome at a thickness of 5-6 µm, then attached to a glass slide that had been treated with albumin-glycerin adhesive. The preparations were then stained using the Hematoxylin-Eosin (HE) method.

Staining begins with rehydration through a decreasing alcohol series, hematoxylin immersion for 4 minutes, rinsing, and then eosin for 1.5 minutes. Dehydration was again done with graded alcohol and clearing with xylene. The preparations were covered using Entellan and cover glass, then dried in an oven. The preparations were observed using an Olympus CX21 light microscope.

2.3.3. Measured Parameters

The parameters measured in this study were clinical symptoms, histopathological examination of gills and kidneys, and water quality

2.4. Data Analysis

Clinical symptom observations were analyzed descriptively. Fish gill and kidney tissue structure data were also analyzed descriptively, with damage scores assigned. Observations of the distance and width of the Secondary Lamella of Fish were tabulated and analyzed using SPSS, including Analysis of Variance (ANOVA). Suppose the treatment showed a significant difference where $P<0.05$, the Newman-Keuls further test was conducted to determine the difference of each treatment. Water quality data were analyzed descriptively.

3. Result and Discussion

3.1. Clinical Symptoms of striped catfish

Clinical symptoms of striped catfish were observed for 14 days after the challenge test. The clinical symptoms observed in fish included behavior during the study, such as movement, body color, appetite, and condition of fins and eyes. Changes in clinical symptoms in post-challenge striped catfish are shown in Table 1.

Table 1. Clinical Symptoms of Post-challenge Striped Catfish

Treatment	Clinical Symptoms				
	Body surface	Movement	Fins	Eyes	Appetite
Kn	Little mucus production, bright body color	Active	Whole	Normal	Normal
Kp	Excess mucus production, skin peeling, ulcers at the injection site, and an enlarged abdomen	Passive	Scrape	Protruding	Decreased
P1	Excessive mucus production, ulcers at the injection site, and an enlarged abdomen	Passive	Intact	Protruding	Declining
P2	Excessive mucus production, ulcers at the injection site	Passive	Intact	Normal	Decreased
P3	Excessive mucus production, ulcers at the injection site, and an enlarged abdomen	Passive	Intact	Protruding	Decreased

Based on the study results, the negative control treatment (Kn) did not show any clinical symptoms, as the test fish were neither treated nor challenged with *A. hydrophila*. In contrast, the positive control (Kp) showed various clinical symptoms, including ulcers, skin peeling, an enlarged abdomen, a damaged tail fin, and protruding eyes. These symptoms are thought to result from *A. hydrophila* infection, which produces toxins, including hemolysin. This condition is consistent with the findings of Pakpahan et al. (2020), who reported that *A. hydrophila* infection can cause fin damage, skin lesions that develop into ulcers, and swelling of the eyes and abdomen. In addition, behavioral changes, such as passive movements and a slow feed response, were observed, indicating physiological stress in the fish (Fitriyanti, 2020; Rosidah et al., 2018).

Treatments P1 and P3 showed similar clinical symptoms, including *ulcer*, protruding eyes, enlarged abdomen, passive movements, and decreased appetite. This is likely because the dose of red dragon fruit peel solution administered is not optimal, so it has not been able to suppress *A. hydrophila* infection effectively. A bacterial infection leads to increased production of the hemolysin toxin, which damages red blood cells and causes bleeding throughout the fish's body (Damayanti, 2024). These clinical symptoms are also consistent with Saputra's (2018) findings, which reported that fish infected with *A. hydrophila* exhibited decreased feed intake, abnormal swimming, and body wounds.

The best results were shown in treatment P2, where clinical symptoms were milder and recovery occurred faster than in other treatments. Fish appetite returned to normal on day 6, fins remained intact, and eye condition was normal. This difference indicates that the dose of red dragon fruit peel solution used in P2 is optimal in helping fish recover from *A. hydrophila* infection. The bioactive compounds in red dragon fruit skin, such as flavonoids, alkaloids, tannins, polyphenols, saponins, and terpenoids, play an important role as antioxidants and antibacterials, inhibiting the growth and death of pathogenic bacterial cells (Juliyanti et al., 2024).

3.2. Histopathological Examination of Gills

Striped catfish gill tissue that has been maintained and challenged with *A. hydrophila* showed abnormalities in some treatments. The abnormalities that occur are telangiectasis, twisted lamella, hypertrophy, and raised epithelium. Immersion treatment with red dragon fruit peel solution at different doses has different effects on the level of damage to striped catfish gill tissue.

Normal gill tissue structure is seen in Kn, where Kn gills do not show any damage to the gill structure, both in the secondary lamella and primary lamella. While the structure of the gill tissue in Kp after the challenge test shows extensive damage, including hypertrophy, telangiectasis, twisted lamellae, and haemorrhage, this damage can disrupt the respiratory system of fish. If it worsens, the risk of death will be high. Hypertrophy of the secondary lamellae occurs when the secondary lamellae experience swelling or enlargement of the cell size in the secondary lamellae. Hypertrophy is thought to result from *A. hydrophila* infection, which damages gill tissue. This is in accordance with the statement by Juanda et al. (2022), which states that cell hypertrophy is known to be caused by several factors, including parasite infestation, exposure to toxic substances, bacteria, and disease. The structure of the striped catfish gill tissue can be seen in Figure 1.

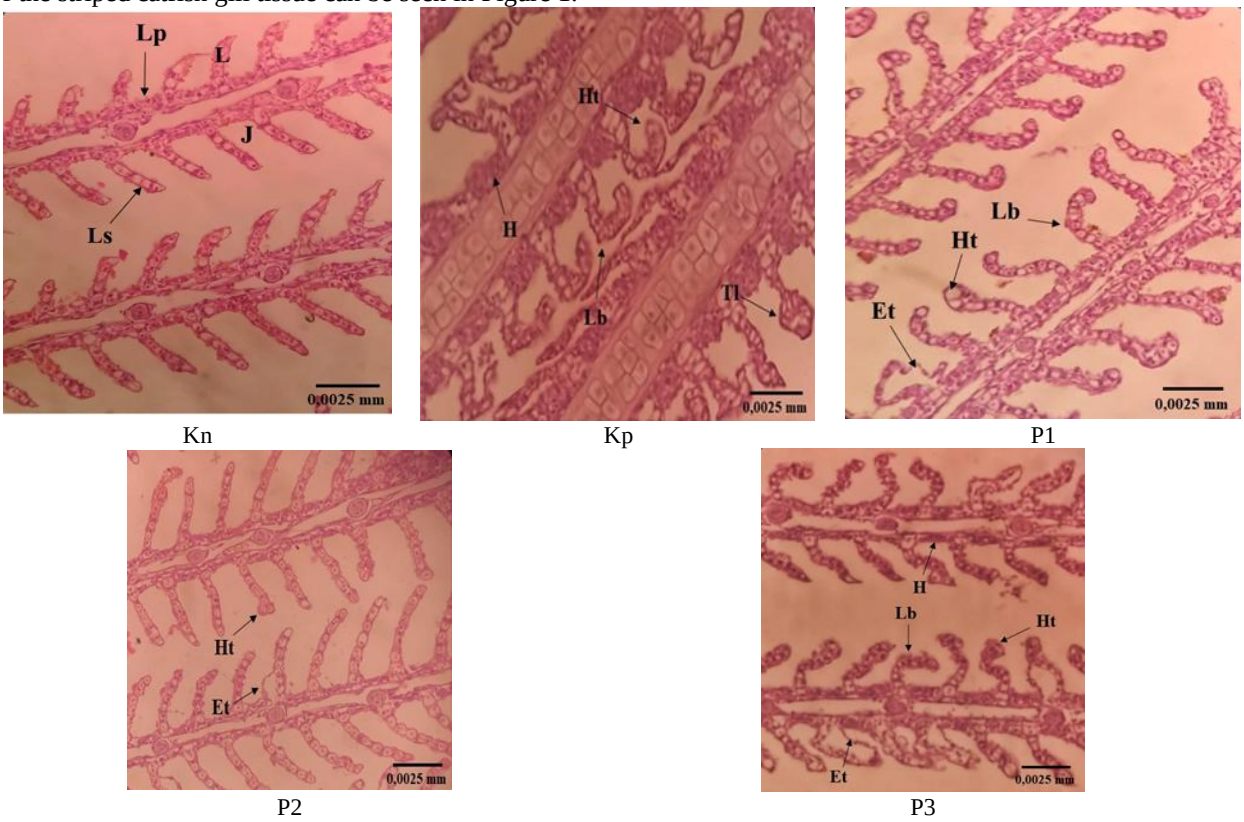

Figure 1. Gill Tissue Structure

Based on histopathological observations, the positive control treatment (Kp) showed severe gill tissue damage, including telangiectasis, haemorrhage, and twisted lamella. Telangiectasis is a permanent condition characterized by dilatation at the ends of the secondary lamellae, making them appear like inflated balloons. This condition is usually associated with poor environmental quality, nutritional deficiencies, or the presence of pathogenic bacteria and viruses (Juanda et al., 2024). In addition, haemorrhage, characterised by rupture of blood vessels and discharge of blood into abnormal tissues, is also observed, manifesting as petechiae and ecchymosis (Juanda & Edo, 2018). The occurrence of these various damages is thought to result from the fact that the fish in Kp were challenged only with *A. hydrophila*, without the red dragon fruit peel immersion treatment, so the infection process could not be suppressed.

In the P1 treatment, the gill tissue damage found was relatively lighter than in Kp, although hypertrophy, raised epithelium, and twisted lamella were still found. Hypertrophy is characterized by the enlargement of epithelial cells in the secondary lamellae, which impairs respiratory function (Sufriyani, 2020). Meanwhile, raised epithelium disrupts oxygen absorption because the epithelial layer is no longer attached to the tissue. This condition can lead to necrosis if it persists (Irene et al., 2021; Juanda & Edo, 2018). However, the damage in P1 can still be recovered, so the severity is lower than in the positive control.

The P2 treatment showed the best results with only mild damage in the form of twisted lamellae and raised epithelium. This is thought to be due to the administration of a red dragon fruit peel solution at an optimal dose, which suppresses *A. hydrophila* development and accelerates recovery of post-challenge gill tissue. Bioactive compounds in red dragon fruit peel, such as flavonoids, alkaloids, terpenoids, and saponins, are known to have antibacterial and antioxidant activities. Flavonoids, for example, can inhibit nucleic acid synthesis, disrupt cell membrane permeability, and inhibit bacterial energy metabolism (Gutiérrez-Venegas et al., 2019; Sarbu et al., 2019).

Meanwhile, the P3 treatment showed more severe gill tissue damage, including hypertrophy, raised epithelium, twisted lamella, and haemorrhage. This is thought to be influenced by two factors: *A. hydrophila* infection and

the use of a high-dose red dragon fruit peel solution, which is toxic. One of the active compounds contained in dragon fruit peel is saponin. At high doses, saponins are toxic to poikilotherm organisms because they can lyse red blood cells, making them harmful to fish (Christien, 2014). Thus, the effectiveness of red dragon fruit peel solution depends heavily on the dose: the optimal dose can suppress bacterial infection, whereas excessive doses can cause toxic effects. The width and distance between lamellae in fish gills greatly affect the process of taking in and exchanging oxygen during respiration. If the width and distance between lamellae are normal, the fish will carry out respiration properly (Table 2).

Table 2. Distance and width of the Secondary Lamella of Striped Catfish

Treatment	Lamella Distance	Lamella Width
Kn	0,038±0,001^{e}	0,012±0,001^{a}
Kp	0,019±0,001^{a}	0,026±0,001^{d}
P1	0,025±0,001^{b}	0,016±0,001^{c}
P2	0,035±0,001^{c}	0,013±0,001^{b}
P3	0,030±0,001^{d}	0,016±0,001^{c}

The results showed that soaking striped catfish in a red dragon fruit peel solution affected the distance and width of the secondary gill lamellae after the challenge test with *A. hydrophila*. The range of secondary lamella distance observed was 0.019-0.038 mm, while the lamella width ranged from 0.012-0.026 mm. The best treatment was shown in P2, with a distance of 0.035 mm and a width of 0.013 mm; this value is close to that of the negative control condition (Kn), with a distance of 0.038 mm and a width of 0.012 mm. According to Safana et al. (2019), the spacing of secondary lamellas that are more tenuous allows fish to breathe better than lamellas that are too close together. This is in line with the statement by Wahyuni et al. (2020) that gills that are close together or fused will reduce the surface area of the lamellae in contact with water, thereby disrupting oxygen uptake. Thus, the results of this study indicate that soaking with red dragon fruit peel solution, especially at the optimal dose (P2), can maintain the structure of secondary lamellae that support the efficiency of post-challenge respiration of striped catfish, in contrast to the positive control (Kp), which was not soaked, so that it suffered more severe damage.

Damage to the gill tissue structure of striped catfish can be classified into 3 levels of damage, namely normal (score 1), mild damage (score 10), and severe damage (score 100) (Windarti et al., 2017). Mild damage can be recovered if the trigger causing it is removed, while heavy damage will be difficult to recover from. The level of damage to the striped catfish gills can be seen using the HAI score (Table 3).

Table 3. HAI Score and Gill Damage Category

Damage level	Type of damage	Kn	Kp	P1	P2	P3
I	Hypertrophy	-	+	+	+	+
	Bent lamella	-	+	+	-	+
	Raised epithelium	-	-	+	+	+
II	Haemorrhage	-	+	-	-	+
III	Telangiectasis	-	+	-	-	-
Histopathological Alteration Index (HAI)		0	112	3	2	13

Notes: (+) there is damage, (-) there is no damage

Based on Table 3, Kn shows no damage, so it is classified as a normal category. In the Kp treatment, damage is observed with a score of 112; according to Windarti et al. (2017), this is classified as very heavy damage and is not recoverable. This damage occurred due to an *A. hydrophila* attack, and in this treatment, the fish were not soaked in a solution of red dragon fruit peel, an immunostimulant that can strengthen the fish's immune system. In the P1 and P2 treatments, scores of 3 and 2 were obtained and still fell within the normal category, whereas in P3, a score of 13 was obtained, which was categorized as a mild level of damage. This shows that striped catfish soaked with red dragon fruit peel solution has a better gill condition than those not soaked with red dragon fruit peel solution (Kp).

3.3. Histological Examination of the Kidney

Based on Figure 2 in the Kn treatment that was not tested with *A. hydrophila*, no damage was observed, and kidney histology was normal, with the Bowman's capsule and glomerulus clearly visible. In accordance with the statement (Laily et al., 2018) that, in general, the histological structure of the kidney consists of the main elements, namely glomeruli, tubules, and blood vessels, in normal fish kidneys, there is also a Bowman's capsule that surrounds the glomerulus.

In addition to hemorrhage, cell degeneration was found, a reversible form of cell damage characterized by swelling of the tubular epithelium. However, if the damage persists, this condition progresses to necrosis, an irreversible form of tissue death (Sulastri et al., 2018). Necrosis in fish tissue is characterized by a change in color to pale, tissue fragility, and loss of cell nuclei. Factors that cause necrosis include trauma and exposure to biological agents, such as bacteria, viruses, fungi, and parasites (Juanda & Edo, 2018).

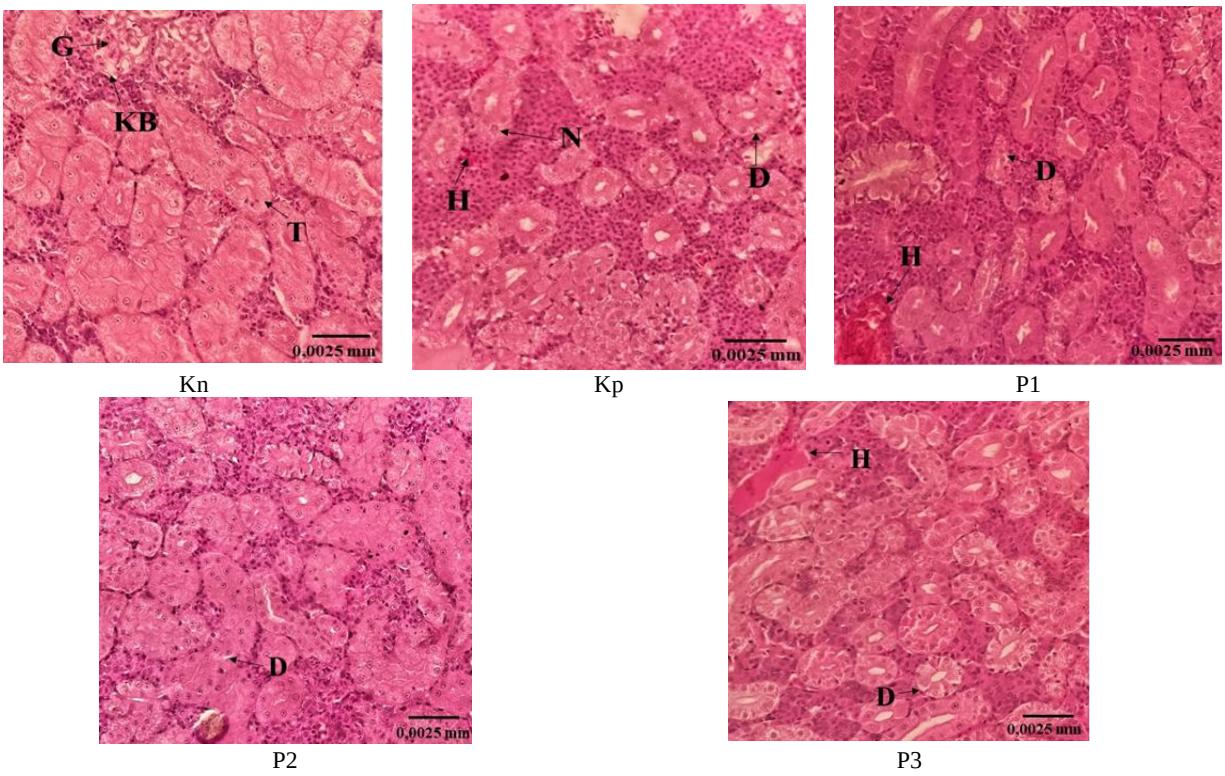

Figure 2. Kidney Histology

In the P1 and P3 treatments, kidney tissue damage was detected in the form of hemorrhage and degeneration, but the level of damage was lighter and still recoverable. Meanwhile, the P2 treatment showed only cell degeneration, indicating that soaking in a red dragon fruit peel solution at the appropriate dose can strengthen the fish's immune system, reducing post-challenge kidney damage. Flavonoid compounds in red dragon fruit peel are known to act as antibacterials by inhibiting cell membrane function, energy metabolism, and bacterial motility.

Overall, P2 treatment with a dose of 1.5 mL/L red dragon fruit peel solution gave the best results compared to other treatments. Histopathological damage to the gills observed included twisted lamellae, hypertrophy, raised epithelium, and telangiectasis, while in the kidney, hemorrhage, degeneration, and necrosis were observed. However, the most severe damage was still observed in the positive control (Kp). This finding confirms that the red dragon fruit peel solution suppressed and even killed *A. hydrophila*, thereby preventing more severe tissue damage in striped catfish.

3.4. Water Quality

Water quality is one of the factors that must be considered in fish rearing to support fish growth and health. The parameters measured in this study are temperature, pH, and dissolved oxygen **(**DO). Measurements were taken twice, at the beginning and at the end of the study. For more details, see Table 4.

Table 4. Water Quality

Parameter	Range of water quality parameters		
	Beginning	End	Quality Standard (SNI 2014)
Temperature(°C)	26-27	27-28	25-32
pH	5,5-6,5	5,5-6,9	6,5-8
DO mg/L	3,9-5,9	4,3-5,8	>3

The fish rearing environment has suitable water quality for fish growth. Water quality that is not suitable for fish can affect their health, development, and growth. When. If the water quality is poor, their appetite decreases, disrupting their metabolic processes and slowing growth. Temperature strongly influences fish growth and health by affecting their appetite. The temperature of the maintenance media when measurements were taken ranged from 26 to 28 (°C), which is included in normal conditions. This is in accordance with the statement of Septimesy et al. (2016), the optimal temperature for striped catfish is 26-30 (°C). Furthermore, Rizki & Sugihartono (2021) stated that for the maintenance of catfish, the temperature of 28-31 °C is still in the normal range.

The degree of acidity (pH) value is a concentration of hydrogen ions that indicates the water is acidic or alkaline. A low pH can cause stress, disrupting the fish's metabolic processes and reducing growth. During the study, the pH ranged from 5.5 to 6.9, which is a suitable range for fish maintenance, in accordance with the

findings of Syaieba et al. (2019), who reported that striped catfish tolerate a pH range of 5.2 to 7.0. Other water quality parameters measured include dissolved oxygen (DO), which is essential to all fish for respiration, metabolic processes, and gas exchange, thereby producing energy for growth and reproduction. The dissolved oxygen value during the study ranged from 3.9 to 5.9, in accordance with the opinion of Kurniawan et al. (2020), who reported that the optimal dissolved oxygen for the maintenance of striped catfish ranges from 4 to 6.5 mg L-1. Furthermore, according to Asis et al. (2017), the ideal dissolved oxygen content in water for fish farming should not be <3.00 mg/L, as levels below this can cause fish death.

4. Conclusions

Based on the research results, soaking striped catfish in a solution of red dragon fruit peel has an effect. The effect of this treatment on the parameters observed, namely clinical symptoms and tissue structure of gills and kidneys of striped catfish after the challenge test with *A. hydrophila.* Fish soaked in a red dragon fruit peel solution showed better results after the challenge test than the positive control (Kp), which showed more severe damage. The best results for soaking striped catfish in a red dragon fruit peel solution across the observed research parameters are observed in the P2 treatment at a dose of 1.5 mL/L.

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