Jurnal Natur Indonesia p-ISSN 1410-9379
21(1), April 2023: 36-41 e-ISSN 2503-0345

Kidney Structure of *Pangasianodon hypophthalmus* Fed with Fermented Red Ginger and Infection by *Aeromonas hydrophila*

Struktur Jaringan Ginjal Ikan Jambal Siam (*Pangasianodon hypophthalmus*) yang Diberi Pakan Mengandung Fermentasi Jahe Merah dan Infeksi dengan *Aeromonas hydrophila*

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ABSTRACT

Red ginger contains antioxidants that can be used to enhance the immune system of fish. A study was conducted from September to December 2021 to understand the kidney structure of *Pangasianodon hypophthalmus* that was fed with red ginger fermented pellets. The fermented red ginger juice was obtained by juicing 500 g of red ginger in 3L water, boiled and mixed with 175 ml molasses, 65 ml yogurt, and 50 mg yeast, and then fermented for seven days. The fermented red ginger juice was obtained by juicing 500 g of red ginger for days. The fermented red ginger juice was mixed with fish feed pellets, namely T1 (150 mL/Kg), T2 (175 mL/Kg), T3 (200 mL/Kg), Cn (no red ginger juice and no infection), Cp (no red ginger juice and infection by *A. hydrophila*). The fish were reared in 30L aquaria. The fish sample was *P. hypophthalmus* fingerling (10-12 cm and 5-7 g), 10 fishes/aquarium. The fish were fed 3 times a day, *adlibitum*. On the 30th day, *A. hydrophila* was injected into the treated fish (0.1 ml of 10^8 cells/ml, subcutan injection). On the 14th after the infection kidney sample was collected and processed for histological study. The result has shown that there were differences in the kidney tissue of fish in each treatment. The worst condition was obtained in Cp, while the best was in T2. The renal structure of Cp showed abnormalities such as hemorrhage, cell necrosis, enlarged Bowman's cyst, and tubular degeneration, while the T2 kidney showed mild lesions specifically hemorrhagically. This fact indicates that fermented red ginger can improve fish immunity against *A. hydrophila* infection.

Keywords: Antibacterial, MAS, Kidney, red ginger, Tubular

ABSTRAK

Abstrak. Jahe merah mengandung antioksidan yang dapat digunakan untuk meningkatkan sistem kekebalan tubuh ikan. Penelitian ini dilakukan pada bulan September sampai Desember 2021 untuk mengetahui struktur jaringan ginjal ikan jambal siam (*Pangasianodon hypophthalmus)* yang diberi pakan mengandung fermentasi jahe merah. Sari fermentasi jahe merah dibuat dengan cara mengambil sari jahe merah dari 500 g jahe merah dan mencampunya dengan 3 L air ditambah dengan 175 ml molase, 65 ml yakult dan 50 mg ragi tapai. Campuran tersebut difermentasikan selama tujuh hari. Perlakuan yang diterapkan adalah Cn (tanpa penambahan ferentasi jahe merah dan tanpa diinfeksi dengan *Aeromonas hydrophila*, Cp (tanpa penambahan fermentasi jahe merah dan diinfeksi dengan *A. hydrophila*). Penambahan fermentasi jahe merah dalam pakan sebanyak 150 ml/Kg (T1), 175 ml/Kg (T2) dan 200 ml/Kg (T3). Ukuran ikan uji yang digunakan adalah 10-12 cm (TL) dan 5-7 g (BW) dengan padat tebat 10 ekor/akuarium. Pemberian pakan ikan sebanyak 3 kali sehari secara *adlibitum*. Setelah diberi perlakuan dengan penambahan fermentasi jahe merah dalam pakan selama 30 hari, kemudian ikan diinfeksi dengan *A. hydrophila* (kepadatan 10^8 sel/ml sebanyak 0,1 ml). 14 hari setelah penginfeksian, organ ginjal ikan diambil dan dibuat preparat histologis. Hasil penelitian menunjukkan bahwa struktur jaringan ginjal setiap perlakuan berbeda. Pada Cp ditemukan kelainan struktur jaringan ginjal yaitu perdarahan, nekrosis sel, pembesaran kista Bowman, dan degenerasi tubular, sedangkan pada T2 menunjukkan adanya lesi ringan yaitu hemoragik. Kesimpulan dari penelitian ini adalah denagn pemberian pakan yang mengandung fermentasi jahe merah dapat meningkatkan daya tahan tubuh ikan terhadap serangan bakteri A. *hydrophila*.

Kata Kunci: Antibakterial, MAS, Ginjal, Jahe Merah, Tubular

Received: 27 Februari 2023
Accepted : 15 March 2023

INTRODUCTION

Pangasianodon hypophthalmus is a member of the Pangasidae family, and this fish can survive in a wide variety of environmental conditions and thrive in high stocking densities (Hossain *et al.,* 2022). Rearing this fish is beneficial as it can grow fast, and there is high market demand on domestic as well as international scales (Nishidonoa *et al.,* 2007; Riauwaty *et al.,* 2020). In *P.hypophthalmus* culture, a problem that commonly occurs is the attack or pathogen microorganisms such as *Aeromonas hydrophila* that causes MAS (*Motile Aeromonas Septicemia*) disease (Riauwaty *et al.,* 2020; Riauwaty *et al.,* 2021a). Fish that is under stress condition or has been wounded due to a parasite attack is vulnerable to an *A. hydrophila* attack. Fish with MAS disease show ulcers in the skin and it might be fatal or at least reduce the fish's economic value. This disease might be cured by using a chemical as well as natural resources antibiotic such as ginger (*Zingiber officinale var.* amarum*).* Ginger has been commonly used as a supplement and medical aid to cure flu-related diseases in human beings. In fish, ginger has been used to improve health and also boost the growth of fish (Kusumawardani *et al.*, 2008). The use of ginger is beneficial as it is cheap, easy to get, and can be planted everywhere. Moreover, the use of ginger is safe as it has no negative effects and is environmentally friendly (Febriani *et al*., 2018).

Ginger contains several types of essential oils and oleoresin that can be used to cure various types of diseases. The red ginger essential oil content ranges from 2.58 to 3.72% of dry weight (Febriani *et al.*, 2018). The oleoresin content in red ginger is higher than that of other types of gingers, which is only around 3% of the dry weight (Nishidonoa *et al.,* 2017). The red ginger extract is powerful to hamper the growth of *A.hydrophila*, with MIC (Minimum Inhibitory Concentration) occurring in the solution with 7.971% or 0.08 g extract/ml solvent, while the MBC (Minimum Bactericidal Concentration) was 31.849% or 0.32 grams extract/ml solvent (Kusumawardani *et al.* 2008). Red ginger is the potential to inhibit *A. hydrophila* growth and it is the potential for curing agents for fish that is infected with those bacteria.

Even though ginger is the potential for curing MAS disease, the hot-stinging taste of the ginger may cause stress in fish. By fermenting the ginger extract, the hot-stinging taste may be reduced and it can be applied to fish easily. By fermenting the ginger extract, the flavor, and taste of the extract will be improved and it may be preferred by fish. Riauwaty *et al.* (2021a) stated that fish pellets enriched with mixed fermented herbal stimulate the appetite and increase the immunity of the fish toward disease, reducing fish stress levels against environmental changes.

As the consumption of extracted ginger may increase fish immunity, it can resist any attack by pathogenic bacteria. The bacteria infection can be identified by the structure of fish organs such as the gill, kidney, liver, skin, and other organs. If the immunity of the fish is well, any attack of the pathogen may not cause serious problems in fish and it can be detected by the fish's tissue structure. To understand the effectiveness of using fermented ginger extract to enhance the immunity of the fish, a study titled "The histopathology of the kidney of *P. hypophthalmus* fed with fermented red ginger enriched pellet and was infected with *A.hydrophila* is necessary to be done.

MATERIALS AND METHOD

Location and Time

This study was carried out from September to December 2021 at the Laboratory of Fish Diseases and Parasites, Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Riau, Indonesia.

Method

Research is conducted by the ethical principles of the research institution and national or international regulations. A Completely Randomized Design was applied in this study. The treatments applied were as follows:

Cn : No *A. hydrophila* infection, the fish were fed fish feed pellets without fermented red ginger extract.
Cp : *A. hydrophila* infection, the fish was fed fish feed pellets without fermented red ginger extract.
T1 : *A. hydrophila* infection, fish was fed with fish feed pellets enriched with fermented red ginger extract, 150 mL/Kg
T2 : *A. hydrophila* infection, fish was fed with fish feed pellets enriched with fermented red ginger extract, 175 mL/Kg
T3 : *A. hydrophila* infection, fish was fed with fish feed pellets enriched with fermented red ginger extract, 200 mL/Kg

Fish rearing

Pangasianodon hypophthalmus was collected from the Marine Fisheries Department's Fishing Unit, Riau Province, Tibun Sub-Region, Kampar. The total length of the fish is about 10-12 cm with a body weight of about 5-7 g. Fish have been acclimatized to laboratory situations for 7 days. After that, the fish were reared in the rearing tank (size 30 x 40 x 40 cm^3), with a density of 10 fish/tank. The aquarium was completed with an aerator and dacron filter. A total of 150 fish were treated in this study (30 fish/tank). The feed provided is an industrial feed containing 35% protein. Lozenges enriched with fermented red ginger juice are given for 30 consecutive days, three times a day, ad libitum.

Fermented Red Ginger Juice Enriched Pellets Preparation

The red ginger (*Z. officinale*) was bought from traditional markets in Pekanbaru. The red ginger was then cleaned, peeled, sliced, and blended. The fermented red ginger juice was obtained by juicing 500 g of red ginger in 3L water and boiling it. As the juice was warm, it is mixed with 175 ml molasses, 65 ml yogurt, and 50 mg yeast and then fermented for seven days. Fermented red ginger enriched pellets were prepared by mixing commercial feed pellets manufactured by PT Central Proteina Prima, Type F-999 (35% protein content) with fermented ginger juice. The red ginger juice enriched pellets were given to the fish just after being prepared.

Aeromonas hydrophila infection

On day 32 after being treated with fermented red ginger juice, fish were injected with *A. hydrophila* (0.1 ml 10^8 cells/ml), subcutaneously. To relieve stress, before injection, the fish has been cooled with clove oil. When the fish showed signs of unconsciousness, the substance *A. hydrophila* was injected using a 1 mL syringe. After infection, fish were kept for 7 days or until clinical symptoms of MAS appeared

Histological study

Histopathological samples were taken on day 45. From each aquarium, 3 fish were taken and the kidneys were removed. The samples were then processed according to standard histological techniques (Riauwaty *et al.*, 2020). Renal tissue was fixed in 10% formalin for 24 h and then converted to 4% formalin for 3 days. The tissue was then treated with an alcohol series, embedded in paraffin, and cut into 6 µ slices using a microtome. The tissue was then stained using Hematoxylin-Eosin (HE) and bound using Entellan neu. Slides were examined using an Olympus CX-21 binocular microscope to assess renal structural changes and then for descriptive analysis.

RESULT AND DISCUSSION

There were differences in clinical signs between the fish that was fed with fermented red ginger and the fish that was not fed with the fermented red ginger juice (Table 1). Clinical signs investigated were balance, appetite, and pigmentation in the tegument. It seems that the fermented ginger-treated fish showed better condition than that of fish with no red ginger juice. The clinical signs of the fish are shown in Table 1.

Table 1. The clinical sign of *Pangasianodon hypophthalmus*

Treatments	Clinical signs on the 7th day after infection		
	Balance	Appetite	Pigmentation in the tegument
Cn	++++	++++	normal
Cp	-	-	darker
T1	++	+++	normal
T2	+	++	normal
T3	-	+	darker

Note: ++++, very good; ++ +, good; ++, bad; -, worst

After 7 days of infection, the clinical signs of MAS diseases have been shown. There was no clinical sign of MAS disease in negative control fish. In contrast, the positive control fish showed strong clinical symptoms of that disease such as loss of balance, no response to food provided, and extra pigmentation in the tegument. The fish fed with fermented red ginger (T1 and T2) showed light symptoms of MAS. There was no extra pigmentation and they responded well to the feed provided. The T3 fish that were fed with high dosages of fermented red ginger had shown the worst clinical signs after being infected with the bacteria. The skin of the

skin getting darker, many ulcer spots are present, no response to feed provided, and has lost balance in swimming. Similar abnormalities and symptoms such as decreased appetite, lethargy, exophthalmia, and swimming imbalance in fish infected with *A. hydrophila* were also reported (Al Mamun *et al.*, 2022; Febriani *et al.*, 2018). Other clinical signs associated with the Aeromonad infection in *P. hypophthalmus* are anorexia, loss of balance with erratic movement, and abnormal coloration along the body with hemorrhage spots (Hossain *et al.*, 2022). The main pathogenic bacteria *Aeromonas* spp. is the causative agent of pasteurellosis, a disease that affects a wide variety of freshwater and marine fish (Irene *et al.*, 2021). The disease is characterized by abdominal swelling, mouth redness, and external and perianal bleeding (Kumalasari & Mahasri, 2020). A bleeding ulcer was also seen at the base of the fin. Consistent with the current findings, hemorrhagic septicemia was also reported in Catfish due to *A. hydrophila* infection, especially when fish are under stress (Yusoff *et al.*, 2020). The kidney structure of *P.hypophthalmus* which was fed with fermented red ginger and infected with *A.hydrophila is* shown in Figure 1.

Table 2. Histopathological changes of *Pangasianodon hypophthalmus*

Treatments	Abnormalities				
	Renal tubules	Bowman's capsule	Space in between glomerulus	Necrotic	Hemorrhage
Cn	++++	++++	++++	-	-
Cp	-	-	++	+++	+++
T1	+++	+++	++	++	++
T2	++++	+++	+++	+	-
T3	++	++	-	++	+

Note: ++++, very good; ++ +, good; ++, bad; -, worst

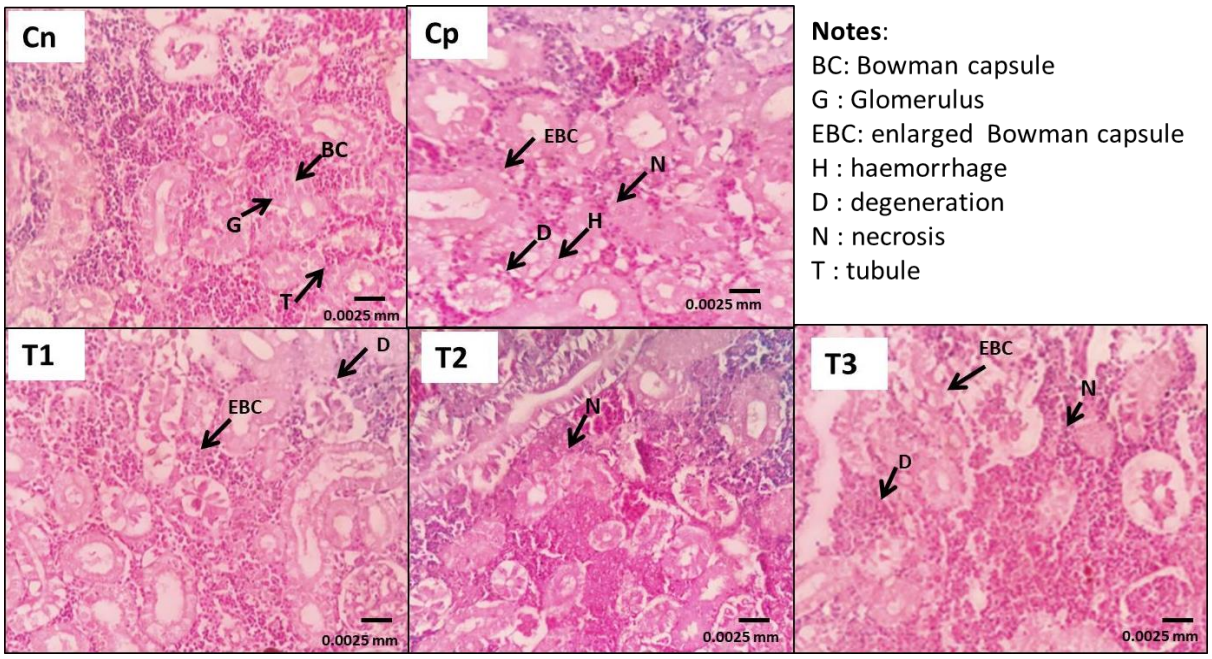

Figure 1. Kidney structure of *P. hypophthalmus* (H&E, 400X).
Note: G: Glomerulus, T: Tubules; BC: Bowman capsule; N: Necrosis; D: Degeneration; EBC: Enlarged Bowman capsule

The results showed that the kidney structure of fish fed with fermented red ginger juice and fish without red ginger juice was different. When fish are infected with *A. hydrophila*, the bacteria multiply in the fish's tissues and produce a toxin (aerolysin) that damages tissues, including kidney tissue. In fish that were not fed fermented red ginger and subsequently infected with *A. hydrophila* (Cp), tissue structural damage was evident. This fact suggests that the fish's immunity cannot cope with bacterial infection and hence hemorrhage, cell necrosis, Bowman's cyst, and tubular degeneration occur (Figure 1).

The fish kidney is the target organ during the period of bacterial sepsis with toxins secreted by bacteria that damage the structure (Yusoff *et al.*, 2020). Severe histopathological changes were observed in the kidney, including tubular degeneration with renal corpus callosum damage, capillary necrosis, and loss of Bowman's capsule when fish were infected with *A. hydrophila* (Mohammadi *et al.*, 2020). Invasion and multiplication of pathogenic bacteria can damage the kidneys and lead to glomerulonephritis (Mohammadi *et al.*, 2020).

According to Hossain *et al.* (2022) the histopathological changes in fish kidney *Notopterus notopterus*, are renal epithelial cell degeneration and dissolution tubules, hypertrophy, vacuolization in the epithelium renal tubular cells, and necrosis. Necrosis in the tubular epithelium may result from the presence of toxins, injury, and infection (Sulodia *et al.* 2014). Insufficient blood supply into the cell may trigger necrosis, and the process is initiated by inflammation reaction and edema in the glomerulus (Riauwaty *et al.*, 2021b).

When renal tissue is infected with *A. hydrophila*, the bacterium produces the Aero and Hyla genes involved in the production of the toxins aerolysin and hemolysin (Nishidonoa *et al.*, 2017; Prayitno *et al.*, 2021). Aerolysins are extracellular proteins produced by certain strains of *A. hydrophila* that are soluble, hydrophilic, and possess hemolytic and cytolytic properties (Mohammadi *et al.*, 2020). Aerolysine from *A. hydrophila* can damage fish cells through its hemolytic and cytolytic activity. This toxin attacks kidney tissue and can cause glomerular abnormalities (Prasad & Patel, 2018).

The kidney is considered a basal excretory organ mainly used for osmotic regulation. It captures the majority of body fluids and post-bronchial blood causing temporary or permanent deterioration of homeostasis and kidney damage that one would expect as a good marker of environmental pollution (Sulodia *et al*., 2014). In this study, the abnormalities present in the kidneys of fish infected with *A. hydrophila* were hemorrhage, cell necrosis, epithelial cell fusion, and hypertrophic chondrocyte. These effects are similar to the renal structures of infected fish studied by (Riauwaty *et al.*, 2020).

The kidney in general or the glomerulus was damaged and the shape of the cells was no longer perfect (Prasad & Patel, 2018; Riauwaty *et al.*, 2021a). The fish with Aeromonas infection showed severe damage such as complete injury to Bowman's capsules, necrosis, and hemorrhage. Riauwaty *et al.* (2021b); Sulodia *et al.* (2014) found renal degeneration in fish exposed to toxic substances, including tubular epithelial necrosis, lumen narrowing, and turbid swelling of tubular epithelial cells, glomerular contraction, and lateral space dilation in Bowman's capsule.

According to Sulodia *et al.* (2014), the case of *Channa punctatus* showed degeneration in renal tubules, hemorrhage, necrosis in the focal area, and atrophy in the glomerulus caused by bacteria infection, and agricultural and industrial wastes. Vacuolization and hypertrophy of the epithelium renal tubular cells, epithelial cell necrosis renal tubules, urinary tubular pyknosis, hypertrophy in the proximal tubule, collecting duct necrosis, rupture of blood vessels will cause bleeding and shrinkage of Bowman's capsule and glomerulus with the concentration of fermented red ginger given in the feed.

The weak immune system of infected fish in this recent study may be due to a lack of nutritional supplements that strengthen the fish's immune system. Healing of kidney structures indicated that the immune system was responding well to bacterial attacks, and the number of bacteria and the adverse effects caused by an attack can be reduced. These results indicate that the addition of red ginger to the diet for 30 days may have stimulated the catfish and generated an immune system against *A. hydrophila*. Red ginger contains active ingredients such as phenols, flavonoids, terpenoids, saponins, and alkaloids (Kumalasari & Mahasri, 2020). On the other hand, two bioactive compounds, namely saponins, and flavonoids, play an important role in *A.hydrophila* (Fazelan *et al.*, 2020). These bioactive compounds denature proteins and damage bacterial cell membranes by inhibiting bacterial cell wall permeability, bacterial cell protein synthesis, bacterial cell nucleic acid synthesis, and bacterial cell metabolism (Mohammadi *et al.* 2020).

The only abnormality present in the kidneys of fish-fed fermented red ginger was mild bleeding. This malformation is classified as the first type of kidney injury (Sulodia *et al.*, 2014; Yusoff *et al.*, 2020) and may heal as water quality improves and bacteria die. Current results show that *Z. officinale var. amarum* extract at 175 ml/kg can effectively enhance the immunity of *P. hypopthalmus*

CONCLUSION

P. hypophthalmus that was fed with fermented red ginger showed an ability to face the *A. hydrophila* attack, as is shown by less damage in the kidney after being infected with that bacteria. The most effective dosage of fermented red ginger to improve the immunity toward *A. hydrophila* attack was P2 (175ml/Kg).

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