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THE USE OF *Kaempferia galanga* AND *Curcuma xanthorrhiza* FERMENTED TO IMPROVE THE IMMUNITY OF *Oreochromis niloticus* AGAINST *Streptococcosis*

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ABSTRACT

Streptococcus agalactiae is a bacterium that causes streptococcosis, which attacks tilapia. This research was conducted from March to June 2022 at the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine, University of Riau. This study aims to understand the best dose of fermented *Kaempferia galanga* and *Curcuma xanthorrhiza*, which are added to the feed to increase *Oreochromis niloticus* immunity. The method used was experimental with a completely randomized design (CRD) with five treatment levels and three replications. The treatments applied were Kn: Negative control (no treatment used), Kp: positive control (no treatment used and infected with *S.agalactiae*), P1 (100 mL/kg feed), P2 (125 mL/kg feed), P3 (100 mL/kg feed) and infected with S. agalactiae. The fish were contaminated on the 32nd day at a density of bacteria 10^8 CFU/mL of 0.1 mL/fish intramuscularly, and then the fish were reared up to the 46th day. The results showed that the fermented *K.galanga* and *C.xanthorrhiza* affected the haematology of *O.niloticus*. The best dose was in P3 with a dose of 150 ml/kg of feed which showed total erythrocytes of 2.01 x106 cells/mm3, hematocrit value of 27.33%, a hemoglobin level of 7.93 g/dL, total leukocytes of 6.59 x10^4 cells/mm^3, lymphocytes 77.33%, monocytes 14.00%, neutrophils 8.67%, growth with a weight of 34.06 g, a length of 7.5 cm and a survival rate of 93.33%. Based on the data obtained, it can be concluded that fermented *K.galanga* and *C.xanthorrhiza* can increase the immunity of *O.niloticus* against *Streptococcosis* disease.

Keywords: *Oreochromis niloticus, Streptococcus agalactiae,* Fermented Herbs, Immunity.

1. INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the most popular freshwater aquaculture fish and has good prospects for cultivation[1]. Several areas of tilapia-producing centers in Indonesia, namely West Java, Central Java, South Sumatra, West Sumatra, North Sumatra, South Kalimantan, and North Sulawesi[2]. Fish cultivators in Indonesia mainly use intensive cultivation systems with high stocking density maintenance. High stocking affects space for movement, competition for food and oxygen, and metabolic activity and often causes stress to fish. Fish that experience stress can reduce their immunity and cause fish death[3]. Fish mortality is often caused by bacteria such as *Streptococcus agalactiae*.

Streptococcosis is caused by the bacterium *S.agalactiae*. The species most commonly found attacking tilapia are *S.iniae* and *S.agalactiae*. *Streptococcosis* infection occurs in several areas in Sumatra, Java, Kalimantan, Sulawesi, Nusa Tenggara, and Papua, with clinical symptoms of meningoencephalitis, pop-eye, loss of appetite, unilateral/bilateral exophthalmus, curvature of the spine forming the letter C, stiffness, swimming erratic, and whirling movements[4].

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Antibiotics are often used to treat *Streptococcosis*. However, antibiotics have side effects because they can increase bacterial resistance and cause residues, so alternative measures are needed to overcome this disease[5]. An alternative way to prevent *Streptococcosis* is using fermented natural ingredients such as *Kaempferia galanga* and *Curcuma xanthorrhiza*[6]. *K.galanga* and *C.xanthorrhiza* have the potential as immunostimulants[7]. Immunostimulants are substance compounds that can increase the activation of the fish's immune system to fight disease agents that infect the host. Active substances in herbal supplements can improve growth, the body's defense system, and fish health[8]. According to Syawal et al.[6], giving fermented herbs in feed can stimulate fish appetite, reduce fish stress levels against environmental changes, increase fish immunity against disease, and stimulate the immune system and organ function related to the formation of blood cells.

Blood is one of the parameters that can be used as a reference for fish health conditions. Hematological studies are an essential criterion for disease diagnosis and determination of fish health[9]. Many studies have been carried out on using herbal ingredients for fish immunity. However, there is still no research on fermented herbs mixed into feed with a mixture of *K.galanga* and *C.xanthorrhiza* to improve the health and growth of tilapia. This study aimed to get the best dose of fermented *K.galanga* and *C.xanthorrhiza* herbs to be added to the feed and to increase the tilapia body's immunity from a hematological perspective. Therefore, the authors are interested in researching the use of fermented *K.galanga* and *C.xanthorrhiza* to increase tilapia (*O.niloticus*) immunity against *Streptococcosis*.

2. RESEARCH METHOD

Time and Place

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

Method

The method used was experimental with one factor Completely Randomized Design (CRD), with five treatment levels and three replications. The modified dose treatment refers to Kurniawan et al.[10]:

Kn : Negative control (given without fermenting herbs and without being challenged with *S.agalactiae*

Kp : Positive control (given without fermented herbs and challenged with *S.agalactiae*

P1 : Feed containing fermented herbs at a dose of 100 mL/kg of feed and challenge tested with *S.agalactiae*

P2 : Feed containing fermented herbs at a dose of 125 mL/kg of feed and challenge tested with *S.agalactiae*

P3 : Feed containing fermented herbs at a dose of 150 mL/kg of feed and challenge tested with *S.agalactiae*

Procedure

Container Preparation

The container preparation starts with cleaning the aquarium, measuring 40x30x30 cm. The maintenance container was cleaned and then filled with water until it was packed, and 25 ppm of $KMnO_4$ (Potassium Permanganate) solution was given to it for 24 hours. After that, the aquarium is rinsed and dried. The water used comes from a drilled well and has been deposited in a tank that is aerated for 2x24 hours. Then, each container is filled with 30 L of water, after which tilapia seeds measuring 6-7 cm with a stocking density of 1 fish/3 L are added.

Fermented Herbal Medicine

Kaempferia galanga and *C.xanthorrhiza* were obtained from the Pekanbaru City Traditional Market, meeting the criteria of being fresh and in good condition. Then, the material is peeled and washed clean, then weighed, and each weighs 100 g. The ingredients were thinly sliced into a blender, added with 500 mL of water, blended until smooth, and then filtered to obtain a solution. The solution is added with clean water to a volume of 3000 mL and boiled over low heat until boiling. After chilling, 175 mg of molasses, 65 mL of probiotic drink, and 50 mg of tapai yeast and stirred until homogeneous. After being homogeneous, put it in jerry cans and tightly closed. Then, it is fermented for 7 days until a distinctive aroma changes, such as a sweet smell and gas is formed[6].

Maintenance of Test Fish

The fish rearing was carried out for 46 days, and during the maintenance, the test fish seeds were given feed that had been added with fermented herbs according to the treatment. Feeding is done as much as 10% of fish biomass and offered thrice daily at 08.00, 12.00, and 17.00 WIB. Every 10 days, fish body weight and length measurements are carried out to adjust the dose amount in the feed given for the next rearing.

Provision of *S.agalactiae* Isolate and Challenge Test

The *S.agalactiae* isolates were obtained from collections at the Gelam-Jambi River Freshwater Aquaculture Fishery Center (BPBAT SG). *S.agalactiae* bacteria were grown on blood agar and incubated for 24 hours. Then, the *S.agalactiae* bacteria were inoculated again into Brain Heart Agar medium and incubated at 29-30^{o}C for 24 hours until a pure culture was obtained. After obtaining a pure culture, the bacterial isolates were cultured onto Brain Heart Infusion media and incubated at 29-30^{o}C for 24 hours. After 24 hours, the culture was centrifuged at 7,000 rpm at 4 °C for 30 minutes to separate the cell pellet and the supernatant. The centrifuged cell pellet was washed twice with Phosphate-Buffered Saline (PBS), and PBS was finally added according to the initial volume. The culture was matched with Mc. Farland No.1 standard or equivalent to $3x10^8$ CFU/mL to obtain bacteria with a specific concentration. Equalizing the turbidity of the bacterial suspension using a double-masked method was carried out by holding the test tubes side by side and looking at them against a white background with black lines. If the turbidity of the bacterial suspension is still not the same, the bacterial suspension can be diluted or added to bacteria[11]. Tilapia were challenged on day 32 with *S.agalactiae* with a break of 10^8 CFU/mL as much as 0.1 mL/fish using intramuscular injection.

Fish Blood Sampling

Blood was collected by first anesthetizing the fish with 0.1 mL/L clove oil for ±1 minute in 5 L of water. Before blood collection, a 1 mL syringe and Ependorf tube were moistened with 10% EDTA to prevent blood clots. Blood collection was carried out in the caudal vein; 0.2 mL of blood was taken, and then the syringe's blood was put into the Eppendorf tube for observation. Blood examination was done 3 times, namely at the beginning before treatment, after 30 days of maintenance (feeding containing fermented herbs), and 14 days after the challenge test with *S.agalactiae*.

Parameters measured

Observation of Clinical Symptoms

Observation of clinical symptoms was carried out for 14 days after the injection of *S.agalactiae* bacteria, including body movements, body color, morphology, or physical condition of the fish's body.

Total Erythrocytes

The method for calculating total erythrocytes is described by way[12]. The results are converted into the formula:

$$\sum \text{Erythrocytes} = \sum n \times 10^4 \text{ cell/mm}^3$$

Description:

$\sum n$ = The number of erythrocytes counted in 5 fields of view

10^4 = Dilution factor

Hematocrit

The method for calculating hematocrit levels refers to the process[13]. Hematocrit levels are expressed in percent as % blood volume.

Hemoglobin levels

The method for calculating hemoglobin levels refers to Blaxhall & Daisley[12]. Hemoglobin levels are expressed in g/dL or g%.

Total Leukocytes

The total leukocyte count method is described by Blaxhall & Daisley[12]. The result is converted into the formula:

$$\sum \text{leukocyte} = \sum n \times 50 \text{ sel/mm}^3$$

Description:

∑ n = The total number of leukocytes in the 4 large squares

50 = Dilution factor

Leukocyte Differentiation

The leukocyte differentiation calculation method is described by Blaxhall & Daisley[12]. The result is converted into the formula:

$$\text{Percentage sel} = \sum n \times 100\%$$

Description:

∑ n = number of cells counted

Absolute Weight Growth

Absolute weight growth is calculated using the formula according to Zissalwa et al.[14] as follows:

$$GR = Wt - Wo$$

Description:

GR = Absolute growth (g)

Wt = The average weight of fish at the end of the study (g)

Wo = The average weight of the fish at the start of the study (g).

Survival Rate

Fish survival during the study was calculated using the formula Zissalwa et al.[14], namely:

$$SR = Nt/N0 \times 100\%$$

Description:

SR = Survival Rate (%)

Nt = Number of fish at the end of the study

N0 = Number of fish at the start of the study

3. RESULT AND DISCUSSION

Clinical Symptoms

Based on the results of tilapia research on the negative control (Kn) under normal conditions, the power was not challenged with *S.agalactiae* bacteria. In contrast, the positive control (Kp) showed more severe symptoms than treatments P1, P2 and P3.

Changes in how the fish swam in the Kp and P1 treatments were most noticeable compared to P2 and P3. The real change occurred on the 3rd post-infection day, namely the irregular swimming pattern of the fish. These symptoms are the symptoms reported by Evans et al.[15] on tilapia infected with *S.agalactiae*, namely swimming weakly and at the bottom of the aquarium, response to food is weak, swimming whirling (floundering/circling), and the body forming the letter "C" post-challenge test in the Kp treatment.

Changes in the appetite of tilapia occurred after the 2nd day after being challenged. It was seen that the fish were slow to respond to the feed given, and the amount of feed eaten also decreased. This is due to the disruption of fish's digestive system due to *S.agalactiae* infection, which attacks the hypothalamus (brain), the center that regulates hunger and fish digestion. While the fish fed with fermented herbs had a normal appetite, treatment P3 showed milder clinical symptoms than treatments P1 and P2, which showed an increase in the body's resistance to tilapia from the provision of fermented herbs added to fish feed. According to Hasibuan et al.[16], the better the response to eating fish, the faster the process of recovering the condition of the fish's body.

Based on the results of the post-challenge test, it was shown that there were injuries at the injection site and bleeding on the body parts at the base of the pectoral fins, abdomen, and tail. Bleeding occurs because it is caused by the hemolysin enzyme produced by bacteria, which breaks down red blood cells, causing a reddish color on the surface of the fish's skin[17]. According to Arief et al.[18], the vitamin C content found in fermented herbs *K.galanga* and *C.xanthorrhiza* can accelerate the healing of ulcers because they have anti-inflammatory properties as antibiotics and pain relievers and stimulate the growth of new cells in the skin to speed up the healing process.

Changes in pathological anatomy of tilapia after being challenged with *S.agalactiae*, tilapia experienced changes in the eyes, operculum, and changes in body color. Clinical symptoms in fish can be associated with the target organs of *S.agalactiae* (eyes, brain, and kidneys). Bacteria in the eye organ can cause changes in the vision (purulent, opacity, exophthalmia, and wrinkling of the eye). Bacteria in the brain organs can cause fish to swim abnormally (gasping, swimming sideways, and even whirling). In contrast, bacteria in fish kidneys can cause body discoloration to become blacker[19].

Total Erythrocytes

At the beginning of the study, the total erythrocytes of tilapia ranged from 1.11-1.12x10^6 cells/mm^3. The increase in total erythrocytes at 30 maintenance days went from 1.26-1.92x10^6 cells/mm^3. The highest total erythrocytes occurred in the P3 treatment, 1.92x10^6 cells/mm^3, while the lowest total erythrocytes occurred in the Kn treatment, 1.26x106 cells/mm^3. The total erythrocytes of tilapia for 30 days of rearing were still relatively normal. This is to the opinion of Royan et al.[20] that in teleost fish, the average number of erythrocytes is 1.05-3.0x10^6 cells/mm^3. The total erythrocytes of tilapia during rearing can be seen in Table 1.

Table 1. Total Erythrocytes of Tilapia (*O. niloticus*)

Treatment	Total erythrocytes (x10^6 cell/mm^3)		
	Early Maintenance	After Maintenance 30 days	After the 14th day of the Challenge Test
Kn	1,12 ± 0.01	1,27 ± 0,01^a	1,36 ± 0,01^b
Kp	1,11 ± 0,01	1,26 ± 0,01^a	0,98 ± 0,01^a
P_1	1,12 ± 0,01	1,58 ± 0,02^b	1,72 ± 0,01^c
P_2	1,11 ± 0,01	1,74 ± 0,02^c	1,85 ± 0,02^d
P_3	1,12 ± 0,01	1,92 ± 0,02^d	2,01 ± 0,02^e

Description: * Different superscripts show significantly different P<0.05.

Based on the statistical test analysis of variance (ANOVA) showed that feeding containing fermented herbs after 30 days of rearing affected total tilapia erythrocytes (P<0.05). The total erythrocytes of tilapia after the *S.agalactiae* challenge test ranged from 0.98-2.01x10^6 cells/mm^3. In the treatment, Kp decreased, namely 0.98 x 10^6 cells/mm^3, because the fish were attacked by *S.agalactiae* bacteria, which can cause *Streptococcosis* disease. When it enters the blood vessels, *S.agalactiae* produces hemolysin enzymes, which are exotoxins. Hemolysin can lyse red blood cells, decreasing their number in blood vessels[21]. Bacterial toxins are soluble toxic components produced by bacteria and cause adverse effects on host cells by altering the normal metabolism of the host cells[22].

The highest increase in total erythrocytes on the 14th day after the challenge test occurred in the P3 treatment, 2.01x10^6 cells/mm^3. This shows that administering fermented *K.galanga* and *C.xanthorrhiza* herbs at a dose of 150 mL/kg of feed can improve the health of tilapia. Fermented herbs contain secondary metabolites, such as curcuminoids, vitamin C, essential oils, quinones, tannins, and flavonoids, which can trigger blood-producing organs, such as the spleen and kidneys, to produce more blood to repair damaged cells and form the immune system[23].

Hematocrit

Hematocrit is the ratio of red blood cells to blood fluids in fish. Hematocrit observation aims to determine the health status of fish, one of which is anemia. Decreased hematocrit levels can be used as an indicator of low protein content in the feed, vitamin deficiency, or fish infection. Tilapia hematocrit values during the study can be seen in Table 2.

Table 2. Tilapia (*O. niloticus*) Hematocrit

Treatment	Hematocrit Levels (%)		
	Early Maintenance	After Maintenance 30 days	After the 14th day of the Challenge Test
Kn	26,33 ± 0,58	32,67 ± 0,58^{a}	36,67 ± 0,58^{b}
Kp	26,00 ± 0,00	32,33 ± 0,58^{a}	30,67 ± 0,58^{a}
P_1	26,00 ± 1,00	34,33 ± 0,58^{b}	37,00 ± 0,00^{b}
P_2	26,33 ± 1,15	35,33 ± 0,58^{b}	37,67 ± 0,58^{b}
P_3	26,33 ± 0,58	36,33 ± 0,58^{bc}	38,33 ± 0,58^{bc}

Description: * Different superscripts show significantly different P<0.05

Based on Table 2. shows that the hematocrit value of tilapia at the beginning of the study ranged from 26.00-26.33%. The hematocrit value of tilapia after 30 days of rearing, which was given fermented herbal feed, increased in each treatment, ranging from 32.3-36.33%. According to the opinion of Riantono et al.[24], this value is still relatively average, which states that the average fish hematocrit is 28-40%.

The results of the Student Newman-Keuls test after 30 days of rearing showed that the Kn and Kp treatments were significantly different from the P1, P2, and P3 treatments. The hematocrit value of tilapia on the 14th day after a challenge test with *S.agalactiae* ranged from 30.67 to 38.33%. The highest increase in hematocrit value occurred in the P3 treatment, which was 38.33%, and then the lowest value was in the Kp treatment, with a discount of 34.67%. The hematocrit in the fish's blood decreases when the fish loses its appetite or gets sick due to stress and the hematocrit value will decrease[25].

Syawal & Ikhwan[3] stated that the decrease in hematocrit levels was due to stress in fish due to environmental changes, so the level of feed consumption decreased. If the fish's appetite decreases, it can result in a lack of nutrients entering the fish's body so that the erythrocyte level decreases because nutrition is essential to help form erythrocyte cells in the body. An increase influences the increase in the value of hematocrit in total erythrocytes. There is a correlation between the value of hematocrit, hemoglobin level, and total erythrocytes because hemoglobin and hematocrit are found in the erythrocyte cells. Meanwhile, the decrease in hematocrit and erythrocyte values indicates that fish are generally stressed. Based on the statistical test analysis of variance (ANOVA) showed that feeding containing fermented herbs had a significant effect on the hematocrit value of tilapia after a 14-day challenge test with *S.agalactiae* (P<0.05).

Hemoglobin

The results of measurements of tilapia hemoglobin levels during the study can be seen in Table 3.

Based on the statistical test analysis of variance (ANOVA) showed that feeding containing fermented herbs after 30 days of rearing affected tilapia hemoglobin (P<0.05). Hemoglobin levels in tilapia-fed fermented herbal diet increased in each treatment. The highest increase in haemoglobin levels at 30 days of maintenance occurred in the P3 treatment, namely 7.20 (g/dL). Then Kp and Kn were not significantly different, namely in the range of 6.00-6.07 (g/dL) because this treatment was not given fermented herbs, so there was no increase in hemoglobin levels

Table 3. Hemoglobin of Tilapia (*O. niloticus*)

Treatment	Hemoglobin Levels (g/dL)		
	Early Maintenance	After Maintenance 30 days	After the 14th day of the Challenge Test
Kn	5,07 ± 0,12	$6{,}00 \pm 0{,}00^{a}$	$7{,}00 \pm 0{,}00^{a}$
Kp	5,07 ± 0,12	$6{,}07 \pm 0{,}12^{a}$	$5{,}67 \pm 0{,}31^{a}$
P_1	5,07 ± 0,12	$6{,}33 \pm 0{,}12^{b}$	$7{,}40 \pm 0{,}20^{b}$
P_2	5,07 ± 0,12	$6{,}87 \pm 0{,}12^{c}$	$7{,}47 \pm 0{,}12^{b}$
P_3	5,07 ± 0,12	$7{,}20 \pm 0{,}20^{d}$	$7{,}93 \pm 0{,}12^{c}$

Description: * Different superscripts show significantly different P<0.05

Based on the statistical test analysis of variance (ANOVA) showed that feeding containing fermented herbs had a significant effect on tilapia hemoglobin after 14 days of challenge test with *S.agalactiae* (P<0.05). The highest increase in hemoglobin levels after the 14th-day challenge test occurred in the P3 treatment, namely 7.93 (g/dL).

The increase in hemoglobin levels is due to the effectiveness of fermented herbs in feed containing flavonoids and tannins, which function as antioxidants, thereby protecting hemoglobin from oxidation[26]. Then, the lowest value was in the Kp and Kn treatments, which were not significantly different in the 5.67 - 7.00 (g/dL) range on feed that was not given fermented herbs. According to Royan et al.[20], the average hemoglobin level of fish is related to anemia and the number of blood cells; an increase in hemoglobin followed by a very rapid decrease occurs due to a bacterial infection. The average hemoglobin level of ordinary tilapia ranged from 6–11.01 (g%), while the fish injected with *S.agalactiae* bacteria ranged from 6–14.4 (g%). An increase influences the occurrence of an increase in hemoglobin in total erythrocytes. Erythrocytes contain hemoglobin, which binds oxygen and is used for food metabolism processes so that energy is produced.

An increase in hemoglobin is closely related to the rise in erythrocytes. This is due to increased iron content in the blood[27]. Hemoglobin determines the level of resistance of the fish's body because of its close relationship with the binding capacity of oxygen by the blood. If the hemoglobin in the blood is low, the fish will lack oxygen[28]. Erythrocytes contain hemoglobin, which binds oxygen and is used for catabolic processes to produce energy. The level of hemoglobin in the blood correlates strongly with the value of the erythrocytes. The lower the number of red blood cells, the lower the hemoglobin level in the blood. Hemoglobin is a substance in blood cells that contains iron and globin proteins, which can combine with oxygen and transport oxygen throughout the body[29].

Total Leukocytes

The results of observing the total leukocytes of tilapia during the study can be seen in Table 4.

Based on the statistical test analysis of variance (ANOVA) showed that feeding containing fermented herbs after 30 days of rearing affected the total leukocytes of tilapia (P<0.05). Total leukocytes in tilapia increased in each treatment. The highest increase in total leukocytes at 30 maintenance days occurred in the P3 treatment, namely 6.15×10^4 cells/mm^3.

Then Kp and Kn were not significantly different, namely in the 3.79-3.86x10^4 cells/mm^3 range because this treatment was not given fermented herbs. The increase in total leukocytes in each treatment was influenced by certain conditions such as stress, age, weight, and physiological activity of fish[30].

Table 4. Total Leukocytes of Tilapia (*O. niloticus*)

Treatment	Total Leukocytes (x10^4 cell/mm^3)		
	Early Maintenance	After Maintenance 30 days	After the 14^{th} day of the Challenge Test
Kn	3,01 ± 0,01	3,79 ± $0,02^a$	4,23 ± $0,02^a$
Kp	3,02 ± 0,01	3,86 ± $0,55^a$	7,22 ± $0,03^e$
P_1	3,01 ± 0,01	4,87 ± $0,02^b$	5,37 ± $0,02^b$
P_2	3,02 ± 0,01	5,07 ± $0,06^b$	5,56 ± $0,01^c$
P_3	3,02 ± 0,00	6,15 ± $0,03^c$	6,59± $0,04^d$

Description: * Different superscripts show significantly different P<0.05

The increase in leukocytes is also thought to be due to the influence of feeding containing fermented *K.galanga* and *C.xanthorrhiza* herbs on tilapia. Silalahi[26] stated that flavonoids can stimulate the immune system by sending signals intracellular to cell receptors so that cell performance is more active. The mechanism of action of flavonoids in boosting the immune system is to accelerate the activation of leukocytes and macrophages so that phagocytosis of foreign bodies can be carried out quickly[31].

Based on the statistical test analysis of variance (ANOVA) showed that feeding containing fermented herbs had a significant effect on the total leukocytes of tilapia after 14 days of challenge test with *S. agalactiae* (P<0.05). The increase and activity of leukocytes can be caused by infections that trigger cell division activity. Evenberg et al.[32] stated that changes in the leukocyte population could be observed 7 days after exposure. As a defense effort, the *S.agalactiae* infection causes the fish to send more leukocyte cells to the infected area. These leukocyte cells work as cells that phagocytize existing bacteria so that they cannot develop and spread virulence in the host's body, so it is often found that the total number of leukocytes has increased after infection by bacteria[20].

The total leukocytes continued to increase after the 14-day challenge test. The highest increase occurred in Kp, 7.22x10^4 cells/mm^3. This was due to fish stress to the environment and bacterial infections. According to A'yunin et al.[33], a high total leukocyte is a sign of a bacterial infection, and fish are trying to increase their resistance to bacterial infection so that the leukocytes move actively toward the infected site. Whereas in treatments P1, P2, and P3, there was an increase in leukocytes but not as high as Kp; this was thought to be due to the influence of herbal medicine containing curcumin and flavonoids, which can normalize body tissue function and antioxidants. The increase in total leukocytes was still within the normal range. According to Putranto et al.[34], the average leukocyte count in tilapia ranges from 20,000 - 150,000 cells/mm^3.

According to Utami et al.[35], an increase in leukocyte cells is a reflection of the success of the fish immune system in developing a cellular (non-specific) immune response as a trigger for an immune response. Suprayudi et al.[36] added that the answer given by fish is to increase their body's resistance by increasing the number of leukocytes that function as defense cells. According to Fajriyani et al.[30], an increase in total leukocytes indicates that flavonoids can increase the production of leukocytes, and flavonoids trigger the immune system because leukocytes, as eaters (phagocytosis) of foreign bodies, are activated more quickly.

Leukocytes are blood cells that play a role in the immune system from foreign bodies, including pathogens that attack fish. The immune system uses antibodies to provide stimulation to identify and neutralize foreign bodies (antigens) that enter, such as bacteria[37].

Absolute Weight Growth

The maintenance of tilapia given feed containing fermented herbs affects the weight of tilapia. Data on the absolute weight growth of tilapia during the study can be seen in Table 5.

Based on the data in Table 5, the average value of tilapia at the beginning of rearing ranges from 5.07-5.21 g/fish. After 46 days of rearing by providing feed containing fermented herbs *K.galanga and C.xanthorrhiza*, the average weight of tilapia has increased to 27.20-39.26 g/fish. There were differences in the absolute weight growth of tilapia in several treatments. It can be seen that the highest average total weight growth of tilapia was in the P3 treatment, with a value of 34.06 g, while the lowest total weight was in the Kn treatment, namely 22.11 g

Table 5. The Absolute Weight Growth of Tilapia (*O. niloticus*)

Treatment	Average Weight (g/fish)		Absolute Weight (g/fish) ± SD
	Initial maintenance	End of maintenance	
Kn	5,07 ± 0,03	29,20 ± 0,06 ^{b}	24,14 ± 0,05 ^{b}
Kp	5,09 ± 0,08	27,20 ± 0,14 ^{a}	22,11 ± 0,07 ^{a}
P_1	5,13 ± 0,03	34,66 ± 0,02 ^{c}	29,53 ± 0,01 ^{c}
P_2	5,14 ± 0,03	34,83 ± 0,04 ^{c}	29,68 ± 0,08 ^{d}
P_3	5,21 ± 0,09	39,26 ± 0,14 ^{d}	34,06 ± 0,10 ^{e}

Description: * Different superscripts show significantly different (P<0.05)

Increasing the dose of fermented herbs in the feed can increase the growth of tilapia. Treatment P3 with 150 mL/kg of fermented meal herbs resulted in a weight growth of 39.26 g/head. Fish can grow optimally if several nutritional intakes are received and absorbed by the body. This growth is due to *C.xanthorrhiza* having antibacterial content, which can lyse toxins attached to the intestinal wall so that the absorption of nutrients becomes better and can trigger growth[38]. The fermentation process has broken down the complex compounds in *K.galanga* and *C.xanthorrhiza* into simpler compounds so that the fish's body quickly absorbs them. In addition, the fermentation process also produces a distinctive aroma that fish like. According to Meilina[39], the fermentation process results in the hydrolysis of complex compounds so that they become more easily absorbed. The curcumin content in fermented herbs can increase fish appetite and improve the work of the digestive organs in digesting carbohydrate, fat, and protein feed ingredients[23]. Thus, the absorption of nutrients becomes better; curcumin and essential oils physically and chemically have the potential as feed additives to increase feed productivity, feed quality, and fish health[10].

The results of the analysis of variance (ANOVA) showed that the administration of fermented herbs affected the absolute weight (P <0.05).

Survival Rate

Survival is the survival rate of fish in the cultivation process from the start of stocking to the end of rearing. Factors that affect fish's high or low survival are biotic factors, including competitors, population density, age, and ability to adapt to their environment[40]. The survival results of tilapia can be seen in Table 6.

Based on Table 6, it can be seen that the lowest percentage of survival was found in the Kp treatment, which was 23.33%. The low survival rate in the Kp treatment was due to post-infection with *S.agalactiae* bacteria, which can inhibit the performance of fish metabolism by damaging cells, tissues, and even organs in fish. Bacteria continue to develop so that they can produce more severe clinical symptoms, cause stress in fish and disease, and even cause death.

Table 6. Survival of Tilapia (*O. niloticus*)

Treatment	Survival Rate (%)		
	After Maintenance 30 days	After the 14th day of the Challenge Test	ANOVA
Kn	100	100	100,00 ± 0,00 ^{d}
Kp	100	23,33	23,33 ± 5,77 ^{a}
P_1	100	66,66	66,66 ± 5,77 ^{b}
P_2	100	86,66	86,66 ± 5,77 ^{c}
P_3	100	93,33	93,33 ± 5,77 ^{d}

Description: * Different superscripts show significantly different P<0.05

Analysis of variance (ANOVA) showed that the administration of fermented herbs affected survival (P<0.05). The highest survival rate for tilapia was found in treatment P3, namely 93.33% bacteria *S.agalactiae* in solid condition and can maintain its viability. The provision of this fermented herbal medicine can increase the immunity of fish so that mortality can be reduced. This is because fermented herbal medicine can boost the body's immunity to tilapia and make it healthier, as shown by the high consumption of feed every day several factors, such as stocking density, water quality, and feed quality, influence fish survival rates.

Syawal et al.[40] stated that giving herbal supplements mixed with pellets could triggerfish growth and reduce mortality; curcumin, flavonoids, and essential oils found in fermented herbs could boost the fish's immune system, function as antioxidants, antimicrobials, anti-inflammatories and be able to maintain and prevent, repair damaged cell tissue due to infection with foreign objects or microbes. Healthy fish conditions will increase fish appetite, weight growth, and fish survival.

4. CONCLUSION

The results showed that the fermented *K.galanga* and *C.xanthorrhiza* affected the hematology of *O.niloticus.* The best dose was in P3 with a dose of 150 ml/kg of feed which showed total erythrocytes of 2.01×10^6 cells/mm^3, hematocrit value of 27.33%, a hemoglobin level of 7.93 g/dL, total leukocytes of 6.59 x104 cells/mm3, lymphocytes 77.33%, monocytes 14.00%, neutrophils 8.67%, growth with a weight of 34.06 g, a length of 7.5 cm and a survival rate of 93.33%. Based on the data obtained, it can be concluded that fermented *K.galanga* and *C.xanthorrhiza* can increase the immunity of *O.niloticus* against *Streptococcus* disease.

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