**Asian Journal of Aquatic Sciences,** August 2025. Vol 8, Issue (2) 240-251
e-issn: 2716-4608
p-issn: 2655-366X

# HEMATOLOGY OF STRIPED CATFISH (*Pangasianodon hypophthalmus*) FED WITH ADDED FERMENTED HERBAL MEDICINE AND TESTED WITH *Aeromonas hydrophila*

**Fharisa Nabila Rizvi[1*], Henni Syawal[1], Morina Riauwaty[1]**
[1]Department of Aquaculture, Faculty of Fisheries and Marine,
Universitas Riau, Pekanbaru, 28293 Indonesia
*fharisanabila10@gmail.com

## ABSTRACT

Fermented herbs made from turmeric, ginger, and kencur have the potential to be antimicrobials and immunostimulants that can increase appetite and reduce fish stress due to changes in water quality. The research was conducted from June 2024 to August 2024 at the Fish Disease and Parasite Laboratory, Faculty of Fisheries and Marine, Universitas Riau. This study aims to determine the best frequency of fermented herbs enriched on the health of striped catfish (*Pangasianodon hypophthalmus)* hematology after challenge with *Aeromonas hydrophila*. The research method used a completely randomized design (CRD) with one factor, five treatments, and three replicates. Treatments consisted of Kn (negative control, no challenge test), Kp (positive control and challenge test), P1 (herbs enriched every day and challenge test), P2 (giving every 3 days and test challenge), and P3 (giving every 5 days and a test challenge). Fish were kept for 46 days and challenged with *A. hydrophila* ($10^8$ CFU/mL) on day 32. Clinical symptoms were observed for 14 days post-challenge. The best results were obtained in treatment P1 with the following hematological parameters: erythrocytes $2.37\times10^6$ cells/mm³, hematocrit 36.00%, hemoglobin 9.60 g/dL, leukocytes $11.14\times10^4$ cells/mm³, glucose 69.33 mg/dL, and normal leukocyte differentiation. The survival rate reached 100%. Feeding fermented herbs effectively improved the health and body endurance of striped catfish.

**Keywords:** *Aeromonas hydrophila,* Hematology, Immunostimulant, Fermented herbs.

## 1. INTRODUCTION

The Striped catfish (*Pangasianodon hypophthalmus*) is one type of freshwater fish that is very popular with the public, especially in Riau Province, because of its good taste, thick meat, and high economic value. Striped catfish is an intensively cultivated fish with high stocking densities and intensive feeding. In addition to its fast growth, this fish is responsive to artificial feed and easy to grow in public waters[1].

Efforts to meet market demand for striped catfish can be carried out through intensive cultivation. However, intensive cultivation systems have disadvantages, including stress on fish caused by high stocking density and decreased water quality so that striped catfish are susceptible to *Motile Aeromonas Septicemia* (MAS) disease caused by *Aeromonas hydrophila*, which can cause mortality rates of up to 80-100% within 1-2 weeks[2].

Farmers still use many antibiotics to control disease. Long-term use of antibiotics tends to be environmentally unfriendly and has adverse side effects on polluted waters. Chemicals can cause pathogenic bacteria to become resistant. In connection with these problems, using natural ingredients around us can be an alternative for handling fish treatment[3].

Prevention or treatment of diseases in fish can be done by utilizing natural ingredients with antimicrobial potential.

Received: 15 June 2025
Accepted: 13 July 2025

Natural ingredients commonly used in fish medicine are turmeric, temulawak, and kencur. These natural ingredients are processed in the form of herbal medicine and made through a fermentation process. According to Syawal et al.[4], the benefits of providing fermented herbal medicine in feed can stimulate fish appetite, increase growth, fish immunity to disease, reduce fish stress levels to environmental changes, and stimulate the immune system and organ functions related to hematological parameters.

Hematological profiles can be used as an indicator to determine the health condition of fish. Haematological profiles play a role in the physiology of metabolism and body activity and are a defence component from disease attacks that enter the fish body[3]. Therefore, the author is interested in researching the haematology of Jambal Fish fed with Fermented Herbs and tested with *A. hydrophila* to determine the best frequency for giving herbs to striped catfish and analyzing the blood of striped catfish tested with *A. hydrophila*.

## 2. RESEARCH METHOD

### Time and Place

This research was conducted from June to August 2024 at the Laboratory of Fish Parasites and Diseases, Faculty of Fisheries and Marine Sciences, Universitas Riau.

### Method

The method used was an experimental method with a one-factor Completely Randomized Design (CRD), namely the frequency of adding fermented herbal medicine to feed, with five treatment levels.

Kn : Negative control (Feeding of commercial pellets without *A.hydrophila* challenge)
Kp : Positive control (Feeding commercial pellets and tested for *A.hydrophila*)
P1 : Giving fermented herbal medicine in feed every day and tested with *A. hydrophila*
P2 : Giving fermented herbal medicine to feed every 3 days and tested with *A. hydrophila*
P3 : Giving fermented herbal medicine on feed every 5 days and tested with *A. hydrophila*.

### Procedures

### Making Fermented Herbs

The procedure for making fermented herbal medicine is turmeric, which involves peeling and washing clean turmeric, temulawak, and kencur, each 400 g. Next, they are sliced thinly and mashed with a blender, after which they are filtered to obtain a solution. The solution was obtained in as little as 12 L, boiled over low heat until boiling, and then cooled.

### Fish Blood Collection

Fish were anaesthetized with 0.1 mL/L clove oil in 5L of water. Then, a 1 mL syringe and an Eppendorf tube were prepared and moistened with 10% EDTA to prevent blood clotting. Blood was drawn from the tail vein using a 45° angle syringe, and then the blood in the syringe was put into an Eppendorf tube. Blood was drawn three times before treatment, on day 30, and 14 days after challenge with *A. hydrophila.*

### Challenge Test

The challenge test was conducted on day 32 with Aeromonas hydrophila with a solution concentration of 0.1 mL/head with a bacterial density ($10^8$ CFU/mL) that had been increased in virulence with Koch's Postulate rules5. Before infection, fish were first anaesthetized using clove oil as much as 0.1 mL/L water to reduce stress in fish. Infection was done intramuscularly using a 1 mL syringe. Post-challenge, the fish were kept for 14 days, fed, and observed for clinical symptoms.

### Measured Parameters

The parameters measured in this study were clinical symptoms, total erythrocytes, hematocrit values, haemoglobin levels,

glucose, total leukocytes, and leukocyte differentiation.

## 3. RESULT AND DISCUSSION

### Clinical Symptoms

Clinical symptoms of striped catfish after the *A. hydrophila* challenge include changes in appetite, movement, body surface, eyes, and fins. It is known that the clinical symptoms of fish on day 14 post-challenge are Kn fish not infected with *A. hydrophila* and without additional treatment. The Kn test fish showed no clinical symptoms of the disease. Kn fish showed normal appetite, regular swimming, bright body surface, no excess mucus production, and intact fins. In contrast to Kp test fish, fish in good and healthy condition after being infected with *A. hydrophila* showed severe clinical symptoms. Kp test fish experienced clinical symptoms that were observed, including decreased appetite and a lack of response to the feed given, passive movement, colour on the body surface is pale, excess mucus; there are ulcers on the body, convex eyes/exophthalmia, and thinning fins.

According to Kurniawan et al.[6], the clinical symptoms of striped catfish after being infected with *A. hydrophila* are decreased appetite, convex eyes, inflammation in the injection area, and ulcers. As a further disease development, the wound festered, causing ulcers, pale body colour, and haemorrhage evenly distributed throughout the body[7]. These symptoms show the direct effects of *A. hydrophila* infection. Thus, from the observations of fish, the Kp test was the worst treatment because the fish were only infected with *A. hydrophila* without being treated to prevent disease progression. Changes in clinical symptoms can be seen in Figure 1.

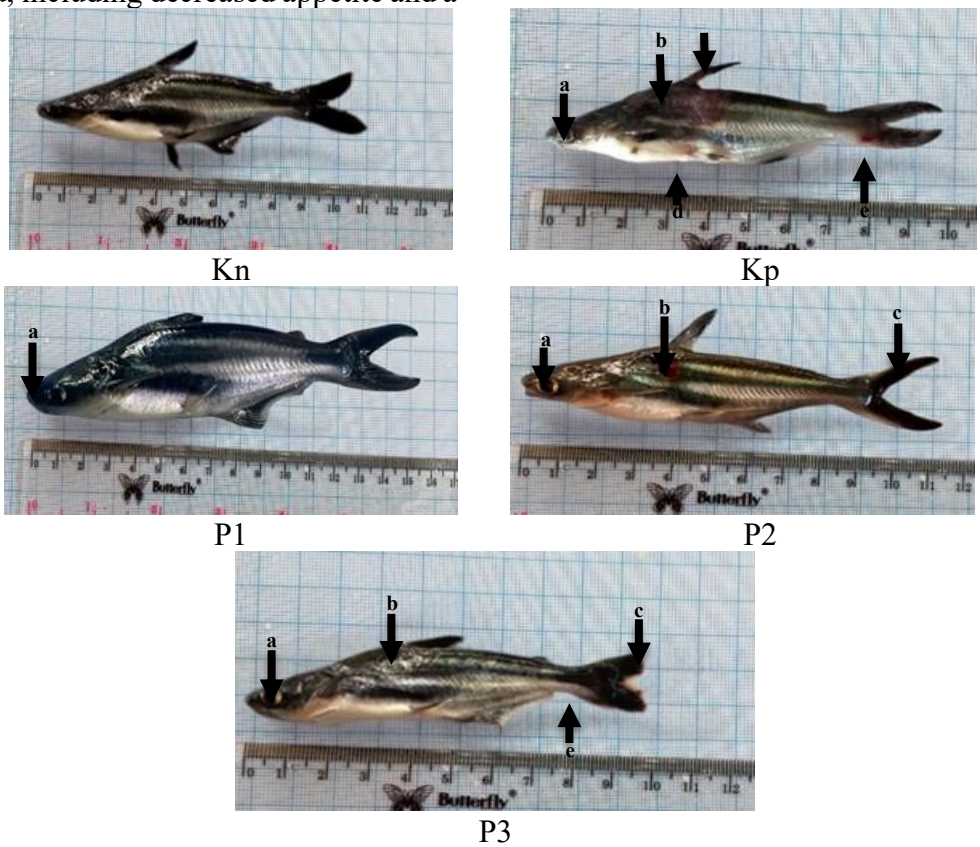


**Figure 1**. Clinical symptoms striped catfish
Description: a. Exopthalmia, b. Ulcer, c. Thinning fins, d. Reddening of the abdomen, e. Bleeding at the base of the tail.

P1 treatment shows that the daily frequency of adding fermented herbal medicine affects the effectiveness as an immunostimulant. This treatment showed

the best results compared to the other treatments, which were close to Kn. P1 test fish did not show severe clinical symptoms generally caused by *A. hydrophila*. In addition, appetite, movement, mucus condition, and body colour were normal. The ulcers slowly shrank on the 5th day post-challenge, and appetite returned to normal. The P1 test fish showed recovery and increased endurance, and experienced faster recovery than the other test fish. The addition of fermented herbs can increase fish immunity after being infected with *A. hydrophila*. The success of the P1 test fish can be explained by the high curcumin compounds in fermented herbs that are more effective in increasing the fish's immune system. Feed containing fermented herbs contains flavonoids and vitamin C. The flavonoid content acts as a cell structure repair process.

**Total Erythrocytes**

Total erythrocytes of striped catfish at the beginning of rearing, day 30, with feed added with fermented herbs, and post-challenge with *A. hydrophila* are presented in Table 1.

**Table 1.** Total of Erythrocytes striped catfish (*P. hypophthalmus*)

| Treatments | Total of Erythrocytes (x $10^6$ sel/$mm^3$) | | |
|---|---|---|---|
| | H-0 | H-30 | H-46 (14 Post-Challenge Day) |
| Kn | 1,61±0,02 | 1,67±0,04[a] | 1,72±0,02[a] |
| Kp | 1,60±0,02 | 1,66±0,05[a] | 1,61±0,02 [a] |
| P1 | 1,65±0,03 | 1,94±0,05[b] | 2,37±0,13[c] |
| P2 | 1,63±0,02 | 1,85±0,05[b] | 2,02±0,10[b] |
| P3 | 1,60±0,02 | 1,69±0,10[a] | 1,89±0,22 [ab] |

Notes :*Different Superscript letters in the same column indicate that between-treatment treatments are significantly different, P<0.05

Based on Table 1, the total erythrocytes of striped catfish obtained after 30 days of maintenance with feed-added fermented herbs ranged from 1.66 - 1.94 x $10^6$ cells/$mm^3$, and post-challenge ranged from 1.61-2.37 x$10^6$ cells/$mm^3$. The treatment with fermented herbal medicine added to the feed showed a significant increase in total erythrocytes. The highest growth in total erythrocytes compared to all test fish treatments was in the P1 treatment, indicating that the length of frequency of daily fermented herbal medicine can have the most significant effect on erythrocyte production.

The results of this study suggest that the average total erythrocytes of jambal siam fish are still classified as usual. Pulungan et al.[8] stated that the total erythrocytes of striped catfish ranged from 1.15 - 2.39 x $10^6$ cells/$mm^3$. Aulia et al.[9] noted that the total erythrocytes of striped catfish ranged from 1.31-2.44 x $10^6$ cells/ $mm^3$. Day 14 post-challenge with *A. hydrophila*, the total erythrocytes of striped catfish in the Kn treatment increased even though it was not as high as the treatment given the addition of fermented herbs, namely 1.72 x $10^6$ cells/$mm^3$.

The Kn treatment of test fish was not infected with *A. hydrophila*. The fish were not given the addition of fermented herbs, so the total erythrocytes could increase because they were fed protein. The Kp test fish treatment experienced a decrease in total post-challenge erythrocytes compared to all treatments, namely 1.61 x$10^6$ cells/$mm^3$. This proves that the condition of the fish after being infected with *A. hydrophila* has decreased, and the immune system has also decreased because no additional feed is given. The number of erythrocytes decreased due to the attack of *A. hydrophila*, which caused damage to cells and organs in the fish's body. According to Zissalwa et al.[10], the content of secondary metabolite compounds in fermented herbs can trigger blood-producing organs to produce more

blood to replace erythrocytes that are lysed due to infection from pathogens.

The content of fermented herbal medicine can act as a natural immunostimulant that can increase erythrocyte production and the fish's immune system. The increase in total erythrocytes can occur due to the content of curcumin and flavonoid compounds in the fermented herbal medicine solution. Adding fermented herbs with different frequencies can increase total fish erythrocytes compared to fish not given the addition of fermented herbs. Zissalwa et al.[10] stated that the increase in total erythrocytes is influenced by the size, feed nutrition, age, parent, physical activity, and environmental conditions of fish.

**Hematocrit Value**

Hematocrit values at the beginning of rearing, after the 30th day of rearing by feeding fermented herbs, and post-challenge with *A. hydrophila,* the hematocrit values of striped catfish during the study (Table 2).

**Table 2.** Hematocrit value of striped catfish (*P. hypophthalmus*)

| Treatment | Hematocrit Value | | |
|---|---|---|---|
| | H-0 | H-30 | H-14 post-challenge |
| Kn | 24,33±0,58 | 30,66±0,57$^{a}$ | 33,66±1,52$^{bc}$ |
| Kp | 23,67±1,15 | 30,66±0,57$^{a}$ | 21,00±3,60$^{a}$ |
| P1 | 24,67±0,58 | 33,66±0,57$^{c}$ | 36,00±1,00$^{c}$ |
| P2 | 24,33±0,58 | 32,33±0,57$^{b}$ | 31,66±1,52$^{bc}$ |
| P3 | 24,00±1,00 | 31,66±0,57$^{ab}$ | 30,00±1,00$^{b}$ |

Notes: *Different Superscript letters in the same column indicate that between-treatment treatments are significantly different, P<0.05

Based on Table 2, the hematocrit value of striped catfish before being treated (H-0) obtained the results of the hematocrit percentage of the test fish 23.67-24.67%, after being fed with the addition of fermented herbs for 30 days of maintenance with different frequency of addition of fermented herbs, the hematocrit value increased ranging from 30.66-33.66%. Post-challenge hematocrit values ranged from 21.00-36.00%. The post-challenge Kp test fish showed the lowest hematocrit value of 21.00%, compared to all treatments. This was due to the fish experiencing stress due to *A. hydrophila* infection.

The highest post-challenge hematocrit value is found in the P1 treatment, which is 36.00% because the P1 fish test fish, so adding fermented herbal medicine daily can increase the body's immunity to *A. hydrophila* attacks when tested. While the lowest hematocrit value is found in the Kp treatment, which is 21.00%, this is because the fish in the Kp treatment are not given additional fermented herbal medicine, but are tested with *A. hydrophila*, so the fish experience stress and do not want to eat. Thus, the fish's body becomes weak. The hematocrit value of post-challenge jambal fish is still within the normal range.

According to Zissalwa et al.[10], a decrease in hematocrit indicates fish experiencing symptoms of anemia. According to Ulandari et al.[11], hematocrit levels of healthy striped catfish range from 20 to 39%. According to Prasetio et al.[12], the supporting statement states that freshwater fish are in normal condition when hematocrit levels range from 22 to 60%. The hematocrit value of the test fish is within the normal range.

**Hemoglobin Level**

Hemoglobin levels are measured by feeding them fermented herbs and testing them with *A. hydrophila*. The hemoglobin levels during the study (Table 3).

**Table. 3** Hemoglobin level of striped catfish (*P. hypophthalmus*)

| Treatment | Hemoglobin Level | | |
|---|---|---|---|
| | H-0 | H-30 | H-46 (14 days post-challenge) |
| Kn | 6,80±0,72 | 6,80±0,20$^{a}$ | 8,86±0,23$^{cb}$ |
| Kp | 6,85±0,72 | 7,00±0,20$^{a}$ | 5,20±0,20$^{a}$ |
| P1 | 6,90±0,73 | 10,60±0,20$^{d}$ | 9,60±0,40$^{d}$ |
| P2 | 6,87±0,73 | 9,53±0,41$^{c}$ | 8,20±0,20$^{bc}$ |
| P3 | 6,89±0,73 | 8,60±0,20$^{b}$ | 7,80±0,72$^{b}$ |

Notes: *Different Superscript letters in the same column indicate that between-treatment treatments are significantly different, P<0.05

Based on Table 3. Hemoglobin levels of striped catfish before maintenance ranged from 6.80-6.90 g/dL. After 30 days of maintenance, the test fish ranged from 6.80-10.60 g/dL, while post-challenge ranged from 5.20-9.60 g/dL. Kn and P1 test fish continue to experience an increase in hemoglobin levels as long as the fish are healthy. In the P1 treatment fish, the highest hemoglobin level compared to all treatments was 10.60 g/dL, indicating that the fish were in normal condition. The frequency of the addition of fermented herbal medicine affects the effectiveness of increasing hemoglobin, where the more often given the addition of fermented herbal medicine is added, the higher the effect on hemoglobin levels. According to Hasibuan et al.[3], normal hemoglobin levels of jambal fish range from 6-13 g/dL. The supporting opinion of Aulia et al.[9] is that the hemoglobin range of striped catfish is 6.60-8.87 g/dL. The hemoglobin level of the test fish shows that it is normal.

After the challenge, the hemoglobin level of Kn test fish continued to increase, with a hemoglobin level of 8.86 g/dL in a healthy state. Kn test fish did not experience stress and were given protein-containing food. The Kp test fish showed a decrease in hemoglobin levels of 5.20 g/dL, which indicates that the test fish is in a state of decreased endurance because the Kp test fish is infected with *A. hydrophila*. Fish immunity is not good, so the fish's blood parameters decrease. This is because the Kp test fish experience injuries or infections caused by pathogens, so their health has been directly reduced. According to Pakpahan et al.[7], hemoglobin in the blood is related to the low value of hemoglobin, which is thought to be due to fish experiencing lysis, which is caused by the rupture of red blood cells due to hemolysis.

**Total Leukocyte**

Total leukocytes at the beginning of maintenance, after the 30$^{th}$ day of maintenance, by giving feed added to fermented herbs, and testing. The total leukocytes of jambal siam fish during the study can be seen in Table 4.

**Table 4.** Total leukocytes of striped catfish (*P. hypophthalmus*)

| Treatments | Total Leukocytes ($10^4$ x sel/ mm$^3$) | | |
|---|---|---|---|
| | H-0 | H-30 | H-14 post-challenge |
| Kn | 7,76 ± 1,00 | 8,58 ± 1,00$^{a}$ | 8,61 ± 2,52$^{a}$ |
| Kp | 7,77 ± 0,58 | 8,58 ± 0,58$^{a}$ | 12,15 ± 2,52$^{e}$ |
| P1 | 7,79 ± 0,58 | 9,42 ± 1,00$^{d}$ | 11,14 ± 3,06$^{d}$ |
| P2 | 7,78 ± 1,00 | 9,34 ± 0,58$^{c}$ | 9,46 ± 2,00$^{c}$ |
| P3 | 7,76 ± 1,53 | 8,73 ± 0,58$^{b}$ | 9,22 ± 3,61$^{b}$ |

Notes: *Different Superscript letters in the same column indicate that between-treatment treatments are significantly different, P<0.05

Table 4 shows that the average total leukocytes of striped catfsh at the beginning of the study ranged from 7.76-7.77 x 104 cells/mm$^3$. The average leukocytes of striped

catfish after 30 days of maintenance ranged from 8.58-9.42 x $10^4$ cells/$mm^3$, and post-challenge ranged from 8.61-12.15 x$10^4$ cells/$mm^3$. Total leukocytes post-challenge with A. hydrophila were highest at Kp 12.15 x$10^4$ cells/$mm^3$. Indicating that the total leukocytes of post-challenge striped catfish Kp are classified as abnormal. This is due to the body of fish Kp test producing many leukocytes due to the infection that occurs due to the attack of *A. hydrophila.*

According to Syawal et al.[4], the greater the antigen stimulus, the more antibodies will be produced, and leukocytes will identify bacteria that enter the fish's body as antigens. Leukocytes are responsible for the immune response of fish; if a foreign substance enters the body, leukocytes will make antibodies and provide body defence.

The immune system will use antibodies to stimulate, identify, and neutralize foreign objects (antigens) that enter the body, such as bacteria. According to Soltani et al.[13], high total leukocytes are a sign of bacterial infection, so fish try to increase their immune system from bacterial infections so that leukocytes move actively towards the site of infection.

According to Pohan et al.[14], the total leukocytes of normal striped catfish are 7.20-10.54 x $10^4$ cells/$mm^3$. Tanjung et al.[15] supporting statement is that the increase in total leukocytes is still within the normal range according to the normal range of striped catfish leukocytes, which is 7.51-10.85 x $10^4$ cells/$mm^3$. The results of the total leukocytes of the test fish show that the total leukocyte value is still relatively normal. The function of leukocytes can be used to detect disease in the fish body[16].

**Blood Glucose Levels**

Blood glucose levels at the beginning of maintenance, after the 30th day of maintenance, were tested by giving feed added to fermented herbs. The blood glucose levels of striped catfish during the study can be seen in Table 5.

**Table 5.** Glucose level of striped catfish (*P. hypophthalmus*)

| Treatment | Glucose Level (mg/dL) | | |
|---|---|---|---|
| | H-0 | H-30 | H-14 post-challenge |
| Kn | 50,00±4,00 | 50,33±29,16$^a$ | 53,33±9,07$^a$ |
| Kp | 51,00±4,00 | 51,00±9,84$^a$ | 79,66±4,04$^c$ |
| P1 | 53,00±4,00 | 63,33±18,03$^a$ | 69,33±3,21$^b$ |
| P2 | 55,00±4,00 | 50,33±10,40$^a$ | 70,33±5,50$^c$ |
| P3 | 51,33±5,00 | 45,33±4,93$^a$ | 77,33±5,03$^c$ |

Notes: *Different Superscript letters in the same column indicate that between-treatment treatments are significantly different, P<0.05

Table 5 shows that the average glucose level of striped catfish at the beginning of the study ranged from 50.00 mg/dL. The average glucose levels of striped catfish after 30 days of maintenance ranged from 50.33 - 63.33 mg/dL, according to Manurung et al.[17], normal fish blood glucose levels range from 40-90 mg/dL. According to Pohan et al.[14], the glucose level of striped catfish is 37-78 mg/dL. These results indicate that the value of glucose levels of the test fish is still classified as usual. The occurrence of an increase or decrease in glucose levels in the plasma indicates that the fish is experiencing stress. One indication of stressed fish is the increase in plasma glucose levels. The glucose concentration in circulating plasma depends on glucose production and its rapid loss in the bloodstream.

Glucose describes the stress response as a result of the action of catecholamines on the glycogen center in the liver and tissues to break down glycogen into glucose so that blood glucose increases[18]. Energy needs from glucose to handle stress can be met if glucose in the blood can immediately enter the cells, which is determined by the performance of insulin. Meanwhile, insulin

inactivation occurs during stress, thus closing the use of glucose in cells[17].

Fish that experience stress result in hyperglycemia, which can interfere with further development and can even be deadly[19]. The mechanism of changes in blood glucose levels in fish begins with the receipt of stress-causing information by the receptor organ, which is then forwarded to the hypothalamus through the nervous system. Furthermore, the hypothalamus commands chromaffin cells to secrete the hormone catecholamine through sympathetic nerve fibres so that catecholamines activate enzymes involved in the catabolism of glycogen stores, which results in increased blood glucose[17].

The increase in glucose levels in striped catfish fed with the addition of fermented herbs has increased due to the content of fermented herbs, which can increase fish appetite, reduce stress in fish, help maintain fish blood glucose levels, and keep the immune system. Fermented herbs play an active role in improving fish health by absorbing nutrients and regulating blood glucose metabolism. Fermented herbs can reduce stress in fish, helping in a more efficient metabolism and increasing immunity. This statement is by Syawal et al.[4], that giving fermented herbal medicine can increase appetite in fish, reduce stress in fish, and increase fish immunity.

The content of secondary metabolite compounds contained in turmeric, temulawak, and kencur, such as flavonoids, maintains the condition of fish blood glucose. As for other supporting opinions Novita et al.[1], flavonoid compounds trigger the release of glucose from the liver, activate insulin receptors, and increase insulin receptors and glucose absorption in insulin tissues and gene expression. Flavonoid administration has significantly reduced freshwater fish's blood glucose levels, inhibiting glucose absorption in the intestine and adjusting their levels in fish blood.

**Leukocyte Differentiation**

Total leukocyte differentiation at the beginning of maintenance, after the 30$^{th}$ day of maintenance, was tested by giving feed added to fermented herbs. The total leukocytes of the striped catfish during the study can be seen in Table 6.

Leukocyte differentiation at the beginning of maintenance, 30 days of maintenance, and post-challenge of *A. hydrophila* was found to increase and decrease in the type of differentiation treated with the addition of fermented herbs. Lymphocytes are white blood cells that play a role in the adaptive immune response, recognizing and destroying specific pathogens, such as viruses and bacteria. There are two main types of lymphocytes: B and T. B lymphocytes are responsible for antibody production, while T lymphocytes destroy infected cells and regulate immune responses. The number of lymphocytes on the 30th day of maintenance ranged from 64.00-76.66%, with the highest lymphocytes found in the P1 treatment, which was 76.66% because the fish in the P1 treatment were given the addition of fermented herbs to the feed to increase lymphocyte levels. The number of lymphocytes in normal striped catfish is, in her opinion of Septiana[20], that the number of lymphocytes in striped catfish ranges from 73.00-80.33%. At the same time, Kurniawan et al.[16] stated that the number of lymphocytes in striped catfish ranged from 66.00-77.67%. In this study, lymphocyte levels were still around normal. Lymphocytes play an important role in antibody formation. After being tested, the number of lymphocytes in this study ranged from 42.66-73.00%, with the best lymphocytes in the treatment, Kn, which is 73.00%, a fish treatment that has not been tested for *A. hydrophila*. Therefore, the number of lymphocytes remains stable/ normal. According to Septiana[20], if the intensity of pathogen infection increases, the need for white blood cells or lymphocytes increases because the need for white blood cells increases.

The number of neutrophils in this study after being tested was 5.33-11.66%; the lowest number of neutrophils was found in the Kn treatment, which was 5.33%, and the highest number of neutrophils was found in the Kp treatment, which was 11.66% because the Kp treatment was tested with *A. hydrophila*. According to Kurniawan et al.[6], the value of fish neutrophils in normal conditions is 5.75-14.94%. Meanwhile, according to Sari et al.[21], the number of neutrophils is 5.67-15.00%. The neutrophil value in this study is within normal conditions. Kp treatment has increased because neutrophil cells are still working to suppress bacterial infections.

**Table 6.** Leukocyte differentiation of striped catfish (*P. hypophthalmus*)

| Treatments | Leukocyte Differentiation (0 days) | | | | |
|---|---|---|---|---|---|
| | lymphocytes | netrofil | basophils | platelets | monocytes |
| Kn | 73,66±1,52 | 8,00±1,00 | 7,33±2,08 | 3,66±1,15 | 7,33±1,52 |
| Kp | 74,66±1,52 | 7,00±1,00 | 7,33±2,08 | 3,66±1,15 | 7,33±1,52 |
| P1 | 75,66±1,52 | 6,00±1,00 | 7,33±2,08 | 3,66±1,15 | 7,33±1,52 |
| P2 | 73,66±1,52 | 8,00±1,00 | 7,33±2,08 | 3,66±1,15 | 7,33±1,52 |
| P3 | 75,66±1,52 | 6,00±1,00 | 7,33±2,08 | 3,66±1,15 | 7,33±1,52 |
| Treatments | Leukocyte Differentiation (30th day of maintenance) | | | | |
| | lymphocytes | netrofil | basophils | platelets | monocytes |
| Kn | 72,66±1,15[a] | 7,00±1,73[a] | 7,00±1,73[a] | 5,00±2,00[a] | 8,33±1,15[a] |
| Kp | 64,00±4,35[a] | 5,33±1,52[a] | 10,00±2,6[a] | 7,33±2,08[a] | 13,33±6,50[a] |
| P1 | 76,66±2,08[a] | 6,66±3,05[a] | 6,66±0,57[a] | 4,33±0,57[a] | 5,66±0,57[a] |
| P2 | 70,66±10,40[a] | 9,33±3,51[a] | 8,66±6,35[a] | 4,66±2,08[a] | 6,66±1,52[a] |
| P3 | 65,66±8,08[a] | 10,33±3,05[a] | 7,33±1,52[a] | 4,66±0,57[a] | 12,00±5,19[a] |
| Treatments | Leukocyte Differentiation (14 days post-challenge) | | | | |
| | lymphocytes | netrofil | basophils | trombosit | monosit |
| Kn | 73,00±1,00[e] | 5,33±0,57[a] | 6,00±1,73[a] | 6,66±0,57[a] | 9,00±1,00[a] |
| Kp | 42,66±2,51[a] | 11,66±1,15[c] | 17,66±0,57[c] | 11,66±3,05[b] | 16,33±0,57[b] |
| P1 | 69,00±1,00[d] | 7,66±0,57[b] | 5,66±2,08[a] | 8,66±0,57 [ab] | 9,00±2,00[a] |
| P2 | 63,66±0,57[c] | 10,00±1,00[c] | 7,33±0,57[a] | 10.33±0,57[ab] | 8,66±0,57[a] |
| P3 | 59,00±1,00[b] | 11,33±1,52[c] | 11,00±1,00[b] | 10.00±1,73[ab] | 8,66±1,15[a] |

The number of basophils in the study after being challenged ranged from 5.66 to 17.66%; the highest number of basophils was found in the Kp treatment, which was 17.66% because the Kp test fish was infected with *A. hydrophila*, while basophils function as an allergic reaction and as inflammation. According to Puspitowati et al.[22], basophil levels in fish are 0-11%. The number of basophils in this study is normal. Basophils are a defence of the immune system to fight infections that attack the fish's body and increase immune cells to reach infected or injured areas.

Platelets assist in the formation of a blood clot to prevent excessive blood loss during a wound. In addition to their primary role of hemostasis (stopping bleeding), platelets are also involved in the immune response and wound healing. The lowest platelet count was found in the Kn treatment, which was 6.66%, because the fish were not tested / or the fish were healthy. According to Gusriansyah et al.[23], the number of normal fish platelets is 6.66-7.67%.

Monocytes are a type of white blood cell that plays a role in phagocytosis (this process is where cells engulf large particles, including pathogens in dead cells). The increase in the number of monocytes in each treatment is thought to be caused by fish in an unhealthy state due to infection entering the body or stimulation from foreign objects to produce new monocytes. Monocyte cells are stimulated by curcumin, which functions as an immunostimulant. The number of monocytes at the time of the challenge test was obtained from 9.00-16.33%; the highest number of monocytes was in the Kp

treatment, 16.33% and the lowest treatment, Kn, 9.00%.

The decrease in the number of monocytes indicates that the fish are healthy. According to the opinion of Kurniawan et al.[6], the decrease in monocyte value is due to fish in a healthy condition, for which monocyte cells are not needed to phagocytize because there is no infection entering the body or there is no stimulus from foreign objects to produce monocytes. According to Sari et al.[21], the Monocyte value of striped catfish is 9.33-32.67%. Meanwhile, according to Kurniawan et al.[6], the value of fish monocytes under normal conditions is 5.67-7.00%. The monocyte value is higher than the normal range, presumably due to the stressful condition of the fish, and the proportion of monocyte cell count has decreased due to the blood balance response to an increase in the proportion of leukocyte cell types, namely monocytes.

**Survival of Striped Catfish (*P. hypopthalmus*)**

The results showed that feeding with the addition of fermented herbal medicine can increase the survival of striped catfish to 100% on day 14 after the *A. hydrophila* challenge. The survival rate of post-challenge striped catfish fed with fermented herbal medicine is higher than that of those not fed with fermented herbal medicine (Kp), which only reaches 33%, as shown in Figure 1.

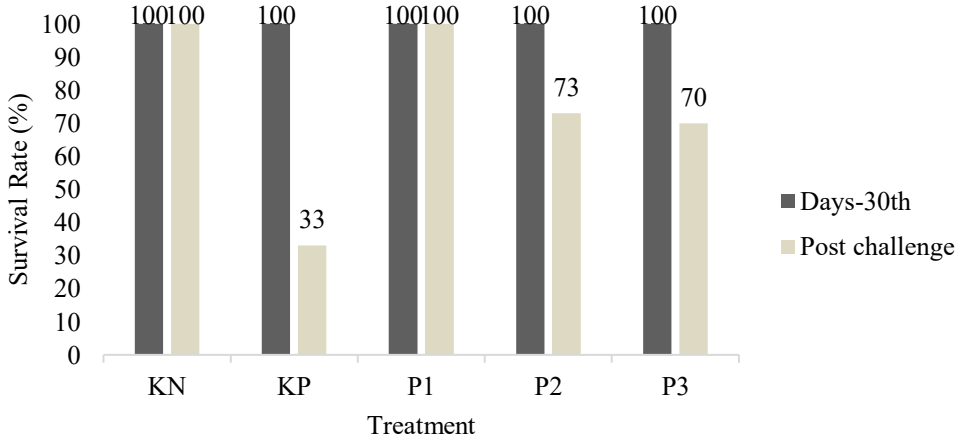


**Figure 2.** Survival rate of striped catfish during maintenance

The lowest survival rate of striped catfish in the Kp treatment is 33%; this is because in the Kp treatment, the fish are given commercial feed and tested with *A. hydrophila*, causing them to be stressed and their immune system to decrease. Therefore, they are susceptible to disease, which causes the fish to die. The highest survival rate in the Kn and P1 treatments is 100%; the high survival rate in Kn and P1 is because the fish are in good health. Fish in the Kn treatment were not tested with *A. hydrohila*, while P1 fish were tested with *A. hydrohila*, but the immunity in P1 fish was strong. Therefore, it could minimize the bacteria that attack the fish's body. According to Syawal et al.[24], giving herbal supplements mixed in pellets can trigger fish growth and reduce mortality rates. The fish survival rate is influenced by good aquaculture management, including stocking density, feed quality, water quality, and disease. Feed that has good nutrition plays a role in maintaining survival and accelerating fish growth[25].

## 4. CONCLUSION

The addition of fermented herbal medicine in feed with different frequencies affects the hematological parameters of striped catfish before and after the *A. hydrophila* challenge test. The best treatment was daily feeding (P1), which showed increased erythrocytes, hemoglobin, hematocrit, leukocytes, and endurance with a survival rate of 100%.

## REFERENCES

1. Novita, H., Riauwaty, M., & Syawal. H. Efek Perendaman pada Ikan Jambal Siam (*Pangasianodon hypophthalmus*) dalam Larutan Kulit Nanas (*Ananas comosus*) Terhadap Gambaran Leukosit. *Jurnal Akuakultur Sebatin,* 2023; 4(1):78-89
2. Gaol, D.P.L., Riauwaty, M., & Syawal, H. Histopatologi Insang Ikan Jambal Siam (*Pangasianodon hypophthalmus*) yang Terinfeksi *Aeromonas hydrophila* dan Diobati dengan Larutan Kulit Kayu Manis (*Cinnamomum burmannii*). *Jurnal Ilmu Perairan* (*Aquatic Science*), 2024; 12(1): 21-29
3. Hasibuan, Y.P., Syawal, H., & Lukistyowati, I. Gambaran Darah Merah Ikan Jambal (*Pangasianodon hypophthalmus*) Siam yang Diberi Pakan Mengandung Jamu Fermentasi untuk Mencegah Penyakit *Motile Aeromonas septicemia*. *Jurnal Ruaya*, 2020; 9(1): 41-55
4. Syawal, H., Effendi, I., & Kurniawan, R. Perbaikan Profil Hematologi Ikan Patin (*Pangasius hypophthalmus*) Setelah Penambahan Suplemen Herbal pada Pakan. *Jurnal Veteriner*, 2021; 22(1).
5. Lusiastuti, A. M., Sumiati, T., & Hadie, W. Probiotik *Bacillus firmus* untuk Pengendalian Penyakit *Aeromonas hydrophila* pada Budidaya Ikan Lele Dumbo, *Clarias gariepinus*. *Jurnal Riset Akuakultur*, 2013; 8(2): 253-264
6. Kurniawan, R., Syawal, H., & Effendi, I. Pengaruh Penambahan Suplemen Herbal pada Pakan terhadap Diferensiasi Leukosit Ikan dan Sintasan Ikan Patin (*Pangasionodon hypopthalmus*). *Jurnal Akuakultur Rawa Indonesia*, 2020; 8(2): 150-163
7. Pakpahan, P., Syawal, H., & Riauwaty, M. Pengaruh Pemberian Kurkumin pada Pakan terhadap Pengobatan Ikan Jambal Siam (*Pangasiodon hypopthalmus*) yang terinfeksi bakteri *Aeromonas hydrophila*. *Jurnal Perikanan dan Kelautan*, 2020; 25(3): 224-231
8. Pulungan, L.A., Riauwaty, M., & Lukistyowati, I. Hematology of *Pangasianodon hypophthalmus* that Were Fed with Containing Fermented Red Ginger (*Zingiber Officinale* Var Rubrum) to Prevent the *Motile Aeromonas septicaemia* Disease. *Asian Journal of Aquatic Sciences*, 2022; 5(2): 291-300
9. Aulia, A.S., Sarjito, S., & Desrina, D. Pengaruh Nanokitosan Ekstrak Daun Bidara Arab (*Ziziphus spinachristi*) dalam Pakan terhadap Gejala Klinis, Kadar Eritrosit, Hemoglobin, dan Pertumbuhan Ikan Patin (*Pangasius* sp.) yang Diinfeksi *Aeromonas hydrophila*. *Sains Akuakultur Tropis: Indonesian Journal of Tropical Aquaculture*, 2023; 8(1): 1-9
10. Zissalwa F., Syawal, H., & Lukistyowati, I. Profil Eritrosit Ikan Jambal Siam (*Pangasianodon hypopthalmus*) yang diberi Pakan Mengandung Ekstrak Daun Mangrove (*Rhizophora apiculata)* dan dipelihara dalam Keramba. *Jurnal Perikanan dan Kelautan,* 2020*;* 25(1): 70-78
11. Ulandari, U., Syawal, H., & Lukistyowati, I. Pencegahan Penyakit *Motile Aeromonas septicemia* pada Ikan Jambal Siam (*Pangasianodon hypophthalmus*) Menggunakan Larutan Kulit Nanas (*Ananas comosus*). *South East Asian Aquaculture*, 2025; 2(2): 68-77
12. Prasetio, E., Fakhrudin, M., & Hasan, H. Pengaruh Serbuk Lidah Buaya (*Aloe vera*) terhadap Hematologi Ikan Jelawat (*Leptobarbus hoevenii*) yang Diuji Tantang Bakteri *Aeromonas hydrophila*. *Jurnal Ruaya: Jurnal Penelitian dan Kajian Ilmu Perikanan dan Kelautan*, 2017; 5(2)
13. Soltani, M., Pakzad, K., Taheri-Mirghaed, A., Mirzargar, S., Shekarabi, S.P.H., Yosefi, P., & Soleymani, N. Dietary Application of the Probiotic *Lactobacillus plantarum* 426951 Enhances Immune Status and Growth of Rainbow Trout (*Oncorhynchus mykiss*) Vaccinated Against *Yersinia ruckeri*. *Probiotics and Antimicrobial Proteins*, 2019; 11: 207-219

14. Pohan, I. F., Riauwaty, M., & Syawal, H. Hematology and Physiology of Striped Catfish (*Pangasianodon hypophthalmus*) with the Addition of Guava Leaf Extract (*Psidium guajava* L). *Jurnal Perikanan dan Kelautan*, 2024; 29(3): 367-374
15. Tanjung, S.A.F., Syawal, H., & Riauwaty, M. Pengkayaan Pakan dengan Larutan Kulit Kayu Manis (*Cinnamomum burmannii*) untuk Mencegah *Aeromoniosis* pada Ikan Jambal Siam (*Pangasianodon hypophthalmus*). *Jurnal Veteriner*, 2023; 24(3).
16. Kurniawan, R., Syawal, H., & Effendi, I. Efektivitas Penambahan Suplemen Herbal pada Pellet terhadap Pertumbuhan dan Kelulushidupan Ikan Patin (*Pangasius hypophthalmus*). *Jurnal Ruaya: Jurnal Penelitian dan Kajian Ilmu Perikanan dan Kelautan*, 2020; 8(1): 69-79
17. Manurung, J., Syawal, H., & Riauwaty, M. Respons Fisiologis Ikan Jambal Siam (*Pangasianodon hypophthalmus*) yang Diberi Pakan Mengandung Larutan Kulit Kayu Manis (*Cinnamomum burmanii*) untuk Mencegah Penyakit Bakterial. *Jurnal Ilmu Perairan (Aquatic Science)*, 2024; 12(1): 96-106
18. Hertika, A.M.S., Diana, A., Evellin, L., Renanda, B., & Dzulhamdi, S.P. Analisis Hubungan Kualitas Air dan Kadar Glukosa Darah *Gambusia affinis* di Perairan Sungai Brantas. *Journal of Fisheries and Marine Research*, 2021; 5(3): 522-530
19. Saputra, A., Mumpuni, F.S., Setiadi, E., & Setiawan, I.D. Kinerja Pertumbuhan dan Sintasan Benih Ikan Baung (*Hemibagrus nemurus*) yang diberi Probiotik Berbeda. *Journal Mina Sains*, 2019; 5(1): 1-12
20. Septiana, L. *Uji Aktivitas Antibakteri Kayu Manis (Cinnamomum burmanni) dalam Menghambat Pertumbuhan Bakteri Aeromonas hydrophila.* Universitas Riau. 2022.
21. Sari, R.M., Lukistyowati, I., & Riauwaty, M. Prevention of *Motile Aeromonas Septicemia* in Striped Catfish (*Pangasianodon hypophthalmus*) using Cherry Leaf Solution. *Jurnal Perikanan dan Kelautan*, 2022; 27(1): 24-31
22. Puspitowati, D., Lukistyowati, I., & Syawal, H. Gambaran Leukosit Ikan Jambal Siam (*Pangasianodon hypophthalmus*) yang diberi Pakan Mengandung Larutan Daun Pepaya (*Carica papaya* L.) Fermentasi. *Jurnal Akuakultur Sebatin*, 2022; 3(1): 78-92
23. Gusriansyah, D., Syawal, H., & Riauwaty, M. The Effect of *Moringa oleifera* Leaf Fermented Enriched Pellets on Hematology of *Pangasianodon hypophthalmus* Reared in Photoperiod System. *Asian Journal of Aquatic Sciences*, 2025; 8(1): 41-52.
24. Syawal, H. Pemanfaatan Pakan Herbal (Jamu) untuk Meningkatkan Produksi Ikan Budidaya. *Dinamisia: Jurnal Pengabdian kepada Masyarakat*, 2019; 3: 188-193.
25. Fratiwi, G., Dewiyanti, I., & Hasri, I. Aplikasi Probiotik dari Bahan Baku Lokal pada Pakan Komersial terhadap Pertumbuhan dan Tingkat Kelangsungan Hidup Benih Ikan Depik (*Rasbora tawarensis*). *Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah*, 2018; 3(1): 46-55