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THE EFFECT OF *Moringa oliefera* LEAF FERMENTED ENRICHED PELLETS ON HEMATOLOGY OF *Pangasianodon hypophthalmus* REARED IN PHOTOPERIOD SYSTEM

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ABSTRACT

Moringa leaf (*Moringa oleifera*) is a herbal plant that has the potential to inhibit bacterial growth and increase immune response. This research was conducted from April to July 2024 at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau. This study aims to obtain the best dose of fermented moringa leaf added to feed to the immunity of Siamese striped catfish reared on a photoperiod system and tested with *A. hydrophila*. The research method is the experimental method applying a completely randomized design (CRD) with four treatments and three replicates. Treatment 0 without adding fermented moringa leaves, P1: 10 g/kg feed, P2: 15 g/kg feed, and P3: 20 g/kg feed and challenged with *A. hydrophila*. The test fish samples measuring 6-7 cm were reared using 100 L buckets of 12 containers with a 30 fish/container stocking density. Feeding was done ad satiation with a frequency of 3 times daily at 08.00, 13.00 and 17.00 WIB. The results showed that adding fermented moringa leaf to the feed affected the immunity of striped catfish. The best dose was found in P1 (10 g/kg feed) with total erythrocytes 2.75 x 10^6 cells/mm^3, hemoglobin level 8.80 g/dL, hematocrit level 34.00%, and total leukocytes 10.86 x 10^4 cells/mm^3, leukocrit level 3.33%, lymphocytes 76.00%, monocytes 7.33%, neutrophils 7.33%, Thrombocytes 9.33%, absolute weight growth 15.02 g/fish, absolute length growth 6.88 cm/fish, 96.67% survival rate. Based on the study's results, feeding added fermented moringa leaves has a significant effect and can improve the immunity of *Pangasinodon hypophthalmus*.

Keywords: *Motile Aeromonas Septicaemia*, Striped Catfish, Aquaculture.

1. INTRODUCTION

Striped catfish (*Pangasianodon hypophthalmus*) is a fish favored by the public because it has economic value, is easy to maintain, exports commodities to foreign countries, and has high demand in the domestic market[1]. To meet these market needs can be done with an intensive cultivation system that cannot be separated from the need for feed and high stocking density, which can cause fish to experience stress so that they are susceptible to disease. One of them is Motile Aerominas Septicaemia[2].

Motile Aeromonas Septicaemia (MAS) disease has symptoms such as hemorrhage, ulcer, abdominal swelling, and loose fins, which can cause mass mortality reaching 80-100% within 1-2 weeks[3]. An *Aeromonas hydrophila* infection in striped catfish occurred in 2019 in Kampung II, Koto Masjid Village, XIII Koto Kampar District[4].

Efforts to control *A. hydrophila* infection can be carried out using antibiotics, such as kanamycin oxytetracycline, oxolinic acid, erythromycin, and streptomycin. However, the continuous use of antibiotics can hurt the

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aquatic environment, and pathogen resistance and its residues can accumulate in waters and humans[5]. Currently, the utilization of herbal plants as an alternative to antibiotics for the control of *A. hydrophila* infection has been widely carried out, one of which uses Moringa leaf (*Moringa oleifera*).

Moringa leaves can inhibit bacterial growth and increase immune response. This is because Moringa leaves contain flavonoids, quercetin, kaempferol, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, vitamins (A, C, E, and K) and there are also alkaloids, anthraquinones, triterpenoids that are good for fish growth[6].

Striped catfish maintenance with 24-hour dark photoperiod manipulation is one alternative maintenance system that can be applied because striped catfish are classified as nocturnal fish that actively move and look for food at night[7]. Light can affect feeding patterns through variations in intensity, wavelength, and polarization; the higher the active period of striped catfish in searching for food, the more food consumed and the higher the growth rate[8].

Based on the description above, research on "The effect of giving fermented Moringa leaf in feed on the hematology of striped catfish in photoperiod system maintenance" needs to be done. This study aims to obtain the best dose of fermented Moringa leaf added to feed on the immunity of striped catfish reared with a photoperiod system and tested with *A. hydrophila*.

2. RESEARCH METHOD

Time and Place

This research was conducted from April to October 2024 at the Integrated Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Riau.

Methods

The research method used in this study is an experimental method that applies a one-factor, Completely Randomized Design (CRD), namely, the dose of fermented moringa leaf in feed with four levels of treatment. Each treatment was repeated 3 times to minimize the error level so that 12 experimental units were obtained. The treatment dose refers to Annavi et al.[9], namely:

- P0 : Feed that is not given fermented moringa leaf
- P1 : Feed given fermented moringa leaf at a dose of 10 g/kg feed.
- P2 : Feed given fermented moringa leaf at a dose of 15 g/kg feed.
- P3 : Feed given fermented moringa leaf at a dose of 20 g / kg feed.

Procedures

Preparation of Fermented Moringa Leaf

The test material used was Moringa leaf obtained from the Traditional Market on Jl. HR. Soebrantas, Tuah Madani District, Pekanbaru City. The fermentation process was conducted at the Biotechnology Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Riau. Moringa leaves were cleaned and separated from the petioles. Next, moringa leaves were boiled for 8-10 minutes and dried for 30-60 minutes. Next, moringa leaf was added with *Rhizopus* sp. in the ratio of 1 kg of dried moringa leaf and 1 g of yeast and then stirred until homogeneous, after which it was put into a plastic seal that had been given a hole to ferment for 2-3 days.

The successful fermentation process is characterized by the growth of hyphae on the surface of the dough. After that, the fermented moringa leaf is steamed to stop the development of the fungus, and then the fermented moringa leaf is cut into small pieces and dried in the sun for 3 days to dry, then mashed using a blender and sieved with a sieve that has holes with a diameter of 60 mesh (0.25 mm) to become fermented moringa flour. Furthermore, the flour can be directly mixed into commercial feed. Mixing fermented moringa leaf using the counting technique, namely, using tapioca flour 20 g/kg, which has been dissolved with 100 mL of boiling water as an adhesive glue for moringa leaf and feed, after mixing the feed is dried in the sun for 4-8 hours to dry, then the feed can be given to the fish.

Fish Blood Collection

Blood collection of test fish was carried out 3 times, namely at the beginning of the study, the 60th day of maintenance, and the 76th day (14 days post-challenge) with *A. hydrophila*. Fish samples were taken as many as three fish from each treatment; before blood collection, a 1 mL syringe and Eppendorf tube were moistened with 10% EDTA. Fish were anesthetized with clove oil at a dose of (1 L water: 0.1 mL clove oil); fish blood was taken using a syringe at the caudal vein, blood taken as much as 0.4 mL and put into an Eppendorf tube and put into a cool box. Blood observations calculate total erythrocytes, hemoglobin levels, hematocrit values, total leukocytes, and differentiation.

Aeromonas hydrophila Challenge Test

Striped catfish were challenged on day 62 with *A. hydrophila* at 10^8 CFU/mL density, as much as 0.1 mL/fish, by intramuscular injection using a 1 mL syringe. Before the fish were infected, they were first anesthetized using clove oil. After infection, fish were returned to the container and maintained for 14 days.

3. RESULT AND DISCUSSION

Total Erythrocytes

Total erythrocytes of striped catfish at the beginning of rearing, 60th day of rearing with feed supplemented with fermented Moringa leaves, and post-challenge with *A. hydrophila* are presented in Table 1.

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

Treatment	Total Erythrocytes (x 10^6 sel/mm^3		
	Start (0 Day)	60th day of maintenance	14 days Post-challenge
P0	2,06 ± 0,05	2,54 ± 0,02^a	3,07 ± 0,06^c
P1	2,06 ± 0,05	2,75 ± 0,09^b	2,76 ± 0,05^b
P2	2,06 ± 0,05	2,55 ± 0,06^{ab}	2,66 ± 0,02^b
P3	2,06 ± 0,05	2,63 ± 0,10^a	2,55 ± 0,07^a

Note: Different *superscript* letters in the same column indicate that the treatment differs significantly ($P < 0.05$); ± Standard Deviation (SD).

Table 1 shows that the total erythrocytes of striped catfish at the beginning of maintenance is 2.06 x 10^6 cells/mm^3. After the 60th day of maintenance with feed added with fermented moringa leaves, the total erythrocytes ranged from 2.54 - 2.75x 10^6 cells/mm^3. The best erythrocyte total in this study is P1, which is 2.75 x 10^6 cells/mm³, while for the same treatment on the addition of moringa leaf flour to the feed, it is 2.13 x 10^6 cells/mm^3[10]. According to Syawal et al.[11], the number of normal erythrocytes in striped catfish ranges from 1.91 - 2.81 x 10^6 cells/mm^3.

After the 60th day of maintenance, the total erythrocytes increased from the beginning of maintenance; this is due to the high appetite of the fish, and the response to the feed given is good; besides that, it is also influenced by environmental factors that support fish growth. Addini et al.[12] states that the increase in erythrocyte levels in the blood indicates that the oxygen content in the blood increases and that it begins to adapt to the environment. Supported by Zissalwa et al.[13], factors that affect the number of erythrocytes are species, parent differences, feed nutrition, size, physical activity, age, and environment.

The total number of erythrocytes added with fermented moringa leaves after being challenged with *A. hydrophila* ranged from 2.55 - 3.07 x 10^6 cells/mm^3, while the normal total erythrocytes of striped catfish without being given additional food and without being challenged are 2.01 x 10^6 cells/mm^3[10]. The best erythrocyte total in this study is P1, which is 2.76 x 10^6 cells/mm^3; compared to the study of Arnoli[10], the erythrocyte total is 2.49 x 10^6 cells/mm^3. The lower total erythrocytes in

Arnoli's[10] study were due to the protein content in fresh Moringa leaves of only about 5.1% per 100 g. In contrast, protein levels can increase significantly after fermentation, reaching 29.69% per 100 g[14]. Increased protein levels can support hemoglobin synthesis and increase the number of erythrocytes[15].

Aeromonas hydrophila infection can cause erythrocyte counts higher than the normal limit in striped catfish, which is 1.91 - 2.81 x 10^6 cells/mm^3. As in P0, which has total erythrocytes that are outside the normal limits of striped catfish. These bacteria infect fish through wounds or the digestive tract, then enter the bloodstream and cause an immune response that increases the number of erythrocytes. Bacterial infection can also cause oxidative stress as the fish is injured, encouraging the fish body to produce more erythrocytes as compensation[16]. After a challenge test with *A.hydrophila*, erythrocytes will increase, indicating the body's response to stress. Erythrocytes carry oxygen throughout the body, which is important to maintain adequate oxygen availability during the body's fight against infection[11].

This increase in total erythrocytes indicates a homeostatic effort in the fish body (pathogen infection). The body produces more blood cells to replace erythrocytes that are lysed due to illness[17]. The fermentation process of moringa leaves increases the bioactive availability of certain compounds, such as polyphenols, flavonoids, and other antioxidant compounds, which help improve the immune response. Flavonoids function as antioxidants, protecting body cells from oxidative damage and impacting the fish's health. According to Fatmawati[18], flavonoids, antioxidants in the polyphenol group, can capture free radicals, act as anti-inflammatories, prevent oxidative damage to cells, and protect body cells from oxidation.

While the decrease in total erythrocytes is thought to be caused by excessive tannin and saponin content, this is because tannins can bind iron and inhibit enzyme activity, which has an impact on reducing erythrocyte production. While saponins are hemolytic, which can damage the membrane of red blood cells (erythrocytes), it is the opinion of Septiana[19] that saponin compounds in fermented moringa leaves can lyse erythrocytes. The higher the dose of fermented moringa leaves, the more toxic they are to fish. In addition, *A. hydrophila* infection decreases the immune system of fish. Furthermore, according to Lase et al.[20], *A. hydrophila* infection produces enzymes that can lyse erythrocytes and disrupt the performance of blood-producing organs so that they cannot produce erythrocytes to replace erythrocytes that come out due to *A. hydrophila* infection.

Hemoglobin Levels

Hemoglobin levels after the 60^{th} day of maintenance with feed-added fermented moringa leaves and post-challenge with *A. hydrophila*. The hemoglobin levels of striped catfish during the study can be seen in Table 2.

Table 2. Hemoglobin level of striped catfish (*P. hypophthalmus*)

Treatment	Hemoglobin Level (g/dL)		
	Start (0 Day)	60^{th} day of maintenance	14 days Post-challenge
P0	7,33 ± 0,30	8,00 ± 0,20^a	9,20 ± 0,11^d
P1	7,33 ± 0,30	8,80 ± 0,20^b	8,53 ± 0,11^c
P2	7,33 ± 0,30	8,33 ± 0,11^a	8,06 ± 0,11^b
P3	7,33 ± 0,30	8,13 ± 0,11^a	7,66 ± 0,11^a

In Table 2, it is known that the hemoglobin level of striped catfish at the beginning of maintenance is 7.33 g/dL. After the 60^{th} day of maintenance, feed-added fermented moringa leaves ranging from 8.00 - 8.80 g/dL will be given. The best hemoglobin level in this study, P1, is 8.80 g/dL. According to Suriyadin et al.[21], normal hemoglobin levels in striped catfish range from 7 - 9 g/dL.

Hemoglobin levels after the 60th day of maintenance have increased; this is thought to be caused by the high appetite of fish so that the process of circulating oxygen in the blood runs smoothly. According to Syawal et al.[11], high hemoglobin levels can help store oxygen and support blood in fish. Hemoglobin levels in fish blood determine the level of fish body resistance related to oxygen binding capacity in the blood[22].

Table 2 shows post-challenge hemoglobin levels with *A. hydrophila* ranged from 7.66 - 9.20 g/dL, with the best treatment being P1 at 8.53 g/dL. Meanwhile, according to Arnoli[10], hemoglobin levels with the same dose were 9.73 g/dL. Hemoglobin levels of striped catfish that are not tested and not given additional feed are 8.33 g/dL. Hemoglobin levels in P1, P2, and P3 decreased; this is thought to be due to fish bleeding. According to Hasibuan et al.[22], the blood of injured fish will experience lysis, which is caused by the rupture of red blood cells due to the presence of a bacterial toxin in the blood called hemolysin. This toxin will lyse hemoglobin and release hemoglobin in the blood. *A. hydrophila* infection can also lead to low hemoglobin levels in the blood, causing stress to the fish and damaging the immune system, which can affect hemoglobin production and stability.

Whereas P0 experienced an increase in hemoglobin levels, which is thought to be caused by stress factors that can trigger the release of stress hormones such as cortisol, which stimulates the release of erythrocytes from storage organs such as the spleen into the blood circulation. This increase in the number of erythrocytes directly contributes to an increase in hemoglobin levels because hemoglobin is the main component in erythrocytes that functions to transport oxygen[20] and added by Hasibuan et al.[22]; there is a relationship between erythrocytes, hematocrit, and hemoglobin where the higher the number of erythrocyte cells, the higher the hematocrit, and hemoglobin content in the blood.

Hematocrit Values

Hematocrit values at the beginning of rearing, after the 60th day of rearing with feed added with fermented moringa leaves, and post-challenge with *A. hydrophila*. The hematocrit values of striped catfish during the study can be seen in Table 3.

Table 3. Hematocrit value of striped catfish (*P. hypophthalmus*)

Treatment	Hematocrit Value (%)		
	Start (0 Day)	60th day of maintenance	14 days Post-challenge
P0	30,33 ± 0,57	32,33 ± 1,52	37,66 ± 0,57^{b}
P1	30,33 ± 0,57	34,00 ± 1,00	32,33 ± 0,57^{a}
P2	30,33 ± 0,57	33,00 ± 1,00	31,33 ± 0,57^{a}
P3	30,33 ± 0,57	31,66 ± 0,57	30,33 ± 0,57^{a}

Table 3 shows that the hematocrit value of striped catfish (at the beginning of maintenance) is 30.33% after the 60th day of maintenance by giving feed added fermented moringa leaves ranging from 31.66 - 34.00%. The best hematocrit value in this study is P1, namely 34.00%, while the hematocrit value, according to Arnoli[10], with the same dose, is 31.33%. According to Suriyadin et al.[21], the normal hematocrit value of striped catfish ranges from 30-44%.

The hematocrit value has increased; this is thought to be caused by fish appetite, which increases in line with the fish's weight, length, and age. Factors affecting hematocrit are nutrition, infection, fish weight, and length. This is in line with the opinion of Maryani et al.[23] that fish hematocrit varies depending on nutritional factors and fish age. According to Hasibuan et al.[22], hematocrit measures the ratio between erythrocytes and plasma.

Table 3 shows post-challenge hematocrit values with *A. hydrophila* ranged from 30.33 - 37.66%, with hemoglobin levels at P1, 32.33%. The normal hematocrit value of striped catfish without any addition to the feed was not tested, namely 32.00[10]. Hematocrit values in P1, P2, and P3 decreased; this is thought to be caused by low total erythrocytes. The challenge test process using *A. hydrophila* causes an imbalance between bacterial activity and increased fish immunity. According to Suriyadin et al.[21], lower hematocrit values indicate fish are anemic and stressed due to bacterial attacks.

According to Zissalwa et al.[13], such stress can result in the release of stress hormones such as cortisol, which can affect the production of red blood cells and increase the metabolic rate of fish, which can reduce the number of red blood cells in the blood circulation and cause a decrease in hematocrit value.

A decrease in fish's hematocrit shows symptoms of anemia, which affects the growth and health of fish. A low hematocrit makes oxygen transportation in the fish body impaired. As a result, oxygen supply to cells, tissues, and vital organs is reduced, resulting in impaired energy metabolism and new tissue synthesis. This condition directly impacts feed efficiency, growth ability, and fish immune response[13]. According to Maryani et al.[23], anemia disrupts nutrient and oxygen transport throughout the body, which impacts fish metabolism and causes physiological stress. This condition worsens fish health and increases the risk of pathogen infection due to a decreased immune system.

Total Leukocytes

Total leukocytes at the beginning of maintenance, after the 60th day with feed, added fermented moringa leaves, and post-challenge. The total leukocytes of striped catfish during the study can be seen in Table 4.

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

Treatment	Total Leukocytes (x 10^4 sel/mm^3)		
	Start (0 Day)	60th day of maintenance	14 days post-challenge
P0	8,55 ± 0,11	9,53 ± 0,16^a	6,56 ± 0,19^a
P1	8,55 ± 0,11	10,86 ± 0,08^c	10,00 ± 0,20^c
P2	8,55 ± 0,11	10,81 ± 0,06^c	9,44 ± 0,09^b
P3	8,55 ± 0,11	10,41 ± 0,15^b	9,33 ± 0,10^b

In Table 4, it is known that the total leukocytes of striped catfish at the beginning of maintenance are 8.55 x 10^4 cells/mm^3. After the 60th day of maintenance by giving feed added fermented moringa leaves ranging from 9.53 - 10.86 x 10^4 cells/mm^3. The best total leukocytes in this study, P1, is 10.86 x 10^4 cells/mm^3, while according to Arnoli[10], the total leukocytes with the same dose is 10.15 x 10^4 cells/mm^3. According to Yusuf et al.[24], the number of white blood cells (leukocytes) in normal striped catfish generally ranges from 6.95 to 11.9 x 10^4 cells/mm^3.

Total leukocytes after 60 days of maintenance have increased; this is thought to be caused by the response of the fish body to environmental conditions, fish weight, and length. A very high increase in the total number of leukocytes indicates an autoimmunity abnormality in the fish body. Meanwhile, the decrease in the total number of leukocytes is due to disturbances in the function of the kidney and lymph organs in producing leukocytes caused by infectious diseases.

After being challenged with *A. hydrophila* for 14 days, total leukocytes ranged from 6.56 - 10.00 x 10^4 cells/mm^3.

The best dose in P1 is 10.00 x 10^4 cells/mm^3. As for normal total leukocytes without being fed additional food or tested, namely, 8.56 x 10^4 cells/mm^3[10].

P0 experienced a fairly high decline; this is likely due to the unsuccessful immune system of fish in developing cellular immune responses as a trigger for immune responses. The decrease that occurred was caused by leukocytes in the blood vessels being greatly reduced because most of the leukocytes moved toward infected tissues to eliminate pathogens and reduce damage to the fish's body. This agrees with Kurniawan et al.[25] that the decrease in leukocytes after the challenge test is due to the leukocytes being active and leaving the blood vessels toward infected tissues. According to Yusuf et al.[24], the fish body reads that the incoming vaccine is an antigen; the body produces leukocytes and forms antibodies.

Leukocyte Levels

Leukocyte levels at the beginning of maintenance, after the 60th day of maintenance with feed, added fermented moringa leaves, and post-challenge with *A. hydrophila*. The total leukocyte of striped catfish during the study can be seen in Table 5.

Table 5. Leukocrit value of striped catfish (*P. hypophthalmus*)

Treatment	Leukocrit value (%)		
	Start (0 Day)	60th day of maintenance	14 days post-challenge
P0	2,33 ± 0,57	3,33 ± 0,57[a]	1,66 ± 0,57[a]
P1	2,33 ± 0,57	3,33 ± 0,57[a]	2,66 ± 0,57[a]
P2	2,33 ± 0,57	3,33 ± 0,57[a]	2,66 ± 0,57[a]
P3	2,33 ± 0,57	2,66 ± 0,57[a]	2,66 ± 0,57[a]

Table 5 shows that the leukocrit value of striped catfish at the beginning of maintenance is 2.33%, and after the 60th day of maintenance, by giving feed, added fermented moringa leaves ranging from 2.66 - 3.33%.

According to Sari et al.[16], the normal leukocrit value of striped catfish ranges from 1-3%. Meanwhile, the leukocyte value in striped catfish after the challenge test is 1.66 - 2.66%; leukocytes are one component of blood cells that function as a non-specific defense that will localize and eliminate pathogens[26]. The weakened pathogenicity of bacteria, because it has passed the stationary phase of life, causes leukocytes to begin to be produced again to restore the health condition of the fish[16].

The percentage of leukocrit in the blood can be used as an indication of the health status of fish. Low leukocrit can be caused by chronic infection, low nutritional quality, and contaminants. Low leukocrit can also be caused by initial infection and stress. Decreased leukocyte values can indicate immunosuppression, where the immune system is not functioning optimally. This can be caused by excessive stress, chronic illness, or a particularly severe infection. Exposure to pathogens or environmental stress conditions such as temperature changes, poor water quality, or physical handling can cause fish to experience physiological stress[21]. Addini et al.[12] show that this stress can result in changes in leukocyte distribution, including a decrease in the number of leukocytes in the blood circulation due to redistribution to other tissues or a decrease in leukocyte production.

Leukocyte Differentiation

Leukocyte differentiation at the start, after the 60th day of maintenance, and post-challenge with *A. hydrophila*. The leukocyte differentiation of striped catfish during the study can be seen in Table 6.

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

Treatment	Leukocyte Differentiation (0 Day)			
	Lymphocytes	Monocytes	Neutrophils	Thrombocytes
P0	70,33 ± 0,57^{a}	10,33 ± 0,57^{a}	9,00 ± 1,00^{a}	10,33 ± 0,57^{a}
P1	70,33 ± 0,57^{a}	10,33 ± 0,57^{a}	9,00 ± 1,00^{a}	10,33 ± 0,57^{a}
P2	70,33 ± 0,57^{a}	10,33 ± 0,57^{a}	9,00 ± 1,00^{a}	10,33 ± 0,57^{a}
P3	70,33 ± 0,57^{a}	10,33 ± 0,57^{a}	9,00 ± 1,00^{a}	10,33 ± 0,57^{a}
Treatment	Leukocyte Differentiation (60th day of maintenance)			
	Lymphocytes	Monocytes	Neutrophils	Thrombocytes
P0	72,33 ± 0,57^{a}	9,00 ± 1,00^{a}	9,00 ± 1,00^{a}	9,66 ± 0,57^{a}
P1	76,00 ± 1,00^{b}	7,33 ± 1,15^{a}	7,33 ± 0,57^{b}	9,33 ± 0,57^{a}
P2	74,00 ± 1,00^{a}	8,66 ± 0,57^{a}	8,33 ± 0,57^{a}	9,00 ± 1,00^{a}
P3	73,00 ± 1,00^{a}	9,00 ± 1,00^{a}	9,00 ± 1,00^{a}	9,00 ± 1,00^{a}
Treatment	Leukocyte Differentiation **(**14 days Post-challenge)			
	Lymphocytes	Monocytes	Neutrophils	Thrombocytes
P0	58,00 ± 4,00^{a}	18,66 ± 2,51^{b}	14,33 ± 1,15^{b}	9,00 ± 1,00^{a}
P1	75,00 ± 1,00^{b}	8,33 ± 0,57^{a}	8,33 ± 0,57^{a}	9,33 ± 0,57^{a}
P2	73,33 ± 0,57^{b}	9,33 ± 0,57^{a}	8,33 ± 0,57^{a}	9,00 ± 1,00^{a}
P3	71,00 ± 1,00^{b}	9,66 ± 1,15^{a}	9,00 ± 1,73^{a}	9,66 ± 0,57^{a}

In Table 6, the number of lymphocytes at the beginning of maintenance was 70.33% after 60 days of maintenance ranged from 72.33 - 76.00%, with the highest number of lymphocytes in P1, namely, 76.00%, while in Arnoli[10], in the same treatment, namely, 83.33%. According to Puspitowati et al.[27], the number of lymphocytes in striped catfish ranges from 73.00 - 80.33%. Meanwhile, according to Kurniawan et al.[25], the number of striped catfish lymphocytes ranged from 66.67 - 77.67%.

Lymphocytes are a type of white blood cell that plays a role in the adaptive immune response, which is involved in 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 in this study after challenge ranged from 58.00 - 75.00%, with the best lymphocytes in P1, 75.00%. According to Arnoli[10], the % of lymphocytes with the same treatment is 83.33% as for the number of lymphocytes in striped catfish that are not given additional feed and are not tested, namely, 79.00%.

P0 has the lowest number of lymphocytes; it is suspected that fish are less able to adapt to incoming bacteria, while P1, P2 and P3 can adapt so that they can form antibodies and produce lymphocytes normally. According to Puspitowati et al.[27], if the intensity of a pathogen infection increases, the need for white blood cells or lymphocytes will increase because the need for white blood cells increases. The number of lymphocytes, the agent cells that provide the body's immune substances, will decrease.

Monocytes are a type of white blood cell that plays a role in phagocytosis (a process in which cells engulf large particles, including pathogens and dead cells)[28]. The number of monocytes at the beginning of maintenance was 10.33%, and after the 60th day of maintenance, it ranged from 7.33 to 9.00%. The number of monocytes in P1 is 7.33%, compared to the number of monocytes in Arnoli[10] with the same treatment, namely 4.33%. The number of monocytes in this study after being tested ranged from 8.33 -18.66%, with the number of monocytes in P1 namely, 8.33%, while monocytes in sambal fish given moringa leaves in P1 namely, 4.33%[10]. For the number of normal fish monocytes without being given additional feed and without being tested, namely, 7.33%.

The increase in the number of monocytes in each treatment is thought to be caused by fish in an unhealthy state due to infections that enter the body or stimulation from foreign objects to produce monocytes. According to Kurniawan et al.[25], the proportion of monocyte cell count increased due to the response of blood balance to an increase in the proportion of other leukocyte cell types, namely lymphocytes, neutrophils and Thrombocytes. The increase in the number of monocytes in small amounts indicates that the compounds in fermented moringa leaves help the monocyte work process.

The number of neutrophils at the beginning of the study was 9.00%. After the 60th day of maintenance, it ranged from 7.33 - 9.00%, with the number of neutrophils of striped catfish in P1, namely, 7.33%, while the number of neutrophils in fish given additional feed moringa leaf flour, namely, 4.67%[10]. In acute or bacterial infections, neutrophil cells function as phagocytic cells; one neutrophil cell can phagocytize 5-20 bacteria. Before the neutrophil cell becomes inactive and dies[23].

The number of neutrophils in this study after being tested ranged from 7.66 - 14.33%, with the number of neutrophils in P1 after being tested, namely, 8.33%, while the number of neutrophils of striped catfish given the addition of moringa leaf flour to the feed with the same treatment, namely, 4.33%. The number of neutrophils in regular striped catfish is 6.67%[10].

In P1, P2 and P3, the number of neutrophils increased slightly because the fish are still trying to fight bacteria that enter their bodies and maintain neutrophil production at a sufficient level. In P0, the number of neutrophils exceeds the standard limit, which is thought to be caused by bacterial infection, which stimulates the leukocyte system to produce many neutrophils to fight bacterial infection. According to Yusuf et al.[24], the range of neutrophils for regular fish is 6-9%. This indicates that the dose added to the feed can help inhibit pathogenic infections. According to Pasaribu & Djonu[5], tannin compounds will bind to cell membranes and extracellular proteins in bacteria to inhibit the growth of microorganisms or enzyme activity. Normal neutrophils cause autolysis after successfully suppressing infection from pathogens that enter the fish body, as well as alkaloids that are toxic to microbes, thus effectively increasing the work of neutrophils to kill bacteria[29].

The number of Thrombocytes at the beginning of the study was 10.33%, while after being maintained for 60 days, it ranged from 9.00-9.66%; the number of Thrombocytes in P1 was 9.33% for the same treatment from Arnoli[10], which was 7.67%. Thrombocytes play a role in the blood clotting process. Thrombocytes help form a blood clot to prevent excessive blood loss during a wound. In addition to its central role in hemostasis (stopping bleeding), Thrombocytes are also involved in the immune response and wound healing process by releasing factors that help tissue repair.

After being challenged, the platelet counts in this study ranged from 9.00 - 9.66%; the platelet count in P1 was 9.33%, while in the same treatment from Arnoli[10], it was 8.67%. The normal platelet count in striped catfish is 7.00%. This stable platelet is caused by the presence of vitamin C content in fermented moringa leaves; vitamin C plays a role in normalizing immune function, reducing stress and helping the wound-healing process in fish. Ridwan et al.[30] also stated that vitamin C can increase the immune system of fish and collagen formation and trigger the process of repairing body tissues. Saponin is one of the compounds that spur the formation of collagen, a protein structure that plays a role in wound healing. Saponin can also be a cleanser, effectively healing open wounds[27].

4. CONCLUSION

Based on the study's results, adding fermented moringa leaves to feed can affect hematological parameters and improve the immune response of striped catfish. Fermentation of moringa leaves can increase the availability of nutrients and bioactive compounds, such as antioxidants, which play an important role in supporting the health and endurance of fish. The results showed that P1 (feed given fermented moringa leaves at a dose of 10 g/kg feed and challenged with *A. hydrophila*) gave the most optimal effect on the parameters measured, such as hematology and immune response of striped catfish.

The researcher suggests that further research can be carried out related to the histology of the intestines, liver and kidneys to determine whether the addition of fermented moringa leaves to this feed can cause damage to the internal organs of fish and a proximate test is carried out to determine the nutrient content in the artificial feed.

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