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Histopathology of Gills and Liver of Tilapia (*Oreochromis niloticus*) Infected with *Streptococcus agalactiae* and Fed with Fermented Herbal Medicine

Histopatologi Insang dan Hati Ikan Nila (Oreochromis niloticus) Terinfeksi Streptococcus agalactiae dan Diberi Pakan Diperkaya Jamu Herbal Fermentasi

Romensius Anggi AW Siallagan[1*], Henni Syawal[1], Morina Riauwaty[1]
[1]Department of Aquaculture, Faculty of Fisheries and Marine,
Universitas Riau, Pekanbaru 28293 Indonesia
**email: romensius.anggi2001@student.unri.ac.id*

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Abstract

Streptococcus agalactiae is a pathogen that causes significant losses in tilapia (*Oreochromis niloticus*) aquaculture. One alternative control is the use of natural ingredients such as fermented herbs. This study aims to analyze changes in the histopathological structure of the gills and liver of tilapia fish fed with feed containing fermented herbs after being infected with S. agalactiae. The research was conducted from March to December 2023 at the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine Sciences, Universitas Riau and Bukittinggi Veterinary Center. The method used was a one-factor, completely randomized design (CRD) with five treatments and three replicates. The treatments consisted of negative control (Kn), positive control (Kp), and three feed treatments with fermented herbal medicine doses of 100 (P1), 125 (P2), and 150 mL/kg feed (P3). *S. agalactiae* infection was carried out on day 31 at a dose of 10^8 CFU/mL. The results showed that the P3 treatment gave the best results, characterized by minimal damage to gill tissue (only hypertrophy) and liver (mild vacuolar degeneration) and the highest survival rate of 83.33%. In conclusion, adding fermented herbal medicine at a dose of 150 mL/kg feed can increase the immunity of tilapia against *S. agalactiae* infection, as shown through the improvement of organ tissue structure and improved survival.

Keywords: Fermented Herbal Medicine, Histology, *Streptococcus agalactiae*.

Abstrak

Streptococcus agalactiae adalah patogen yang menyebabkan kerugian signifikan dalam budidaya ikan nila (*Oreochromis niloticus*). Salah satu kontrol alternatif adalah penggunaan bahan-bahan alami seperti herbal fermentasi. Penelitian ini bertujuan untuk menganalisis perubahan struktur histopatologi insang dan hati ikan nila yang diberi pakan yang mengandung ramuan fermentasi setelah terinfeksi *S. agalactiae*. Penelitian dilakukan pada bulan Maret hingga Desember 2023 di Laboratorium Parasit dan Penyakit Ikan, Fakultas Perikanan dan Kelautan, Universitas Riau, dan Pusat Veteriner Bukittinggi. Metode yang digunakan adalah Rancangan Acak Lengkap satu faktor dengan lima perlakuan dan tiga ulangan. Perlakuan tersebut terdiri dari kontrol negatif (Kn), kontrol positif (Kp), dan tiga perlakuan pakan dengan dosis obat herbal fermentasi 100 (P1), 125 (P2), dan 150 mL/kg pakan (P3). Infeksi *S. agalactiae* dilakukan pada hari ke-31 dengan dosis 10^8 CFU/mL. Hasil penelitian menunjukkan bahwa pengobatan P3 memberikan hasil terbaik, ditandai dengan kerusakan minimal pada jaringan insang (hanya hipertrofi) dan hati (degenerasi vakuolar ringan), dan tingkat kelangsungan hidup tertinggi sebesar 83,33%. Kesimpulannya, penambahan obat herbal fermentasi dengan dosis 150 mL/kg pakan dapat meningkatkan kekebalan ikan nila terhadap infeksi *S. agalactiae*, seperti yang ditunjukkan melalui perbaikan struktur jaringan organ dan peningkatan kelangsungan hidup.

Kata kunci: Jamu fermentasi, Histologi, *Streptococcus agalactiae*

1. Introduction

Tilapia (*Oreochromis niloticus*) is a fishery commodity that has economic value. This fish is very popular among Indonesians because of its delicious taste, thick meat, and high nutritional content (Yaningsih et al., 2018). A consistent increase in tilapia production can be achieved through intensive cultivation by paying attention to various aspects that support the survival of the fish. However, there are obstacles to tilapia cultivation, including the spread of *Streptococcus agalactiae* and the disease it causes, *streptococcosis*. Cases of mass tilapia deaths due to *S. agalactiae* infection in tilapia (Sirimanapong et al., 2018; Doan et al., 2019).

Alternatives that can be used to control fish diseases include using natural ingredients, such as temulawak and kencur. The advantages of using medicinal plants include being relatively safer, easy to obtain, not causing resistance, and relatively harmless. According to Syawal et al. (2019), giving fermented herbal supplements into feed can stimulate fish appetite, improve fish health against disease, and reduce fish stress against environmental changes, resulting in mortality in fish of 5% and an absolute weight of 95.5 g. According to Puspitasari (2017), active substances in herbal supplements can improve the body's defense system, growth, and fish health.

Histopathology has also been used as an important tool in environmental monitoring to examine specific target organs. One of the tissues that can be used as an observation indicator is the gills and liver (Safratilofa, 2017). Based on the description above, the author is interested in researching the histopathology of the gills and liver of tilapia infected with *S.agalactiae* and fed with fermented herbal medicine.

This study aimed to analyze the histological structure of the gills and liver of tilapia-fed fermented herbal medicine after being infected with *S. agalactiae*. Getting the best fermented herbal medicine to control *S. agalactiae* bacterial infection in tilapia.

2. Materials and Methods

2.1. Time and Place

This research was conducted from March to December 2023. Maintenance and sample fixation processes were performed at the Fish Parasites and Diseases Laboratory, Faculty of Fisheries and Marine, Universitas Riau, while histology preparations were carried out at the Bukit Tinggi Veterinary.

2.2. Methods

The method used is an experimental method with a Completely Randomized Design (CRD) of one factor, five treatment levels, and three repetitions. The treatments used are by Syawal et al. (2018). The treatment levels determined in this study were Kn (without giving fermented herbal medicine and without *S. agalactiae* challenge test), Kp (without fermented herbal medicine challenged with *S. agalactiae*), P1 (fermented herbal medicine dose of 100 mL/kg feed), P2 (fermented herbal medicine dose of 125 mL/kg feed), and P3 (fermented herbal medicine dose of 150 mL/kg feed). Fish maintenance was carried out for 45 days, and during maintenance, the test fish seeds were given feed that had been added with fermented herbal medicine according to the dose. Feeding was 10% of body weight, given thrice daily at 08.00, 13.00, and 18.00 WIB. Every 10 days, the fish's length and weight are measured to adjust the amount of feed given in the next maintenance.

2.3. Procedures

2.3.1. Container Preparation

The maintenance container used is a 40x30x30 cm aquarium, 15 pieces. The aquarium is then filled with water from a drilled well. First, it is deposited in the tank, which is given aeration for 2 x 24 hours to increase oxygen levels (DO) and pH. Furthermore, each tank is filled with 30 L of water, after which 4-5 cm of tilapia seeds are inserted with a stocking density of 1 fish/3 L.

2.3.2. Making Fermented Herbal Medicine

The manufacture of fermented herbal medicine follows the procedure of Syawal et al. (2017). Temulawak and kencur are peeled and washed clean, and drained until dry. Then, we weighed 100 g each. Then, blend until smooth, then filter to obtain a solution of temulawak and kencur, then add clean water to the solution at as much as 3 L and boil until boiling. After cooling, add 175 g of molasses, 65 mL of probiotic drink (Yakult), and 50 mg of tape yeast and stir until smooth. Once smooth, put it in a jerry can and close it tightly. Next, it is fermented for 7-10 days until the aroma changes and it is no longer in gas form.

2.3.3. Streptococcus agalactiae Isolates and Challenge Tests

The *Streptococcus agalactiae* isolate was obtained from the collection at the Balai Perikanan Budidaya Air Tawar (BPBAT) Sungai Gelam, Jambi. *S. agalactiae* was grown on Blood agar media and incubated for 24 hours. Then, *S. agalactiae* was re-inoculated into the media (Brain Heart Agar), BHA, and incubated at 29- 29-30ºC for 24 hours, carried out until a pure culture was obtained. After the culture was obtained Pure, bacterial isolates were cultured into BHI (Brain Heart Infusion) media and incubated at 29-30ºC for 24 hours. After 24 hours, two-fold dilutions were performed, and the turbidity was compared with McFarland No. 1 until *S. agalactiae* was obtained with a density of 10^8 CFU/mL. After being maintained with herbal-enriched feed, fermentation occurs. Tilapia fish were challenged on the 31st day with *S.agalactiae* bacteria with a density of 10^8 CFU /mL, as much as 0.1 mL/fish by intramuscular injection (Selvi et al., 2016)

2.3.4. Preparation of Fish Tissue Structure Preparations

Sample preparations were carried out according to Windarti et al. (2017). The tilapia fish organs were fixed in 10% formalin for 24-48 hours and transferred to 4% formalin. Then, the liver and gills of the fish were cut into ± 0.5 cm thick and put into a tissue cassette for dehydration.

Dehydration started with entering samples into a bottle alcohol series, starting with 70%, 80%, 90%, and 96% each for 1 hour. The aim was to remove water content from the cells/tissues and replace it with alcohol. Immersion in absolute alcohol is done 2 times, each for 1 hour. Furthermore, the sample is put back into the pure xylol solution twice for 1 hour. After that, the sample is placed in a solution of Xylol: paraffin (1:1) for 1 hour (the process is carried out in a 58ºC oven). Then, the sample is placed in pure paraffin twice, and each time, it is done for 1 hour. Next, the sample is embedded in paraffin using a mold (thick paper) and allowed to harden at room temperature. Then, the sample is cut with a microtome with a thickness 5µ. Tape paraffin containing the sample was placed in a water bath at 45ºC. After the sample expands, it is attached to a glass object smeared with glycerin-albumin. Furthermore, the sample is incubated in an oven at 45ºC. Then, the sample is stained with Haematoxylin and Eosin (HE).

The procedure for staining histological preparations of the gills and liver of tilapia with HE (Hematoxylin-Eosin) is the first to do paraffin. Binding samples were dissolved in Xylol I and Xylol II for 3 minutes each and then rehydrated in the descending alcohol series (Absolute Alcohol I, Absolute II, 96%, 2 times, 80%, 70%) for 3 minutes each and washed with running water for 3 minutes. Then, the sample is soaked in hematoxylin solution for 6-8 minutes and washed with sufficient running water. Next, the sample is soaked in eosin for 6-8 minutes, after which the sample is washed with adequate running water until transparent. Then, the sample is dipped in a series of alcohols. Go on (70% alcohol, 80%, 90%, absolute alcohol 2 times) every 20 seconds and soak in Xylol I and Xylol II for 5 minutes each. Next, the closing (mounting) is carried out.

2.4. Data Analysis

Data on observation parameters of clinical symptoms of fish and water quality are tabulated in a table form. Meanwhile, the reading of histology preparations on changes in the histopathological structure of the gill and liver organs was analyzed using damage scoring value data; if the treatment shows a significant difference, where $P < 0.05$, then a further Newman-Keuls test is carried out to determine the differences between each treatment and discussed descriptively.

3. Result and Discussion

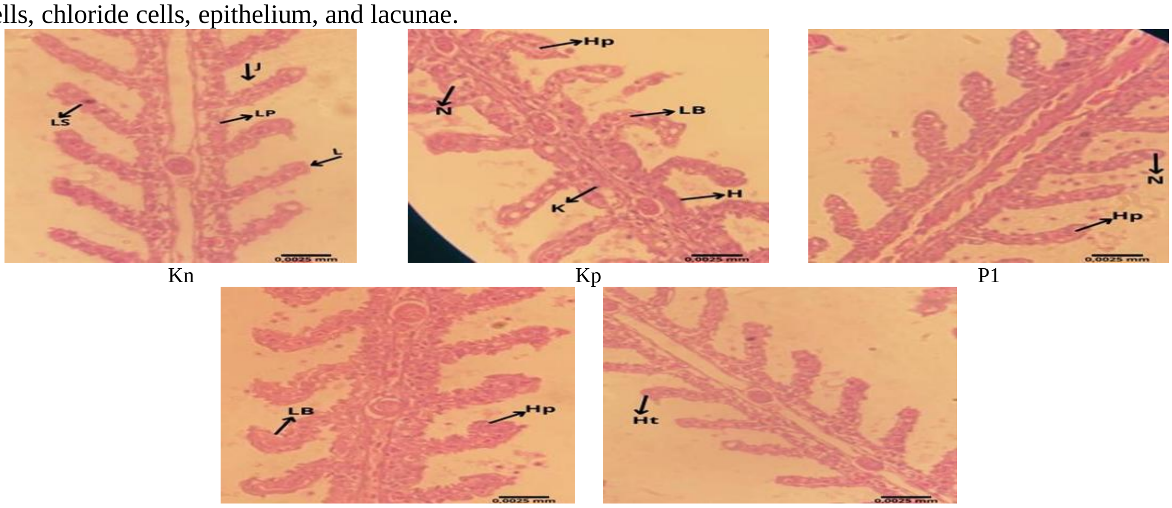
3.1. Clinical Symptoms

Based on the results of the study of tilapia fish in Kn (Negative Control), the condition of the fish was normal because it was not challenged with *S. agalitiae*. Positive control of the condition of the fish was the worst because the fish were challenged and not given feed mixed with herbal medicine, so their movements were passive. They swam sideways close to the aeration, for their appetite decreased (poor appetite), and the body's surface also experienced ulcers/wounds, excessive mucus, pale body color, protruding eyes, and fins were thin. According to Suhermanto et al. (2019), the disease caused by *S. agalactiae* infection causes changes in the color of the fish's body, swimming in circles erratically, and cloudiness in the eyes.

The clinical symptoms in treatments P1 and P2 include fin "*gripis*," loose scales, ulcers, decreased appetite, and fish behavior that swim alone at the aquarium's bottom and often approach the aeration than in the treatment. Clinical symptoms in treatment P3 were milder than in treatments Kp, P1, and P2; only the dorsal fins were flaky after the challenge test. Feeding enriched with fermented ginger and kencur herbs at 150 ml/kg increases fish immunity. The content of antibacterial compounds, such as flavonoids, in ginger and kencur prevents bacterial activity from infecting/damaging fish organs.

3.2. Tilapia Fish Gill Tissue Structure

The standard gill tissue structure of tilapia fish is characterized by primary and secondary lamellae, which are arranged neatly and regularly. The secondary lamella comprises mucus cells, which are still clearly visible, pillar cells, chloride cells, epithelium, and lacunae.



Kn Kp P1

P2 P3

Figure 1. Structure network gill tilapia given fermented herbal medicine and infected with *S. agalactiae,* HE staining (400x). Description: L: Lamella width; J: Lamella distance; Lp (Primary lamella); Ls (Secondary lamella); N (Necrosis); H (Hemorrhage); Ht (Hypertrophy); Et (Epithelium raised); Hp (Hyperplasia); Lb (bent lamella); K (Congestion).

Based on Figure 1, there is much damage to the gill structure in Kp, including hyperplasia, hemorrhage, congestion, and necrosis with severe intensity, so damage to this tissue structure can inhibit the respiratory process. If the damage to the gills increases, it can cause death in the tilapia. According to Juanda & Edo (2018), hyperplasia is the beginning of necrosis. Damage can be characterized by a very dense accumulation of red blood cells in the blood vessels, indicating abnormal conditions in the fish's gills (Rifa'i, 2018). P1 shows that the structure of the tilapia gill tissue was found to experience hypertrophy and necrosis.

Hypertrophy is characterized by the size of the primary lamella and secondary lamella, which are enlarged due to the cells in the secondary lamella enlarging, but their number has not increased. P2 indicates that the structure of the fish gill tissue experiences hyperplasia and bent lamellae. P3 secondary lamellae are arranged in an orderly manner in the primary lamellae, and one defect, namely hypertrophy, is found. In the treatment given, fermented herbal medicine was in the feed, and damage occurred because the dosage was not optimal. The distance and width between the secondary lamellae of tilapia can also determine the survival rate of the fish. The average distance and width between secondary lamellae can be seen in Table 1.

Table 1. Distance and width between secondary lamella of tilapia

Treatment	Lamella Distance (mm)	Lamella Width (mm)
K_n	$0.02{\pm}0.003^{b}$	$0.0276{\pm}0.003^{ab}$
K_p	$0.01{\pm}0.001^{a}$	$0.023{\pm}0.002^{a}$
P_1	$0.01{\pm}0.002^{ab}$	$0.027{\pm}0.0026^{ab}$
P_2	$0.01{\pm}0.003^{ab}$	$0.028{\pm}0.003^{ab}$
P_3	$0.02{\pm}0.003^{ab}$	$0.032{\pm}0.0025^{b}$

Description: Superscript letters listed in the column indicate significant differences in all treatments ($P<0.05$).

Based on Table 1, the width of the secondary lamella after being challenged with *S. agalactiae* was between 0.023-0.032 mm, and the distance of the secondary lamella after being challenged with *S. agalactiae* ranges from 0.01-0.02 mm. Gaol et al. (2024) stated that fish that have a vast lamella distance can usually live better than fish that have a close secondary lamella distance because the lamella that are close to each other can cause the lamella surface to narrow, which makes it difficult for fish to breathe.

The distance of secondary lamellae >0.013 mm is a lamella distance that is considered normal in fish respiration. If the distance between the gills is close to each other (<0.007), it will cause the area of the gill lamellae that is in contact with the water to become narrow, causing the fish to have difficulty breathing (Wahyuni et al., 2017). According to Windarti et al. (2017), the level/class of damage to gill tissue is divided into three levels, namely normal (Score 1), light damage (Score 10), and heavy damage (Score 100). The level of damage to tilapia can be seen using the HAI score (Table 2).

Based on Table 2, Kn shows no damage and is classified as usual. In Kp and treatment P 1, there is severe damage with an HAI score (> 100). Windarti et al. (2017) stated that gill tissue damage with a total score of > 100 is categorized as severe damage. Meanwhile, treatments P2 and P3 show HAI scores ranging from 1 to 2; this total score is still in the normal category.

Table 2. HAI score and gill damage category

Level of Damage	Types of Damage to Gill Tissue	Kn	Kp	P_1	P_2	P_3
I	Hypertrophy	-	-	+	-	+
	Hyperplasia	-	+	-	+	-
	Bent lamella	-	-	-	+	-
	edema	-	-	-	-	-
	Congestion	-	+	-	-	-
	Raised epithelium	-	-	-	-	-
	Lamella fusion	-	-	-	-	-
II	Hemorrhage	-	+	-	-	-
III	Necrosis	-	+	+	-	-
Histopathological Alteration Index (HAI)		0	112	101	2	1

3.3. Tilapia Liver Tissue Structure

The liver tissue of tilapia fish comprises cells that form a functional unit. The standard liver tissue structure is indicated by a picture of liver cells that are still good, namely hepatocyte cells that are still neatly arranged and cell nuclei and sinusoids that are visible. The structure of the liver tissue of tilapia after the *S. agalactiae* can be seen in Figure 2.

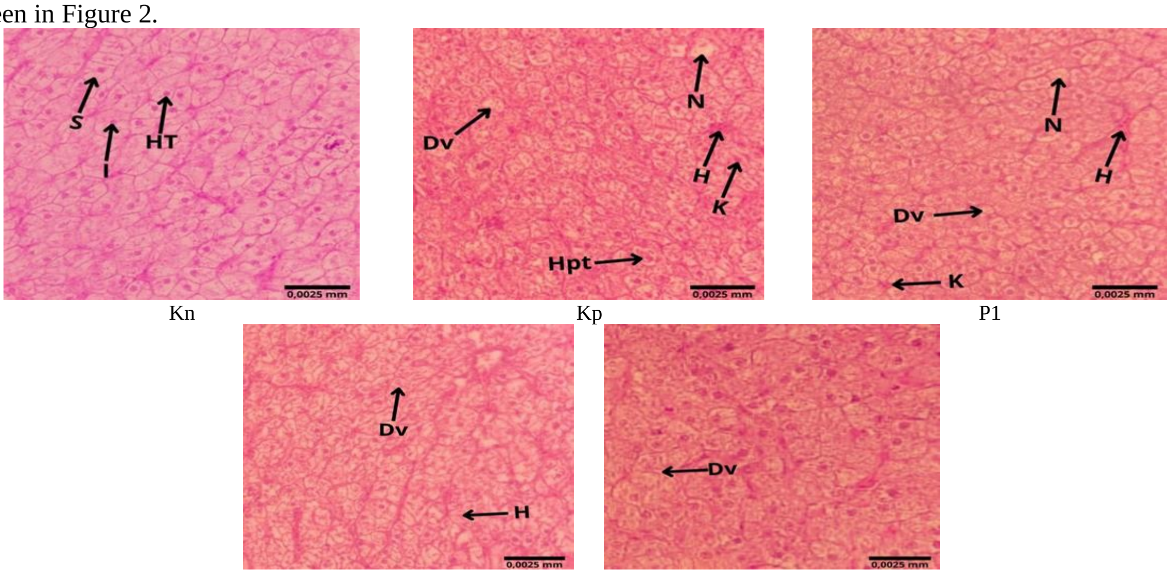


Figure 2. Structure network heart tilapia post in test challenge *S. agalactiae*
Description: Core cell (I), Hepatocytes (Ht), Sinusoid (S), Hemorrhage (H), Necrosis (N), Hypertrophy (Hpt), Vacuole degeneration (Dv).

Figure 2 shows the results of observations of the structure of the liver tissue of tilapia fish after the challenge test with *S. agalactiae*. The liver tissue structure of fish that were not challenged (Kn) looks normal, which is indicated by clearly visible hepatocytes and sinusoids. Hepatocytes in the heart are highly active cells, so they are easily damaged. However, they also have the property of being easily regenerated to replace damaged cells (Wahyuningtyas et al., 2018). Kp found much liver tissue damage, including necrosis, hemorrhage, hypertrophy, vacuole degradation, and congestion. Necrosis is a continuation stage of lysis in tissue, where cells will burst, and the cell nucleus disappears. According to Sulastri et al. (2018), necrosis is the death of cells or tissue accompanying cell degeneration in every animal's life and is the final stage of irreversible degeneration.

According to Juanda & Edo (2018), hemorrhage (bleeding) is a condition characterized by the release of blood (leakage) or the presence of red streaks or red spots from within the vacuole, spreading damage to the vacuole. According to Fitriani et al. (2020), Cell swelling is one indication of reversible damage (can still return to normal)

Besides that, there is damage in the form of vacuole degeneration. This will appear like empty spaces, and it will appear like fine threads. Oktafa et al. (2017) stated that vacuole degeneration occurs due to the accumulation of fat (neutral fat) with damage to the cell nucleus and shrinking of liver cell tissue. Degeneration vacuole. This is reversible so that the liver tissue structure can return to normal. Congestion on a network is the disturbance that happens in blood circulation due to the volume of blood in the capillaries experiencing an increase. Disturbed circulation of the blood clot is a blockage of blood circulation due to swelling of one part of the tissue and narrowing of another part of the tissue so that blood accumulates in a particular area of the tissue.

The condition of liver tissue in P1 shows tissue damage in necrosis, hemorrhage, vacuole degeneration, and congestion. In the P2 treatment, damage was found in the form of hemorrhage and vacuole degeneration. Conditions in P 3 found vacuole degeneration, damage, and congestion. An attack by *S. agalactiae* causes damage to the structure of the liver tissue of tilapia fish. It enters the fish's body and results in an abnormality affecting its liver histological structures. It contains turmeric, ginger, and galangal in fermented herbal medicine, which can increase the fish's body's resistance to bacterial attacks without causing damage to the liver, which is the center of metabolism (Fransiska et al., 2019).

Based on the results observation, the structure network heart tilapia fish post-challenge test *S. agalactiae* in treatment P3 with a dose of 150 mL/kg experienced less damage, whereas in this treatment, the structure of the liver tissue experienced much healing, sinusoids had begun to form clearly, and hepatocytes were slowly filled and visible. This is likely due to the influence of the addition of fermented herbal medicine to the feed, which has many antibacterial compounds. In turmeric, temulawak, and kencur, there are active ingredients, including curcumin, flavonoids, saponins, and tannins, which can inhibit *S. agalactiae* attacks so that there is not much damage to the liver tissue of tilapia.

The histological structure of the liver of tilapia fed with fermented herbal medicine and challenged with *S. agalactiae* found several types of tissue damage, including hemorrhage, hypertrophy, vacuole degeneration, and necrosis. The mouth of the pikehead has a large opening that is useful for capturing live prey such as insects or small fish and reflects an adaptation to a carnivorous diet. This distinctive mouth structure is well suited for capturing prey in calm water environments and dense vegetation. The relatively fast movement of the pikehead reflects its adaptation to capture prey efficiently. The pikehead moves at high speed to prey on fish and will protrude the premaxilla, where this movement is triggered by the mauthner cells to open the jaws (cranial elevation). The jaws' rapid movement involves extreme premaxillary protrusion and cranial elevation (the ability of the head to bend/lookup) (Table 3).

Table 3. Level of liver damage in tilapia fish (*O. niloticus*)

Treatment	Liver Damage					Level of Damage	Information
	Hpt	H	Dv	N	K		
Kn	-	-	-	-	-	0	Normal
Kp	+	+	+	+	+	5	Damaged
P1	-	+	+	+	+	4	Damaged
P2	-	+	+	-	-	2	Damaged
P3	-	-	+	-	-	1	Normal

Description: (+) there is damage, (-) there is no damage, (Ht) Hypertrophy, (H) Hemorrhage, (Dv) Vacuole degeneration, (N) Necrosis, (K) Congestion

Based on the observation data in Table 3, damage categories can be seen from the types of liver tissue damage in each treatment. Most damage is found in the Kp treatment; there are five types of damage, ranging from hemorrhage, hypertrophy, vacuole degeneration, necrosis, and congestion. This is followed by treatment P1, which has four types of liver damage, namely hemorrhage, necrosis, vacuole degeneration, and congestion. In treatment P2, there are two types of damage, namely hemorrhage and vacuole degeneration, and the last treatment, P3, only has 1 type of damage, namely vacuole degeneration.

Vacuole degeneration was found in the Kp, P1, P2, and P3 treatments. Vacuole degeneration occurs because hepatocytes swell, which causes the narrowing of the sinusoids. According to Juanda & Edo (2018), vacuole degeneration in fish liver tissue is a further response due to excessive fat accumulation in cells, so cells lose their ability to break it down. Necrosis was found in the Kp and P1 treatments. This necrosis or cell death is caused by infection with toxins or organisms that are very severe, such as loss of cell nuclei and rupture of the plasma membrane so that cells die and cannot be repaired (irreversible). Congestion was found in the Kp and P1 treatments. Congestion in a tissue is a disorder that occurs in blood circulation due to increased blood volume in the blood capillaries.

3.4. Water Quality Parameters

Water quality greatly influences the growth and survival of tilapia fish. Water quality during the study can be seen in Table 4.

Table 4. Water quality during research

Parameter	Beginning	End	Quality standards
Temperature (°C)	28 – 29	28 – 30	24 – 30
Ph	6.3 – 6.9	6.5 – 7	6 – 9
DO (ppm)	4.4 – 5	4.5 – 5.2	>3 mg/L
NH_3 (mg/L)	0.02	0.02 – 0.03	<0.3 mg/L

Based on Table 4, the range of tilapia water quality during the study showed that the water quality was normal for fish growth. The temperature during the study ranged from 28-30 °C. If the temperature increases, it can also reduce the solubility of oxygen in water and directly affect fish activity, increasing the toxicity of a pollutant to aquatic organisms (Koniyo, 2020). The pH value obtained during the study included the standard limit ranging from 6.3 to 7. According to the statement of Indriati & Hafiludin (2022), the normal pH range for tilapia growth is 7-8, but tilapia can survive and tolerate pH values ranging from 5 to 11. DO or dissolved oxygen during the study ranged from 4.4 -5.2 mg/L. Sa'adati & Andayani (2022) state that good dissolved oxygen for koi fish growth is 4 - 8 mg/L. According to PP Number 22 of 2021, the DO value at good water quality standards is > 3 mg/L, so it can be concluded that the dissolved oxygen in this study is ideal for living tilapia.

During the study, ammonia levels (NH_3) ranged from 0.019 to 0.027 mg/L. Moreover, they are still within the normal range for fish maintenance. Tilapia fish, such as tilapia and nila, have acute ammonia levels or above their tolerance threshold of around 1 mg/L, but some types of tilapia with good body health conditions can even tolerate ammonia levels reaching 1.5 mg /L.

3.5. Survival Rate

The survival rate of fish at the start of rearing reaches 100%. The survival of tilapia fish enriched with fermented herbal medicine and after being challenged with *S. agalactiae* is presented in Table 5.

Table 5. Survival rate of tilapia fish after challenge test

Treatment	Survival Rate (0%)		
	Initial Maintenance	After 14 days of Challenge Testing	ANOVA Results
Kn	100	86.66	86.66 ± 5.77^b
Kp	100	33.33	33.33 ± 5.77^a
P_1	100	70	70.00 ± 10.00^b
P_2	100	76.66	76.66 ± 5.77^b
P_3	100	83.33	83.33 ± 5.77^b

Description: Superscript letters listed in the column indicate significant differences in all treatments (P<0.05).

Table 5 shows that the survival rate of tilapia after the *S. agalactiae* challenge test only reached 33.33 -83.33%. Based on the results of the Analysis of Variance (ANOVA) test, P <0.05 means that adding fermented herbal medicine with different doses in the feed affects the survival of tilapia. The Kp treatment showed the lowest survival rate, which was 33.33. The low survival rate in the Kp treatment was due to the absence of additional fermented herbal medicine in the feed, resulting in infection with bacteria. According to Fakhrudin (2017), stress that occurs in fish will cause the fish to be sensitive to disease, thus affecting the survival rate of the fish. The highest survival rate of tilapia was in the P3 treatment, which was 83.33%; the high survival rate in the P3 treatment was due to the presence of active compounds, namely curcumin and essential oils, which were able to increase the immune system so that the fish's immune system when infected with S.agalactiae was in a strong condition and could maintain its survival. According to Dai et al. (2022), curcumin can inhibit bacteria by damaging the permeability of cell membranes, both gram-positive and gram-negative bacteria, which causes bacterial death. This is also supported by the statement of Syawal et al. (2019) that giving fermented herbal medicine mixed with pellets can stimulate fish growth and reduce mortality rates.

4. Conclusions

The study results showed that feeding containing fermented herbal medicine affected the immunity of tilapia after being challenged with *S. agalactiae*. The histological structure of the gills and liver changed after being fed with fermented herbal medicine. The best dose of fermented herbal medicine added to the feed was 150 mL/Kg of feed (P3), with a survival rate of 83.33 %. Furthermore, the structure of the gill tissue in the P3 treatment experienced hypertrophy and vacuole degeneration in the liver.

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