The replacement of fish meal with fermented Moringa leaves meal and its effect on the immune response of red tilapia (Oreochromis sp.)

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Abstract. Moringa (Moringa oleifera) has frequently been studied as an alternative and promising protein source in fish feed. Moringa leaves is among the potential and affordable plant-based protein source ingredient, as it protein, vitamin, beta-carotene, and various kind of minerals. Flavonoid, saponin, vitamin and alkaloids reportedly exhibit immune-stimulating activity which increase immune response. This study aims to evaluate the replacement of fish meal with fermented Moringa leaves meal in feed on the immune response of red tilapia (Oreochromis sp.). The research was conducted experimentally using a completely randomized design by four treatments in triplicate. The treatments include replacement of the fish meal with fermented moringa leaves meal at 0, 10, 20, and 30 %. Red tilapia with total length of 9 to 10 cm was fed twice daily for two months at a feeding rate of 3 % of total biomass. The results showed that replacement fish meal with fermented Moringa leaves meal as much as 20 % increases the percentage values of hematocrit and leukocrit, stimulates phagocytic activity and phagocytic index, suppresses monocyte and increases lymphocytes, and increases total plasma protein.

1. Introduction
Red tilapia (Oreochromis sp.) is one of freshwater cultivation main commodities in Indonesia, reared intensively in high density and fed commercial feed. As aquaculture is very dependent on feed quantity and quality, fish feed demand increases with aquaculture development. Large portion of total production cost, around 60-70, is spent on feed. Due to the high price of commercial fish feed, cultivators prepare their own homemade feed using more affordable materials.

Moringa leaves (Moringa oleifera) is among the potential and affordable plant based protein source ingredient, as it contains 30.3 % protein that consist of 19 amino acids, such as methionine, cystein, and triptophan [1] [2], vitamin B, C, K, betacarotene [3], various kind of minerals [4]. Flavonoid,
saponin, vitamin A, B, C, E and alkaloids reportedly exhibit immune-stimulating activity which increase immune response [5]. However, Moringa leaves also contain antinutrient compounds [6] [7], such as phytase, tannin, saponins, and crude fiber which reduce nutrient bioavailability [8]. Moringa leaves can be improved through fermentation [9], using microorganism in dry or semi-dry condition.

Hematology profile represents animal physiological response to internal or external environment, including feed and eating habits. Feed compositions, metabolic adaptation, and activity are the main factors responsible for changes in fish hematology profile [10]. Other influencing factors are fish species, size, age, physiological status, environment condition, as well as feed profile, such as quality and quantity, feed composition, protein, vitamin, and probiotic content [11] [12]. Hematology profile analysis, an important and effective method to measure sensitivity index that represents fish’s physiology and pathology state, consists of total red blood cell, hematocrit (PVC), hemoglobin concentration (Hb), erythrocyte constants (mean corpuscular volume–MCV, mean corpuscular hemoglobin–MCH, mean corpuscular hemoglobin concentration–MCHC), total white blood cell, and thrombocyte [13], as erythrocyte, leukocytes, and thrombocyte are critical in oxygen transportation, immunity, and platelet clot formation. Fish health status can be analyzed using blood biochemical index (the level of glucose, cortisol, and total protein). There are hematology parameter differences among species, but haematocrit can be used as a general indicator to define fish health [14]. Moreover, total protein of serum is the most important factor to measure nutrition’s biochemical status and fish health [13].

Substitution of fish meal using fermented plant-based protein concentrate up to 40 % had no negative impact on the immune system of Paralichthys olivaceus [15]. [16] reported that Moringa leaves addition in feed promoted O. niloticus immunity indicated by increasing white blood cells. Higher white blood cell level indicates stronger immunity of fish fed Moringa leaves meal feed thus effectively fight infection [17]. Moringa leaves have therapeutic activity that promotes O. mossambicus health [5]. [18] noted that fish meal substitution using 25 % Kikuyu leaf meal (Pennisetum clandestinum) had no negative effect on Tilapia rendalli. Therefore, this research aimed to evaluate the effect of replacement of fish meal with fermented Moringa leaves meal in feed on immune response red tilapia (Oreochromis sp.).

2. Materials and methods

The research was conducted at the Research Station in the Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia. Moringa leaves were obtained from gardens around the districts of Bantul and Kulon Progo, Special Region of Yogyakarta. Moringa leaves are separated from the stems, then the leaves are dried in an oven at a temperature 40 to 50°C for 6 to 8 hours. The dried leaves are then ground and sieved using a sieve with mesh size 1mm.

The bacteria used in this research were T2A (Bacillus sp.), T3P1 (Bacillus sp.) and JAL11 (Lactococcus raffinolactis), which previously were grown in a sterile Tryptic Soy Broth (TSB) medium for 24 hours and added with 450 mL of sterile Phosphate Buffer Saline, homogenized, mixed evenly with 500 g of MLM that was airtight, and fermented at a temperature of 34 to 37 °C for 168 hours. The sample was dried at a temperature of 50 to 60 °C for 7 hours, refined, and kept in an airtight plastic bag. Based on the calculation of Total Plate Count (TPC) on TSB culture media, the density of the bacterial mixture was $2.16 \times 10^9$ CFU/mL.

The treatments involved replacing fish meal with fermented Moringa leaves meal (FMLM) at 0, 10, 20, and 30 %, and each was subjected to proximate analyses (Table 1).
Table 1. Ingredient and proximate composition (% on dry weight basis) of diets containing different level of FMLM

| Ingredient          | Fermented Moringa Leaves Meal |
|---------------------|------------------------------|
|                     | 0 % | 10 % | 20 % | 30 % |
| Fish meal           | 42.00 | 32.00 | 22.00 | 12.00 |
| Soybean meal        | 35.00 | 35.00 | 35.00 | 35.00 |
| FMLM                | 0.00 | 10.00 | 20.00 | 30.00 |
| Rice bran           | 10.00 | 10.00 | 10.00 | 10.00 |
| Starch              | 8.00 | 8.00 | 8.00 | 8.00 |
| Mineral mix         | 2.00 | 2.00 | 2.00 | 2.00 |
| Vitamin C           | 2.00 | 2.00 | 2.00 | 2.00 |
| Fish oil            | 1.00 | 1.00 | 1.00 | 1.00 |

Proximate analysis

| Component           | 0 %       | 10 %      | 20 %      | 30 %      |
|---------------------|-----------|-----------|-----------|-----------|
| Moisture            | 15.41±3.08 | 14.79±3.90 | 14.16±0.02 | 13.25±0.12 |
| Protein             | 31.32±1.43 | 30.41±0.00 | 27.24±1.39 | 29.23±0.30 |
| Total lipid         | 13.74±0.07 | 14.55±0.42 | 14.01±0.13 | 15.43±0.12 |
| Ash                 | 13.16±0.03 | 12.42±0.39 | 10.65±0.25 | 11.19±0.14 |
| Crude fiber         | 3.09±0.31  | 4.14±0.32  | 5.44±0.41  | 4.65±0.24  |
| NFE                 | 23.28±0.98 | 23.69±1.00 | 28.50±0.44 | 26.25±0.18 |
| Energy (kcal DE 100 g⁻¹) | 347.45±0.82 | 352.16±0.47 | 349.82±0.65 | 363.82±0.20 |

NFE = nitrogen-free extract; calculated = 100(% CP+ % CL+ % moisture+ % ash+ % CP); Energy = \(\{(\text{protein} \times 4.5 \text{ kcal g})+(\text{lipid} \times 9.1 \text{ kcal g})+(\text{NFE} \times 3.5 \text{ kcal g})\}/100\) [19]; DE = digestible energy.

The research was conducted experimentally using a completely randomized design by four treatments in triplicate. The treatments include replacement of the fish meal with FMLM at 0, 10, 20, and 30 %. Feeding trial in red tilapia was done for 60 days. The red tilapia with total length of 9 to 10 cm were reared in a fiber tank measuring 50 x 50 x 70 cm³ with 15 units with rearing density of 15 fish/tank. The fish was fed twice daily at 8:00 am and 2:00 pm with feeding rate of 3 % of total biomass.

Red tilapia blood sampling was carried out on the day-30 and day-60 of maintenance. Blood samples were taken from the caudal vein using a syringe that had been moistened with 10% EDTA and accommodated in a microtube. The blood samples were used for testing hematocrit, leukocrit, phagocytic activity, phagocytic index, extracellular respiration blast activity (NBT), superoxide dismutase (SOD) activity, leukocyte differentiation, and total plasma protein.

Leukocrit and hematocrit were calculated using the formula by [20]. Blood samples were inserted into the hematocrit after one side of the vitrex hematocrit capillary was closed using paraffin. The other side was then closed before being centrifuged at 3000 rpm for 5 minutes to separate erythrocytes, leukocytes, and plasma. The length of storage of erythrocytes, leukocytes, and plasma was measured using a caliper and expressed as a percentage.

\[
\text{Leukocrit (})\% = \frac{\text{The length of leukocytes deposit in capillary tube}}{\text{The length of total blood cells and serum in capillary tube}}\times 100\%
\]

\[
\text{Leukocrit (})\% = \frac{\text{The length of erythrocyte deposit in capillary tube}}{\text{The length of total blood cells and serum in capillary tube}}\times 100\%
\]

Aeromonas hydrophila antigen preparation was carried out by fifty microliters of leucocytes was poured into 96-well microplate and filled in the same volume of 10⁸ cells mL⁻¹ formalin killed A. hydrophila. After incubation at 30 °C for 30 minutes, each sample was smeared on object-glass,
fixation with ethanol 96 %, air-dried, and stained with 10 % Giemsa. A. hydrophila bacteria culture was prepared in Tryptone Soya Agar (TSA) medium for 24 hours and harvested using sterile Phosphate Buffer Saline (PBS) solution. After inactivation using formalin until a final concentration of 2 % and homogenization using a vortex, bacteria were washed using PBS solution for three times, and centrifuged at 3,000 rpm for 5 minutes. Formalin-killed A. hydrophila was prepared by its 24 hours incubation in TSB medium at room temperature. The inoculum was inactivated by 24 hours incubation in 2 % formalin solution at room temperature placed in a rotary shaker, then centrifuged at 3,500 g for 10 minutes. After supernatant removal and pellet three-time washing using vortex-shaking in PBS solution, the pellet was centrifuged at 3,500 g for 10 minutes. The concentration of the weakened A. hydrophila was measured by the TPC method, then prepared into ready-to-use 10⁷ cell mL⁻¹ formalin-killed A. hydrophila stock.

For calculate phagocytic activity (PA) and phagocytic index (PI), a blood sample was put into a capillary tube then centrifuged at 1,500 rpm for 5 minutes to separate erythrocyte, leucocytes, and plasma. Hematocrit capillary was then cut at the borderline that separated leukocytes and erythrocytes [20]. Leucocytes were collected in micro-tube and put into a microplate well, 50 µL each, added 50 µL antigen A. hydrophila (density of 10⁶ cell mL⁻¹), homogenized by pipetting, and incubated at 30 °C for 30 minutes. Approximately 5 µL sample was taken from microplate well, put on object glass to make blood smear then left to dry, then fixed by dipping in 96 % ethanol for 5 minutes, dried, and stained using 10 % Giemsa solution for 15 minutes. The PA and PI was observed under microscope at 1,000x magnification from 100 phagocytes each side. The PA and PI index was calculated using the equation below.

\[
PA (\%) = \frac{Number\ of\ active\ phagocyte\ cell}{Number\ of\ observed\ phagocyte\ cell} \times 100%
\]

\[
PI (\%) = \frac{Total\ number\ of\ engulfed\ bacteria}{Number\ of\ active\ phagocyte\ cell} \times 100%
\]

Nitroblue tetrazolium (NBT) activity test is carried out by preparing heparinized 15 µL of blood was put in a microtube and poured with 15 µL of 0.2 % NBT in 0.85 % NaCl then incubated for 30 minutes in room temperature incubation. N, N-dimethylformamide (DMF) at a volume of 1,000 µL was added and then centrifuged at 3,000 rpm for 5 minutes. The absorbance of the supernatant was determined using a spectrophotometer at 540 nm.

SOD activity test was carried out using NBT by adding riboflavin. A total of 100 L of heparin-treated blood was mixed with 0.5 mL of phosphate buffer (50 mM; pH 7.8) until homogeneous, then centrifuged at 6,000 g, at 4 °C, for 5 minutes. The supernatant was discarded, heated at 65 °C for 5 minutes and centrifuged to obtain the supernatant. One hundred microliters of the supernatant were added to 20 L of a mixture of NBT (0.1 mM EDTA; 13 M methionine; 0.75 mM NBT and 20 M riboflavin in 50 mM phosphate buffer, pH 7.8), placed under a fluorescent lamp for 2 min, then the absorbance of the sample was measured at 630 nm. The result is expressed as relative enzyme activity.

The preparation of leucocyte was conducted according to the method previously published by [21]. Red tilapia blood was taken using spuit previously dipped in 10 % EDTA solution, collected in microtube then centrifuged to separate erythrocyte, leucocytes, and plasma. Approximately 5 µL of leucocytes were taken using a white tip and micropipette, put on object glass to make blood smear then left to dry. After fixation using 96 % ethanol for 5 minutes, prep was left to dry, stained using 10 % Giemsa for 10 minutes, and washed with clean water. Observation on blood smear was conducted using a microscope at 1,000 magnification. Approximately 100 leucocytes cells were classified into lymphocytes, monocytes, neutrophils, eusinophils, basophils [21].

Total protein plasma was spectrophotometrically analyzed using the Bradford method with Bovine Serum Albumin (BSA) as standard [22]. A standard curve was prepared in various concentrations by serial dilution. Approximately 798 µL redistilled water was put into microtube before the addition of 2 µl BSA solution at 25, 50, 75, 100 µg µL⁻¹ and another microtube for control. Subsequently, 200 µL
reagent from the protein test kit (Biorad) was added, homogenized, and incubated for 15 minutes. Absorbance was measured at 600 nm. Similar steps were applied to samples. Results from BSA were made into a standard curve which was then used to analyze samples [21].

All data were statistically analyzed using one-way variant analysis (ANOVA) by SPSS version 20. Dunnet Test was used to compare significant differences to the control treatment. The treatment effects were considered significant at $p < 0.05$.

3. Result

Hematocrit level at day-30 was at range of 16.84±3.08 to 26.33±0.57 %, increased to 22.48±0.98 to 29.05±1.64 % at day-60. Based on 60 days observation, replacement fish meal with FMLM in feed promoted hematocrit level of red tilapia. The highest percentage was obtained by 20 % FMLM among others, at 26.33±0.57 to 29.05±1.64 % (Figure 1).

Leukocrit level was also visible increased from 0.76±0.13 to 1.55±0.95 % at day-30 and 1.59±0.80 to 4.54±3.10 % at day-60. From 60 days observation, FMLM increased leukocrit percentage in red tilapia (Figure 2).

![Figure 1. Hematocrit percentage of red tilapia](image1)

![Figure 2. Leukocrit percentage of red tilapia](image2)

Replacement fish meal with 10 to 30 % FMLM increased phagocytic activity during 60 days red tilapia rearing. The PA and PI index generally increased during the observation (Table 2).
Table 2. Phagocytic activity and phagocytic index of red tilapia fed with FMLM

| FMLM level (%) | 30 days | 60 days | 30 days | 60 days |
|---------------|---------|---------|---------|---------|
| 0             | 24.00±4.58<sup>b</sup> | 23.33±3.51<sup>d</sup> | 1.10±0.03<sup>a</sup> | 1.16±0.05<sup>b</sup> |
| 10            | 14.33±0.57<sup>a</sup> | 17.33±1.52<sup>c</sup> | 1.13±0.01<sup>b</sup> | 1.14±0.02<sup>b</sup> |
| 20            | 11.67±0.57<sup>a</sup> | 14.33±1.15<sup>b</sup> | 1.13±0.03<sup>b</sup> | 1.11±0.03<sup>a</sup> |
| 30            | 10.00±1.00<sup>a</sup> | 12.33±1.15<sup>a</sup> | 1.13±0.02<sup>b</sup> | 1.13±0.02<sup>a</sup> |

NBT level during day-30 was higher than those at day-60, thus extracellular respiratory burst (NBT activity) in red tilapia tend to decrease. Similarly, SOD activity during day-30, higher than those at day-60. NBT activity decreased, while the value of SOD activity varied (Table 3).

Table 3. NBT and SOD activity of whole blood of red tilapia fed with FMLM

| FMLM level (%) | NBT activity of whole blood | SOD activity of whole blood |
|---------------|----------------------------|-----------------------------|
|               | 30 days | 60 days | 30 days | 60 days |
| 0             | 0.59±0.14<sup>a</sup> | 0.26±0.07<sup>a</sup> | 0.07±0.01<sup>a</sup> | 0.40±0.23<sup>b</sup> |
| 10            | 0.58±0.04<sup>a</sup> | 0.40±0.04<sup>a</sup> | 0.06±0.005<sup>a</sup> | 0.12±0.01<sup>a</sup> |
| 20            | 0.33±0.10<sup>a</sup> | 0.24±0.14<sup>a</sup> | 0.05±0.01<sup>a</sup> | 0.04±0.04<sup>a</sup> |
| 30            | 0.38±0.16<sup>a</sup> | 0.37±0.16<sup>a</sup> | 0.07±0.005<sup>a</sup> | 0.07±0.04<sup>a</sup> |

Leukocytes members found during observation were lymphocytes, monocytes, neutrophils, eosinophil and basophils. On day 30, monocytes increased, while on day 60, monocytes decreased followed by an increase in lymphocytes.

Figure 3. Leukocytes differentiation of red tilapia at day-30
Total protein plasma during 60 days of fish rearing generally increased, except in the treatment of replacing fish meal with FMLM as much as 10%.

4. Discussion

Hematocrit percentage is one of haematology profile to measure physiological condition of red tilapia, indicated by erythrocyte volume percentage in blood. During 60 days observation, hematocrit percentage of red tilapia was at 16.84±0.31 to 29.05±1.73 %, which can be classified as normal. [23] mentioned that normal hematocrit of fish is at 15 to 45 % range. *O. niloticus* fed Moringa leaves meal substituted feed as protein source had hematocrit level at range of 13.42 to 15.28 % [16].

Replacement fish meal with 10 to 20 % FMLM in feed increased hematocrit percentage, which indicates higher erythrocyte or lower blood plasma. FMLM provides natural protein that reduces physiological stress [18]. Increasing hematocrit percentage can be resulted by improved protein absorption. Protein is important for both growth and cells rejuvenation, particularly red blood cells [24]. Low level of hematocrit percentage might be the result of stress inducing condition during initial handling for blood sampling.
Leukocrit represents leukocytes volume percentage in blood and physiology condition of red tilapia. Leukocyte’s important function is phagocytosis that localize and eliminates infectious pathogen. Leukocrit percentage of red tilapia during 60 days observation was at range of 0.76±0.13 to 4.54±3.10 %. [25] mentioned normal leukocrit percentage of fish is at 1 to 2 % range, and at 1.22 to 1.27 % for tilapia [21]. Blood leukocrit percentage showed body immune mechanism against antigen. Leukocytes percentage above normal indicates defense response against infections [26], thus increasing leukocrit level is a parameter to evaluate red tilapia resistance against pathogen [21]. During 60 days rearing, replacement fish meal using 10 to 30 % FMLM resulted low leukocrit percentage. This indicated that FMLM promoted white blood cells, with immunomodulator activity that improve immune system protection. The cells responsible for phagocytosis, a process where foreign objects are engulfed by the cells. Based on [27], increasing number of white blood cells or leukocytes is an indicator of immune system activity.

The PA is also an indicator to define pathogenicity of engulfed bacteria, as it represents phagocytic cells for phagocytosis. Phagocytosis is indicated by the presence of phagocytic cells (macrophag, monocytes and granulocyte) engulfing A. hydrophila antigen. It was observed by the number of active phagocytic cells reducing antigen. The PA was at a range of 10.00±1.00 to 25.66±2.08 %. Normal PA for tilapia is at a range of 21 to 36 % [28], [29], [30]. Increasing PA indicated that replacement fish meal with FMLM improved red tilapia immunity. [31] mentioned that higher levels of PA showed improved immune function.

Beside the activity, PI is another criteria that represents the number of bacteria engulfed by one macrophage. The index is used to evaluate PA by calculation of average number bacteria ingested per phagocyte. The PI of tilapia was ranged at 1.11±0.03 to 1.21±0.08. Generally, the index tends to decrease, indicating that 20 % FMLM in feed stimulated PA in 30 days. The PI is affected by PA, complementary stimulated by higher attachment frequency of antigen-antibody complex to bind to phagocytic cells receptor and also stimulate opsonization of foreign cell coated by antibody [32].

Extracellular respiratory burst is the destruction process of foreign particles by phagocytes that generates free radical. It was observed using NBT test through measurement of free radicals generated by phagocytes. Extracellular respiratory burst of tilapia was range at 0.33±0.10 to 0.83±0.13. During 60 days observation, FMLM resulted in decreasing NBT activity. This indicated that reduction rate of oxygen radical production to kill bacteria, or in other words, indicated contamination or chronic infection in red tilapia, in stressed conditions [31].

SOD activity was measured using photoreduction of NBT induced by sensitizer. Superoxide dismutase (SOD) is an enzyme produced by the body during extracellular respiratory burst as an antioxidant against free radicals, and was spectrophotometrically measured by NBT reduction by O2 into blue formazan. SOD activity of tilapia was at 0.05±0.01 to 0.40±0.23. The treatment of replacement fish meal with FMLM has not been able to increase NBT activity. NBT activity does not produce excessive free radicals so that the response to SOD activity is relatively low.

However, higher percentage replacement of FMLM in feed had no effect on SOD activity. SOD is an antioxidant enzyme against free radicals that prevent animal tissue damage to organisms through netralization. Immune parameter represents the effect of nutrient in feed on fish immunology status [15]. Other factors that might affect SOD activity are fish oxidative stress and nutritional status. Oxidative stress is the manifestation of imbalance level of free radical and antioxidant enzymes due to enzyme inactivity.

Based on observation of leukocytes differentiation in red tilapia fed with FMLM, lymphocytes, monocytes, neutrophils, eosinophils and basophils were identified. This was in accordance to the finding of [12], reported on five leukocytes types of lymphocytes, neutrophils, monocytes, basophils and eusinophils identified in tilapia blood circulation. Among them, the highest was lymphocytes, followed by monocytes, neutrophils, basophils and eusinophils. This was similar to previous report on lymphocytes domination in leukocytes differentiation, with it as the dominant component of red tilapia white blood cells [12]. It was also comparable to the report by [33], that leukocytes profile of O. mossambicus consists of 69.5 % lymphocytes, 7.8 % neutrophils, 1.3 % eosinophil, and 21.5 %
monocytes. In this research, lymphocytes was at range of 64.00±1.00 to 95.67±4.04 %, and tend to increase with higher percentage of FMLM substitution in feed during rearing. Lymphocytes generates antibody that bind to antigen to stimulate its complement activity, induces agglutination due to the binding of complement to part of carbohydrate in microbia [34].

The number of monocytes was range at 1.00±0.00 to 33.00±0.00 %, and the number was out of normal range. The normal level of fish monocytes ranged between 14.5 to 21.71 %. Generally, monocytes number in animal is usually below 10 % of total white blood cells production. In this research, monocytes decreased after 30 days rearing. Monocyte presence indicates that a species has well developed immunology system. Increasing rate of monocytes indicates intensive defense mechanism [35], while the otherwise might indicates impaired defense mechanism against pathogen. Monocytes or macrophage and neutrophils are critical for foreign object phagocytosis. Replacement fish meal with FMLM in feed increased neutrophils after 30 days rearing, ranged between 0.33±0.57 to 2.67±2.51 %. Neutrophils is the main phagocytic leukocytes, which proliferates in blood circulation as response toward infection, inflammation and stress. Neutrophils number represents highest sensitivity induced by environmental changes and plays important role in leukocytes formation [36]. The increase in monocytes is thought to be due to stress at the beginning of the treatment, so that monocyte and lymphocyte phagocytic cells will increase. Lymphocytes in the fish body are not phagocytic but play an important role in the formation of antibodies. The presence of eosinophil and basophils indicates inflammation as biomarker of health status [15], a part immune system to fight parasite. The number of eosinophile in this research was range at 0.33±0.57 to 5.00±8.66 %. Replacement fish meal with FMLM up to 10 % in feed increased number neutrophil.

During 60 days rearing, total plasma protein tends to increase as much as 20 % of FMLM. [31] mentioned that increasing total protein plasma indicates response due to infection, stimulant, or vaccine, while its reduction indicates acute infection or low protein level of feed. Increasing total protein plasma in red tilapia fed FMLM in feed showed positive effect of non-specific defense system of red tilapia.

5. Conclusion
Replacement fish meal with FMLM as much as 20% increases the percentage values of hematocrit and leukocrit, stimulates PA and PI, suppresses monocyte and increases lymphocytes, and increases total plasma protein.

Suggestion
Immunostimulant and other active ingredients in FMLM need to be further evaluated, particularly to support hematology performance of red tilapia.

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