

HEMATOLOGY OF *Osphronemus goramy* FED CONTAINING FERMENTED TURI (*Sesbania grandiflora*) LEAF MEAL and CHALLENGED WITH *Aeromonas hydrophila*

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ABSTRACT

Turi leaf (*Sesbania grandiflora*) is a natural ingredient with antibacterial compounds. This research was conducted from April to June 2022 at the Fish Nutrition Laboratory and the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine, Universitas Riau. This study aimed to obtain the best dose of fermented turi leaves in feed seen from hematology before and after the challenge test with *A. hydrophila*. The method used in this study is an experimental method using a one-factor Completely Randomised Design (CRD) with five treatment levels, namely Kn: (no treatment), Kp: (no cure and infected with *A. hydrophila*), P1: (fermented feed containing 15% turi leaves, 85% soy flour), P2: (30% turi leaves, 70% soy flour), and P3: (45% turi leaves, 55% soy flour). Fish were reared for 46 days. The challenge test was conducted on day 32 with *A. hydrophila* bacteria at a density of 10^8 CFU/mL, as much as 0.1 mL/head. The results showed that fermented feed contained 45% turi leaves and 55% soybeans (P3). Wheat flour obtained total erythrocytes 2.51×10^6 cells/mm³, hematocrit 40.33%, hemoglobin 10.40 g/dL, total leukocytes 10.13×10^4 cells/mm³, phagocytosis activity 21.13%, survival rate 90%, and absolute weight growth 9.52g. Water quality parameters were temperature ranging from 27-30°C, pH 5.9-6.2, DO 5.5-5.9 mg/L, and NH₃ 0.015-0.017 mg/L. Based on the results of the study, it can be concluded that there is an effect of adding fermented turi leaves to feed on the hematology of gourami before and after the *A. hydrophila* bacterial challenge test has a significant impact.

Keywords: *Osphronemus goramy*, Fermentation, *Sesbania grandiflora*.

1. INTRODUCTION

Gourami (*Osphronemus goramy*) is a freshwater fish that has an economic value whose price is quite high¹. Gourami is a freshwater fish that is popular with the public and is one of twelve types of commodities to fulfill community nutrition². Fish disease caused by bacteria is a problem that often arises in aquaculture, one of which is the *Motile Aeromonas Septicemia* (MAS) disease caused by *Aeromonas hydrophila* bacteria. The pathogenicity of *A. hydrophila* can cause mortality in cultured fish up to 80-100% within 1-2 weeks³. Symptoms of

MAS are red spots on the surface of the fish body (hemorrhagic septicemia), ulcers, abscesses, and flatulence⁴. Panda et al.⁵ states that turi leaves contain active ingredients such as flavonoids, tannins, minerals, and saponins. Turi (*Sesbania grandiflora*) leaf flour has quite the potential to be used as an alternative fish feed ingredient. This is very possible to be used for the cultivation of omnivorous fish that tend to be herbivorous so that they are more adaptable to the type of feed mixed with vegetable sources such as turi leaf flour. According to Aryani et al.⁶, feeding fermented turi leaf flour has a significant

effect on the growth rate of carp $3.21 \pm 0.17\%$ /day.

Kombucha is a symbiosis between colonies of yeast, fungi, and bacteria composed of gelatinous mass that resembles a hard membrane⁷. Kombucha also contains various cellulase and protease enzymes that can break down complex molecules in feed ingredients into simpler compounds. The results of research Priskila⁸ show that the use of kombucha in the taro leaf fermentation process can increase the crude protein content from 29.35% to 32.71% and reduce the crude fiber content from 22.17% to 15.78%. So far, the fermentation of turi leaves has used fermenters such as *Rhizopus* sp, *Aspergillus* sp, and *Trichoderma* sp. Fermentation of turi leaves with kombucha has not been found, for this reason, this research was conducted.

Fish blood chemistry parameters can be used as indicators to determine the health condition of fish. These parameters can provide important information about the physiological status of fish, both influenced by processes within the fish body and from the observed living environment⁹.

Based on the description above, the author is interested in researching the "Hematology of gourami fed with feed containing fermented turi leaf flour tested against *A.hydrophila*".

2. RESEARCH METHOD

Time and Place

This research was conducted from April to June 2022 at the Fish Nutrition Laboratory, and the Fish Parasites and Diseases Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

Method

The method used in the research is an experimental method using a one-factor Completely Randomised Design (CRD) with 5 levels of treatment and 3 replications so that 15 experimental units are needed. The treatment refers to Yuni¹⁰ which uses

25% fermented turi leaf flour in feed to produce the best feed efficiency and growth in gourami.

Kn :turi leaf meal and not challenged with *A.hydrophila* bacteria)

Kp :turi leaf meal and challenged with *A.hydrophila* bacteria)

P1 :leaves, and 85% soya flour and tested with *A. hydrophila* bacteria.

P2 :leaves, and 70% soya flour and tested with *A. hydrophila* bacteria.

P3 :thorn leaves, and 55% soya flour and was challenged with *A.hydrophila* bacteria.

Procedure

Container Preparation

The fish-rearing containers used were 15 aquariums measuring 40x30x30 cm. Before the aquarium is used, it is first cleaned and filled with water to the brim then given a solution of KMnO_4 (Potassium Permanganate) as much as 25 ppm for 24 hours so that the aquarium is free from pathogenic microorganisms, then the aquarium is rinsed with water until clean then dried for 24 hours. Once clean, the aquarium was filled with water from a borehole that was first settled in a tank.

Kombucha Starter Preparation and Fermentation of Turi Leaf with Kombucha

Water is boiled until it boils, then 500 mL of water is taken, then put into 2 cups of tea bags until the water is brown. Then put 100 g sugar into the tea solution, stirring until the sugar dissolves. Then the Kombucha was weighed as much as 50 g and then cut and put into the tea solution, stirred until smooth then put in a glass jar and closed tightly, stored at a temperature of $23-27^\circ\text{C}$ for 8-12 days, without being exposed to sunlight. After undergoing the fermentation process for 8-12 days, the

water from the Kombucha solution was used for the fermentation process of turi leaves.

The leaves were first soaked in water for 24 hours to reduce anti-nutrient levels, then dried in the sun until dry, after which the leaves were ground into flour. Turi leaf flour is mixed with water in a ratio of 1:1 until homogeneous, then steamed for 30 minutes. After that, it was dried for 15 minutes. Then the turi leaf flour was weighed as much as 10 g, mixed with kombucha solution as much as 30% of turi leaf flour, then added molasses solution as much as 2% of the weight of turi leaf flour. Then the ingredients were put into a plastic jar and sealed and stored in a dark place. After 7 days of successful fermentation marked the characteristics: the growth of white fungus and sour aroma like tape and ready to be used for mixing formulations into feed.

Preparation of Test Feed

The test feed to be made previously determined the formulation and composition of each ingredient following the expected protein requirement of 30%. The ingredients used were weighed as needed. The mixing of the ingredients is done in stages, starting from the lowest to the highest. Furthermore, the homogeneous ingredients were added with cooked water as much as 25-30% of the total weight of the ingredients. The addition of water is done while the material is stirred evenly so that lumps can be made. Then the pellet dough is moulded in the mill and dried by drying in the sun. After drying the pellets are ready to be used for fish feed.

Test Fish Preparation

The fish used in this study were 150 gouramy with a length of 8-10 cm. The fish used came from a fish hatchery in West Sumatra. The test fish fry was acclimatized for 15 minutes and adapted for 7 days. On the 6th day after adaptation, the fish were fed for 1 day to empty the stomach of the fish to respond to the new feed. The test

fish fry was first weighed, and then each aquarium was stocked with fish at a stocking density of 1 fish/3 L.

Challenge test

The challenge test with *A. hydrophila* bacteria was carried out on day 32 of rearing intramuscularly with a bacterial density of 10^8 CFU/mL at a dose of 0.1 ml/head using a 1 mL syringe. After the challenge test, the fish were returned to the aquarium and reared for 14 days.

Fish blood Collection

Blood collection was carried out by anesthetizing the test fish first by dissolving clove oil in water as much as 0.1 mL/water so that the fish were not stressed. After that, fish blood was taken with a 1 mL syringe that had been rinsed with 10% EDTA. Blood was taken from the caudal vein, and then the blood in the syringe was put into an Eppendorf tube that had been moistened with 10% EDTA to prevent blood clotting.

Observation of Clinical Symptoms

Observation of clinical symptoms includes body movement, morphology, or physical condition of the fish. Observation of clinical symptoms is carried out every day during the maintenance period until after the challenge test. The results of observations of clinical symptoms are described in tabular form.

Total erythrocytes

Total erythrocytes were calculated following the procedure of Klontz *in* Kurniawan¹¹. The results were converted into the formula below:

$$\text{Erythrocyte count} = \sum n \times 10^6 \text{ cells/mm}^3$$

Description:

n = Number of erythrocyte cells present in 5 small boxes
 10^6 = Dilution factor

Hematocrit

Hematocrit percentage was calculated following the procedure of Anderson and

Siwicki *in* Kurniawan¹¹, as % of blood cell volume.

$$SR = \frac{N_t}{N_o} \times 100\%$$

Hemoglobin

Calculation of hemoglobin levels was performed using the Sahli method. Hemoglobin levels are expressed in g/dL or g% (Wedemeyer and Yasutake *in* ¹¹).

Total Leukocytes

The procedure for calculating total leukocytes refers to Klontz *in* Kurniawan¹¹, the total number of leukocytes is counted using a microscope on 4 large boxes of hemocytometer with the following formula:

$$\sum \text{Leukocytes} = \sum n \times 50 \text{ cells/mm}^3$$

Description:

$\sum n$ = Total number of leucocytes in 4 large boxes

50 = Dilution factor

Phagocytosis Activity

Phagocytic activity is measured by the following formula:

$$\text{Phagocytosis} = \frac{\sum \text{Sel Fagosit active}}{\sum \text{Sel Fagosit observed}} \times 100\%$$

Survival Rate

According to Effendie¹², the survival rate can be calculated using the following formula:

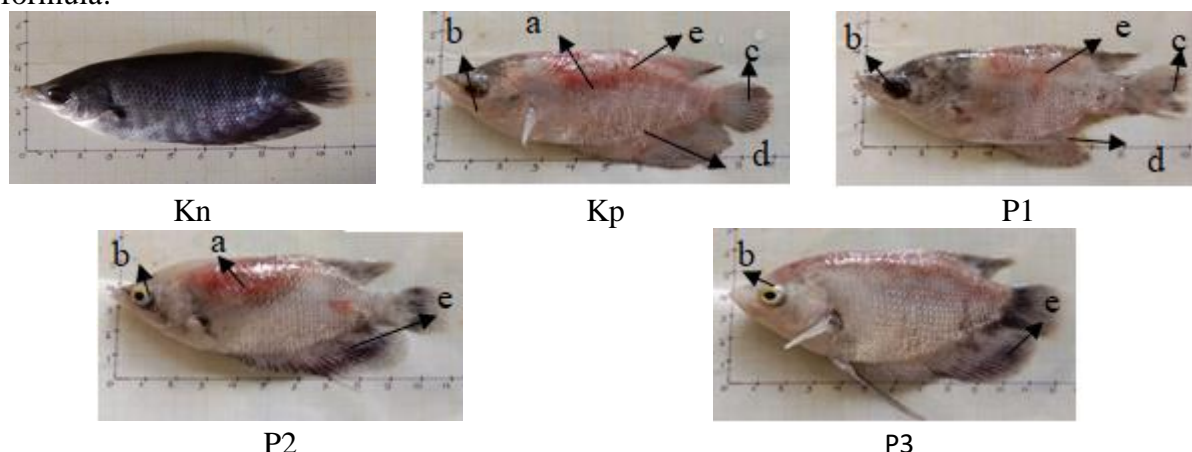


Figure 1. Clinical Symptoms of Gouramy after Challenge Test Infected with *A. hydrophila* Bacteria

Description: a). ulcer, b). Protruding eyes, c). Thinning fins, d). Bleeding at the base of the fin, e). Red spots on the fish's body

Clinical symptoms of gouramy infected with *A. hydrophila* bacteria are pale

Absolute weight growth

Absolute weight growth was calculated using the formula according to Effendie¹² as follows:

$$W_m = W_t - W_o$$

Description:

W_m = Absolute growth (g)

W_t = Average weight of fish at the end of the study (g)

W_o = Average weight of fish at the beginning of the study (g)

Water Quality

The water quality measured was temperature, pH, DO, and NH_3 . Water quality measurements were carried out twice, namely at the beginning of maintenance.

3. RESULT AND DISCUSSION

Clinical symptoms of gouramy (*O. goramy*)

Clinical symptoms of gourami after the challenge test with *A. hydrophila* bacteria can be seen in Figure 1.

body, exophthalmia, peeling of some scales, loose tail fin, and sudden death. *A. hydrophila* produces hemosilin which breaks down and lyses red blood cells, causing them to escape from blood vessels and cause reddening of the skin surface.

In the Kn treatment, the fish were normal, because the fish were not challenged. When compared to KP after the challenge test, the fish had ulcers, protruding eyes, thinning fins, bleeding at the base of the fins, and red spots on the body of the fish. P1 After the challenge test the fish had protruding eyes, a fin grip, bleeding at the base of the fin, and red spots on the body of the fish. P2 post-challenge fish had ulcer/borrow, protruding eyes, and red spots on the body of the fish. P3 after the fish challenge test experienced protruding eyes and red spots on the body of the fish. this is thought to be the content of feed containing 45% fermented turi leaves has a greater ability because the dose of fermented turi leaves is 45% and turi leaves contain secondary metabolic content including triterpenoids, tannins, flavonoids, and saponins more when compared to treatment P₁ and P₂ so that the P₃ treatment dose is higher to increase its endurance. Flavonoids act as antioxidants in neutralizing free radicals and act to repair cell structure¹³.

The ability of *A. hydrophila* to invade fish occurs through the mechanism of action of extracellular toxins, which are products released when the bacteria are still alive and attached to fish organs. Several types of toxins contained in *A. hydrophila* bacteria in addition to haemoxins also produce chitinase and lecithinase where these enzymes can damage fish body tissues¹⁴.

The positive control had more severe clinical symptoms compared to the other treatments, namely, ulcers, exophthalmia/protruding eyes, thinning fins, and red spots. This is because the fish were challenged with *A. hydrophila* so these pathogenic bacteria degrade organ tissues and release toxins that are spread

throughout the body through the bloodstream, causing a reddish color on the fish body. The bacterial attack causes clinical symptoms of hyperemia (redness) after 4 hours in the injection area, then develops into inflammation after 9 hours. The second day after the injection of Kp, there is a former injection of necrosis so that the movement of the fish becomes slow, even silent and the fish can not survive so it dies (Lukistiyowati and Kurniasih in¹⁵.

The content of tannin and saponin compounds in turi leaves when interacting with bacteria, the bacterial wall will break or lysis. Anti-bacterial substances will be able to easily enter into bacterial cells and will interfere with bacterial metabolism so that eventually the bacteria die¹⁶.

According to Nur et al.¹⁷, vitamin C content can accelerate ulcer healing, because vitamin C has anti-inflammatory properties that have the ability as antibiotics and painkillers and stimulate new cell growth in the skin to accelerate the healing process. According to Hasibuan¹⁵, the better the fish's feeding response, the faster the recovery process of the fish's body condition.

Total Erythrocytes of Gourami (*O.goramy*)

The average erythrocytes of gourami at the beginning of the study ranged from 1.21-1.36x10⁶ cells/mm³. The average gourami erythrocytes after 30 days of maintenance ranged from 1.53-1.95 x 10⁶ cells/mm³ and after the challenge test ranged from 1.42-2.51x10⁶ cells/mm³. According to Hastuti & Karoror¹⁸, the number of normal erythrocytes in teleostei fish is about 1.05x10⁶ cells/mm³. The results of analysis of variance (ANOVA) showed that feeding fermented turi leaves with different doses had a significant effect on the total erythrocytes of gourami after being treated for 30 days (p<0.05).

The results of this study indicate that the total number of erythrocytes of gourami fish during 30 days of maintenance has

increased, this is thought to be due to the response of the body's defense system to the entry of flavonoid compounds. P₃ showed higher total erythrocytes compared to other treatments, this is due to the higher response of fish eating. Turi leaf flour fermented using kombucha is preferred by gourami because feed ingredients that undergo the fermentation process

experience the breakdown of complex compounds so that they are easily digested by fish. This follows the statement by Adelina et al.¹⁹, which states that fermentation is a process of increasing the digestibility of materials because fermented materials can convert indigestible plant material substrates into single-cell proteins.

Table 1. Total Erythrocytes of Gouramy (*O. goramy*)

Treatment	Total Erythrocytes (x10 ⁶ cells/mm ³)		
	Day 0	After 30 days of Maintenance	Post challenge day 14th
Kn	1,28	1,53 ± 0,02 ^a	1,75 ± 0,01 ^b
Kp	1,21	1,65 ± 0,01 ^b	1,42 ± 0,02 ^a
P1	1,36	1,89 ± 0,01 ^c	1,95 ± 0,01 ^c
P2	1,35	1,91 ± 0,00 ^c	2,01 ± 0,07 ^c
P3	1,34	1,95 ± 0,01 ^d	2,51 ± 0,04 ^d

Note: *Different superscript in the same column indicates that between treatments are significantly different (P < 0.05)

The total erythrocytes of gourami after the *A.hydrophila* challenge test ranged from 1.42-2.51x10⁶ cells/mm³ still within normal limits indicating an effort to homeostasis in the fish body due to pathogen infection so that the body produces more blood cells to replace erythrocytes that are lysed due to infection²⁰. The lowest total erythrocytes are found in Kp which is 1.42x10⁶ cells/mm³. This is because in Kp fish attacked by *A.hydrophila* bacteria spread toxins throughout the body through blood circulation so that bacterial toxin activity destroys red blood cells formed. Prasetyo et al.²¹ state that the reduced number of erythrocytes in fish is caused by bleeding that occurs due to infection with *A.hydrophila* bacteria that damage the external organs and cause wounds.

Cahyani²² stated that the attack of *A.hydrophila* bacteria can cause damage to organs and blood-forming organs such as the kidneys, resulting in a decrease in erythrocytes. The presence of the hemolysin enzyme in *A.hydrophila* bacteria can lyse red blood cells and white blood cells cause tissue necrosis and cause

infected fish to experience anemia and necrosis or ulcers in infected organs¹⁴.

The highest erythrocyte total was found in P₃ which was 2.51 x 10⁶ cells/mm³. According to Grant²³ shows that feeding fermented turi leaves can improve fish health. The increase in total erythrocytes is thought to be due to the presence of saponin and flavonoid compounds present in active turi leaves in improving health in gourami fish, but saponin compounds can also have a negative impact and cause low total erythrocytes in fish. According to Fajriani et al.¹³ the low total erythrocytes are thought to be due to saponin compounds being able to lyse red blood cells, so the total erythrocytes of gourami in the Kp treatment are lower.

The main function of erythrocytes is to transport Hb and to carry oxygen from the gills to the tissues. In addition to transporting Hb, erythrocytes also contain large amounts of carbonic acid which functions to catalyze the reaction between carbon dioxide and water, so that blood can transport carbon dioxide from tissues to the gills²⁴.

Hematocrit of Gouramy (*O.goramy*)

Calculation of hematocrit values was carried out to see changes in hematocrit that occurred after the maintenance of test fish

by feeding fermented turi leaves with 30 days of maintenance and 14 days after the challenge test. The average hematocrit value can be seen in Table 2.

Table 2. Hematocrit Values of Gouramy (*O.goramy*)

Treatment	Hematocrit Value (%)		
	Day 0	30-day maintenance	Post-challenge test day 14
Kn	26,33	32,33 ± 0,57 ^a	37,67 ± 0,57 ^b
Kp	27,33	35,00 ± 1,00 ^a	34,33 ± 0,57 ^a
P ₁	28,33	37,00 ± 1,73 ^b	38,00 ± 1,00 ^b
P ₂	28,00	37,67 ± 0,57 ^{bc}	39,33 ± 0,57 ^{bc}
P ₃	28,33	39,33 ± 0,57 ^c	40,33 ± 1,52 ^c

Note: *Different superscript in the same column indicates that between treatments are significantly different (P < 0.05)

Based on Table 2. The average hematocrit value of gourami at the beginning of the study ranged from 26.33 to 28.33%. The hematocrit value of gourami after 30 days of maintenance ranged from 32.33-39.33%. According to Dopongtonung²⁵ which states that if the hematocrit value of fish is below 22%, it indicates that the fish is anemic and may have a bacterial infection. The results of the calculation of the hematocrit value of gourami in this study are still within normal limits. According to Riyantono & Putra²⁶, the hematocrit value of freshwater fish ranges from 26-40%. The results of the analysis of variance (ANOVA) showed that feeding fermented turi leaves with different doses had a significant effect on the hematocrit value of gouramy after 30 days of maintenance (p<0.05).

The hematocrit value of gouramy after the challenge test with *A.hydrophila* bacteria ranged from 34.33-40.33%. The highest hematocrit value fed with fermented turi leaves was found in P3 40.33%. While the lowest value of hematocrit was found in Kp 34.33%. The results of analysis of variance (ANOVA) showed that feeding fermented turi leaves with different doses had a significant effect on the hematocrit value of gourami after being treated after the challenge test with *A.hydrophila* bacteria (P <0.05).

The hematocrit value after the challenge test with *A.hydrophila* bacteria on gourami fish increased in all treatments except Kp. This is because the fish were tested with *A.hydrophila* bacteria and were not fed fermented turi leaves so there was an imbalance between bacterial activity and increased fish body resistance which caused bacterial activity to be stronger than the activity of increasing the natural immunity of the fish body so that the fish's body defense was weak due to *A.hydrophila* infection.

The highest hematocrit value was found in P3 (40.33), namely, gourami fish fed with fermented turi leaves and tested for *A.hydrophila* fish are still in normal condition seen from the total erythrocytes which are increasing followed by increased blood hematocrit values. Feeding fermented turi leaves can increase body resistance because fermented turi leaves contain antioxidants that can improve cell structure in the blood. Fajriani et al.¹³ stated that flavonoids act as antioxidants in neutralizing free radicals and play a role in the process of repairing blood cell structures.

The reduction in hematocrit value was also due to anemia in the test fish. Anemia can inhibit the supply of food to fish cells, tissues, and organs due to low blood levels so that the metabolic process of fish becomes inhibited. When

erythrocyte levels decrease, the hematocrit value will also decrease. Farouq²⁷ stated that if the number of erythrocytes is low, the hematocrit value will be low, on the other hand, if the erythrocytes are high, the hematocrit value will also be high. Yulistia et al.²⁸ also stated that there is a correlation between erythrocytes, hematocrit, and hemoglobin where the higher the number of erythrocyte cells, the higher the hematocrit and hemoglobin content in the blood.

The increase in hematocrit value is due to the presence of flavonoids, tannins, and vitamin C content that can trigger the work of blood-producing organs, such as the kidneys to increase hematocrit levels in the blood¹¹. Vitamin C is useful for

strengthening the immune system and can absorb iron from food needed to prevent anemia, Vitamin C acts as an equivalent reduction donor in collagen synthesis (skin tissue constituent protein), iron absorption, and antioxidants¹⁶. Antioxidants play a role in neutralizing free radicals for the blood cell repair process.

Hemoglobin of Gouramy (*O.goramy*)

The calculation of hemoglobin levels was carried out to see changes in hemoglobin that occurred after feeding fermented turi leaves during the study. The results of the measurement of gourami hemoglobin levels during the study can be seen in Table 3.

Table 3. Haemoglobin of Gouramy (*O.goramy*)

Treatment	Haemoglobin Value (g/dL)		
	Day-1	After Treatment (30 Days)	Post Challenge Test (14 Days)
Kn	6,00	8,33 ± 0,58 ^a	8,33 ± 0,11 ^b
Kp	6,67	8,47 ± 0,42 ^a	7,33 ± 0,30 ^a
P1	6,25	9,33 ± 0,76 ^{ab}	9,86 ± 0,11 ^c
P2	6,00	9,67 ± 0,70 ^{ab}	9,86 ± 0,20 ^c
P3	6,33	10,27 ± 0,31 ^b	10,40 ± 0,30 ^d

Note: *Different superscript in the same column indicates that between treatments are significantly different (P < 0.05)

Based on Table 3, it can be seen that the hemoglobin value of gourami fish at the beginning of the study ranged from 6.00-6.75 g/dL. After 30 days of maintenance, the hemoglobin value increased to 8.33-10.27 g/dL. The highest hemoglobin level was found in P3, which was 10.27 g/dL. The lowest hemoglobin level was found in Kn, which was 8.47 g/dL, so it can be stated that the hemoglobin level of gourami in this study is still in the normal range. According to Hastuti & Subandiyono⁹, normal fish hemoglobin ranges from 8-13 g/dL.

The highest hemoglobin level after the challenge test, P3 (10.40 g/dL), increased due to the effectiveness of turi leaves in the diet such as flavonoid and tannin activity which functions as an antioxidant to protect hemoglobin from oxidation. According to Purwanti²⁹, the

more the number of erythrocytes increases, the more hemoglobin level also increases. Haemoglobin physiologically determines the level of endurance of the fish body due to its close relationship with oxygen binding capacity in the blood. The results of the analysis of variance (ANOVA) showed that the provision of fermented turi leaves had a significant effect on the hemoglobin level of gourami fish during 30 days of rearing (p<0.05).

Fish hemoglobin levels are related to anemia and blood cell counts, with rapid increases and decreases in hemoglobin occurring due to infection. The increase in hemoglobin is closely related to the increase in the number of erythrocytes, this condition is due to the increase in iron content and serum concentration in the blood. The increase in hemoglobin levels is thought to be due to the activity of tannins

in turi leaves that function as antioxidants, protecting hemoglobin from oxidation.

Increased hemoglobin in the blood means that food intake and oxygen in the blood can be circulated to all body tissues which in turn can support the survival and growth of fish. high levels of hemoglobin can help in oxygen storage and carry out blood buffer functions in fish³⁰. Based on hemoglobin after the *A.hydrophila* bacteria challenge test, the range was between 7.33-10.40 g/dL. The highest haemoglobin level is found in P3 which is 10.40 g/dL and the lowest is found in Kp 7.33 g/dL. According to Nursatia et al.³² hemoglobin levels after the challenge test decreased this is related to the low erythrocytes and hematocrit. The

decrease in hemoglobin levels in the blood is related to the low value of erythrocytes because the fish blood is lysed due to the rupture of red blood cells due to the presence of bacterial toxins in the fish body called hemolysin. Based on analysis of variance (ANOVA) shows that there is an effect of feeding fermented turi leaves on the hemoglobin value of gourami after being challenged by *A.hydrophila* ($p < 0.05$).

Total Leukocytes of Gourami (*O.goramy*)

Leukocyte observations were made to see changes in the number of leukocytes during the study. The total leucocytes of gourami fish during the study can be seen in Table 4.

Table 4. Total Leukocytes of Gourami (*O.goramy*)

Treatment	Total Leukocytes ($\times 10^4$ cells/mm ³)		
	Day 0	30-day maintenance	Post challenge day 14th
Kn	8,25	8,59 \pm 0,02 ^a	8,90 \pm 0,01 ^a
Kp	8,66	9,61 \pm 0,03 ^a	11,23 \pm 0,11 ^b
P ₁	8,61	8,85 \pm 0,08 ^b	9,38 \pm 0,03 ^c
P ₂	8,56	8,32 \pm 0,03 ^c	9,42 \pm 0,18 ^d
P ₃	8,79	8,46 \pm 0,06 ^d	10,13 \pm 0,07 ^e

Note: *Different superscript in the same column indicates that between treatments are significantly different ($P < 0.05$)

Based on Table 4, it is known that the average number of leukocytes in gourami at the beginning of the study ranged from 8.25-8.79 $\times 10^4$ cells/mm³, at 30 days of maintenance ranged from 8.59-9.61 $\times 10^4$ cells/mm³, and after the challenge test ranged from 8.90-11.23%. According to Hartika et al.³², the number of white blood cells (leukocytes) in normal fish generally ranges from 20,000-150,000 cells/mm³. Leukocytes are blood components that play an important role in fighting infections caused by bacteria, viruses, and toxin metabolic processes.

The highest leucocyte count after the challenge test was found in Kp (11.23 $\times 10^4$ cells/mm³) while the lowest was found in Kn (8.90 $\times 10^4$ cells/mm³). The increase in the number of leucocytes occurs due to the response of the fish body to disease infection, poor environment, and stress

factors. The Kp treatment experienced a significant increase due to the total leucocytes indicating that the fish responded to the presence of foreign bodies entering the blood cells. The high total leucocytes are one of the signs of bacterial infection so fish try to increase the body's resistance to bacterial infection so that the leucocytes move actively to the site of infection.

An increase in the number of leucocytes indicates that the cellular immunity (non-specific immunity) of fish does not improve³³. Total leucocytes in P3 are higher than P1 and P2, due to the presence of flavonoids in turi leaves that respond faster to increase the work of blood-producing organs, so that white blood cell production increases. Flavonoids can increase leukocyte production and trigger the immune system because

leukocytes as eaters (phagocytosis) of foreign objects are more quickly activated¹³.

The increase in total leucocytes indicates that flavonoids can increase leucocyte production and flavonoids trigger the immune system because leucocytes as foreign body eaters (phagocytosis) are activated faster. The mechanism of action of active ingredients, especially flavonoids, in triggering the immune system is to accelerate the activation of leukocytes and macrophages so that the process of phagocytosis of foreign bodies can be carried out in a fast time. Leukocytes are blood cells that play a role in the immune system, leukocytes help rid the body of foreign bodies, including pathogen invasion

through the immune response system. Diseased fish will produce many leucocytes to phagocytose bacteria and synthesize antibodies³⁴.

Phagocytic Activity

Phagocytosis is a normal process in the body but is usually triggered by bacteria entering the body, and the body seeks to defend itself non-specifically. In phagocytosis, macrophages play an important role as well as neutrophil cells, eosinophil cells, and monocytes³⁵. The results of the observation of the phagocytosis activity of gourami leukocyte cells during the study can be seen in Table 5.

Table 5. Phagocytosis Activity of Gouramy (*O.goramy*)

Treatment	Phagocytosis Activity (%)		
	Day 1	After Treatment (30 Days)	Post Challenge Test (14 Days)
Kn	19,67	18,66 ± 0,57 ^a	19,66 ± 0,57 ^a
Kp	19,00	18,66 ± 0,57 ^a	18,66 ± 1,15 ^{ab}
P1	21,33	19,66 ± 0,57 ^{ab}	20,33 ± 0,57 ^b
P2	21,33	20,33 ± 0,57 ^{bc}	20,66 ± 0,57 ^b
P3	22,67	19,73 ± 1,16 ^c	21,13 ± 1,12 ^b

Note: *Different superscript in the same column indicates that between treatments are significantly different (P < 0.05)

Based on Table 5, it is known that the range of phagocytosis activity in gourami at the beginning of rearing ranged from 19.00-22.67%, at 30 days of rearing it became 18.66-20.33%, and after the challenge test ranged from 18.66-21.13%. The phagocytosis activity of gourami after the challenge test with *A.hydrophila* ranged from 18.66-21.13%, where the lowest was in the Kp treatment, namely 18.66%, and the highest was in the P3 treatment, namely 21.13%. The Kp treatment showed a decrease in the number of cells that perform phagocytosis activity, and the P1, P2, and P3 treatments showed an increase. Turi leaf fermentation can stimulate the phagocytic activity of phagocytic cells. According to Utami et al.³⁶, the increased activity of phagocytic cells from the blood indicates

that the immune system or body defense of the fish also increases.

The increase in the percentage of phagocytosis activity is due to the flavonoid compounds contained in turi leaf fermentation. This is because flavonoid compounds have the potential to work on lymphokines produced by T cells so that they will stimulate phagocytic cells to carry out phagocytic responses³⁷. According to Nurhayati et al.³⁸, an increase in the percentage of phagocytosis activity indicates an increase in leukocyte cells. The antioxidant content of flavonoids can also improve the performance of the immune system. According to Hurriyani³⁹ antioxidants from flavonoids are known to be able to increase phagocytosis activity which is a function of non-specific immune response. The antioxidant mechanism in

flavonoids in increasing phagocytosis activity is by inhibiting the release of oxygen anions and releasing free radicals. Tannin compounds are also the cause of the increase in the percentage of phagocytosis activity. Tannin compounds can stimulate phagocytic cell activity, as well as polyphenols and flavonoids, these compounds are destructive to the cell walls of invading bacteria⁴⁰. These potential compounds will make it easier for phagocytic cells to perform their function in phagocytosing antigens due to the opsonin function. Opsonin is a substance that functions to increase phagocytosis activity (Jawetz *et al.* In⁴¹.

The results of this study indicate that the addition of fermented turi leaves to fish feed can increase phagocytosis activity. That phagocytosis activity increases in line with the increasing dose of fermented turi leaves added, this indicates that a body defense system has been formed in gourami.

Phagocytosis is the first step for the next immune response mechanism, namely the formation of a specific response in the form of antibodies, this can be seen in the treatment of P₃ there is an increase in immune response indicated by an increase in the population of leukocytes and phagocytosis activity, this is also shown by closing the injection wound in gourami.

According to Utami *et al.*³⁶, phagocytosis of pathogens through the activity of the process of swallowing, destruction, and digestion of pathogens, There are three main phases in phagocytosis, namely the attachment of pathogens to the surface of blood cells, swallowing through phagosome formation and destruction of particles in phagosomes, Attachment of a pathogen to the phagocyte membrane is a prerequisite for facilitating and usually relatively passive.

Calculation of phagocytosis activity is done to see the ability of leucocyte cells to eat foreign bodies, especially bacterial attacks. This is by Sulistriani *et al.*⁴² that when there is contact from the pathogen

with the surface of the cell membrane phagocytosis cells then undergo imagination where two cytoplasmic arms swallow particles so that they are trapped in the cytoplasm which is located in a membrane-coated vacuole (phagolysosome).

Feeding containing fermented turi leaves can increase phagocytosis activity because the elements contained in fermented turi leaves such as flavonoids, saponins, and tannins can activate cells and macrophages directly and flavonoid compounds can increase phagocytosis activity and stimulate immunity and produce antibodies⁴³.

Survival of Gourami (*O. goramy*)

The lowest survival rate of gourami fish is in the Kp treatment (27%) this is because, in the Kp treatment, the fish are challenged with *A. hydrophila* bacteria and are not fed fermented turi leaves so the fish experience stress due to *A. hydrophila* bacteria that spread throughout the body of the fish becoming weak so that they experience death (Figure 2).

The highest survival rate is found in the negative control treatment and P₃, namely 100% and 90%, the high survival rate in the P₃ treatment is due to the active compounds contained in turi leaves, namely flavonoids that can increase the immune system of striped catfish. The increase in the immune system of fish treated with turi leaves is known from the survival value of fish at the end of the study which is higher than those not treated with a low survival rate¹³.

Gourami fry can utilize feed well so that it can produce high growth compared to the treatment that does not give turi leaves. This is due to the enzyme activity that occurs in the fermentation process of turi leaf flour with Kombucha fermentation so that it can produce nutrients that are simpler and easier to digest for gourami fish seeds. This is by Adelina *et al.*¹⁹ which states that fermentation is a process of increasing the digestibility of materials

because fermented materials can convert indigestible plant material substrates into single-cell proteins. Fermentation in fish feed makes the nutritional content of the feed better and easily utilized by fish. The easier the fish utilize the feed given, the higher the protein absorbed by the fish body for growth. According to Robiansyah et al.⁴⁴ the higher the digestibility of feed consumed by fish, the higher the available nutrients that can be absorbed by the fish body, and the fewer nutrients are wasted

through feces so that fish can meet their survival needs, repairing body tissues and for better growth. According to Munisa et al.⁴⁵, the content of vitamin C that enters the feed can increase and normalize the immune system to prevent stress on fish seeds. The increase in the immune system of fish treated with fermented turi leaves is known from the value of fish survival at the end of the study higher than those not treated have a low survival rate¹³.

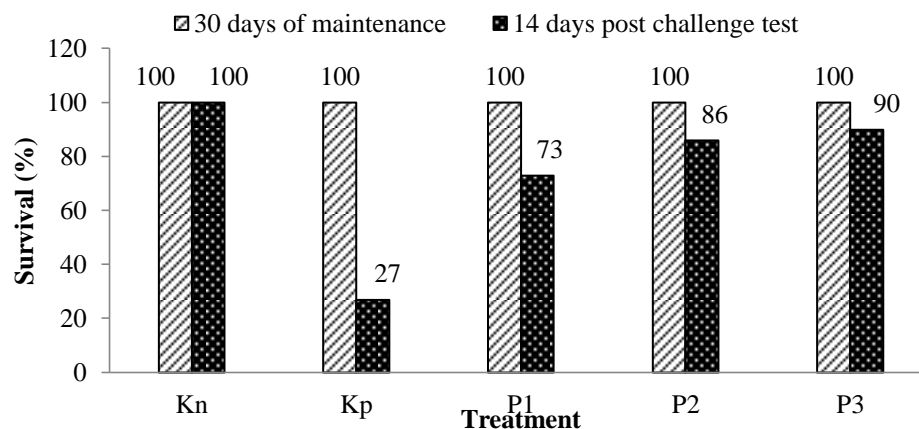


Figure 2. Survival Rate of Gourami (*O.goramy*)

Based on the results of the analysis of variance (ANOVA) test, it shows that feeding fermented turi leaves is significantly different from the survival of gourami ($P < 0.05$). The addition of fermented turi leaf feed infected with *A.hydrophila* can increase the survival rate to 100%. According to Kordi⁴⁶ fish survival can be influenced by biotic and abiotic factors. Biotic factors consist of age and the

ability of fish to adapt to the environment. Abiotic factors include food availability and quality of living media.

Absolute Weight Growth of Gourami (*O.goramy*)

The results of the measurement of the growth rate of gourami fish reared with feed containing fermented turi leaves can be seen in Table 6.

Table 6. Results of Absolute Weight Measurement of Gourami (*O.goramy*)

Treatment	Initial (g)	End (g)	Absolute weight (g/head) \pm SD
Kn	14,62	21,47	6,65 \pm 0,09 ^a
Kp	14,68	21,44	6,43 \pm 0,19 ^{ab}
P ₁	15,13	21,34	6,95 \pm 0,02 ^b
P ₂	14,83	21,96	7,77 \pm 0,03 ^c
P ₃	15,04	24,39	9,52 \pm 0,29 ^d

Note: *Different superscript in the same column indicates that between treatments are significantly different ($P < 0.05$)

Based on Table 6, it is known that the average weight value of gourami at the beginning of rearing was 14.62-15.13

g/fish. After 46 days of maintenance by giving feed containing fermented turi leaves, the average weight value of gourami

fish has increased, namely 21.34-24.39 g/fish. The highest absolute weight growth was found in the P₃ treatment, namely 9.52 g/fish, and the lowest in the Kp treatment, namely 6.43 g/fish. The maintenance of gourami given feed containing fermented turi leaves affects the growth of gourami weight.

This is due to the enzyme activity that occurs in the fermentation process of turi leaf flour with Kombucha fermentation so that it can produce simpler and easily digestible nutrients for gourami fish seeds. This is by the statement Adelina et al.¹⁹

which states that fermentation is a process of increasing the digestibility of materials because fermented materials can convert indigestible plant material substrates into single-cell proteins.

Water Quality

Water quality can affect fish health conditions if water conditions are not by fish needs. The measured water quality parameters include temperature, pH, dissolved oxygen (DO), and ammonia (NH₃). The range of gourami water quality during the study can be seen in Table 7.

Table 7. Water Quality of Gourami (*O. gouramy*)

Parameters	Initial	End	Quality Standard ⁴⁶
Temperature (° C)	27 - 28	27 - 30	24 - 30
pH	5,8 - 6,2	6,4 - 7	6 - 7,34
DO (ppm)	5,5-5,9	5,5 - 5,8	4,00 - 5,95
NH ₃ (mg/L)	0,015 - 0,017	0,020 - 0.023	0,015 - 0,028

The water quality results obtained during the 46 days of research were classified as good because these figures met the standard values for the life of gourami. The temperature obtained during the study ranged from 27-30°C and was still in the optimal range. This is following the opinion of Asis et al.⁴⁷ that the optimum water temperature for the growth of gourami is 24-28°C. The temperature difference that does not exceed 10°C is still classified as good and the temperature range is good for organisms in the tropics 25-32°C. Temperature greatly affects fish growth because temperature affects the appetite of gourami. Temperature greatly affects fish growth because temperature affects fish appetite. If the temperature increases but is within the tolerance limit, it will increase the metabolic rate so that the fish's appetite can also increase but if the temperature changes drastically, it will cause fish stress so that it can reduce fish appetite and be susceptible to disease⁴⁸.

Dissolved oxygen at the beginning of the study ranged from 5.5-5.9 ppm and the end of the study ranged from 5.5-5.8 ppm, this condition indicates that the dissolved

oxygen content is still within the normal range (4.0-5.95 ppm). pH is one of the chemical properties of water that affects the life of aquatic organisms, so it can be used as a good or bad indication of the state of a body of water. pH is also a limiting factor that affects and determines the speed of metabolic reactions in fish which in turn will affect fish growth⁴⁹. If the pH is low, the dissolved oxygen content will decrease, as a result, oxygen consumption decreases, respiratory activity increases and appetite will decrease so that growth will be inhibited and fish are susceptible to infection with bacteria and parasites⁵⁰.

Ammonia levels at the beginning of the study ranged from 0.015-0.017 mg/L and at the end of the study ranged from 0.020-0.023 mg/L. This ammonia range is not too high and is good for fish farming. According to Sutama⁵⁰, the ammonia content that is good for fish farming is <1 mg/L.

4. CONCLUSION

Based on the results of the study, it can be concluded that there is an effect of adding fermented turi leaves in feed on the

hematology of gourami before and after the *A. hydrophila* bacterial challenge test. The best dose in this study was treatment P₃ (Fermented feed containing 45% turi leaves, 55% soybean flour and tested with *Aeromonas hydrophila* bacteria) with the best results with total erythrocytes 2.51×10^6 cells/mm³, hematocrit value 40.33%,

hemoglobin level 10.40 g/dL, total leucocytes 10.13×10^4 cells/mm³, phagocytosis activity 21.13%, survival 90% and absolute weight growth 9.52 g/head. Water quality during the study was temperature ranging from 27-30⁰ C, pH 5.8-6.2, DO 5.5-5.9 mg/L, and NH₃ 0.015-0.017 mg/L.

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