

Physiological Responses and Nonspecific Immunity in Striped Catfish (*Pangasianodon hypophthalmus*) Fed Fermented Herbal Medicine

*Respons Fisiologis dan Kekebalan Non Spesifik Ikan Jambal Siam (*Pangasianodon hypophthalmus*) yang Diberi Pakan Jamu Fermentasi*

Wiwik Yuliana^{1*}, Henni Syawal², Morina Riau waty², Fahrurrozi³

¹Department of Marine Science, Postgraduate, Universitas Riau, Pekanbaru 28293 Indonesia

²Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Riau, Pekanbaru 28293 Indonesia

³Dar Aswaja Islamic Institute Rokan Hilir, Kubu Babussalam 282991 Indonesia

*email: yulianawiwik@gmail.com

Abstract

Received
17 May 2026

Accepted
17 June 2026

Aeromonas hydrophila infections in aquaculture can cause mass mortality of up to 80–100% of striped catfish. Adding fermented herbal medicine containing essential oils, curcumin, flavonoids, and xanthorrhizol to feed can boost the fish's immunity. This study aims to analyze the optimal frequency of administering fermented herbal supplements in feed to enhance fish immunity, conducted from June 2024 to June 2025. A Completely Randomized Design (CRD) was used with 5 treatments and 3 replicates: Kn (no supplement, no infection), Kp (no supplement, infected with *A. hydrophila*), with 200 mL/kg of herbal supplement administered daily (P1), once every 3 days (P2), and once every 5 days (P3). The fish (*P. hypophthalmus* fingerlings, 8–12 cm, 20 fish/tank) were reared in 100-liter plastic buckets for 46 days. On day 32, the fish were infected with *A. hydrophila* (intramuscularly, 0.1 mL, 10⁶ CFU/mL), and they were maintained for 14 days thereafter. Each container was equipped with an aerator and a Dacron filter. The best results were obtained in P1, which showed a survival rate (SR) of 100%, a post-infection survival rate of 100%, a weight gain of 109.33 ± 2.51 g, serum protein of 3.94 ± 0.06 g/dL, albumin 3.76 ± 0.05 g/dL, globulin 0.21 ± 0.04 g/dL, lysozyme activity 373.33 ± 15.27 U/mL, and phagocytosis activity 51.11 ± 16.44%. Based on the data obtained, it can be concluded that the daily administration of 200 mL/kg of fermented herbal supplement in the diet yields the best results in enhancing the immune response and growth of *P. hypophthalmus* infected with *A. hydrophila*.

Keywords: Striped Catfish, Globulin, Herbal, Immunity, Lysozyme

Abstrak

Serangan *Aeromonas hydrophila* pada budidaya perikanan dapat menyebabkan kematian massal hingga 80–100% ikan jambal siam. Penambahan jamu fermentasi yang mengandung minyak atsiri, kurkumin, flavonoid, dan xanthorrhizol dalam pakan dapat meningkatkan daya tahan ikan. Penelitian ini bertujuan untuk menganalisis frekuensi terbaik pemberian jamu fermentasi pada pakan untuk meningkatkan daya tahan tubuh ikan, dilaksanakan pada Juni 2024–Juni 2025. Metode Rancangan Acak Lengkap (RAL) dengan 5 perlakuan dan 3 ulangan, yaitu Kn (tanpa jamu, tanpa infeksi), Kp (tanpa jamu, diinfeksi *A. hydrophila*), penambahan 200 mL/kg pakan yang diberikan setiap hari (P1), sekali 3 hari (P2), dan sekali 5 hari (P3). Ikan (benih *P. hypophthalmus* 8-12 cm, 20

ikan/wadah) dibudidayakan dalam ember plastik 100L selama 46 hari. Pada hari ke-32 ikan diinfeksi dengan *A. hydrophila* (intramuskular, 0,1 mL, 10^8 CFU/mL) selanjutnya ikan dipelihara selama 14 hari. Setiap wadah dilengkapi dengan aerator dan filter dakron. Hasil terbaik diperoleh pada P1 yang menunjukkan SR 100%, RPS 100%, pertumbuhan bobot $109,33 \pm 2,51$ g, serum protein $3,94 \pm 0,06$ g/dL, albumin $3,76 \pm 0,05$ g/dL, globulin $0,21 \pm 0,04$ g/dL, aktivitas lisozim $373,33 \pm 15,27$ U/mL, aktivitas fagositosis $51,11 \pm 16,44\%$. Berdasarkan data yang diperoleh, dapat disimpulkan bahwa pemberian jamu fermentasi 200 mL/kg pakan setiap hari memberikan hasil terbaik dalam meningkatkan respons imun dan pertumbuhan ikan jambal siam yang terinfeksi *A. hydrophila*.

Kata kunci: Ikan patin, Globulin, Herbal, Imunitas, lisosim

1. Introduction

The striped catfish (*Pangasianodon hypophthalmus*) is one of the leading commodities in the freshwater aquaculture industry, accounting for approximately 14% of global fish production and reaching 15.4 million tons. This fish is well-known and in high demand among the public, leading to an increase in demand (Nam et al., 2020). Efforts to meet the growing market demand have driven farmers to adopt intensive aquaculture practices. Intensive fish farming often faces various challenges that can lead to losses, such as high feed costs (60–70%), deteriorating water quality, slow growth, and mass mortality due to stress and disease outbreaks (Suhenda et al., 2017).

One of the diseases that frequently affects striped catfish is Motile Aeromonas Septicemia (MAS), caused by the bacterium *Aeromonas hydrophila*, which can result in mortality rates of 80–100% within approximately 1 week of infection (Tamba et al., 2021). The Bogor Regency Livestock and Fisheries Service reported that carp mortality due to this bacterial infection reached 200 tons. In contrast, in West Sumatra Province, gurami mortality reached up to 47 tons, and the disease also extended to the Koto Panjang hydroelectric dam reservoir in Riau Province, causing significant losses for catfish farmers. Currently, disease treatment in cultured fish typically involves chemical agents or antibiotics. However, antibiotics are not recommended because they can lead to antibiotic resistance and harm the environment. Therefore, an alternative disease prevention method based on natural, environmentally friendly, and sustainable ingredients is needed to enhance the physiological response and nonspecific immunity of striped catfish.

The administration of herbal remedies made from natural ingredients, such as fermented turmeric, temulawak, and kencur, can improve feed efficiency, accelerate growth, boost fish immunity against free radicals, and improve metabolism (Ismail et al., 2017; Syawal et al. 2019), promote growth, reduce fat content, and enhance immune function in striped catfish (Zhang et al., 2022). Adding 200 mL/kg of a fermented herbal supplement to commercial feed can improve striped catfish health and appetite, resulting in an absolute weight gain of 119.07 g, an absolute length of 15.45 cm, and a feed efficiency of 82.93% (Syawal et al., 2020). Additionally, it can inhibit *A. hydrophila* infection in striped catfish with phagocytosis activity of 36.33%, a relative protection score of 90%, and a survival rate of 93.33% (Lestari et al., 2021).

Beyond the significant health benefits of fermented herbal supplements for fish, the optimal administration frequency can also influence the supplement's concentration in the body, thereby affecting its effectiveness in preventing pathogen attacks. Administering the supplement at the appropriate frequency is crucial for maintaining an effective concentration in the fish's body to prevent pathogen attacks, as each ingested dose has a specific half-life for digestion. The half-life of herbal ingredients is relatively short, particularly for curcumin compounds found in turmeric and temulawak, which have a half-life of 1–2 days (Kurniawan et al., 2022). To maintain consistent levels of fermented herbal supplements in the body, an appropriate administration frequency is necessary to optimize the striped catfish's immune response. Administering herbal medicine at the appropriate frequency can prevent stress and metabolic disorders, ensure the supplement's effectiveness, and improve cost and labor efficiency. Based on the above discussion, the researchers were motivated to conduct this study because research on the optimal administration frequency of fermented herbal medicine for striped catfish remains very limited.

2. Material and Method

2.1. Time and Place

This study was conducted from June 2024 to June 2025 at the Fish Parasitology and Disease Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau. Total protein, globulin, and albumin analyses were performed at the Nutrition Laboratory. At the same time, lysozyme levels were measured at the Health Laboratory of the Faculty of Fisheries and Marine Sciences (FPIK) at Bogor Agricultural University.

2.2. Methods

The method used was an experimental design with a Completely Randomized Design (CRD), consisting of five treatment levels (different frequencies of herbal feed administration) and three replicates, resulting in 15 experimental tanks. The five treatment levels were as follows:

- Kn : No addition of fermented herbal medicine and not challenged with *A. hydrophila*
- Kp : No addition of fermented herbal medicine and not challenged with *A. hydrophila*
- P1 : Daily addition of fermented herbal medicine and challenged with *A. hydrophila*
- P2 : Addition of fermented herbal medicine every three days and challenged with *A. hydrophila*
- P3 : Addition of fermented herbal medicine every five days and challenged with *A. hydrophila*

The dose of fermented herbal medicine added was 200 mL/kg of feed. On days when fermented herbal medicine was not administered, the fish were still fed wet feed by spraying clean water onto the feed, with feedings administered three times a day at 5% of the test fish's body weight.

2.3. Procedures

2.3.1. Preparation of Fermented Herbal Medicine

The ingredients used to make the fermented herbal mixture consisted of turmeric, temulawak, and kencur, each at 400 g; molasses, 700 mL; probiotic drink, 260 mL (equivalent to four bottles); and yeast, 2 g. The preparation procedure began by thoroughly washing all the main ingredients, then slicing them thinly and blending them until smooth. The blended mixture is strained, 12 L of water is added, then it is brought to a boil and cooled to room temperature. Once cooled, 700 mL of molasses, 260 mL of probiotic drink, and 2 g of yeast are added. The mixture is stirred until homogeneous, transferred into a jerrycan, and tightly sealed. The fermentation process lasts 7 days, during which a distinct, more fragrant aroma develops. The gas produced during fermentation is released daily by opening the jerrycan lid for five minutes (Kurniawan et al., 2020).

2.3.2. Preparation of Herbal Feed

The feed used during the rearing period consists of FF 999 Hiprovite pellets with a protein content of 35%. The fermented herbal supplement is administered at a dose of 200 mL per kilogram of feed, mixed with 300 mL of clean water per kilogram of feed. The mixture is sprayed evenly onto the pellets, then stirred until homogeneous and dried for approximately 10 minutes to ensure the herbal supplement solution is well absorbed (modified from Syawal et al., 2019).

2.3.3. Blood Sampling

Blood sampling was conducted three times: at the start of the experiment (H-0), on day 30 (H-30), and 14 days post-challenge. Blood was collected using two methods: with an anticoagulant for phagocytosis activity analysis and without an anticoagulant for total protein, albumin, globulin, and lysozyme enzyme activity analysis. For each blood sample collection, blood was drawn from five fish from each treatment replicate. Before blood collection, the fish were anesthetized using clove oil at a dose of 0.1 mL/L of water. Blood was drawn from the caudal vein using a 1 mL syringe and placed into a microtube (Laltanmawia et al., 2023).

2.3.4. Challenge Test with *Aeromonas hydrophila*

The challenge test was conducted to determine the nonspecific immune response of striped catfish fed fermented herbal feed and to assess the fish's survival ability following infection with *A. hydrophila*. The challenge test procedure involved intramuscular injection of 0.1 mL of a 10^8 CFU/mL *A. hydrophila* suspension, except for the negative control group (Riauwaty et al., 2020).

3. Result and Discussion

3.1. Clinical Symptoms in Striped catfish (*P. hypophthalmus*)

After being challenged with *A. hydrophila*, striped catfish exhibited clinical symptoms including changes in body surface, movement, appetite, eyes, and fins (Figure 1).



KN



KP



Figure 1. Clinical symptoms of striped catfish (*P. hypophthalmus*)
Description: a. Ulcer, b. Exophthalmos, c. Frayed fins, d. Bleeding at the base of the fins, e. Pale color.

Based on Figure 1, fish in the negative control (Kn) group showed no clinical signs of disease because they were not exposed to *A. hydrophila*; thus, the fish were active, had intact bodies, bright body color, clear eyes, and bright gills. In contrast, fish in the positive control (Kp) exhibited several clinical symptoms, such as the appearance of ulcers on the back on the second day, protruding eyes (*exophthalmia*), damaged fins, as well as hemorrhage at the fin bases and pale coloration on the third day. The *A. hydrophila* infection is suspected to spread through the circulatory system and cause organ tissue damage, as evidenced by reddish discoloration and hemorrhage in the epithelial layer and mucosal cells.

Bleeding and tissue damage in the Kp treatment group were caused by stress, which lowered their immune resistance and allowed *A. hydrophila* to release hemolytic toxins and aerolysin, which can lyse red blood cells. The Kp was not given fermented herbal medicine and therefore could not counteract the toxins produced by *A. hydrophila*, resulting in the fish becoming weak and dying. *A. hydrophila* infection in catfish (*Clarias* spp.) causes inflammation and hemorrhage, leading to 80–100% mortality within 2–14 days, as it attacks the skin's epithelial surface and mucosal cells the outermost layer of the fish's nonspecific defense system. This bacterium has a high capacity to cause damage, such as hemorrhage, ulcerative lesions, frayed fins, red spots at the base of the fins, slightly protruding eye damage, pale coloration, loss of appetite, bleeding wounds, reduced swimming ability, and behavior of rubbing the body against the tank, allowing wounds and bacteria to enter the bloodstream and leading to mass mortality easily (Myszor & Gudmundsson, 2023; Tanjung et al., 2023).

Clinical symptoms in the P1 were the mildest among the other treatment groups. This is suspected to be due to the effect of the daily administration of fermented herbal medicine in the feed. The half-life of herbal ingredients is relatively short; thus, by administering fermented herbal medicine daily, the concentration of fermented herbal medicine in the blood can be maintained, thereby enhancing the fish's immunity and resistance to *A. hydrophila* infection. This is evidenced by the increased phagocytosis activity reaching $51.11 \pm 16.44\%$ in P1. Syawal et al. (2020) found that administering fermented herbal medicine can reduce stress levels in fish, as it contains secondary metabolites such as essential oils, curcuminoids, flavonoids, and xanthorrhizol, which function as antibacterial, antioxidant, and anti-inflammatory agents.

Lestari et al. (2024) reported that regular administration of fermented herbal medicine can enhance immune function, thereby mitigating the severity of clinical symptoms associated with *A. hydrophila* infection. Since fermented herbal medicine contains flavonoids and vitamin C, which can boost immune function and increase fish appetite, the fish become more resistant to *A. hydrophila* infection, as evidenced by improved phagocytic activity. Natural flavonoids can enhance fish growth and appetite, while vitamin C acts as an immunostimulant and antioxidant.

Flavonoids and vitamin C in fermented herbal medicine work synergistically to strengthen the fish's immune system, making the fish better able to fight infections and accelerate post-challenge recovery. As noted by Hasibuan et al. (2021), flavonoids possess strong antioxidant properties that help protect fish cells from damage caused by free radicals generated during infection. Additionally, flavonoids can act as immunostimulants, enhancing the fish's immune system activity against *A. hydrophila* infections. Meanwhile, the vitamin C content in the fermented herbal remedy helps accelerate wound or ulcer healing in fish infected with *A. hydrophila*. By the fifth day, the wounds had begun to improve with the formation of an epithelial layer, so the ulcers had started to close. Vitamin C possesses anti-inflammatory properties, which have potential as both an antibiotic and an anti-inflammatory agent, and can stimulate the growth of epithelial cells in the skin. Furthermore, vitamin C plays a

crucial role in enhancing the fish's nonspecific immune system and exhibits antibacterial activity, helping inhibit the growth of *A. hydrophila* (Pakpahan et al., 2020)

3.2. Survival Rate

The survival rates of striped catfish during the study varied. The ANOVA test results indicated that treatment P1 (feed enriched with 200 mL/kg of fermented herbal medicine administered daily) showed $P < 0.05$, meaning the addition of fermented herbal medicine to the feed had a significant effect on the survival rates of striped catfish. The lowest survival rate of striped catfish was observed in the Kp at 33%. The low survival rate was due to the fish not being fed a diet containing fermented herbal medicine, which does not increase the fish's immune system. Consequently, when the fish were challenged with *A. hydrophila*, they were unable to combat the bacterial infection effectively and exhibited severe clinical symptoms, even leading to death. *A. hydrophila* infection can affect fish survival rates, as the activity of *A. hydrophila* can suppress the fish's immune system.

Table 1. Survival rate of striped catfish (*P.hypophthalmus*)

Treatment	Initial (%)	Final (%)
Kn	100	100.00±0.00 ^d
Kp	100	33.33±0.58 ^a
P1	100	100.00±0.00 ^d
P2	100	73.33±0.58 ^c
P3	100	70.00±0.00 ^b

Note: Different superscript letters in the same column indicate a significant difference between treatments ($P < 0.05$).

Based on Table 1, the survival rates of striped catfish during the study varied. The ANOVA test results indicated that P1 (feed enriched with 200 mL/kg of fermented herbal medicine administered daily) showed $P < 0.05$, meaning the addition of fermented herbal medicine to the feed had a significant effect on the survival rates of striped catfish. The lowest survival rate of striped catfish was observed in the Kp, at 33%. The low survival rate was due to the fish not being fed a diet containing fermented herbal medicine, which does not increase the fish's immune system. Consequently, when the fish were challenged with *A. hydrophila*, they were unable to combat the bacterial infection effectively and exhibited severe clinical symptoms, even leading to death. *A. hydrophila* infection can affect fish survival rates, as the activity of *A. hydrophila* can suppress the fish's immune system.

The highest survival rates were observed in the Kn and P1 at 100%. The high survival rate in the Kn was due to the fish not being challenged with *A. hydrophila*, keeping them in a healthy state. Meanwhile, fish in the P1 exhibited high survival rates due to their strong immune resistance. This is evident in the high phagocytosis index of $51.11 \pm 16.44\%$. The fish's high immunity is likely due to a diet containing 200 mL/kg of fermented herbal extract daily. This aligns with the rapid half-life of the herbal extract (1–2 days), ensuring that the bioavailability of the herbal components, including curcumin, flavonoids, and essential oils, remains relatively stable within the body. This is consistent with the findings of Kurniawan et al. (2020), who reported that adding 200 mL/kg of fermented herbal medicine daily to fish can increase survival rates to 100%.

The highest survival rate of striped catfish was observed in P1. This was because the fish were fed fermented herbal medicine daily, which boosted their immune systems, increased their resistance to bacterial infections, and helped prevent mortality. Feeding fermented herbal supplements rich in curcumin, which possesses antibacterial and anti-inflammatory properties, is highly effective for post-infection treatment against bacterial pathogens. This occurs because the fish's immune response is well-established, enabling it to fend off pathogen attacks and improve survival rates. Fermented herbal medicine possesses antimicrobial, antiviral, and antifungal properties and can also lower blood sugar levels. These effects primarily stem from the essential oils in turmeric, which act as active antimicrobial compounds (Rizki & Panjaitan, 2018). Additionally, herbal ingredients can prevent bacterial attacks, enhance immune responses, and increase appetite, thereby improving the survival rate of striped catfish (Mengi et al., 2022).

The flavonoid content in the fermented herbal remedy aligns with the fish's physiological needs and can be effectively utilized to protect cellular tissues. Curcumin also plays a role in boosting the immune system, acting as an antioxidant, antimicrobial, and anti-inflammatory agent, as well as maintaining, preventing, and repairing tissues damaged by infection (Hoseinifar et al., 2018). The active compounds in fermented herbal medicine can stimulate the formation of antibodies (IgM), thereby enhancing the fish's resistance to repeated attacks by *A. hydrophila*; furthermore, the fermentation process produces metabolites (organic acids, bioactive peptides, probiotics) that strengthen the mucosal immune system (in the gut, skin, and gills), enabling the fish to survive well even when infected with *A. hydrophila*.

3.3. Relative Protection Survival (RPS)

RPS (Relative Percent Survival) is used to indicate the effectiveness of fermented herbal medicine in protecting striped catfish infected with *A. hydrophila* (Figure 2).

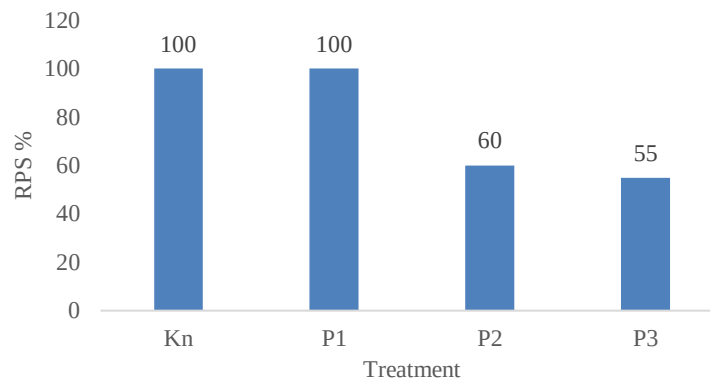


Figure 2. RPS of striped catfish

Based on Figure 2, P1, with a daily administration frequency of 200 mL/kg of feed, showed the best relative protection at 100%, as the administration was consistent every day, ensuring that the intake of fermented herbal medicine remained stable at 200 mL/kg in the body. The half-life of herbal supplements is 1–2 days in enhancing fish immunity. Meanwhile, P2 received the fermented herbal supplement every 3 days, and treatment P3 only every 5 days; consequently, the intake of the herbal supplement in the body was inconsistent at the 200 mL/kg feed dose daily, leading to suboptimal immune response. In contrast, the RPS value for Kn was 100%, as no *A. hydrophila* challenge test was performed; the fish remained healthy and did not die. This indicates that administering fermented herbal medicine can enhance nonspecific immunity, thereby mitigating the impact of *A. hydrophila* infection; the fish can recover and do not die.

Turmeric, a key ingredient in the fermented herbal remedy, contains active compounds, including essential oils and curcumin, with antibacterial properties. According to Lestari et al. (2021), natural ingredients such as turmeric, kencur, and temulawak used in the preparation of fermented herbal medicine at a dose of 200 mL/kg possess antibacterial and antimicrobial properties, thereby enhancing fish appetite, promoting efficient nutrient absorption, stimulating growth, and increasing RPS values. This occurs because fermented herbal medicine contains vitamin C, which plays a role in wound healing in fish, aiding post-injury recovery and boosting the fish's immune system.

According to Komalasari et al. (2017), high RPS values indicate that adding fermented herbal medicine to feed can enhance fish growth and resistance to *A. hydrophila* infection. Based on the RPS values obtained from the three treatments, which ranged from 55% to 100%, these values align with the guidelines set by the Ministry of Marine Affairs and Fisheries (KKP), which state that supplements suitable for distribution to aquaculture farmers must have an RPS value of >50% (Sugiani et al., 2018).

3.4. Absolute Weight Growth (Growth Rate)

The ANOVA test results show $P < 0.05$, indicating that the administration of fermented herbal medicine has a significant effect on the absolute weight gain of striped catfish (Table 2). The highest absolute weight was observed in P1, with daily administration of fermented herbal medicine, at 109.33 ± 2.51 g, compared with the other treatments. This is because in treatment P1, with daily administration of fermented herbal medicine, provides a consistent, optimal intake of bioactive compounds to enhance immune function. In contrast, in P2 and P3, where herbal medicine was administered every 3 and 5 days, respectively, this does not align with the herbal supplement's half-life of 1–2 days.

Table 2. Absolute weight of striped catfish (*P. hypophthalmus*)

Treatment	Initial (g)	Final (g)	Absolute (g)
Kn	163	237	73.67 ± 1.15^c
Kp	163	213	50.00 ± 3.60^a
P1	165	275	109.33 ± 2.51^d
P2	162	237	75.00 ± 0.00^c
P3	165	227	62.00 ± 3.00^b

Note: The same superscript letters in a column indicate no significant difference among treatments ($P > 0.05$), while different letters indicate a highly significant difference ($P < 0.05$).

Based on Table 2, the ANOVA test results show $P < 0.05$, indicating that the administration of fermented herbal medicine has a significant effect on the absolute weight gain of striped catfish. The highest absolute weight was observed in P1, with daily administration of fermented herbal medicine, at 109.33 ± 2.51 g, compared with the other treatments. This is because in P1, with daily administration of fermented herbal medicine, provides a consistent, optimal intake of bioactive compounds to enhance immune function. In contrast, in P2 and P3, where herbal medicine was administered every 3 and 5 days, respectively, this does not align with the herbal supplement's half-life of 1–2 days. According to Syawal et al. (2020), adding fermented herbal medicine to commercial feed

at a dose of 200 mL/kg daily can increase the appetite of striped catfish, as measured by absolute weight gain. The bioactive compounds in the fermented herbal medicine that influence body weight gain in Striped catfish in treatment P1 include curcumin, tannins, flavonoids, vitamin C, alkaloids, and saponins, which play a role in enhancing metabolic processes, improving the fish's immune system performance, and increasing appetite. The bioactive compounds in the fermented herbal medicine can improve gut microflora, thereby facilitating efficient digestion and nutrient absorption (Syawal et al., 2024). Additionally, curcumin, a compound derived from turmeric, has a positive effect on fish appetite, thereby promoting weight gain (Iman et al., 2019).

According to Rahmadona et al. (2020), the tannin content in the fermented herbal remedy also possesses antibacterial properties; furthermore, it can help improve feed digestibility and fish health, thereby supporting better growth. Furthermore, flavonoids are compounds with antioxidant and antibacterial properties that help boost the fish's immune system, which is crucial for optimal growth (Hasibuan et al., 2021). Additionally, the essential oil content supports fish health through its antibacterial effects and its ability to increase appetite (Lestari et al., 2021). It has been proven that administering fermented herbal medicine daily at a dose of 200 mL/kg of feed can promote optimal fish growth.

3.5. Total Serum Protein, Albumin, and Globulin

The results of the total protein, albumin, and globulin tests on Striped catfish are presented in Table 3.

Table 3. Serum total protein, albumin, and globulin of Striped catfish (*P. hypophthalmus*)

Parameter (mg/dL)	Treatment				
	Kn	Kp	P1	P2	P3
Initial Protein	3.48 ± 0.57	3.47 ± 0.75	3.47 ± 0.75	3.47 ± 0.75	3.47 ± 0.75
Final Protein	3.92 ± 0.05 ^b	3.98 ± 0.12 ^b	3.94 ± 0.06 ^b	3.93 ± 0.11 ^b	3.60 ± 0.02 ^a
Initial Albumin	3.46 ± 0.02 ^a	3.29 ± 0.51 ^{ab}	3.49 ± 0.05 ^{ab}	3.63 ± 0.03 ^{ab}	3.86 ± 0.02 ^b
Final Albumin	3.69 ± 0.02 ^b	3.71 ± 0.01 ^{bc}	3.76 ± 0.05 ^{cd}	3.81 ± 0.01 ^d	3.49 ± 0.03 ^a
Initial Globulin	0.08 ± 0.10	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Total Globulin	0.22 ± 0.05 ^{ab}	0.31 ± 0.09 ^b	0.21 ± 0.04 ^a	0.23 ± 0.02 ^{ab}	0.16 ± 0.01 ^a

Based on Table 3, the total protein content in the striped catfish increased. The highest total protein was observed in the Kp, which did not involve administering fermented herbal medicine or the *A. hydrophila* challenge test. Fish that were not given the fermented herbal supplement were unable to enhance their immune system, thus failing to prevent *A. hydrophila* infection. Chronic inflammation resulting from *A. hydrophila* infection can increase total protein levels, which are involved in the fish's nonspecific defense mechanisms. Meanwhile, the lowest total protein value was observed in P3, indicating insufficient intake of additional nutrients from the fermented herbal supplement due to its once-every-5-days administration frequency, resulting in suboptimal intake to boost immunity against *A. hydrophila* infection. This aligns with the statement by Munabari & Syaputra (2022) that total protein levels can be influenced by the body's pathological condition and the addition of fermented herbal medicine to the feed. Lower total protein levels can lead to chronic kidney disease and severe injuries. Conversely, an increase in total protein levels may occur due to severe dehydration and respiratory distress resulting from *A. hydrophila* infection, as observed in the Kp.

The changes in total protein levels observed in the Kp were caused by physiological disturbances in the body resulting from an *A. hydrophila* infection, indicating activation of the humoral immune system through stimulation of immunoprotein production (primarily globulins). Additionally, the increase in total protein in the P1 treatment was due to the daily administration of fermented herbal supplements and optimal fermented probiotics. Kurniawan et al. (2020) stated that striped catfish fed a diet supplemented with 200 mL/kg of fermented herbal medicine exhibited optimal increases in serum total protein, keeping the fish in a normal condition, total protein levels in healthy striped catfish range from 3.5–4.5 g/dL; this value can be influenced by feed nutrition and fish health, environmental conditions (temperature, water quality), exposure to pathogens or immunostimulants, and developmental stages (larvae, fry, adults).

The increase in total protein demonstrates the effectiveness of fermented herbal medicine in enhancing fish immunity. This is because fermented herbal medicine contains bioactive compounds such as curcumin, which can optimize the utilization of dietary nutrients, including in the body's protein synthesis process. Additionally, the active compounds in fermented herbal supplements have the potential to strengthen the nonspecific immune system, for example, by increasing globulin levels. Furthermore, fermented herbal supplements can also enhance protein retention in fish and improve their immune response (Munabari & Syaputra, 2022).

Albumin is an important liver-produced protein that helps maintain plasma osmotic pressure and transport various substances in the blood, playing a significant role in health and wound healing. Albumin is a key component in fish nutrition and health. Based on Table 3, the analysis of variance (ANOVA) for albumin in striped catfish showed $P < 0.05$. This indicates a significant effect of adding fermented herbal supplements at different frequencies to the feed on albumin levels. Adding fermented herbal supplements to the feed not only serves as a feed supplement but also improves overall fish health.

The greatest increase in albumin content was observed in P2, indicating that adding fermented herbal medicine every 3 days is sufficient to increase albumin levels in striped catfish optimally. Meanwhile, the lowest albumin content was found in P3, where fermented herbal supplements were administered every 5 days; thus, the immunostimulatory and antioxidant effects of the herbal supplements were not optimal because they did not align with the optimal half-life of herbal supplement administration, which is only 1–2 days. The decrease in albumin from 3.86 to 3.49 g/dL indicates impaired liver function or reduced protein metabolism, likely due to uncontrolled *A. hydrophila* infection, which is causing physiological stress and metabolic disorders in the fish.

P2 and P1 showed a significant increase in albumin content, indicating good physiological health following infection with *A. hydrophila*, as the administration of fermented herbal medicine was more consistent and aligned with the half-life of the herbal supplement, ensuring that the concentration of fermented herbal medicine at 200 mL/kg of feed remained stable within the body. This stability enhances the fish's physiological response and helps combat pathogen attacks. The increase in serum albumin levels in striped catfish is attributed to the bioactive compounds in the fermented herbal medicine, such as curcumin, gingerol, and xanthorrhizol, which possess antioxidant and anti-inflammatory properties and can enhance hepatocyte activity in albumin synthesis. Additionally, probiotic bacteria produced during fermentation can improve gut health, nutrient absorption, and protein digestibility, thereby indirectly strengthening the fish's metabolic and immune status. These compounds are known to enhance fish health by boosting the immune system and improving metabolism.

Increased albumin levels may also result from stress experienced by fish during rearing, which affects protein metabolism, as observed in the Kp, in which no fermented herbal supplement was administered. However, the fish were challenged with *A. hydrophila*. Stress resulting from *A. hydrophila* infection can increase stress hormone levels such as cortisol, which play a role in regulating protein metabolism. However, administering fermented herbal supplements can minimize the negative effects of this stress, thereby supporting recovery and increasing albumin levels (Kurniawan et al., 2019). All test fish in this study were still in normal condition because the normal albumin range for healthy catfish fry is 3.5–3.9 g/dL. Globulins are a group of proteins comprising 40% of the blood and performing various important functions, including acting as enzymes, transport proteins, and components of the immune system. Globulins are vital components of the fish's physiological system, functioning in the transport of essential substances and providing protection against pathogenic infections.

Based on Table 3, each treatment showed an increase in globulin levels in response to *A. hydrophila* infection, indicating that the fish's immune system responded to the stress caused by the bacterial infection; however, the differences were not significant, as indicated by the ANOVA analysis followed by Duncan's multiple range test, which showed $P > 0.05$. This is attributed to the administration of fermented herbal medicine, which increased total protein, albumin, globulin, and lysozyme levels in striped catfish, thereby helping reduce stress levels and physiological disturbances, including clinical symptoms, survival rate, relative protection, and absolute weight in the fish. Increased globulin levels are part of the fish's defense mechanism, activating both nonspecific and specific immune systems, indicating that the administration of fermented herbal medicine strengthens the immune response in striped catfish (*P. hypophthalmus*).

The Kp showed the highest globulin levels, ranging from 0.07 to 0.31 g/dL. This occurs because *A. hydrophila* infection triggers a very strong immune response, causing the immune system to work hard in producing antibodies and other defense factors; this is the fish's natural reaction to stress or severe infection caused by *A. hydrophila*, as no preventive measures were taken with the fermented herbal supplement. According to Mu et al. (2022), *A. hydrophila* infection can trigger physiological stress and activate the innate and adaptive immune systems in fish. Additionally, bacterial infection can increase immunoglobulin production, particularly IgM, as part of the humoral immune response.

Meanwhile, the lowest globulin content was found in P3, where fermented herbal feed was administered every 5 days. The infrequent administration schedule resulted in suboptimal performance of the fermented herbal feed as an immunomodulator in maintaining the balance of the immune response to combat pathogen attacks and reduce stress in the fish. In addition to the infrequent or inappropriate administration schedule of the fermented herbal feed, the low globulin content in P3 was also attributed to *A. hydrophila* infection. The globulin levels in each treatment in this study indicate that the fish were in a state of low immunity or vulnerability, as the normal globulin levels in uninfected striped catfish fry should range around 0.5 g/dL.

The administration of fermented herbal medicine as a feed supplement has been proven to enhance nonspecific immune responses in striped catfish. Bioactive compounds in fermented herbal medicine, such as flavonoids, tannins, and saponins, possess immunostimulatory properties that can enhance macrophage phagocytic activity and the production of immune proteins, such as globulin (Rauta et al., 2013). According to Li et al. (2015), fish fed a diet based on natural ingredients exhibit increased immune enzyme activity and serum protein levels (albumin and globulin), making them more resistant to bacterial infections. This is particularly relevant for managing fish health in intensive aquaculture, where *A. hydrophila* infections are often a major challenge in freshwater fish farming, leading to high mortality rates; thus, the use of fermented herbal feed is an effective strategy to enhance fish immunity without antibiotics and supports sustainable farming practices.

3.6. Lysozyme Enzyme Activity

An increase in lysozyme activity in response to infection indicates that the fish's immune system is functioning properly. The results of the lysozyme enzyme activity test in this study are presented in Table 4.

Table 4. Lysozyme enzyme activity in striped catfish

Treatment	Initial Lysozyme Enzyme Content	
	Initial (g/dL)	Final (g/dL)
Kn	290.00±10.00	343.33 ± 20.81 ^a
Kp	290.00±10.00	410.00±10.00 ^c
P1	303.33 ± 15.27	373.33±15.27 ^b
P2	296.67±5.77	376.67 ± 15.27 ^b
P3	296.67 ± 20.81	390.00 ± 10.00 ^{ab}

Note: The same superscript letters in a column indicate no significant difference between treatments ($P>0.05$), while different letters indicate a highly significant difference ($P<0.05$)

Based on Table 4, lysozyme enzyme activity indicates that P1, with daily administration of fermented herbal medicine, showed the lowest value (373.33 g/dL), approaching the control (Kn) level, compared to the every three days and every five days treatments. This suggests that the protective effect of 200 mL/kg of fermented herbal medicine in P1 was sufficient, so the immune system did not have to work hard to produce antibodies and defend against *A. hydrophila* infection, thereby not triggering a significant increase in the lysozyme response. This indicates that the immune system was not significantly disrupted and remained stable.

This is because the fish were in good health and were not challenged with *A. hydrophila*. Meanwhile, the Kp showed the highest lysozyme enzyme levels. This increase in lysozyme activity was due to environmental factors or minor stress that triggered its natural activation in response to severe infection by *A. hydrophila*. The high lysozyme activity in the Kp indicates that the fish's body has responded to stress caused by rearing conditions or a bacterial infection; this suggests that the body's defense mechanisms are functioning effectively, as evidenced by the increase in lysozyme activity. High lysozyme activity indicates that the fish's innate immune system is functioning optimally to combat pathogens, such as bacteria. Lysozyme degrades bacterial cell walls, thereby enhancing the fish's resistance to *A. hydrophila* infection (Syawal et al., 2024).

Feeding fermented herbal supplements infrequently results in suboptimal immune enhancement, as the dose in the body is not consistently optimal, leading to minor immune disruption during *A. hydrophila* infection. Similarly, in Kp, there was a very significant increase in lysozyme, indicating a high immune response to *A. hydrophila* infection due to the absence of immune protection from the fermented herbal feed, thereby increasing lysozyme production to combat *A. hydrophila* infection. After the *A. hydrophila* challenge test, all fish in this study experienced health issues because the normal range of lysozyme activity in striped catfish (*P. hypophthalmus*) should range from 290–310 U/mL, and the increased lysozyme levels exceeding 400 U/mL following the *A. hydrophila* challenge represent an acute immune response. Lysozyme is a component of the nonspecific immune system that plays a crucial role in the fish's defense mechanism. This enzyme functions by breaking the β -1,4-glycosidic bonds in the peptidoglycan structure of the bacterial cell wall, causing cell membrane damage and fluid influx into the cell, leading to cell swelling, rupture, and ultimately lysis of the pathogen cell until it dies.

Lysozyme enzyme activity increases in response to infection by *A. hydrophila*. In studies on saltwater tilapia, increased lysozyme activity correlates with the fish's ability to combat bacterial infection. Thus, a significant increase in lysozyme activity contributes to the fish's resistance to bacterial infection. In addition to being caused by *A. hydrophila* infection, the increase in lysozyme enzyme activity was also influenced by the age of the test fish used; in this study, the fish used were fingerlings and still in the developmental stage, so that in the Kn treatment, which did not undergo a challenge test, an increase in lysozyme enzyme activity could also occur.

According to Nasrullah et al. (2019), increased lysozyme activity is influenced by several factors, including age and developmental stage. Lysozyme activity tends to be higher in fish larvae and fry compared to adult fish. This indicates that lysozyme plays a crucial role in early defense. Stressed fish exhibit higher lysozyme levels. Stress and pathogen infection can trigger increased lysozyme activity as part of the fish's immune response.

In P1 (daily administration of herbal medicine) and P2 (administration every three days), the increase in lysozyme was not significant or did not differ significantly. This occurred because administering fermented herbal medicine enhanced the fish's nonspecific immune system and reduced stress in the fish. This aligns with the findings of Hadie et al. (2015), who stated that supplementing feed with natural ingredients, such as fermented herbal supplements or plant extracts, can increase lysozyme activity. This is attributed to the bioactive compounds in the supplements, which stimulate the striped catfish's immune system. Fermented herbal medicine administered daily (P1) can enhance the nonspecific immune system earlier before challenge testing because the concentration of fermented herbal medicine in the fish's body remains at an optimal level daily, so that when the fish are infected with *A. hydrophila*, the effects are not too severe on the fish's body. There is no need to produce large amounts of lysozyme, as the administration of the fermented herbal supplement aligns with the optimal frequency and effectively prevents the pathogen.

3.7. Phagocytosis Activity

Observations at the start of the rearing period (Day 0) showed that phagocytosis activity in striped catfish remained stable because the fish were healthy and had not been challenged with *A. hydrophila*. Phagocytosis activity on Day 30 showed that there were no significant differences among treatments ($P > 0.05$); similarly, on Day 46 (14 days post-challenge), there were no significant differences; however, the highest value was observed in P1 (51.11 ± 16.44), which was nearly comparable to Kn (61.11 ± 15.00) (Table 5), the standard for healthy fish not infected with *A. hydrophila*. This indicates optimal phagocytic activity, suggesting no significant disruption to the immune response. Meanwhile, in P2 and P3, there was no significant increase; in fact, the values were nearly identical to those in the Kp control group which received no fermented herbal supplement and was challenged with *A. hydrophila* indicating a low immune response because the administration of fermented herbal medicine was only performed every 3 and 5 days, respectively; thus, the essential oils, curcumin, flavonoids, and xanthorrhizol which function as antibacterial, antioxidant, and anti-inflammatory agents did not work optimally within the fish's body. This indicates that P1, with daily administration of fermented herbal medicine, exhibited the best phagocytosis activity among all treatments, as the relatively stable increase suggests no significant decline in the immune response within the fish's body.

Table 5. Total phagocytosis activity of Striped catfish (*P. hypophthalmus*)

Treatment	Total Phagocytic Activity (%)		
	H-0	H-30	H-46
Kn	33.33 $\pm$ 0.00	42.22 $\pm$ 16.44	61.11 $\pm$ 15.00
Kp	33.33 $\pm$ 0.00	40.10 $\pm$ 10.00	40.00 $\pm$ 3.33
P1	33.33 $\pm$ 0.00	25.55 $\pm$ 8.38	51.11 $\pm$ 16.44
P2	33.33 $\pm$ 0.00	25.55 $\pm$ 8.38	42.22 $\pm$ 8.39
P3	33.33 $\pm$ 0.00	31.11 $\pm$ 9.62	41.10 $\pm$ 5.09

Note: The same *superscript* letters in a column indicate that there is no significant difference between treatments ($P > 0.05$), and no further tests were conducted.

Observations at the start of the rearing period (Day 0) showed that phagocytosis activity in striped catfish remained stable because the fish were healthy and had not been challenged with *A. hydrophila*. Phagocytosis activity on Day 30 showed that there were no significant differences among treatments ($P > 0.05$); similarly, on Day 46 (14 days post-challenge), there were no significant differences; however, the highest value was observed in P1 (51.11 ± 16.44), which was nearly comparable to Kn (61.11 ± 15.00), the standard for healthy fish not infected with *A. hydrophila*. This indicates optimal phagocytic activity, suggesting no significant disruption to the immune response. Meanwhile, in P2 and P3, there was no significant increase; in fact, the values were nearly identical to those in the Kp control group which received no fermented herbal supplement and was challenged with *A. hydrophila* indicating a low immune response because the administration of fermented herbal medicine was only performed every 3 and 5 days, respectively; thus, the essential oils, curcumin, flavonoids, and xanthorrhizol which function as antibacterial, antioxidant, and anti-inflammatory agents did not work optimally within the fish's body. This indicates that P1, with daily administration of fermented herbal medicine, exhibited the best phagocytosis activity among all treatments, as the relatively stable increase suggests no significant decline in the immune response within the fish's body.

According to Lestari et al. (2021), all treatments in this study remained within normal parameters, as the daily addition of 200 mL/kg of fermented herbal medicine to the striped catfish feed is considered optimal if it can inhibit *A. hydrophila* and achieve a phagocytosis activity of $>36.33\%$. This indicates that fermented herbal medicine can enhance phagocytosis in striped catfish, which is highly beneficial for boosting the immune system in striped catfish (Alifuddin, 2002). The phagocytosis process in P1 is illustrated in Figure 3.

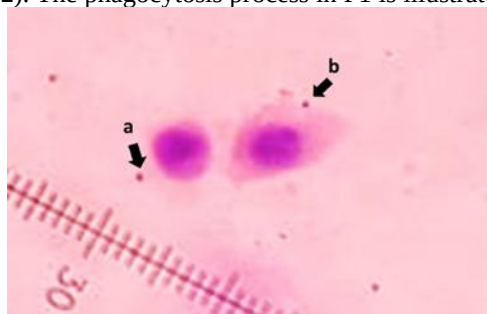


Figure 3. Stages of phagocytosis in striped catfish (1000x magnification)

Note: a. Adhesion of macrophages to bacteria, b. Ingestion of bacteria

According to Safaruddin & Ilimu (2021), phagocytosis is a critical process in the fish immune system, in which phagocytic cells, such as macrophages and neutrophils, eliminate pathogens and foreign bodies that enter the body. This process not only plays a role in the body's defense but also serves as an indicator of fish health and

their immune response to pathogenic infections. Phagocytosis involves several stages: adhesion, ingestion, and digestion. Phagocytic cells migrate to the site of infection, attach to foreign particles, ingest them, and then digest them with lysosomal enzymes. Phagocytosis is the first line of defense in the cellular response carried out by monocytes (macrophages) and granulocytes (neutrophils).

According to Saparuddin & Ilimu (2021), administering probiotic feed supplements or plant extracts, such as fermented herbal remedies, can enhance phagocytic activity. The administration of probiotics can increase macrophage numbers and phagocytic activity in striped catfish. Adding plant-derived β -glucan to fish feed can significantly enhance phagocytic activity. Phagocytic activity can be influenced by various factors, namely stress, infection, and deficiencies in protein and vitamins, which can reduce phagocytic activity and impair immune system function. Therefore, meeting optimal nutritional requirements is crucial for maintaining fish health. Additionally, the presence of *A. hydrophila* in fish can trigger a drastic increase in phagocytic activity as a form of nonspecific immune response; alternatively, a drastic increase in phagocytic activity may indicate a bacterial infection. Phagocytic activity can serve as an indicator of fish health. This indicates that the fish's immune system is functioning properly and can combat *A. hydrophila*. Conversely, a decrease in phagocytic activity may indicate health issues or stress in the fish; thus, phagocytic activity is a crucial component of the striped catfish's immune system, functioning to protect the body against *A. hydrophila* infection.

4. Conclusions

Based on the research results, feeding striped catfish a diet supplemented with fermented herbal medicine at a dose of 200 mL/kg and administered daily showed a significant effect on the physiological response and nonspecific immunity of the fish before and after challenge with *A. hydrophila*, with a survival rate of 100%, RPS of 100%, absolute weight gain of the fish reaching 109.33 ± 2.51 g, total serum protein of 3.94 ± 0.06 g/dL, albumin 3.76 ± 0.05 g/dL, globulin 0.21 ± 0.04 g/dL, lysozyme enzyme 373.33 ± 15.27 u/ml, and phagocytosis activity $51.11 \pm 16.44\%$.

Based on the results of this study, it is recommended that striped catfish farmers administer fermented herbal medicine at a dose of 200 mL/kg of feed daily. Further research is recommended to observe the feed efficiency of feed supplemented with fermented herbal medicine, to test fermented herbal medicine with different types of ingredients, and to conduct histological analysis of the intestines, liver, kidneys, and gills of fish fed fermented herbal medicine.

5. References

- Alifuddin, M. (2002). Imunostimulasi pada hewan akuatik. *Jurnal Akuakultur Indonesia*, 1(2): 87–92.
- Hadie, W., Sularto, S., Hadie, L.E., Lusastuti, A.M., Alimuddin, A., Tahapari, E., & Marnis, H. (2015). Inseri gen lisozim pada ikan patin siam *Pangasianodon hypophthalmus* (Sauvage, 1878) untuk membentuk galur tahan penyakit. *Jurnal Iktiologi Indonesia*, 15(2): 119–127.
- Hoseinifar, S.H., Sun, Y.Z., Wang, A., & Zhou, Z. (2018). Probiotics as means of disease control in aquaculture, a review of current knowledge and future perspectives. *Frontiers in Microbiology*, 9, 2429.
- Iman, KN., Riauwaty, M., & Syawal, H. (2019). Leukocytes differentiation of *Pangasius hypophthalmus* that were with curcumin extract from *Curcuma domestica* V. *JOM FAPERIKA UNRI*, 4(1): 1–14.
- Ismail, H.F., Hashim, Z., Soon, W.T., Rahman, N.S., Zainudin, A.N., Majid, F.A.A. (2019). Comparative study of herbal plants on the phenolic and flavonoid content, antioxidant activities and toxicity on cells and zebrafish embryo. *Journal of Traditional and Complementary Medicine*, (7): 452–465.
- Komalasari, S.S., Subandiyono, S., & Hastuti, S. (2017). Pengaruh vitamin C pada pakan komersil dan kepadatan ikan terhadap kelulushidupan serta pertumbuhan ikan nila (*Oreochromis niloticus*). *Sains Akuakultur Tropis*, 1(1): 31–41.
- Kurniawan, R. (2019). *Profil Hematologis dan Fisiologis Ikan Patin Pangasianodon hypophthalmus yang Diberi Pakan dengan Penambahan Suplemen Herbal*. Fakultas Perikanan dan Ilmu Kelautan. Universitas Riau. p85.
- Kurniawan, R., Syawal, H., & Effendi, I. (2020). Pengaruh penambahan suplemen herbal pada pakan terhadap diferensiasi leukosit ikan dan sintasan ikan patin (*Pangasianodon hypophthalmus*). *Jurnal Akuakultur Rawa Indonesia*, 8 (2): 150–163.
- Lestari, J., Syawal, H., & Riauwaty, M. (2021). Diferensiasi leukosit ikan jambal siam (*Pangasianodon hypophthalmus*) yang diberi pakan mengandung jamu permentasi. *Jurnal Ruaya*, 9 (1): 41–55.
- Mengi, L., Jasmanindar, Y., & Liufeto, F.C. (2022). Pencegahan infeksi bakteri *Vibrio* sp. pada ikan bandeng (*Chanos chanos*) dengan pemberian rebusan kayu manis (*Cinnamomum burmannii*). *Jurnal Vokasi Ilmu-Ilmu Perikanan (JVIP)*, 3(1): 9–12.

- Mu, Q.-J., Kong, W.-G., Qin, D.-C., Zhou, Y., Wang, X.-Y., Yu, Y., et al. (2022). High levels of specific IgM in serum and gut mucus of common carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. *Frontiers in Immunology*, 13:1037517.
- Myszor, I.T., & Gudmundsson, G.H. (2023). Modulation of innate immunity in airway epithelium for host-directed therapy. *Frontiers in Immunology*, 14: 1197908.
- Nam, P.V., Van Hoa, N., Anh, T.T.L., & Trung, T.S., (2020). Towards zero-waste recovery of bioactive compounds from catfish (*Pangasius hypophthalmus*) by-products using an Enzymatic method. *Waste Biomass. Valoriz*, 11(8): 4195–4206.
- Nasrullah, N. (2019). *Identifikasi dan analisis ekspresi gen lisozim pada ikan lele dumbo Clarias gariepinus yang diinfeksi Aeromonas hydrophila*. Library of IPB University. Bogor
- Pakpahan, P., Syawal, H., & Riauwaty, M. (2020). Effect of curcumin feeding in feed for treating jambal siam fish (*Pangasius hypophthalmus*) infected by *Aeromonas hydrophila*. *Jurnal Perikanan dan Kelautan*, 25(3): 224–231.
- Rahmadona, Z., Syawal, H., & Lukistyowati, I. (2020). Description of leukocytes *Pangasius hypophthalmus* which is fed with extracts of mangrove leaf (*Rhizophora apiculata*) and maintained in the floating cages. *Jurnal Perikanan dan Kelautan*, 25(1): 79–87
- Rauta, P.R., Mohanty, J., Garnayak, S.K., & Sahoo, P.K. (2013). Characterization of serum immunoglobulin in *Channa striata* (BLOCH) and kinetics of its response to *Aeromonas hydrophila* antigen. *J Immunoassay Immunochem*. 34(3):283-93.
- Rizki, S.M., & Panjaitan, R.S. (2018). Efektivitas antifungi dari minyak atsiri kulit batang kayu manis (*Cinnamomum burmanni*) terhadap *Candida albicans*. *Jurnal Kimia dan Pendidikan*. 3(2): 172.
- Saparuddin, S., & Ilimu, E. (2021). Peningkatan respon imun ikan nila (*Oreochromis niloticus*) dengan pemberian ekstrak daun *Macaranga tanarius*. *Jurnal Biotek*, 9(2): 186–195.
- Sugiani, D., Taukhid, T., Purwaningsih, U., & Lusiastuti, A.M. (2018). Vaksin kering beku sel utuh bakteri *Aeromonas hydrophila* untuk pencegahan penyakit *Motile Aeromonads Septicemia* pada ikan lele, nila, dan gurami. *Jurnal Riset Akuakultur*, 13(2): 159–167.
- Suhenda, N., Setijaningsih, L., & Suryanti, Y. (2017). Penentuan rasio antara kadar karbohidrat dan lemak pada pakan benih ikan patin jambal (*Pangasius djambal*). *Jurnal Penelitian Perikanan Indonesia*, 9 (1): 2-30.
- Syawal, H., Effendi, I., & Kurniawan, R. (2020). Pengaruh pemberian suplemen herbal dan padat tebar berbeda terhadap laju pertumbuhan ikan jambal siam *Pangasianodon hypophthalmus* (Sauvage, 1878). *Jurnal Iktiologi Indonesia*, 20(2): 143-153.
- Syawal, H., Riauwaty, M., Nuraini, N., & Hasibuan, S. (2019). Pemanfaatan pakan jamu untuk meningkatkan produksi ikan budidaya. *Dinamisia-Jurnal Pengabdian Kepada Masyarakat*, 3: 188-193.
- Tamba, J.M., Syawal, H., & Lukistyowati, I. (2021). Identifikasi bakteri patogen pada ikan jambal siam (*Pangasionodon hypophthalmus*) yang dipelihara di kolam budidaya. *Jurnal Perikanan dan Kelautan*, 26(1): 40-46.
- Tanjung, S.A.F., Syawal, H., & Riauwaty, M. (2023). Pengkayaan pakan dengan larutan kulit kayu manis (*Cinnamomum burmannii*) untuk mencegah aeromoniosis pada ikan jambal siam (*Pangasianodon hypophthalmus*). *Jurnal Veteriner*, 24(3):346–356.
- Zhang, X., Zhang, B., Li, L., Li, X., Zhang, J., & Chen, G. (2022). Fermented noni (*Morinda citrifolia* L.) fruit juice improved oxidative stress and insulin resistance under the synergistic effect of Nrf2/ARE pathway and gut flora in db/db mice and HepG2 cells. *Food Function*, 13: 8254–8273.