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Evaluasi Suplementasi Analog Kurkumin dalam Pakan terhadap Respons Hematologi dan Performa Pertumbuhan Ikan Nila Merah (*Oreochromis niloticus*)

(*Evaluation of Curcumin Analog Supplementation in Diet for Hematological Response and Growth Performance of Red tilapia* (*Oreochromis niloticus*))

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ABSTRAK

Nilai hematologi dapat digunakan untuk mengevaluasi respon fisiologi pada ikan. Suplementasi bahan herbal ke dalam pakan merupakan salah satu cara meningkatkan produksi akuakultur melalui peningkatan kekebalan dan fungsi fisiologis yang berhubungan dengan kesehatan ikan. Penelitian ini bertujuan untuk mengevaluasi pengaruh pemberian pakan dengan suplementasi analog kurkumin terhadap nilai hematologi ikan nila merah (*Oreochromis niloticus*). Rancangan yang digunakan adalah rancangan acak lengkap dengan tujuh perlakuan dan tiga kali ulangan. Sampel yang digunakan berupa induk nila betina dengan bobot badan 294,11±51,40 g. Pemeliharaan dilakukan selama 6 minggu pada jaring masing-masing berukuran 2x1x1m, dengan kepadatan 5 ekor/jaring. Pengambilan sampel darah sebanyak tiga kali, yaitu pada minggu ke-2, minggu ke-4, dan minggu ke-6. Hasil penelitian menunjukkan bahwa pemberian analog kurkumin memengaruhi respon hematologi dan kinerja pertumbuhan ikan nila merah. Berdasarkan penelitian yang telah dilakukan, suplementasi analog kurkumin 2,4 mg/100 g pakan adalah dosis yang terbaik untuk peningkatan kesehatan dan pertumbuhan ikan nila.
Kata kunci: Analog kurkumin; ikan nila merah (*Oreochromis niloticus*); hematologi; performa pertumbuhan

ABSTRACT

The physiological response of fish can be evaluated from its hematological aspect (hematological index). For example, to increase aquaculture production, herbal supplementation was used as a feed mixture to increase the immunity and physiological functions of fish. The study aimed to evaluate the effect of feeding with curcumin analog supplementation on the hematological index of red tilapia (*Oreochromis niloticus*). This study used a randomized complete design with seven treatments and three repetitions. The sample used was a red tilapia with a body weight of 294.11±51.40 g. The fish observation was carried out for 6 weeks on fishing nets sized 2x1x1 m each, with a density of 5 fishes/nets. Fish blood was collected three times, in the second week, the fourth week, and the sixth week. The results revealed that curcumin administration affected the hematological response and growth performance of red tilapia. It concluded that supplementation of curcumin analogs of 2.4 mg/100 g is the most appropriate dose for improving the health and growth of red tilapia.
Keywords: Curcumin analog; red tilapia (*Oreochromis niloticus*); hematology, growth performance

INTRODUCTION

The marine and fisheries sector is currently a mainstay to support food security in Indonesia and even the world. Based on a report from FAO (Food and Agriculture Organization), fishery products are a universal source of animal protein, can improve physical condition, increase intelligence, and are healthy. Tilapia (*Oreochromis niloticus*) is one of the fishery commodities and the mainstay of aquaculture products. This is due to the increasing demand for fish consumption along with the increasing population. Tilapia is a freshwater fish that is widely cultivated and consumed by Indonesians because it has a fast growth rate and occupies a strategic position in the export market. The color and body shape of this fish, which is similar to red snapper, make it a primadonna of importing countries and is considered a substitute for red snapper (Rukmana & Yudirachman, 2015).

Fish feed is one of the important components of aquaculture because it is a source of materials and energy to sustain the survival and growth of fish. The content in the fish feed is a determining factor in the quality of feed so that it can support the growth, survival, and health conditions of fish (Fawole *et al*., 2021). Medicinal plants or phytogenic feed supplements consist of bioactive ingredients, antimicrobial, and antioxidant properties that are usually added to animal feeds to improve health performance and growth (Yang *et al*., 2015). Turmeric (*Curcuma longa*) is a herbaceous plant that belongs to the Zingiberaceae family and is often used as a feed supplement in aquaculture production. Turmeric contains substances including curcumin, artumerone, and zingiberene that possess antioxidant properties (Amalraj *et al*., 2017). Studies have shown that turmeric produces a wide variety of pharmaceutical potentials including antitoxic, antitumor, hepatoprotective, immunomodulatory, and antibacterial agents in biological systems (Arulkumar *et al*., 2017). Curcumin in turmeric can be the main treatment against bacterial diseases in aquaculture production (Sahu *et al*., 2008). Turmeric supplementation in *Oreochromis niloticus* feed improves growth performance and protects fish against Pseudomonas fluorescent infection, (Mahmoud *et al*., 2014) boosts the nonspecific immune system, and protects fish against *Aeromonas hydrophila* infection (Sahu *et al*., 2008). Its application is not only for fish. Kasiyati *et al*. (2018) report that the administration of curcumin in Magelang duck feed can reduce saturated fatty acids and triglyceride concentrations.

Efforts to improve the immune system and growth rate of tilapia are by providing other feed additives that can increase digestibility. A potential alternative additive to improve fish health and growth conditions is curcumin. Curcumin is a phenolic compound found in turmeric (*Curcuma longa* L.) with biological activity as antioxidant, anti-inflammatory, chemopreventive, and chemotherapy (Chattopadhyay *et al*., 2004). Modification of curcumin compounds in the aromatic ring can produce curcumin analog compounds that have better biological activity than curcumin. Curcumin has undergone many modifications to obtain stable compounds, has a specific activity to the target protein, and can inhibit the interaction of androgen hormones with its receptors (Fitriasari *et al*., 2013). Lawang oil is one of the most potent essential oils and is produced in eastern Indonesia, which are Maluku and Papua. The reactivity of the dioxolane ring in safrole contained in palm oil can be utilized by converting it into an analog derivative anticancer compound product of curcumin. Curcumin analog has pharmacological properties similar to or even better than parent compounds (Yang *et al*., 2013) and can exhibit high biological activity and bioavailability without increasing toxicity (Liu *et al*., 2012). The analog synthesis of curcumin aims to increase the stability, potency, and selectivity of its biological activity (Rahmawati *et al*., 2018) and is a synthetic product made to provide effects such as curcumin products. Curcumin, which is a group of flavonoids, has antioxidant, anti-inflammatory, antifungal, and antibacterial activity (Niranjan & Prakash, 2008), which can increase the body's resistance and appetite for fish (Mahmoud *et al*., 2017).

Curcumin can regulate fat metabolism. The cholagogues activity of curcumin can stimulate bile to secrete more bile fluid which helps break down fat. The secretion of bile fluid into the small intestine also improves the digestion of proteins and carbohydrates so that the absorption activity of food substances increases (Nelson *et al*., 2017). The hematological index is a prominent method to evaluate the nutritional status and health of fish in reaction to different feeds. The study was designed to evaluate curcumin's administration of blood parameters and tilapia growth performance. Fish blood parameters can indicate the health condition of fish. This parameter provides vital information on the physiological status of fish, which is influenced by processes in the fish's body and the observed living environment (Hastuti & Subandiyono, 2020). The blood profile plays a strong role in the metabolic and physiological activities of the body as well as the defense component against any diseases that enter the fish's body (Anene *et al*., 2021).

MATERIALS AND METHODS

Location and time of study

This research was conducted from May to July 2021 at the Fish Breeding Research Center (BRPI), Sukamandi, Subang Regency, West Java. The analysis of the hematological index of tilapia was carried out at the Aquatic Organism Health Laboratory, FPIK, IPB University.

Experimental Animals, Breeding Management, and Research Design

The experimental animals in this study were tilapia (*Oreochromis niloticus*) totaling 105 fish, with total body lengths ranging from 14-20 cm and body weight ranging from 200-350 g/head. The research container was three outdoor maintenance ponds. The pool was insulated using 21 nets measuring 2x1x1 m^3, each net contains five fishes.

The experimental tilapia was acclimatized for one month before being supplemented with supplementation feed. Feed treatment was supplemented for six weeks of observation. During observation, the fish were supplemented with commercial feed with 33% protein supplemented with analogs curcumin, turmeric powder, and commercial curcumin based on the treatment dose. Analog curcumin was obtained from the process synthesis of palm oil (*Cinnamomum cullilawan* Blume), while turmeric powder was obtained from the Research Center for Medicinal and Aromatic Plants (BALITRO) Cimanggu, Bogor. The commercial curcumin used was produced by Xi'an Day Natural Inc. The amount of feed supplemented was 3% of body weight twice a day, in the morning and evening. The feed coating process used carboxymethyl cellulose (CMC) as a binder for 3% of the amount of feed supplemented.

The study used a complete randomized design with seven treatments and three repetitions. The treatment supplemented were P0 (control/without curcumin supplementation), P1 (2.4 mg analog curcumin/100 g feed), P2 (4.8 mg analog curcumin/100 g feed), P3 (25 mg turmeric powder/100 g feed), P4 (50 mg turmeric powder/100 g feed), P5 (2.4 mg commercial curcumin/100 g feed), and P6 (4.8 mg commercial curcumin/100 g feed).

Sampling and laboratory procedures

The hematological index was measured using tilapia blood samples. The observed parameters were the total number of red blood cells, hemoglobin level, hematocrit values, the total number of white blood cells, and phagocytic index, as well as fish growth performance. One fish was taken randomly every two weeks on all treatment group replays. Before taking blood, the fish was anesthetized using tricaine methanesulfonate (MS-222) in 1 mL/liter of water. Blood was drawn through the caudal vein using a 4 mL syringe, then placed on a tube vacutainer containing anticoagulant ethylene diamine tetraacetic acid (EDTA). The blood sample was accommodated in a polyethylene tube and stored on ice. It was then centrifuged at 3000 rpm for 20 minutes at 4°C to obtain serum.

All procedures that involved the experimental fish handling and treatment have been approved by the Animal Ethics Commission, Faculty of Veterinary, Bogor Agricultural University, Number: 004/KEH/SKE/II/2021.

Red blood cells

The procedure applied in calculating the RBCs is referred to method by Blaxhall & Daisley (1973). The blood treated with supplemented EDTA 10% was absorbed with a hemocytometer pipette containing red stirring threads upscale 1, then added to Hayem's solution to scale 101. Stirring blood in the pipette was done by swinging the hand holding the pipette as it formed the number eight for 3-5 minutes so that the blood was well-mixed. The first two drops of the blood solution in the pipette were removed to clear the air cavity, then dripped on a Neubauer-type hemocytometer and covered with a glass cover. Then, RBCs was calculated with a 400x magnification microscope.

Hemoglobin level

The calculation of hemoglobin levels was carried out using the Sahli method. The Salinometer tube was placed between two tubes of standard color. Then, the Salinometer tube was filled with a solution of HCl 0.1 N to the number 0 (the bottom scale line on the Salinometer tube), then the fish's blood was taken from the microtube with a dropper of 0.02 mL and inserted into the Salinometer tube. Before being let stand for three minutes, the end of the pipette was cleaned. Next, aquadest was added with drip pipettes little by little while being stirred with a stirring glass until the color was precisely the same as the standard color. Hemoglobin levels were expressed in g/dL or g% (Wedemeyer, 1996).

Hematocrit Value

The hematocrit measurements were performed according to Anderson & Siwicki (1993). The blood sample was inserted into the hematocrit capillary tube until approximately 2/3 of the length of the capillary pipe (done by touching the end of the capillary pipe that was red-striped on the blood, while the other end of the pipe was closed with the fingertips and perform a closed opening motion to draw blood into the capillaries). Once the blood reached the desired volume, the end of the capillaries was stuffed with cystoceles. After that, the microhematocrit tube was centrifuged (microhematocrit centrifuge Model SH120-1) for three minutes at a speed of 11,000 rpm with the same volume tube position facing each other so that the centrifuge rotation was balanced. After that, the percentage of the hematocrit value was measured on a special reading device (microhematocrit reader).

The total number of white blood cells

The procedure used to calculate the WBCs, is referred to the method by Blaxhall & Daisley (1973). The blood samples were sucked from microtubes using leukocyte pipettes up to a scale of 0.5 and added Turk solution to line 11, after which it was homogenized by shaking the leukocyte pipette to form a number eight for five minutes. Once homogeneous, two drops of blood were removed to remove air, then the blood was dripped onto the hemocytometer box and covered with a glass cover. It was further observed under a light microscope with a magnification of 10x40.

Phagocytic Index

The procedure for measuring phagocytic activity was according to Anderson & Siwicki (1993). The blood sample was taken 50 µL and inserted into the Eppendorf tube. After that, a 50 µL *Streptococcus agalactiae* suspension was added with a density of 107 cells/ml. Then, the suspension was homogenized and incubated at room temperature for 20 minutes. 5 µL of the suspension was taken and made for blood review preparations. A blood sample was taken and dripped on the object-glass on the right side. The glass of another object was placed on the right side of the blood-forming at an angle of 30°. The glass of the object was pulled to the left by still touching the blood until it formed a blood review preparation that was thin enough to be easily observed. After that, the review preparation was dredged air. Dried review preparations were then fixed in a methanol solution for 5-10 minutes. After that, the review preparation was dredged air. Review preparations were soaked in Giemsa solution for 10-15 minutes. The review preparation was then rinsed with aqua dest and re-dredged air. After that, the review preparation could be observed under a microscope. The percentage of phagocytic cells could be calculated by observing the number of cells that made bacteria up to 100 cells. The calculation method was as follows: phagocytic index = number of phagocyte cells / 100 x 100.

Daily long growth rate (LPPH)

The growth rate of daily length was calculated based on the below formula:

Information:

LPPH = Daily long growth rate (%/day)
Lo = Average length of fish at the beginning of observation
It = Average length of fish at the end of observation (mm)
T = Observation duration (day)

Daily weight growth rate (LPBH)

The daily weight growth rate was calculated based on the below formula:

Information:

LPBH = Daily weight growth rate (% day-1)
Wo = Average weight of fish at the beginning of observation (g)
Wt = Average weight of fish at the end of observation (g)
T = Observation duration (day)

Data analysis

The data obtained was analyzed using Microsoft Excel 2010 and Rstudio 1.3.1093 programs. The difference between the middle values of the treatment was tested using the Tukey Test.

RESULT

Hematology index

The measurement results of the RBCs, hematocrit value, and hemoglobin level parameters during the study are presented in Table 1. An increase in the total number of red blood cells after administration of curcumin at different doses, when compared to controls, showed that analog curcumin supplementation improved the hematological profile of fish.

Table 1 The red blood cells, hemoglobin levels, and hematocrit values in red tilapia for 6 weeks of observation.

Treatments	Parameters		
	RBCs (x 10^6 sel/mm^3)	Hematocrit (%)	Hemoglobin (g/dL)
P0	2.83±0.33^a	47.91±3.66^b	9.27±0.91^b
P1	3.13±0.91^a	56.98±3.17^a	10.36±0.93^a
P2	3.11±0.88^a	53.46±6.70^{ab}	10.16±0.41^a
P3	3.07±0.15^a	53.77±2.89^{ab}	10.18±0.13^a
P4	3.04±0.53^a	52.94±3.23^{ab}	10.15±0.43^a
P5	3.10±0.27^a	51.72±6.35^{ab}	10.02±0.50^{ab}
P6	3.07±0.28^a	50.92±1.92^{ab}	10.00±0.43^{ab}

The data is the average value of ± standard deviation. Different superscript letters on the same column show a noticeable difference (P<0.05). (P0 = Control, P1 = 2.4 mg analog curcumin/100 g feed, P2 = 4.8 mg analog curcumin/100 g feed, P3 = 25 mg turmeric powder/100 g feed, P4 = 50 mg turmeric powder/100 g feed, P5 = 2.4 mg commercial curcumin/100 g feed, and P6 = 4.8 mg commercial curcumin/100 mg feed).

The WBCs of tilapia fed with curcumin analog supplementation at different doses for 6 weeks range from 7.86-8.92 x 10^4 cells/mm^3 (Table 2).

Table 2 Measurement of the total number of white blood cells, and phagocytic index in red tilapia for 6 weeks of observation.

Treatments	Parameters	
	WBCs (x 10^4 sel/mm^3)	Phagocytic index (%)
P0	7.86±0.55^b	41.55±2.60^a
P1	8.92±1.01^a	47.33±7.21^a
P2	8.11±0.65^{ab}	45.33±5.76^a
P3	8.10±0.45^{ab}	44.22±5.11^a
P4	8.07±0.62^{ab}	44.77±4.89^a
P5	8.89±0.46^a	44.44±4.79^a
P6	8.78±0.40^a	44.22±3.19^a

The data is the average value of ± standard deviation. Different superscript letters on the same column show a noticeable difference (P<0.05). (P0 = Control, P1 = 2.4 mg analog curcumin/100 g feed, P2 = 4.8 mg analog curcumin/100 g feed, P3 = 25 mg turmeric powder/100 g feed, P4 = 50 mg turmeric powder/100 g feed, P5 = 2.4 mg commercial curcumin/100 g feed, and P6 = 4.8 mg commercial curcumin/100 mg feed).

Growth Performance

The results of the observations of tilapia growth after supplementation of curcumin analogs with different doses are presented in Table 3.

Table 3 Daily long growth rate (LPPH) and daily weight growth rate (LPBH) of red tilapia supplemented with different doses of curcumin analogs in feed for 6 weeks.

Treatments	Parameters	
	LPPH (%)	LPBH (%)
P0	0,025±0,001^{d}	0,03±0,04^{c}
P1	0,094±0,003^{a}	0,37±0,003^{a}
P2	0,046±0,003^{bc}	0,16±0,05^{b}
P3	0,054±0,003^{b}	0,19±0,004^{b}
P4	0,025±0,002^{d}	0,32±0,01^{a}
P5	0,042±0,002^{c}	0,34±0,04^{a}
P6	0,027±0,002^{d}	0,05±0,05^{c}

The data is the average value of ± standard deviation. Different superscript letters on the same column show a noticeable difference (P<0.05). (P0 = Control, P1 = 2.4 mg analog curcumin/100 g feed, P2 = 4.8 mg analog curcumin/100 g feed, P3 = 25 mg turmeric powder/100 g feed, P4 = 50 mg turmeric powder/100 g feed, P5 = 2.4 mg commercial curcumin/100 g feed, and P6 = 4.8 mg commercial curcumin/100 mg feed).

DISCUSSION

Fish are known to live in a very close relationship with their environment and are highly dependent on it. Therefore, hematological parameters are widely used as an early signal of changes in fish health status and have been proven as an approach to monitoring the effects of habitat changes on fish biology (Gabriel *et al*., 2004; Sheikh & Ahmed, 2016). Variations in blood parameters depend on fish species, aquatic biotope, health, nutritional status, age, and sexual maturity (Parrino *et al*., 2018).

The results of statistical analysis showed that the analog administration of curcumin in all treatment groups had no significant effect (P>0.05) on the RBCs. However, the results of the study in Table 1 found the highest RBCs during 6 weeks of observation, which was in the group of fish treated with 2.4 mg analog curcumin of 3.13 x 106 ± 0.91 cells / mm3, while the lowest value in the control group was 2.83 x 106 ± 0.33 cells / mm3. In teleost fish species, such as tilapia, the RBCs is still within the normal range. As reported by Prasetyo *et al*. (2021), the RBCs is between 20,000-3,000,000 cells/mm3. According to Abu-Elala *et al*. (2021), factors affecting the RBCs are species, age, feed nutrition, brood, size, physical activity, and environmental conditions. RBCs can also describe the condition of the fish because they can indicate the defense of the fish's body against pathogenic bacteria. In addition, the blood parameters of fish are very sensitive to changes in the environment. Water quality, oxygen, temperature, and salinity are reflected in blood parameters (Sheikh & Ahmed, 2016) as well as factors such as diet and solid spread (Parrino *et al*., 2018).

Hematocrit is a percentage of the volume of red blood cells in the fish's body. Hematocrit is commonly used to measure the comparison between erythrocytes and plasma. Thus, providing the ratio of total erythrocytes to the total volume of blood in the body. Hematocrit values are influenced by the size and amount of RBCs (Parrino *et al*., 2018). In this study, the hematocrit value of tilapia during observation ranges from 47.91-to 56.98%. The highest score was obtained in the fish group with a treatment of 2.4 mg of curcumin analogs, which was 56.98 ± 3.17%. Nevertheless, the results of this study are still within the normal range of hematocrit levels in fish which is 5-60% (Anderson & Siwicki, 1993).

Tilapia hemoglobin levels during the 6 weeks of observation range from 9.27-10.36 dg/dL. According to Wedemeyer & Yasutake (1977), normal hemoglobin levels in tilapia range from 10-11.1 g/dL. The hematocrit value and hemoglobin levels were also affected by an increase in RBCs. The correlation between hematocrit values, hemoglobin levels, and RBCs, is that there are hemoglobin and hematocrit in erythrocyte cells. The increase in tilapia RBCs observation is caused by several factors, including older age and bigger size of fish, as well as the amount of feed nutrition (De *et al*., 2019). An increase in the age and size of the fish affects the need for oxygen. Oxygen is needed by fish for respiration, blood circulation, and metabolism, so larger fish have more erythrocytes than smaller fish. Hemoglobin (Hb) levels are affected by the RBCs and hematocrit. The correlation between hemoglobin and hematocrit is that erythrocytes contain Hb which serves to bind oxygen used for catabolism processes so that energy is generated. The increase in Hb follows the increase in the RBCs, and it is due to the increase

of iron content in the blood (Parrino *et al*., 2018). Hemoglobin determines the degree of resistance of the fish's body because it corresponds to the binding power to oxygen by the blood. The lower the number of red blood cells, the lower the level of hemoglobin is in the blood (De *et al*., 2019).

Feeding nutrients such as proteins that meet the needs of fish help the process of erythrocyte formation (erythropoiesis). Erythrocyte formation is stimulated by the hormones glycoprotein and erythropoietin in the kidneys and requires precursors to synthesize new cells. Precursors need iron, vitamins, and amino acids (Njieassam, 2016). Curcumin supplementation in feed improves the health of fish because, in addition to being antibacterial, curcumin contains flavonoid compounds and essential oils so that it can trigger blood-producing organs such as the spleen and kidneys to produce more blood to repair damaged cells and form the immune system.

Curcumin increases fish appetite and improves their digestive organs, stimulates the bile wall to excrete fluid, and the discharge of pancreatic sap containing amylase enzymes, lipase, and proteases to improve digestion of carbohydrate, fat, and protein feed ingredients (Ulum *et al*., 2018). Thus, the absorption of nutrients is enhanced.

Curcumin also provides antioxidant effects on the cell membrane. It can reduce the damage to the erythrocyte cell membrane due to oxidation. According to Njieassam (2016), erythrocyte cell membrane damage affects the lifespan of erythrocyte cells. Furthermore, curcuminoids can increase the work of blood-producing organs such as the spleen and kidneys. Flavonoid content supports the blood-producing organs (lymph myeloid) so that blood production increases. In addition, flavonoids serve to protect cell structure and increase the effectiveness of vitamin C as an anti-inflammatory and antibiotic (Tung *et al*., 2019). Flavonoids are also one of the natural antioxidants, which can alter or reduce free radicals and anti radicals (Skibola & Smith, 2000). Hafiz *et al*. (2017) reported that tannins stimulate the rejuvenation of cells, including erythrocytes (red blood cells). Vitamin C is an antioxidant that prevents the break of fatty acid chains into various compounds that are toxic to cells, such as aldehydes, ethane, and pentane. They cause severe damage to cell membranes including erythrocyte membranes. Curcumin supplementation in feed improves the performance of the immune system to fight pathogens entering the fish's body. By increasing the hemoglobin in the blood, the intake of food and oxygen can be circulated throughout body tissues which can support the survival and growth of fish.

Leukocytes in teleost fish are a part of the body's defense system that is non-specific (Chan *et al*., 2022). If there is a viral infection in the fish and an effort from the body to fight back, the production of WBCs will be high. According to Osman *et al*. (2018), the number of tilapia WBCs ranges from 35,670 to 50,650 cells /mm3. Many factors can cause the escalation of leukocyte cells. The WBCs fluctuations in each treatment group are affected by certain conditions such as stress, age, weight, and physiological activity. Adult fish have stronger body defense systems than larvae or juveniles. The WBCs in the study still belongs to the normal range.

Leukocytes are responsible for the immune response. If foreign substances are entering the body, then leukocytes will make antibodies. Antibodies are useful for the immune system to provide stimulation, identify, and neutralize incoming foreign bodies (antigens) such as bacteria. The greater the antigen stimulation, the more antibodies will be produced. Bacteria that enter the body of the fish are identified by leukocytes as antigens (Chan *et al.,* 2022).

Administration of analog curcumin in feed indicates a higher leukocyte value than other groups. Curcumin analogs improve nonspecific immune reactions by increasing the WBCs due to the active substance curcumin, which is antibacterial and anti-inflammatory (Mahmoud *et al.,* 2014).

Chan *et al*. (2022) reported that the increase WBCs is the success of the fish immune system in developing cellular (nonspecific) immune responses as the trigger. According to Yuandani *et al.* (2021), the flavonoid in curcumin serves as immunomodulation or an ingredient that affects the quality and intensity of the immune response. Flavonoids stimulate the immune system by sending signals intracellularly to cell receptors so that cell performance is more active. The mechanism of action of active additives, especially flavonoids, in spurring the immune system is to accelerate the activation of leukocytes and macrophages so that the process of phagocytosis of foreign bodies can be done faster (Chan *et al*., 2022).

Phagocytosis is the process of absorption and elimination of microbes or other particles by phagocytic cells namely monocyte cells and neutrophils. The results of the average percentage observation of phagocytosis index in tilapia for 6 weeks of analog curcumin administration of in the feed are shown in Table 2. The results showed that the average percentage of phagocytosis index ranged from 41.55 to 47.33%.

One of the immune response mechanisms formed by the fish body in defending itself from the attack of pathogenic microorganisms is through

the phagocytosis process (Biller & Takahashi, 2018). The increased phagocytosis index during the study is caused by the stimulation of antibodies of the fish body during the administration of antimicrobial compounds in curcumin. The compound works as an immunostimulant against leukocytes of immunostimulant cells and is a natural or synthetic substance that improves the body's defense system. According to Purwanto *et al*. (2021), low phagocytosis activity is caused by contaminants, stress, chronic infections, and a lack of protein and vitamins. High phagocytosis activity is generally caused by immunostimulants, early vaccination, or early response to infection. The administration of curcumin analog in the feed stimulates the natural immune system so that It fights foreign objects.

According to Zhang *et al*. (2019), the increase in the phagocytosis index is caused by an increase in the percentage of leukocyte cell types in lymphocytes, monocytes, and neutrophils. Activated lymphocytes differentiate the cognitive cells that recognize antigens from effector cells which can get rid of antigens (Yang *et al*., 2021). Differentiated T-itolic cells possess more cytoplasmic granules that contain proteins used for lysing the target cell. Meanwhile, B lymphocytes recognize plasma cells that produce antibodies.

The administration of curcumin in feed has a significant effect (P<0.05) on the daily long growth rate (LPPH) of tilapia. The highest daily length growth rate was found in the fish group with a treatment of 2.4 mg analog curcumin (P1) with an average length growth of 0.094 ± 0.003%, followed by the fish group with a treatment of 25 mg of turmeric powder (P3) of 0.054 ± of 0.003%, the fish group with a treatment of 4.8 mg analog curcumin (P2) of 0.046 ± 0.003%, the fish group with a treatment of 2.4 mg commercial curcumin (P5) of 0.042 ± 0.002%, The fish group with a treatment of 4.8 mg of commercial curcumin (P6) of 0.027 ± 0.002%, and the smallest value was found in the fish group treated with 50 mg of turmeric powder (P4) and the Control group with values of 0.025 ± 0.002% and 0.025 ± 0.001%.

The results of statistical analysis showed that the administration of curcumin analog in the feed had a significant effect (P<0.05) on the daily weight growth rate (LPBH) of tilapia. The highest daily weight growth was found in the fish group with a treatment of 2.4 mg analog curcumin (P1) with an average value of 0.37 ± 0.00%3, this value was markedly different from the group of fish treated with 4.8 mg analog curcumin (P2) with an average daily weight growth of 0.16 ± 0.05%, the group of fish treated with 25 mg turmeric powder/100 g feed (P3) with an average daily weight growth of 0.19 ± 0.004%, the fish group treated with 4.8 mg of commercial curcumin (P6) with an average daily weight growth of 0.05 ± 0.05%, and the control treatment group with an average daily weight growth of 0.03 ± 0.04%.

The length growth and daily weight of tilapia fish indicate an increase in the length and weight of the fish after being supplemented with curcumin analogs in the feed for 6 weeks. The highest increase in length and weight was found in the group of fish supplemented by 2.4 mg analog curcumin/100 g, which was a length increase of 0.094% and a weight gain of 0.37%. Meanwhile, the smallest increase in length and weight was found in the control group at 0.025% and 0.03%. During the observation, the experimental fish experienced an increase in length and body weight. The increase in body length and weight is correlated with the growth process in fish. The results showed that curcumin supplementation in tilapia increases the rate of body length and weight as a form of a metabolic process of the body.

During the growth of fish, there is an increase in body tissue characterized by an increase in body length and weight gain. Curcumin stimulates the walls of the gallbladder to secrete bile fluid into the small intestine (Mooraki *et al*., 2019). This can improve the digestion of fats, proteins, and carbohydrates so that the absorption of food substances increases. In addition, the essential oil in turmeric serves to prevent excessive discharge of stomach acid in the absorption of food substances by the small intestine. It is supported by Ulum *et al*. (2018), who reported that curcumin can stimulate the walls of the gallbladder to secrete bile fluid and revive the release of pancreatic sap containing amylase, lipase, and protease enzymes into the small intestine. Therefore, it improves the digestion of fats, proteins, and carbohydrates resulting in an increased absorption activity of food substances. In addition, curcumin as an antibacterial can lyze toxins attached to the intestinal wall, so that the absorption of nutrients improves and triggers growth. This was due to the acceleration of the digestive system's maturation and the increase of nutrient digestion caused by the nutrients and bioactive compounds in curcumin analogs. In addition the role of curcumin and the content of essential oils can accelerate the emptying of the stomach. It triggers the increase in eating behavior due to signals that enter the brain when the stomach is empty so that fish will experience increased feed consumption (Kaselung *et al*., 2014).

The study concludes that the parameter measurement data proves that feeds supplemented by curcumin analog increase the RBCs, hemoglobin levels,

hematocrit values, the WBCs, and phagocytic index of red tilapia observed for 6 weeks. The administration of curcumin in feed improves the growth performance of red tilapia. Analog supplementation of curcumin with doses of 2.4 mg/100 g of feed provides the best hematological index and growth performance of tilapia compared to other treatments for each of the parameters.

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