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Structure of Intestinal and Kidney Tissue of Carp (*Cyprinus carpio*) Maintained in Salinity Media and Feed Enriched with Guava Leaf Flour (*Psidium guava*)

Struktur Jaringan Usus dan Ginjal Ikan Mas (*Cyprinus carpio*) yang Dipelihara pada Media Bersalinitas dan Diberi Pakan Diperkaya Tepung Daun Jambu Biji (*Psidium guajava*)

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ABSTRACT

Carp (*Cyprinus carpio*) is one of the freshwater fishery commodities with high economic value. Keeping carp in saline media can affect the osmoregulation system, impacting the performance of vital organs such as the intestines and kidneys. Such disturbances can potentially reduce fish's health and survival rate. One strategy that can be used to increase the fish's immune system is through feed supplementation with natural immunostimulants, one of which is guava leaf flour (*Psidium guajava*), which is known to contain bioactive compounds such as flavonoids, tannins, and saponins. This study aims to evaluate the effectiveness of guava leaf flour-enriched feed on the histological structure of the intestines and kidneys of carp raised in saline media and exposed to *Aeromonas hydrophila* bacterial infection. The study was conducted from August to October 2024 at the Biotechnology Laboratory, Faculty of Fisheries and Marine, Universitas Riau. The method used was an experiment with a completely randomized design (CRD) consisting of five guava leaf flour dosage treatments with three replicates. The results showed that the histological structure of the intestine in all treatments was relatively normal, with neatly arranged villi and round to oval goblet cells. However, in the guava leaf powder supplementation treatment, several abnormalities were found in the kidney tissue, including inflammation, hemorrhage, and necrosis. This indicates that the salinity factor has a greater effect on kidney damage than the effect of immunostimulant administration. Nevertheless, the administration of guava leaf powder still positively improved the immune response and survival rate of fish. The optimal dose was obtained in treatment P2 (15 g/kg feed) with a survival rate of 83.33–93.33%. The water quality parameters during the study were still within the acceptable range for aquaculture, namely temperature 28–31°C, DO 3.7–7.4 mg/L, and pH 5.5–7. Thus, it can be concluded that feeding fish with guava leaf flour-enriched feed is effective in maintaining intestinal histology and increasing the survival rate of carp in saline media, although it cannot completely prevent kidney damage caused by environmental factors

Keywords: *Cyprinus carpio*, Guava Leaf Flour, Histology, Intestinal, Kidney

ABSTRAK

Ikan mas (*Cyprinus carpio*) merupakan salah satu komoditas perikanan air tawar yang bernilai ekonomis tinggi. Pemeliharaan ikan mas dalam media bersalinitas dapat memengaruhi sistem osmoregulasi, sehingga berdampak pada kinerja organ penting seperti usus dan ginjal. Gangguan tersebut berpotensi menurunkan kesehatan dan tingkat kelulushidupan ikan. Salah satu strategi yang dapat dilakukan untuk meningkatkan daya tahan tubuh ikan adalah melalui suplementasi pakan dengan imunostimulan alami, salah satunya tepung daun jambu biji (*Psidium guajava*) yang diketahui mengandung senyawa bioaktif seperti flavonoid, tanin, dan saponin. Penelitian ini bertujuan untuk mengevaluasi efektivitas pemberian pakan yang diperkaya tepung daun jambu biji terhadap struktur histologi usus dan ginjal ikan mas yang dipelihara pada media bersalinitas serta menghadapi tantangan infeksi bakteri *Aeromonas hydrophila*. Penelitian dilaksanakan pada bulan Agustus–Oktober 2024 di Laboratorium Bioteknologi Fakultas Perikanan dan Kelautan, Universitas Riau. Metode yang digunakan adalah

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eksperimen dengan Rancangan Acak Lengkap (RAL) yang terdiri atas lima perlakuan dosis tepung daun jambu biji dengan tiga ulangan. Hasil penelitian menunjukkan bahwa struktur histologi usus pada semua perlakuan relatif normal dengan vili yang tersusun rapi dan sel goblet berbentuk bulat hingga oval. Namun, pada jaringan ginjal ditemukan beberapa kelainan, meliputi inflamasi, hemoragi, dan nekrosis, termasuk pada perlakuan dengan suplementasi tepung daun jambu biji. Hal ini mengindikasikan bahwa faktor salinitas memiliki pengaruh lebih besar terhadap kerusakan ginjal dibandingkan dengan pengaruh pemberian imunostimulan. Meskipun demikian, pemberian tepung daun jambu biji tetap memberikan dampak positif terhadap peningkatan respons imun dan kelulushidupan ikan. Dosis optimal diperoleh pada perlakuan P2 (15 g/kg pakan) dengan tingkat kelulushidupan mencapai 83,33%–93,33%. Parameter kualitas air selama penelitian masih berada dalam kisaran layak untuk budidaya, yaitu suhu 28–31 °C, DO 3,7–7,4 mg/L, dan pH 5,5–7. Dengan demikian, dapat disimpulkan bahwa pemberian pakan yang diperkaya tepung daun jambu biji efektif dalam mempertahankan kondisi histologi usus serta meningkatkan kelulushidupan ikan mas pada media bersalinitas, meskipun tidak sepenuhnya mampu mencegah kerusakan ginjal yang dipengaruhi oleh faktor lingkungan

Kata Kunci: *Cyprinus carpio*, Tepung Daun Jambu, Histologi, Usus, Ginjal

INTRODUCTION

Carp (*Cyprinus carpio*) are freshwater fish with high economic value and are widely farmed due to their rapid growth and high market demand (Tatipata et al., 2024). Although known as freshwater fish, carp can also live in low-salinity environments. However, these environmental changes can affect the fish's osmoregulation system, causing physiological stress, metabolic disorders, decreased immunity, and an increased risk of disease infection. Osmoregulation is a mechanism that regulates fluid balance in an organism's body by controlling the amount of fluid that enters and exits, so that the osmotic pressure in the body remains stable and supports normal physiological functions (Pamungkas, 2012). One of the pathogens that often attacks carp is *Aeromonas hydrophila*, a bacterium that can cause wounds, swelling, and even fish death.

To reduce the impact of stress and increase the immune system of fish, a natural approach that is safe and environmentally friendly is needed. According to Safitri et al. (2023), one potential alternative is using immunostimulants from natural ingredients such as guava leaves (*Psidium guajava*). Guava leaves contain various bioactive compounds, including flavonoids, tannins, and saponins, which have antibacterial, antioxidant, and immunostimulant properties (Jannah et al., 2024). Several studies have shown that guava leaf extract can boost the fish's immune system and help fight pathogenic infections.

Considering this potential, this study aims to determine the effectiveness of administering immunostimulants in the form of feed enriched with guava leaf powder on carp's intestinal and kidney structure in the face of attacks from the bacterium *A. hydrophila*. The results of this study are expected to provide scientific information on the benefits of guava leaves as a natural feed additive in increasing fish resistance to challenging environmental conditions and bacterial infections.

MATERIALS AND METHODS

Time and place of the research

The research was conducted from August to October 2024 at the Biotechnology Laboratory of the Faculty of Fisheries and Marine, Universitas Riau.

Research Method

This study used an experimental method with a completely randomized design. The treatment followed Effendi et al. (2022) study, with five treatments with three replicates. The treatments were without the addition of guava leaf powder (control) and with the addition of guava leaf powder. The doses used were 10 g/kg of feed (P1), 15 g/kg of feed (P2), 20 g/kg of feed (P3), and 25 g/kg of feed (P4).

Preparation of Fish Containers

This study used 15 basins with a diameter of 1 m as rearing containers, which were washed clean and filled with 80 L of brackish water with a salinity of 5 ppt, obtained from a mixture of 66.7 L of fresh water and 13.3 L of seawater. Each container was filled with 20 goldfish with a density of 1 fish per 4 L of water.

Fish Feed Production

Feed production began with collecting the third or fourth guava leaves from the shoots, which were dried in the sun, blended, and sieved (100-200 mesh). The feed is made by crushing the pellets using a blender, mixing them with guava leaf flour according to the dosage, adding water, and then molding them into pellets with a diameter of 0.5 mm. The pellets are dried in the sun before use.

Fish Maintenance

Fish fry measuring 5.00 ± 1.00 cm and weighing 4.00 ± 1.00 g were obtained from farmers at the KJA Waduk PLTA Koto Panjang. Before maintenance, the fish were adapted for 2 days without feed, then fed a mixture of guava leaf flour according to the treatment dosage for 60 days at the Biotechnology Laboratory of the Faculty of Fisheries and Marine, Universitas Riau. Feed was given at 5% of the fish's body weight, three times a day (08:00 WIB: 1%, 13:00 WIB: 1%, 17:00 WIB: 3%). Any leftover feed was discarded, and water quality was maintained using a Dacron filter, which was cleaned daily in the afternoon and replenished if water levels decreased. After the first stage, the fish were challenged with *A. hydrophila* and reared for another 14 days with the same feed and water management.

Bacterial Challenge Test

A challenge test was conducted to assess the effectiveness of phytoimmunostimulants against *A.hydrophila* infection. The bacteria were obtained from the Biotechnology Laboratory of the Faculty of Fisheries and Marine, Universitas Riau, and had been tested for pathogenicity. All fish were intramuscularly injected with *A. hydrophila* (10^8 CFU/ml, 0.1 ml/fish) in the dorsal area after being anesthetized with ice. After injection, the fish were kept for 14 days and observed daily to evaluate clinical symptoms and mortality. On day 14, intestinal and kidney tissue samples were taken for histological analysis.

Fish Tissue Structure Sampling Test

Tissue sampling was performed twice, on day 60 and after the bacterial challenge test. The process involved dissecting the fish abdomen to remove the intestine and kidney, which were then stored in bottles containing 10% formalin for further analysis.

Preparation of Fish Tissue Structure Specimens

The preparation of intestine and kidney specimens followed the method of Sumiwi et al. (2023). The samples were fixed in 10% formalin for 24-48 hours, then transferred to 4% formalin, cut (2-3 mm), and packaged before being sent to IPB. The preparation process included:

1) Dehydration – Immersion in a series of alcohols (70%–absolute) for 1 hour at each stage.
2) Clearing – Immersion in pure xylol twice for 1 hour each.
3) Embedding – Immersion in xylol-paraffin (1:1) for 1 hour, then in pure paraffin twice, followed by casting and cooling.
4) Cutting – Samples cut with a microtome (5 µm), placed in a water bath, then glued to glass objects using glycerine-albumin and dried (45°C, 24 hours).
5) Staining – The sample is immersed in xylol, rehydrated with descending alcohols, stained with hematoxylin (4 minutes) and eosin (1.5 minutes), then dried and covered with entellan neu. The preparation is stored in an oven for 2–3 days before being observed under a microscope.

Survival Rate

According to Effendi (1997), the survival rate can be calculated using the following formula:

$$SR = \frac{Nt}{No} \times 100\%$$

Description:

SR = Survival rate (%)
Nt = Number of fish alive at the end of the study (fish)
No = Number of fish alive at the beginning of the study (fish)

Fish Tissue Structure

Fish histology observations include analysis of abnormalities in the intestines and kidneys, cell damage, cell shape and nuclei, and the condition of the glomerulus and Bowman's capsule, including possible bleeding. In the intestines, observations focus on the condition of the villi and goblet cells to compare the intestinal structure of fish fed with or without guava leaf extract to assess its impact on tissue health.

Water Quality Measurements

Water quality measurements included temperature, pH, and dissolved oxygen (DO), taken every morning and evening when sampling fish once every 10 days.

Data Analysis

Histopathological data were analyzed using ANOVA, and if there were significant differences ($p < 0.05$), the Student Newman Keuls (SNK) test was used to compare treatments. The results were then analyzed descriptively based on changes in intestinal and renal tissue damage.

RESULT AND DISCUSSION

Survival Rate

The survival rate in carp before the bacterial challenge test ranged from 86% to 93%. However, after the challenge test using *A.s hydrophila* bacteria, the SR decreased to 68-83%. In the control group (P0), which was not given guava leaf powder, there was 100% mortality within 15 days after infection. The survival rate data can be seen in Table 1.

Table 1. Survival rate (SR) of goldfish treated with guava leaves and kept in saline media

Treatment	SR Parameter (%)	
	Day 60	Post challenge test
P0 (control)	86.67±2.89	0.00±0.00
P1	91.67±2.89	68.33±2.89
P2	93.33±2.89	83.33±2.89
P3	91.67±2.89	76.67±2.89
P4	88.33±2.89	68.33±2.89

After injection with *A. hydrophila*, control fish not given guava leaf powder showed red wounds around the injection site. The wounds worsened over several days, resulting in gradual death until, by day 10, no fish had survived. This occurred because the control fish did not have sufficient immune protection to fight bacterial infection. According to Chen et al. (2018), *A. hydrophila* can cause damage to vital organs such as the liver, kidneys, and spleen, as evidenced by swelling and cell necrosis. This bacterial infection can also cause physiological disturbances in fish, thereby worsening their health condition (Jaya et al., 2023).

Conversely, fish fed with guava leaf powder had a higher survival rate. In the best treatment (P2), the survival rate after the challenge test reached 83.33%, indicating that guava leaf powder was able to increase the fish's resistance to *A. hydrophila* infection. Omitoyin et al. (2019) stated that guava leaf extract contains secondary metabolites such as flavonoids, which enhance the immune response of fish by stimulating the production of immunomodulators. In addition to increasing resistance to infection, feeding guava leaf powder also affects the fish's appetite. According to Mangat & Hundal (2014), high feed consumption indicates that the fish's metabolism can adapt to certain salinity levels. If fish cannot adapt, mortality will increase due to disruption of the metabolic system.

Intestinal Tissue Structure of Carp

The results showed that the intestinal structure of carp fed guava leaf flour and control fish (P0/control) did not change significantly. The intestinal villi appeared neat and undamaged in the P0 treatment, which was not fed guava leaf flour (Figure 2). Goblet cells also appeared spherical or oval in shape and were located at the edge of the villi, indicating normal intestinal conditions (Astuti et al., 2024). This is in accordance with the research by Gauthier & Landis (1972), which states that a normal intestine has a mucosal layer with structured villi and contains goblet cells as lubricants for the digestive tract wall.

In treatments P1, P2, P3, and P4 (given guava leaf powder), the intestinal structure showed the same

condition as P0, where the intestinal villi remained neat and the goblet cells remained normal (Figure 2). This condition indicates that the administration of guava leaf powder did not cause histological abnormalities in the intestines of carp. Nafis et al. (2017) stated that healthy villi allow optimal digestion and nutrient absorption, which was also observed in this study.

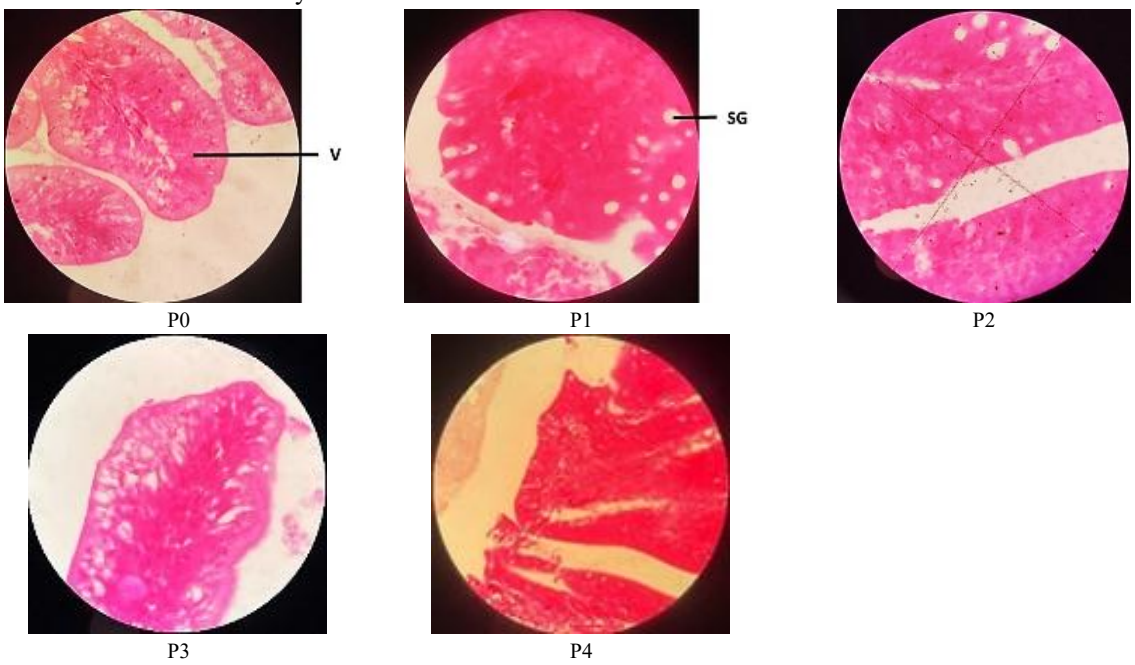


Figure 1. Structure of the intestines of goldfish kept at a salinity of 5 ppt on day 60

Description: P_0: Without guava leaf powder addition (control), P_1: 10 g/kg feed, P_2: 15 g/kg feed, P_3: 20 g/kg feed, P_4: 25 g/kg feed. V (Villi); SG (Goblet cells)

After testing with *A. hydrophila* bacteria, only the intestines of fish from treatments P1, P2, P3, and P4 could be observed because all fish in P0 experienced 100% mortality before tissue sampling. The histological structure of the intestines in these treatments remained normal, with active goblet cells producing mucus and villi showing no degeneration. The intestinal structure can be seen in Figure 2.

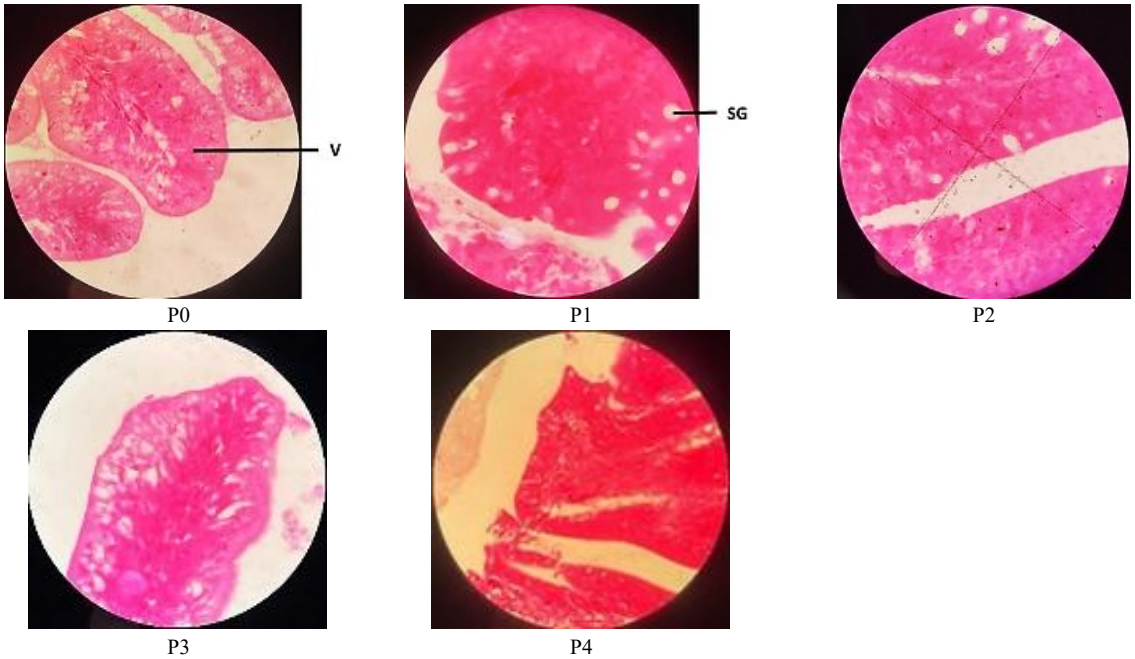


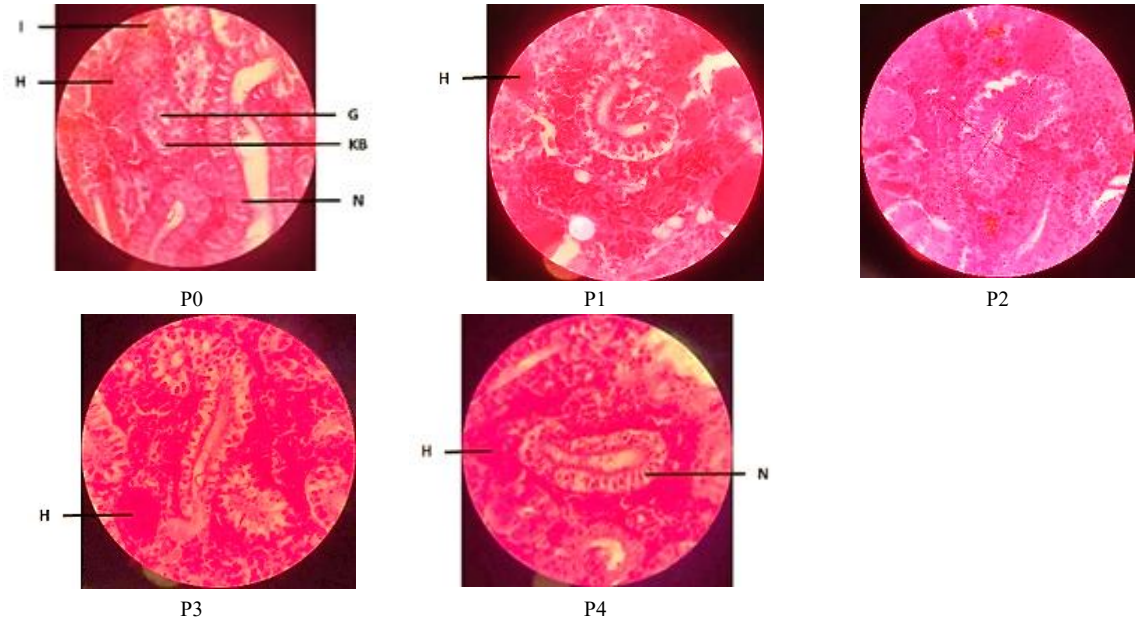
Figure 2. The structure of the intestines of carp was reared at a salinity of 5 ppt after the challenge test.

Description: P_1: 10 g/kg feed, P_2: 15 g/kg feed, P_3: 20 g/kg feed, P_4: 25 g/kg feed. V (Villi); SG (Goblet cells)

Structure of the Kidney Tissue of Carp

In this study, carp were kept in water with a salinity of 5 ppt. The kidneys of carp play an important role in regulating water and salt balance (osmoregulation), which is greatly influenced by the salinity of the environment in which the fish live. Keeping them in a saline medium causes changes in the performance of the kidneys of goldfish that naturally live in fresh water. The impact of these changes is a decrease in the fish's immune system, making them more susceptible to disease.

Normal carp kidneys have a distinct spherical glomerulus structure, surrounded by a neatly arranged cup-shaped Bowman's capsule. Al-Hatem et al. (2025) stated that the glomerulus and Bowman's capsule structures work efficiently in the blood filtration process under normal conditions. However, observations show that both fish fed guava leaves and control fish experienced similar damage to the glomerular cells and Bowman's capsule and hemorrhaging. The following image shows the condition of carp kidneys after being kept in a medium with a salinity of 5 ppt:



P0 P1 P2

P3 P4

Figure 3. Kidney structure of carp reared at a salinity of 5 ppt on day 60

Description: P_0: Without guava leaf powder addition (control), P_1: 10 g/kg feed, P_2: 15 g/100 kg feed, P_3: 20 g/kg feed, P_4: 25 g/kg feed. G (Glomerulus); KB (Bowman's capsule); I (Inflammation); H (Haemorrhage); N (Necrosis); P (Pigmentation)

Since damage was found in all treatments, it can be concluded that the main factor causing kidney damage was not the administration of guava leaves, but rather the salinity of the culture medium. If there were significant differences between the control and treatment groups regarding the level of kidney damage, then guava leaves would most likely be the cause. However, in this study, guava leaves did not negatively affect kidney structure. In treatment P0, abnormalities in the form of inflammation and necrosis were found. In P1, there was severe bleeding and perforated kidney cells. Treatment P2 showed bleeding, pigmentation, and mineralization. Meanwhile, in P3, there was severe bleeding with quite severe kidney damage. In P4, bleeding (hemorrhage) and necrosis were found.

Table 2. Damage to the renal tissue structure of fish found in each treatment

No	Abnormality	Treatment				
		P0	P1	P2	P3	P4
1.	Inflammation	√	-	-	-	-
2.	Necrosis	√	-	-	-	√
3.	Haemorrhage	√	√	√	√	√

After the fish were injected with *A.hydrophila* bacteria, the control fish experienced 100% mortality before kidney sampling, so they could not be observed. Austin & Austin (2007) stated that *A. hydrophila* infection can cause death if not treated quickly, especially in fish with weak immune systems. The observation showed that in the treated fish (P1, P2, P3, and P4), damage was found in the glomerular cells, Bowman's capsule, and hemorrhages in all treatments. The following image shows the structure of the kidney of the carp after testing with *A. hydrophila* bacteria.

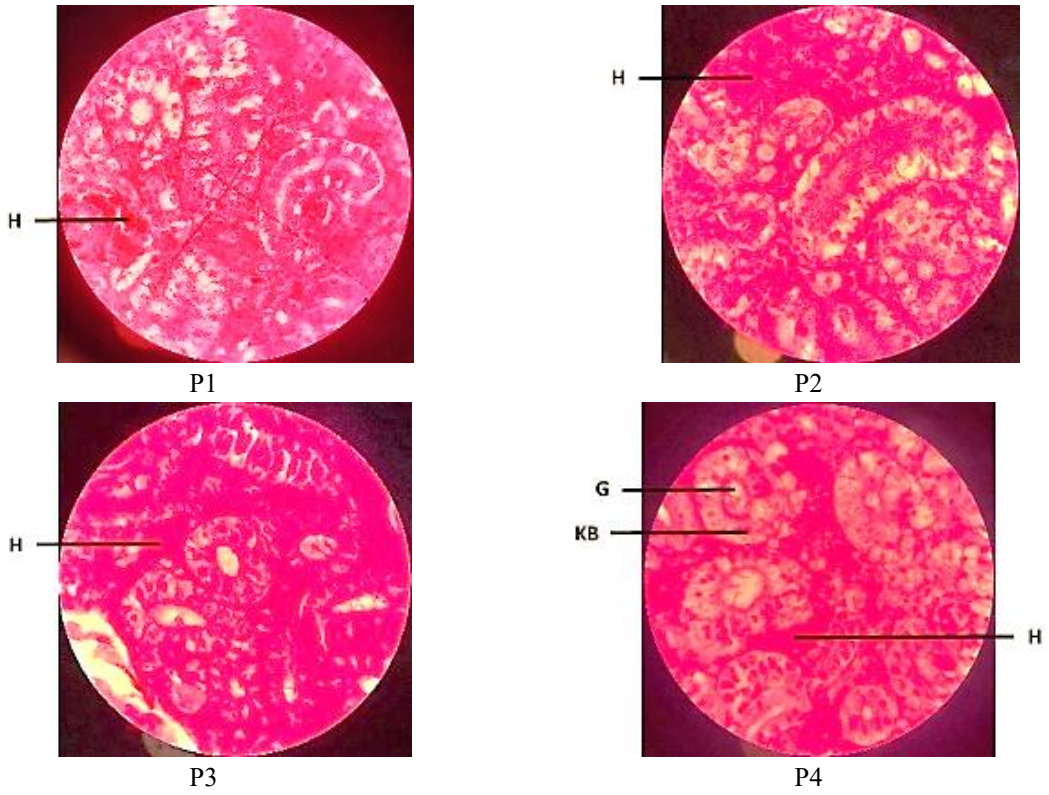


Figure 4. Structure of the kidneys of carp raised at a salinity of 5 ppt after the challenge test

Description: P_1: 10 g/kg feed, P_2: 15 g/kg feed, P_3: 20 g/kg feed, P_4: 25 g/kg feed. G (Glomerulus); KB (Bowman's Capsule); H (Hemorrhage)

Water Quality

Water quality greatly affects the growth and survival of carp. The parameters measured include temperature, dissolved oxygen (DO), pH, and salinity. The measurement results are presented in Table 3.

Table 3. Water quality during the study

Parameter	Value
Temperature (°C)	28-31
Dissolved oxygen (mg/L)	3.7-7.4
pH	5.5-5.77
Salinity	5

The temperature during the study ranged from 28 to 31°C, which is still within the optimal range for carp, generally between 25 and 32°C (Effendi et al., 2016). Dissolved oxygen ranged from 3.7 to 7.4 mg/L, which is still tolerable for fish growth. The pH ranged from 5.5 to 7.0, which is still suitable for carp life (Widiastuti, 2009). Thus, the water quality during the study remained within the optimal range for carp survival.

CONCLUSION

Based on the study's results, the administration of guava leaf powder affected carp's survival and tissue structure. Maintaining fish in a saline medium caused changes in the kidney tissue's structure, while the intestinal tissue's structure remained normal with neat villi cells and spherical or oval goblet cells. However, the kidney structure experienced several abnormalities, such as inflammation, hemorrhage, and necrosis, which were found in both the control fish and those fed guava leaf flour. Feeding guava leaf flour at a dose of 15 g/kg of feed (P2) showed the best results with survival rates ranging from 83.33% to 93.33%. Water quality during the study remained within a range that supported the growth and survival of carp, with temperatures ranging from 28-31°C, dissolved oxygen (DO) between 3.7-7.4 mg/L, and pH between 5.5-7.

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