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EFEKTIVITAS EKSTRAK DAUN KOMAK (*LABLAB PURPUREUS*) TERHADAP SISTEM IMUN IKAN NILA (*OREOCHROMIS NILOTICUS*) YANG DIINJEKSI BAKTERI *AEROMONAS HYDROPHILA*

Effectiveness Of Komak (Lablab Purpureus) Leaf Extract On The Immune System Of Tila Fish (Oreochromis Niloticus) Which Was Injected With Aeromonas Hydrophila Bacteria

Rabi'atussamiah[1], Fariq Azhar[1], Zaenal Abidin[1], Dewi Nur'aeni setyowati[1]

[1]Program Studi Budidaya Perairan, Jurusan Perikanan dan Ilmu Kelautan, Fakultas Pertanian, Universitas Mataram

Jl. Majapahit No.62, Selaparang, Mataram, Nusa Tenggara Barat 83115 Indonesia

*Alamat Korespondensi: fariqazhar@unram.ac.id

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak daun komak terhadap sistem imun ikan nila yang diinjeksi bakteri *A. hydrophila*. Penelitian ini dilaksanakan selama 60 hari di Laboratorium Produksi dan Reproduksi Ikan dan Laboratorium Kesehatan Ikan Budidaya Perairan, Program Studi Budidaya Perairan, Jurusan Perikanan dan Ilmu Kelautan, Fakultas Pertanian, Universitas Mataram. Metode yang digunakan eksprimen Rancangan Acak Lengkap (RAL) dengan lima perlakuan dan tiga ulangan yaitu Dimana K- tidak diberi ekstrak daun komak dan diinjeksi NaCl 0.9%, K+ tidak diberi ekstrak daun komak dan diinjeksi bakteri *A. Hydrophila*, P1 (0.5%) ekstrak daun komak dan diinjeksi bakteri *A. hydrophila*, P2 (1%) ekstrak daun komak dan diinjeksi bakteri *A. hydophila*, P3 (2%) ekstrak daun komak dan diinjeksi bakteri *A. hydrophila*. Parameter penelitian yang diamati berupa total eritrosit, total leukosit, haemoglobin, hematokrit, differensial leukosit, aktivitas fagositosis, total bakteri dan kelangsungan hidup (SR). Data yang diperoleh dianalisis secara deskriptif dan ANOVA dengan uji lanjut Duncan. Hasil penelitian didapatkan nilai rata-rata pada total eritrosit sebesar 1.22-1.82 $x10^6$ sel/mm^3, total leukosit 2.08-2.8 $x10^4$ sel/mm^3, hemoglobin 3.4-5.5 %, hematokrit 14.1-17.3 %, differensial leukosit terdiri dari 4 jenis sel yaitu limfosit, monosit, neutrofil dan trombosit. Limfosit 60.7-72.3%, monosit 4.3-7.3%, neutrofil 17-24.3%, dan trombosit 6.3-7.7%, aktifitas fagositosis 44.8-66.3%, total bakteri (TBC) OD_{620} 3.39-3.84, kelangsungan hidup (SR) 46.7-66.7%. Penambahan ekstrak daun komak sebanyak 2% pada pakan mampu meningkatkan system imun ikan nila yang diinjeksi dengan bakteri *A. hydrophila.*

ABSTRACT

This study aims to determine the effect of comma leaf extract on the immune system of tilapia injected with *A. hydrophila* bacteria. This research was conducted for 60 days at the Fish Production and Reproduction Laboratory and Aquaculture Fish Health Laboratory, Aquaculture Study Program, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram. The method used was a completely randomized design (CRD) experiment with five treatments and three replications, namely where K- was not given comma leaf extract and was injected with 0.9% NaCl, K+ was not given comma leaf extract and was injected with *A. Hydrophila* bacteria, P1 (0.5%) extract comma leaf and injected with *A. hydrophila* bacteria, P2 (1%) of komak leaf extract and injected with *A. hydophila* bacteria, P3 (2%) of komak leaf extract and injected with *A. hydrophila* bacteria. The observed parameters were total erythrocytes, total leukocytes, hemoglobin, hematocrit, differential leukocytes, phagocytosis activity, total bacteria and survival (SR). The results showed that the average value of total erythrocytes was 1.22-1.82 $x10^6$ cells/mm^3, total leukocytes were 2.08-2.8 $x10^4$ cells/mm^3, hemoglobin was 3.4-5.5%, hematocrit was 14.1-17.3%, differential leukocytes consisted of 4 types of cells, namely lymphocytes. , monocytes, neutrophils and platelets. Lymphocytes 60.7-72.3%, monocytes 4.3-7.3%, neutrophils 17-24.3%, and platelets 6.3-7.7%, phagocytosis activity 44.8-66.3%, total bacteria (TB) OD_{620} 3.39-3.84, survival (SR) 46.7-66.7 %. The addition of 2% of comma leaf extract to the feed can improve the immune system of tilapia injected with *A. hydrophila* bacteria.

Kata Kunci	*Lablab purpureus, Aeromonas hydrophila, Ikan nila*
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INTRODUCTION

Tilapia is one of the fish most popular with the wider community. Therefore, tilapia cultivation can be a very promising business. To increase tilapia production, it is necessary to pay attention to several things in terms of environment, feed, reproduction and even diseases that can attack fish, especially bacteria. According to Ashari *et al.,* (2014) the bacteria that usually attack tilapia are A. Hydrophila bacteria and cause the disease Motile Aeromonas Septicemia (MAS) which can reduce the growth rate of fish and even cause 80%-100% death within 1 year. -2 weeks.

One of the treatments used is antibiotics. However, it is less efficient when used on a large scale because it is not economical, it can give rise to new bacteria that are resistant to antibiotics and can pollute the environment (Maisyaroh *et al.,* 2018). So using natural materials is the right alternative because it is more effective and efficient.

One of the natural ingredients that can be used to prevent disease in tilapia, especially those attacked by A. hydrophila bacteria, is Komak leaves (Lablab purpureus). Because as we know, komak leaves contain secondary metabolites which can be used as inhibitors of bacterial growth. According to Parvin et al. (2013) that based on the results of initial phytochemical screening of methanol extract from komak leaves stated the presence of alkaloids, saponins, tannins.

METHODS

This research was carried out for 60 days starting from May to July 2022 at the Fish Production and Reproduction Laboratory and Aquaculture Fish Health Laboratory, Aquaculture Study Program, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram. The method used was an experimental method with a Completely Randomized Design (CRD) consisting of 5 treatments and 3 replications. The treatments include:

K- : No extract was given and 0.9% NaCl was injected
K+ : Not given extract and injected with A. hydrophila
P1 : 0.5% extract and injected with A. hydrophila
P2 : 1% extract and injected with A. hydrophila
P3 : 2% extract and injected A. hydrophila

Research Procedure

1. Media Preparation and Test Fish

The media used were 15 containers. Each container is filled with a stocking density of 20 birds. The fish used as test fish were tilapia measuring 6-7 cm long. The tilapia fish are acclimatized first so they get used to living in a test container for 3 days and are only given commercial feed.

2. Making Komak Leaf Extract

Komak leaves are washed clean and dried. Then it was ground using a blender and the powder was weighed as much as 1 kg and extracted with a 96% ethanol solution with a ratio of 1:3. This extract is made by maceration for 3x24 hours and stirring for 6 or 8 hours (2 times a day). Next, it is filtered using Whatman Number 1 filter paper and evaporated in a Rotary evaporator.

3. Feeding Komak Leaf Extract

Feeding in the form of komak leaf extract is done by spraying it into commercial feed according to the concentration, namely 0% K+, 0% (injection with NaCl), 0.5%, 1%, 2%. Providing commercial feed that has been mixed with extracts amounting to 5% of the fish's body weight and given for 50 days of rearing period with administration times 3 times a day, namely morning, afternoon and evening.

4. Bacterial Preparations

Preparation of A. hydrophila bacteria with a density of 10-8 to infect fish using NA media as a growth medium, then culturing was carried out by taking 1 sample of A. hydrophila bacteria on slanted agar media then streaking on NA media and incubating for 1x24 hours. Next, for the challenge test, use TSB media as a bacterial

growth medium. Meanwhile, the bacterial preparation Streptococcus sp. with a density of 10-6 for phagocytic activity and using TSB media as a growth medium.

5. Challenge Test

Fish injection is carried out using the injection method. Where the bacteria in the TSB media are taken using a syringe and then injected 0.1 mL into each test fish intramuscularly.

6. Parameters

Total Erythrocytes

Total erythrocytes were counted using a haemocytometer by counting the 5 small boxes inside. The total erythrocyte formula according to Fitria *et al.,* (2019) is as follows:

$$SDM = \frac{\text{Ne x 200}}{\text{0.02}}$$

Information :

SDM = Red blood cells (sel/mm^3)
Ne = The number of erythrocytes in 5 counting boxes
P = Dilution factor (200 times)
0.02 = The total blood volume in 5 boxes is calculated (mm^3)

Total Leukocytes

Blood was taken using a pipette with a scale of 0.5 and added Turks solution up to a scale of 11 to the leukocyte pipette with a retail factor of 20 times. Next, homogenize by shaking your hands in a number 8 shape so that it is evenly mixed. After that, the blood was dropped into a haemocytometer and covered using a cover glass and then observed under a microscope with 40X magnification (Putranto *et al.,* 2019). The total leukocyte formula according to Fitria *et al.,* (2019) is as follows:

$$SDP = \frac{\text{Nl x 20}}{\text{0.4}}$$

Information :

SDP = White blood cells (sel/mm^3)
Nl = The number of leukocytes in 5 counting boxes
P = Dilution factor (20 times)
0.4 = The total blood volume in 5 boxes is calculated (mm^3)

Hemoglobin

First of all, put 0.1 HCl solution into a 2 mm3 dilution tube. Next, blood is taken using a pipette. Hb up to a volume of 20 mm3 is mixed with a stirrer and then inserted into a block comparator to see the comparison with the standard solution. If it is still different, distilled water can be added until the color is the same as the solution to obtain the Hb level (%)(Alipin, 2020).

Hematokrit

Hematocrit measurements are carried out by taking a blood sample in a syringe and inserting it into a capillary tube and the end of the capillary tube which has a red mark is covered with plasticine. After that, the capillary tube was arranged in a centrifuge and spun for 5 minutes at a speed of 5000 rpm. Hematocrit results are calculated using a ruler (Pratiwi, 2019). The hematocrit formula is as follows:

$$Hematokrit\ (\%) = \frac{\text{panjang endapan kapiler pipa eritrosit (mm)}}{\text{panjang total}} \times 100\%$$

Leukocyte Differential

Differential leukocyte observations used Giemsa solution and the cells were observed in a microscope with 40X magnification according to their type, namely lymphocytes, monocytes, neutrophils and platelets, then counted until there were 100 cells (Hartika, 2014). The formula is as follows:

$$\%limfosit\ (L) = \frac{\text{L}}{100} \text{x } 100$$

$$\%monosit\ (M) = \frac{\text{M}}{100} \text{x } 100$$

$$\%neutrofil\ (N) = \frac{\text{N}}{100} \text{x } 100$$

$$\%trombosit\ (T) = \frac{\text{T}}{100} \text{x } 100$$

Phagocytic Activity

Observation of phagocytosis activity using Streptococcus sp bacteria. and the formula according to Mardiana & Budi, (2017) is as follows:

$$Aktivitas\ fagositosis = \frac{\text{jumlah sel fagosit yang melakukan fagositosis}}{\text{jumlah sel fagosit yang teramati}} \text{ x } 100\%$$

Total Bacteria in Fish Guts

The total bacteria count in the fish intestines was carried out on the 60th day of the rearing period. Calculation of total bacteria in the fish intestines using the Total Bacterial Count (TBC) method and calculated using a spectrophotometer with OD (Oprical Density) units (Rosmania & Yanti, 2020).

Post-Injection Survival (SR)

The percentage of tilapia fish that lived after being given pellets was calculated at the end of the research (Mardiana, 2017). The survival calculation of these fish was carried out on the 10th day after the challenge test with A. hydrophila bacteria. The formula is as follows:

$$SR\ (\%) = \frac{\text{jumlah ikan pada waktu t}}{\text{jumlah ikan pada waktu awal}} \text{ x } 100$$

7. Data Analysis

The data analyzed in this research is water quality data analyzed descriptively. Meanwhile, for blood tests, survival rates and total bacteria were analyzed using diversity analysis. If the results are significantly different then a further Duncan test is carried out (Purnamasari, 2015).

RESULT

Total Erythrocytes

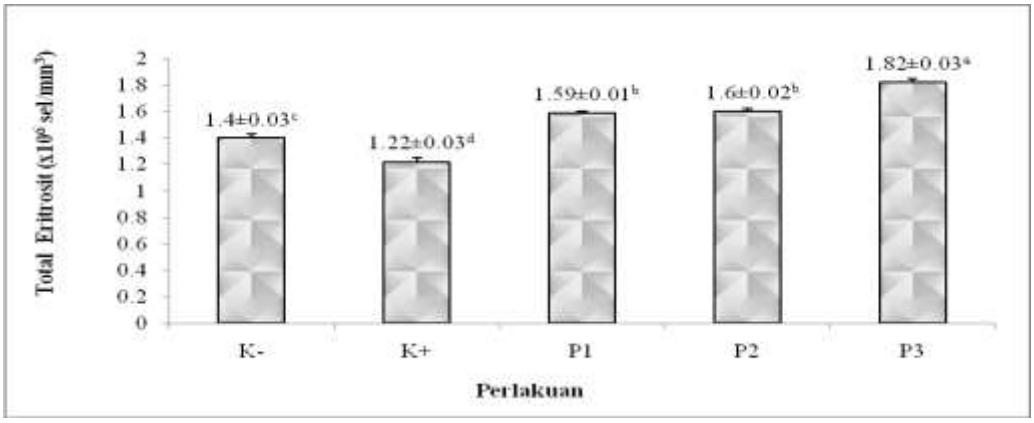

Figure 1. Average Total Erythrocytes of Nile Tilapia (*O. Niloticus*)

The results obtained by adding komak leaf extract to feed at different doses had an effect ($p<0.05$) on the total erythrocyte value in tilapia fish injected with A. hydrophila bacteria. Figure 1 shows that P3 has the highest total erythrocyte value and is different ($p<0.05$) from all treatments, namely 1.82×106 cells/mm3, but P1 and P2 have a total erythrocyte value that is not different ($p>0.05$) from values are 1.59×106 and 1.6×106, then K+ has a total erythrocyte value lower than K- and is different ($p<0.05$) with all treatment, namely values respectively 1.22×106 and 1.4×106 cells/mm3.

Total Leukocytes

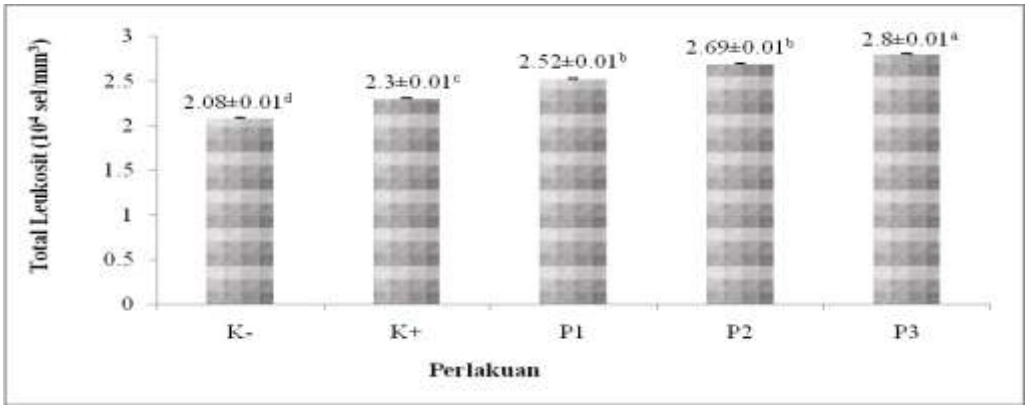

Figure 2. Average Total Leukocytes of Tilapia Fish (*O. niloticus*)

Figure 2 shows that the addition of komak leaf extract to feed at different doses had an influence ($p<0.05$) on the total leukocyte value in tilapia fish injected with A. hydrophila bacteria. Based on the diagram data above, it shows that P3 has the highest and different total leukocyte value ($p<0.05$) with all treatments, namely 2.8×104 cells/mm3, but P1 and P2 have no different total leukocyte value ($p>0.05$), namely 2.52×104 and 2.69×104. Meanwhile, K+ had the lowest total leukocyte value and was different ($p<0.05$) with all treatments, namely 2.3×104 cells/mm3.

Hemoglobin

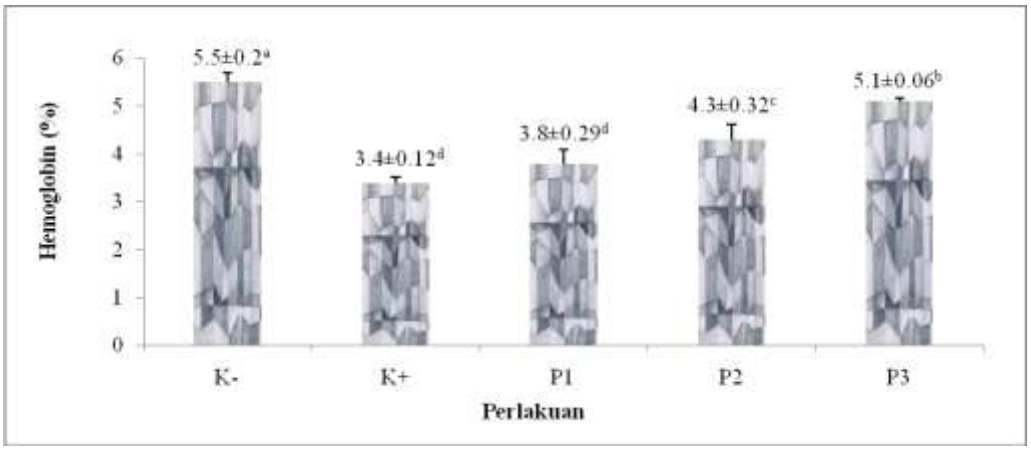

Figure 4.3. Average Hemoglobin of Tilapia (*O. niloticus*)

The results obtained from mixing feed with komak leaf extract using different doses had an influence ($p<0.05$) on the hemoglobin value of tilapia injected with A. hydrophila bacteria. It can be seen in Figure 3 that P3 has the highest hemoglobin value and is different ($p<0.05$) from all treatments, namely 5.1%. P2 and K- have different hemoglobin values ($p<0.05$), namely 4.3% and 5.5%, but K+ has the lowest hemoglobin values and is not different ($p>0.05$) from P1, namely 3.4% and 3.8%.

Hematokrit

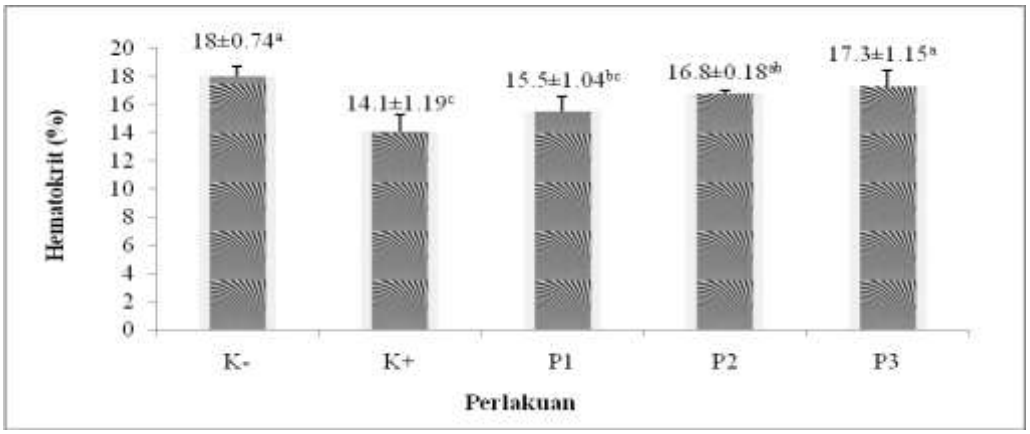

Figure 4. Average Hematocrit of Tilapia (O. niloticus)

The addition of komak leaf extract to feed at different doses is thought to have an influence ($p<0.05$) on the hematocrit value in tilapia fish injected with A. hydrophila bacteria. Where P3 has the highest hematocrit value and is not different

(p>0.05) with K-, namely 17.3% and 18%. Then P1 and P2 have hematocrit values that are not different (p>0.05) with values of 15.5% and 16.8%, then K+ has the lowest hematocrit values and is not different (p>0.05) with P1, namely 14.1% and 15.5%.

Leukocyte Differential

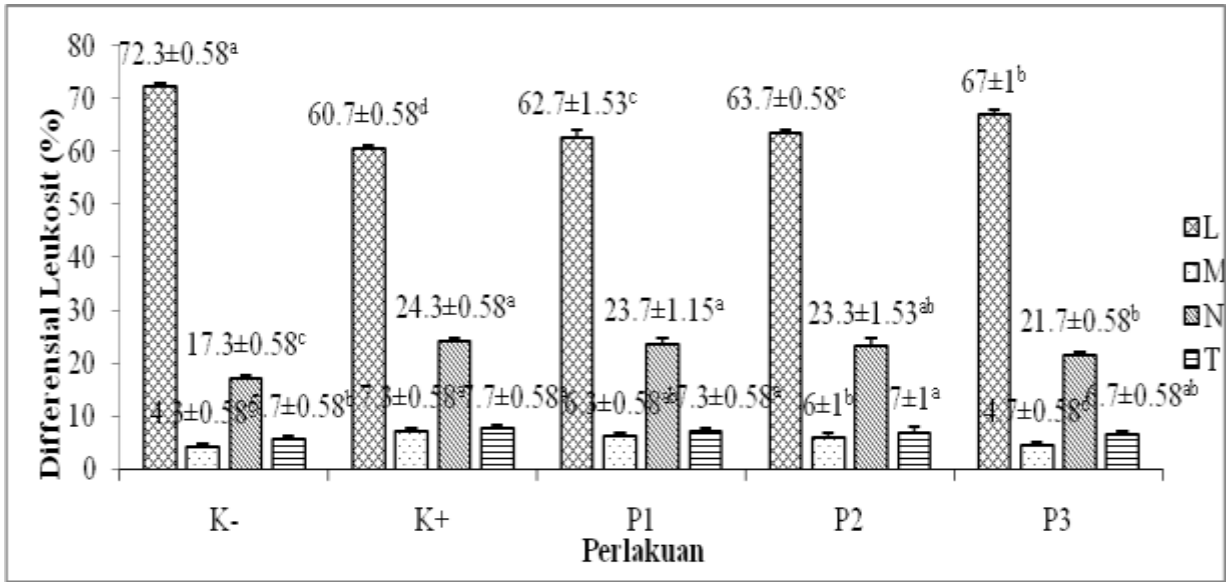

Figure 5. Average Differential Leukocytes in Tilapia (*O. niloticus*)
Superscript: Values with different superscripts in the same cell type show different results (p<0.05)

Differential observations of leukocytes in tilapia consist of 4 types of cells, namely lymphocytes, monocytes, neutrophils and platelets. In Figure 5. it can be seen that feeding in the form of adding komak leaf extract using different doses had an effect (p<0.05) on the differential value of leukocytes in tilapia fish that were injected with A. hydrophila bacteria, both in lymphocytes, monocytes, neutrophils and platelets. In lymphocyte cells, P3 had the highest and different lymphocyte value (p<0.05) with all treatments, namely 67%, however P1 and P2 had no different lymphocyte value (p>0.05) with values of 62.7% and 63.7%, then K+ had the lowest lymphocyte value and was different (p<0.05) from all treatments, namely 60.7%. In monocyte cells, K+ has the highest monocyte values and is not different (p>0.05) with P1, namely 7.3% and 6.3%, then P1 and P2 have monocyte values that are not different (p>0.05) with values of 4.3% and 6.3%. . Then P3 and K- had the lowest monocyte values and were not different (p>0.05), namely 4.7% and 4.3%. Neutrophil cells in K+ had the highest neutrophil values and were not different (p>0.05) from P1 and P2, namely values of 24.3, 23.7, and 23.3% respectively. Then P3 has the lowest value and is not different (p>0.05) from P2, namely 21.7% and 23.3%. Platelet cells in K+ had the highest platelet values and were not different (p>0.05) from treatments P1, P2 and P3, namely values respectively 7.7, 7.3, 7 and 6.7%, but P3 had the lowest platelet values and was not different (p>0.05) with K- namely 6.7% and 6.3%.

Phagocytic Activity

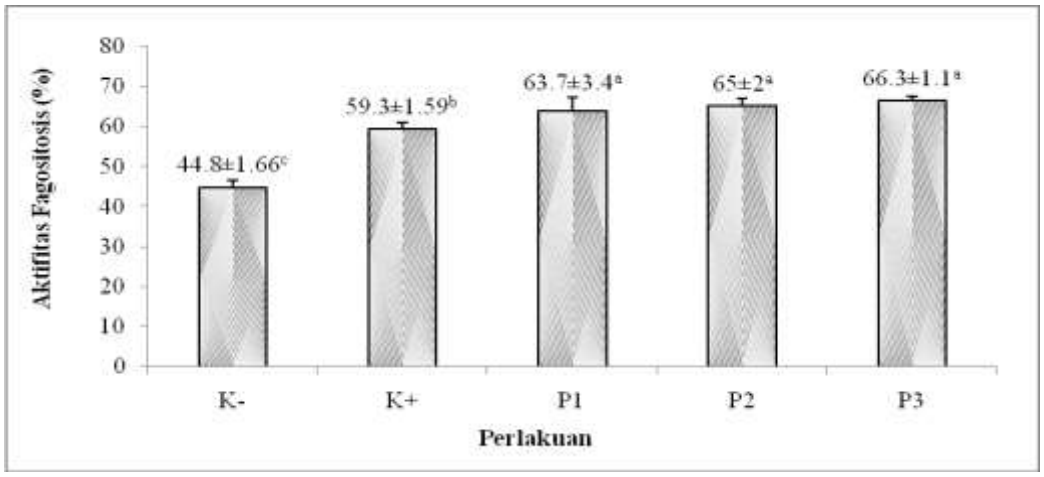

Figure 6. Average phagocytic activity of Tilapia (*O. niloticus*)

The results obtained in Figure 6 show that the addition of komak leaf extract to feed at different doses can have an effect ($p<0.05$) on the value of phagocytic activity in tilapia injected with A. hydrophila bacteria. P3 has the highest phagocytic activity value and is not different ($p>0.05$) from P2 and P1, namely values of 66.3%, 65% and 63.7% respectively. Then K+ has the lowest phagocytic activity value and is different ($p<0.05$) from K-, namely 59.3% and 44.8%.

Total Bacteria

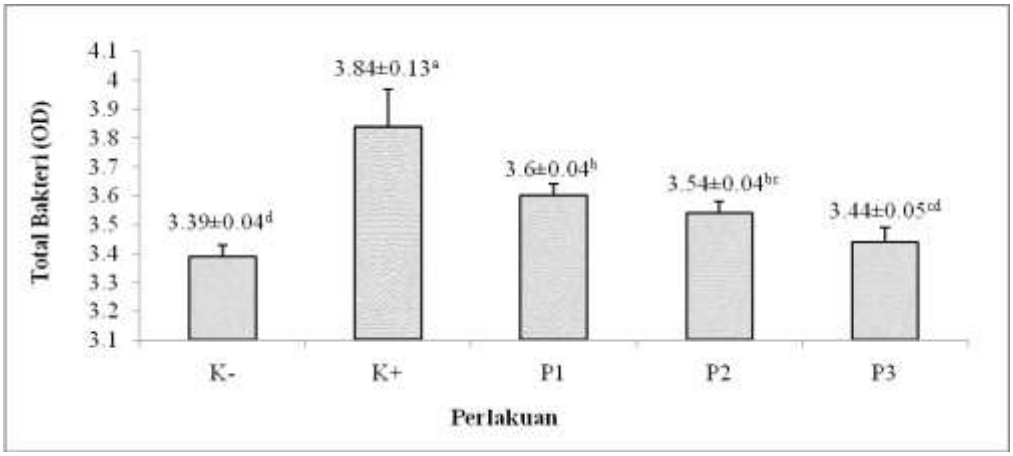

Figure 7. Average Total Bacteria (TBC) for Tilapia (*O. niloticus*)

Figure 7 explains that feeding supplemented with komak leaf extract had an influence ($p<0.05$) on the total bacterial value of tilapia injected with A. hydrophila bacteria. Where K+ has the highest TBC value and is different ($p<0.05$) from all treatments, namely OD620 3.84. P1 and P2 have no different TBC values ($p>0.05$), namely OD620 3.6 and OD620 3.54. Furthermore, P2 had a TBC value that was not different ($p>0.05$) from P3, namely OD620 3.54 and OD620 3.44. Then P3 has the lowest TBC value and is not different ($p>0.05$) from K-, namely OD620 3.44 and OD620 3.39.

Survival Rate (SR)

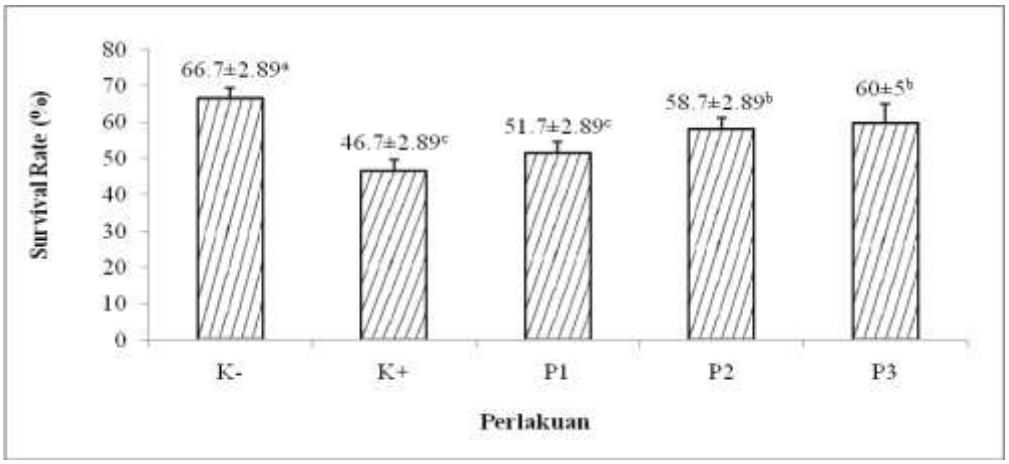

Figure 8. Average Survival Rate (SR) of tilapia

Feeding by adding komak leaf extract at different doses had an influence ($p<0.05$) on the SR value of tilapia that were injected with A. hydrophila bacteria. In Figure 8, it can be seen that P3 has the highest SR value and is not different ($p>0.05$) from P2, namely 60% and 58.3%. Then K+ has the lowest SR value and is not different ($p>0.05$) from P1, namely 46.7% and 51.7%.

DISCUSSION

Red blood cells (erythrocytes) are one of the components of blood cells in tilapia with a higher number of cells than other cells and are used as an indicator of fish health (Putranto *et al.,* 2019). The normal number of erythrocytes in teleost fish is between 1.05–3.0 x106 cells/mm^3 (Matofani *et al.,* 2013). After the A. hydrophila challenge test, the total erythrocyte K+ was lower than when treated with komak leaf extract. According to Matofani *et al.,* (2013) that if bacteria enter the fish's body, a phagocytosis process will occur which causes a decrease in red blood cells. Meanwhile, P3, which was given 2% extract, had a higher total erythrocyte value than other treatments because the komak leaf extract contained phytochemical compounds. According to Putranto *et al.,* (2019) that plant leaves which contain tannin, flavonoid and saponin compounds can be used as an immunostimulant and are indicated to improve the blood profile of fish.

White blood cells (leukocytes) are the body's defense system in tilapia which function as antibodies when bacteria attack the fish's body (Putranto *et al.,* 2019). From the research results, it was found that the total leukocyte value in the bacteria injected treatment had increased compared to K-. According to Dawan *et al.,* (2021) the number of leukocytes will increase if the fish is sick to produce antibodies to phagocytose bacteria. Apart from that, at P3 (2%) leukocytes increased because komak leaf extract contained flavonoid compounds. According to Maryani *et al.,* (2021) who stated that the increase in the number of leukocytes in fish blood is influenced by the presence of flavonoid compounds.

Hemoglobin (Hb) is a part of blood plasma cells which has a function in blood circulation and determines the level of body resistance of fish which is closely related to erythrocytes (Alipin & Sari, 2020). K+ has the lowest Hb value compared to the

treatment given the extract, namely 3.4%. According to Putranto et al., (2019), normal hemoglobin levels in tilapia range from 5.05-8.33%. This low Hb value is caused by bacterial infection in the fish's body and this is in accordance with the opinion of Hardi et al., (2013) who stated that low Hb is caused by hemolysin originating from bacteria which can result in lower blood plasma osmolarity. Meanwhile, P3 (2%) has a Hb value that is still within normal levels because the extract contains flavonoids which have a positive influence on increasing Hemoglobin in fish blood. According to Saparuddin, (2018), increasing hemoglobin levels in treatments given ethanol extract and mixed feed with higher doses means hemoglobin levels in fish also increase.

The hematocrit level in fish is useful for assessing the health condition of the fish, namely by looking at the comparison of the volume value of erythrocytes with blood plasma, which is closely related to red blood cells (Pratiwi & Eddiwan, 2019). K+ has the lowest hematocrit value compared to the treatment given the extract. According to Maryani et al., (2021) that hematocrit has decreased due to fish experiencing stress and also fish being infected with pathogenic bacteria and not being given immunostimulants. However, in the P3 (2%) treatment given komak leaf extract, the hematocrit value was not much different from K- because komak leaf extract has secondary metabolite compounds which can be used as anti-bacterial. According to Saparuddin, (2018) that giving ethanol extract mixed with commercial feed can increase blood hematocrit levels in infected fish.

Differential observations of leukocytes in tilapia fish are divided into four types, namely lymphocytes, monocytes, neutrophils and also platelets. Lymphocyte cells are a system that plays an important role as the immune system in tilapia (Devitha et al., 2013). The lymphocyte cell values obtained in the study ranged from 60.7-72.3% and were considered normal in accordance with the statement by Utami et al., (2013) that the lymphocyte values for tilapia fish were between 60-86%. In P3, by administering 2% extract, the lymphocyte value increased because the komak leaf extract contained active compounds which could be used as immunostimulants in defending the fish's body from pathogen attacks and could also increase lymphocyte cells. According to Riswan et al., (2021) that several bioactive compounds found in several types of plants, such as flavonoids, are thought to be able to improve the immune system and act as antibacterials by disrupting the organism's system.

Monocyte cells are cells that play a role in providing information to leukocytes regarding the presence of foreign substances entering the fish's body (Utami et al., 2013). K+ has a higher monocyte value compared to K- because the working system of monocyte cells increases to detect foreign objects entering the fish's body and transmits this information to leukocytes. According to Rustikawati, (2012) that monocyte cells increase because when infected by foreign substances the monocyte cells will move away from the blood vessels to carry out phagocytosis in areas infected by bacteria. Meanwhile, P3 (2%) given komak leaf extract had a low monocyte value due to the presence of phytochemical compounds as antibacterials so that if infection occurred, the monocyte cells responded to carry out phagocytosis against the A. hydrophila bacteria. According to Riswan et al., (2021) that the active compounds contained in the extract in the form of flavonoids can function as antibacterials by forming complex compounds that can disrupt the integrity of bacterial cell membranes.

Neutrophil cells are cells that play an important role in the body's defense system in tilapia (Utami *et al.,* 2013). The neutrophil K+ is higher than K- due to the presence of pathogens that enter the fish's body. According to Rustikawati, (2012) that the increase in the number of neutrophil cells occurs as a result of the accumulation of macrophages in the infected area and functions to destroy foreign objects. Meanwhile, in P3 (2%) the neutrophil value is low because it contains phytochemical compounds which work actively in areas infected with bacteria so that there are few neutrophil cells in the blood. According to Riswan *et al.,* (2021) the phytochemical compounds contained in the extract can reduce clinical symptoms due to bacterial infections so that the lymphoid organs do not produce neutrophil cells in large numbers.

Platelet cells are cells that play an important role in the blood clotting process (Salim *et al.,* 2016). K+ has a higher value compared to K- because in this treatment A. hydrophila is injected and causes injury to the fish's body so that the number of platelets increases. According to Salim *et al.,* (2016) that platelets play an important role in the blood clotting process and function to prevent greater loss of body fluids due to infections that occur on the surface of the fish's body. Meanwhile, P3, which was given komak leaf extract at a dose of 2%, had the same platelet value as K- because of the secondary metabolite compounds contained in the extract and was used as an antibacterial in healing fish wounds, resulting in a reduced platelet working system. According to Kurniawan *et al.,* (2020) who stated that the decrease in platelet numbers occurred due to the secondary metabolite content in the extract which plays a role in wound healing activity and can be used as an antimicrobial.

Phagocytic activity (AF) is a process of absorption or elimination of foreign objects that enter the fish's body by phagocyte cells (Payung & Manoppo, 2019). Based on the results of observations after the challenge test with A. hydrophila bacteria, it was found that the AF value for K+ was higher than K- due to the attack of A. hydrophila bacteria on the tilapia's body and causing the cell's work system to increase to phagocyte the bacteria. According to Saparuddin, (2021) that the process of phagocytosis is contact between phagocytosed cells and particles with cell membranes experiencing invagination that engulf foreign objects. Apart from that, the treatment given the extract at P3 (2%) had the highest AF value of all treatments because the high dose can increase the body's immune system in phagocytizing foreign objects that enter it and there are phytochemical compounds contained in the komak leaves which can be used as immunostimulants. by blood cells in attacking bacteria. According to Mardiana & Budi, (2017) that increased phagocytic activity can occur at the beginning of the response to administration of immunostimulants or at the beginning of infection.

Total bacteria (TBC) is the percentage of the number of bacteria found in the fish's body using a spectrophotometer. The TBC results obtained after the challenge test on K+ had the highest TBC values compared to the treatment given the extract. As in the P3 treatment given the extract at a dose of 2%, the TBC value was low and not much different from K-. The low TBC value in P3 is due to the content of secondary metabolite compounds which can play an important role in preventing the spread of bacteria due to A. hydrophila infection. According to Parvin et al., (2013) who stated that in the methanol extract from komak beans there are alkaloids, saponins, tannins which function as antibacterial.

Survival rate (SR) is the percentage of the number of fish that live in a certain period of time, the aim of which is to see the number of fish that live from the start of the study to the end of the study (Mardiana, 2017). Based on observations after injection of bacteria into fish, it was found that the SR value of K+ was lower compared to the treatment given with komak leaf extract. The best treatment is in the P3 treatment which is given extract at a dose of 2% because the extract contains active ingredients in the form of flavonoids which are used as antibacterials which are useful in inhibiting the growth of Aeromonas hydrophila bacteria in the host. According to Maryani *et al.,* (2021), secondary metabolite compounds in the form of flavonoids and steroids are thought to have an immunomodulatory effect to inhibit the growth of the pathogenic bacteria A. hydrophila, which is characterized by high tilapia survival.

CONCLUSION

The addition of 2% komak leaf extract to feed can improve the immune system of tilapia fish that are injected with A. hydrophila bacteria. The results obtained for total erythrocytes were 1.82x106 cells/mm3, total leukocytes 2.80x104 cells/mm3, hemoglobin 5.1%, hematocrit 17.3%, differential value of leukocytes in lymphocyte cells 67%, monocyte cells 4.7%, neutrophil cells 21.7% and platelets 6.7%, phagocytic activity value 66.3%, TBC OD620 value 3.44, survival rate (SR) 60%.

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