JMAI Jurnal Media Akuakultur INDONESIA
https://journal.unram.ac.id/index.php/jmai/index. E-ISSN : 2798-0553
Volume 4, Number 4, November 2024

EFEKTIVITAS EKSTRAK DAUN KAWISTA *(Limonia acidissima)* TERHADAP SISTEM IMUN IKAN NILA *(Oreochromis niloticus)* YANG DIINJEKSI BAKTERI *Aeromonas hydrophila*

Effectiveness of kawista leaf extract (Limonia acidissima) against the immune system tilapia fish (Oreochromis niloticus) injection bacteria Aeromonas hydrophila

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ABSTRAK

Ikan nila *(Oreochromis niloticus)* merupakan salah satu komoditas air tawar yang memiliki prospek tinggi pada sektor perikanan. Bakteri *A. hydrophila* adalah bakteri patogen yang dapat menyebabkan kematian, sering ditemukan pada ikan air tawar. Tanaman kawista mengandung senyawa alkaloid, tanin, flavonoid, streoid dan polifenol. Tujuan penelitian adalah untuk mengetahui efektivitas ekstrak daun kawista *Limonia acidissima)* terhadap sistem imun ikan nila *(Oreochromis niloticus)* yang diinjeksi bakteri *A. hydrophila.* Penelitian ini bersifat eksperimental dengan menggunakan metode Rancangan Acak Lengkap (RAL) yang terdiri dari 5 perlakuan dengan 3 ulangan yaitu K- (Tidak diberikan ekstrak daun kawista dan diinjeksi NaCl 0.9%), K+ (Tidak diberikan ekstrak daun kawista dan diinjeksi bakteri *A. Hydrophila*), P1 (Penambahan ekstrak daun kawista pada pakan dengan dosis 0.5% dan diinjeksi bakteri *A.hydrophila*, P2 (Penambahan ekstrak daun kawista pada pakan dengan dosis 1% dan diinjeksi bakteri *A. Hydrophila*), P3 (Penambahan ekstrak daun kawista pada pakan dengan dosis 2% dan diinjeksi bakteri *A. Hydrophila*). Hasil penelitian menunjukkan bahwa pemberian ekstrak daun kawista dengan dosis yang berbeda mempengaruhi sistem imun pada ikan nila dan kelangsungan hidup ikan. Pada penelitian ini diperoleh perlakuan terbaik terdapat pada perlakuan 2 dengan nilai total eritrosit sebesar $1.92x10^6$sel/mm^3, total leukosit $2.58x10^4$sel/mm^3, differensial leukosit terbagi menjadi 4 bagian yaitu limfosit 66.7%, monosit 5.33%, neutrofil 21.7%, dan trombosit 6.33%, hemoglobin 6.6%, hematokrit 17.33%, aktivitas fagosistosis 69.93%, dan *Total Bakteri Count* (TBC) sebesar OD_{620} 3.53 dan survival rate sebesar 68%. Adapun kesimpulan yang diperoleh bahwa penggunaan esktrak daun kawista dengan dosis 1% pada pakan mampu meningkatkan sistem imun ikan nila yang diinjeksi bakteri *A. hydrophila*.

ABSTRACT

Tilapia (*Oreochromis niloticus*) is a freshwater commodity that has high prospects in the fisheries sector. A. hydrophila bacteria is a pathogenic bacteria that can cause death, often found in freshwater fish. The kawista plant contains alkaloids, tannins, flavonoids, steroids and polyphenols. The objective of the study was to determine the effectiveness of kawista leaf extract Limonia acidissima) against the immune system of tilapia (Oreochromis niloticus) injected with A. hydrophila bacteria. This study was experimental using a completely randomized design (RAL) method consisting of 5 treatments with 3 replications namely K- (Not given kawista leaf extract and injected with 0.9% NaCl), K+ (Not given kawista leaf extract and injected with A. Hydrophila bacteria), P1 (Addition of kawista leaf extract to feed with a dose of 0.5% and injection of A. hydrophila bacteria, P2 (Addition of kawista leaf extract to feed with a dose of 1% and injection of A. Hydrophila bacteria), P3 (Addition of kawista leaf extract to feed with dose of 2% and injection of A. Hydrophila bacteria).The results showed that giving kawista leaf extract with different doses affected the immune system in tilapia and fish survival.In this study the best treatment was obtained in treatment 2 with a total erythrocyte value of 1.92 x10^6 cell/mm^3, total leukocytes 2.58x10^4 cell/mm^3, differential leukocytes are divided into 4 parts, namely lymphocytes 66.7%, monocytes 5.33%, neutrophils 21.7%, and platelets 6.33%, hemoglobin 6.6%, hematocrit 17.33%, phagocytosis activity 69.93%, and a Total Bacteria Count (TBC) of OD_{620} 3.53 and a survival rate of 68%. The conclusion was obtained that the use of kawista leaf extract at a dose of 1% in feed could improve the immune system of tilapia injected with A. hydrophila bacteria.

Kata Kunci	*Ikan nila, A. hydrophila, Ektrak daun kawista*
Keywords	*Tilapia, A. hydrophila, Kawista leaf extract*
Tracebility	Submission: 22/5/2024. Published : 4/11/2024
Panduan Kutipan (APPA 7th)	Febriana, N., Azhar, F., Abidin, Z., & Setyowati, D. N. (2024). Efektivitas Ekstrak Daun Kawista (*Limonia acidissima*) terhadap Sistem Imun Ikan Nila (*Oreochromis niloticus*) yang Diinjeksi Bakteri *Aeromonas hydrophila*. *Jurnal Media Akuakultur Indonesia*, 4(4), 191-204. http://doi.org/10.29303/mediaakuakultur.v4i4.6139

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a freshwater commodity that has high prospects in the fisheries sector. Currently, market demand for tilapia is increasing. This fish has quite high economic value in Asia, including Indonesia. Tilapia fish was imported to Indonesia in 1969. According to the Ministry of Maritime Affairs and Fisheries (2020), tilapia production in 2013 targets tilapia production of 1.1 million tons and in In 2014 fish production reached 1.25 million tons. According to the Food and Agriculture Organization (FAO), tilapia production continues to increase in 2010, estimated at 2.5 million tonnes.

While cultivation is ongoing there are still obstacles faced such as disease. Disease is a serious problem that is often encountered by cultivators because it can result in losses. A. hydrophila bacteria is a pathogenic bacteria that causes disease, which is often found in freshwater fish. Clinical symptoms include red spots, destruction of the skin, gills and internal organs of the fish. The way to deal with A. hydrophila bacterial attacks is to give antibiotics, however antibiotics cause pathogens that can pollute the environment, so they are not effective. To overcome this problem, natural materials found in the surrounding environment are used. The kawista plant has medicinal properties ranging from its fruit, seeds, thorns, bark, roots and leaves. Kawista leaves contain secondary metabolite compounds that are beneficial for health. Kawista plants are still not utilized optimally by the community in the fisheries sector, especially kawista leaves.

Kawista leaves are a plant belonging to the Rutaceae member. According to Nugroho *et al.,* (2020) based on previous research, kawista contains phytochemical substances such as alkaloids, tannins, saponins, flavonoids and phenols which function as antibacterials. Antibacterial is a substance that can interfere with the growth or even kill bacteria by interfering with bacterial metabolism. This research was conducted with the aim of determining the effectiveness of kawista (*Limonia acidissima*) leaf extract on the immune system of tilapia (*Oreochromis niloticus*) which were injected with A. hydrophila bacteria..

METHODS

Place and Time

This research was carried out for 60 days from 25 May - 24 July 2022, which took place at the Fish Production and Reproduction Laboratory and Fish Health Laboratory, Aquaculture Study Program, Faculty of Agriculture, Mataram University. Phytochemical tests and making kawista leaf extract were carried out at the Analytical Chemistry Laboratory and Basic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University.

Tools and Material

The tools used in this research include; autoclave, blender, bunsen, petri dish, cover glass, DO meter, Erlenmeyer, haemocytometer, haemometer, hematocrit, hot place, ose needle, glass slide, container, filter paper, miktoscope, micropipette, microtube, ruler, pH meter, measuring pipette, rotary evaporator, centrifugator, spectrophotometer, 1 ml syringe, analytical balance, falcon tube and vortex. The materials used include; Tilapia fish seeds, kawista leaf extract, Aeomonas hydrophila bacteria, Streptococcus sp. bacteria, 95% alcohol, distilled water, anticoagulant, 96% ethanol, fish blood, 0.9% NaCl, NA (Nutrient Agar) Media, TSB (Tryptic Soy Borth) Media , Giemsa solution, Turk's solution, 0.1 N HCL solution, Hayem solution, 95% methanol, HI Pro Vite FF-999 feed, and microhematocrit tube.

Research methods

The method used in this research was a Completely Randomized Design method using 5 treatments with each treatment carried out 3 times, so that 15 experimental units were obtained, including::

K- : Kawista leaf extract was not given and NaCl 0.9% was injected
K+ : Kawista leaf extract was not given and A. hydrophila bacteria were injected
P1 : Kawista leaf extract was added to the feed at a dose of 0.5% and A. hydrophila bacteria were injected
P2 : Kawista leaf extract was added to the feed at a dose of 1% and bacteria were injected
A. hydrophila
P3 : Kawista leaf extract was added to the feed at a dose of 2% and A. hydrophila bacteria were injected

Procedure

Preparation of Containers and Test Animals

The maintenance containers used in this research were 15 containers measuring 45 cm x 30 cm x 28 cm. Before use, the container is cleaned first using detergent and dried in the sun. After that, the containers were arranged neatly according to the experimental design plan on the research shelf. Then it is filled with 20 liters of water and an aeration hose is installed which aims to increase the oxygen supply to the fish. The test animals used in this research were 300 tilapia fish measuring 6-7 cm which were obtained from the Batu Kumbung Fish Seed Center, Lingsar, West Lombok. The tilapia seeds that have been obtained are then acclimatized for 3 days so that they can adapt to new environmental conditions. The fish are stocked into containers with a stocking density of 20 fish/container.

Making Kawista Leaf Extract

Kawista leaf extract is made using the meseration method with a ratio of 1: 4 (1 kg of kawista leaves: 4 liters of ethanol) for 3 x 24 hours. During soaking, stir twice a day to mix thoroughly. After that, the material is filtered using Whatman Number 1 filter paper to obtain a solution without dregs. Then the filter results are put into an evaporator flask, then evaporated in a rotary evaporator at a temperature of 50°C or until a concentrated extract is obtained using the evaporation method. The resulting extract is put into a sample bottle.

Preparation of Feed With Kawista Leaf Extract

The addition of extract to feed is done by mixing the extract using a micropipette (20-200 μ) according to the predetermined treatment dose, namely K-, K+, P1 (0.5%), P2 (1%), and P3 (2%) however No extract is given to K- and K+. The weighed feed is added with extract, then stirred evenly and air-dried for 5 minutes, and stored at room temperature without humidity to avoid the growth of mold on the feed (Fadillah *et al.,* 2019).

Tilapia Fish Maintenance

Tilapia fish are kept for 50 days. Tilapia are fed 3 times a day, namely morning, afternoon and evening. The feeding method used is the Restricted feed method, which is given according to the fish's body weight at 5% of the fish biomass given during rearing. The amount of water taken in siphoning activities is 30% of the total water. Water changes are carried out every 10 days by doing a total change. Every 10 days sampling and water quality measurements are carried out.

Preparation of test bacteria

Bacterial preparation was carried out on days 49-50 before the challenge test. In this research activity, A. hydrophila bacteria were used for the challenge test and Streptococcus sp. for testing phagocytic activity. The stages of bacterial preparation carried out are making the media, rejuvenating the bacteria, and diluting the bacteria. For the rejuvenation of A. hydrophila and Streptococcus sp bacteria. NA (Nutrient Agar) media was used, and for dilution of bacteria TSB (Tryptic Soy Borth) media was used.).

Challenge Test

After the fish were kept for 50 days, on day 51 a challenge test was carried out. Before carrying out the challenge test, prepare a 1 mL syringe, then fill it with A.

hydrophila bacteria that have grown on TSB media. Fish that were challenged with A. hydrophila bacteria were injected intramuscularly into the dorsal fin of the fish at a dose of 0.1 mL/fish in all treatments, except in the K- treatment because it was injected with 0.1 mL NaCl/fish (Maisyaroh *et al.,* 2018).

RESULT AND DISCUSSION

Total Erythrocytes

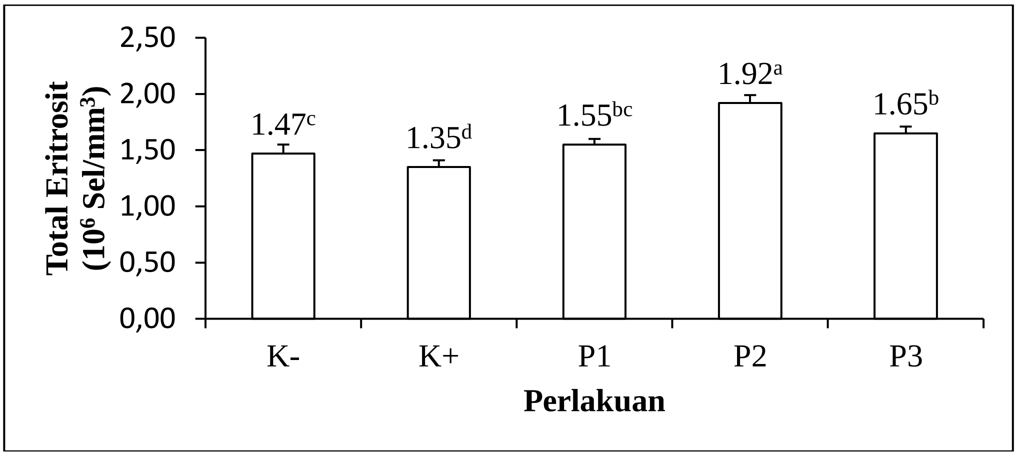

Figure 1. Average Total Value of Tilapia Erythrocytes

The results obtained in Figure 1 show that the addition of kawista leaf extract to feed at different doses had an effect ($p<0.05$) on the total value of erythrocytes injected with A. hydrophila bacteria. The highest total erythrocyte value was found in P2 which was different ($p<0.05$) from all treatments, namely 1.92x106 cells/mm3, however P3 and P1 had total erythrocyte values that were not different ($p>0.05$) with values respectively 1.65x106 cells /mm3 and 1.55x106 cells/mm3, but P1 has a value that is not different ($p>0.05$) with K- with values of 1.55x106 cells/mm3 and 1.47x106 cells/mm3 respectively, then K+ has a total erythrocyte value that is lower and different ($p<0.05$) from all treatments, namely 1.35x106 cells/mm3.

Erythrocytes are the main blood cells that make up the health of fish organisms. One measure of healthy and sick fish can be seen from the number of erythrocytes. The total erythrocyte value obtained was still within the normal range, namely between 1.35-1.92 x106 cells/mm3. This refers to the statement by Maryani *et al.,* (2021) which states that the number of normal erythrocytes in teleost fish ranges from 1.05-3.00 x106 cells/mm3. The high value of total erythrocytes in P2 is thought to be due to the content of kawista leaf extract which plays an important role as an immunostimulant that enters the fish's body, so it can increase the number of erythrocytes. According to Ridwanuloh's (2018) statement, kawista leaves contain phytochemical compounds in the form of alkaloids, flavonoids, tannins, phenols, polyphenols and steroids. According to Putranto *et al.,* (2019) that the flavonoid content can increase the production of fish blood profiles so that it can stimulate the fish's immune system. The low total erythrocytes are caused by fish experiencing stress caused by pathogens entering the fish's body in the form of A. hydrophila bacteria. According to Sukandar's statement (2019), the decrease in the number of erythrocytes is influenced by A. hydrophila infection, causing the liver, spleen and spinal cord to function as erythrocyte cell formation.

Total Leukocytes

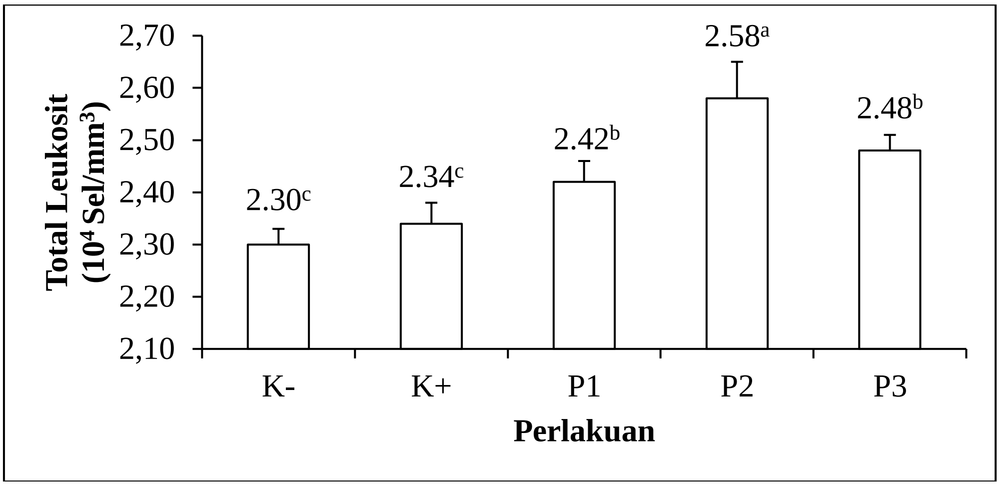

Figure 2. Average Total Leukocyte Value for Tilapia

The results obtained in Figure 2 show that administration of kawista leaf extract in feed at different doses had an influence ($p<0.05$) on the total value of leukocytes injected with A. hydrophila bacteria. Where the highest total leukocyte value was found in P2 which was different ($p<0.05$) from all treatments, namely 2.58x104 cells/mm^3, however in P3 and P1 the total leukocyte value was not different ($p>0.05$) with each value being 2.48x104 cells/mm^3 and 2.42x104 cells/mm^3, and the lowest value for total leukocytes is in K- which has a value that is not different ($p>0.05$) with K+, namely with values of 2.30x104 cells/mm^3 and 2.34x104 cells/mm^3.

Leukocytes are an indicator in the body's defense system which provides defense against pathogenic infections. The main function of leukocytes is to protect the fish's body from damage caused by microorganisms and various other foreign substances. The total leukocyte value obtained shows that it is still within the normal range, namely 2.30-2.58x104 cells/mm^3. According to Putranto *et al.,* (2019) the normal leukocyte value in tilapia is between 20,000-150,000 cells/mm3. The highest percentage value of total leukocytes was found in treatment P2, this is thought to be due to the presence of compounds that play a role in kawista leaf extract and the lowest value was found in K- which was thought to be because bacteria were not injected but instead NaCl was injected and kawista leaf extract was not given, but the lowest value was for those Injected bacteria contain K+. Giving kawista leaf extract can increase the number of leukocytes, because it is caused by the presence of flavonoid compounds. Flavonoid compounds are able to stimulate the number of leukocytes in the fish's body defense. According to the statement by Maryani *et al.,* (2021), the increase in leukocyte production value is due to the presence of flavonoid compounds. This compound functions to improve the immune system because leukocytes act as fast eaters of foreign objects. According to Azhar (2014) The increase in leukocytes is related to the immune system reducing pathogen attacks. The higher the pathogen attack, the higher the fish leukocyte production value.

Hemoglobin

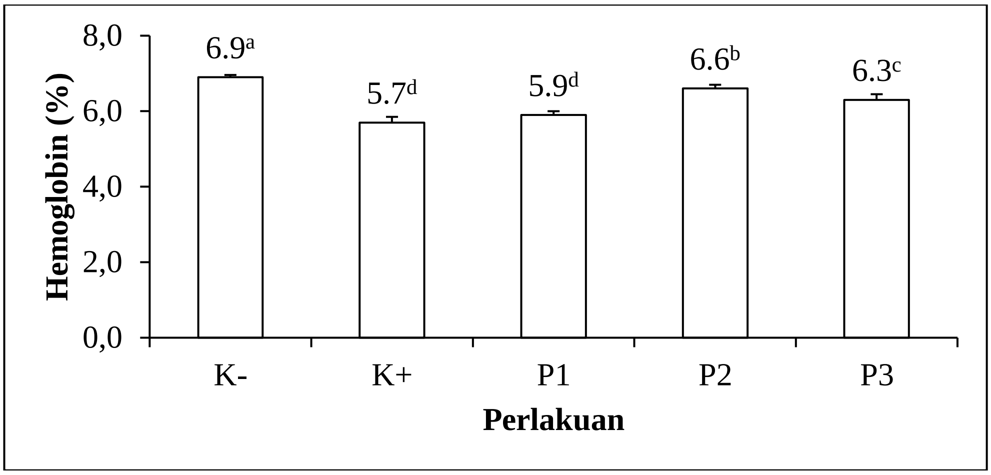

Figure 3. Average Hemoglobin Value for Tilapia

The results obtained in the study of adding kawista leaf extract to feed at different doses had an influence ($p<0.05$) on the hemoglobin value injected with A. hydrophila bacteria. Figure 3 shows that P2 has the highest hemoglobin value which is different ($p<0.05$) from K-, namely with values of 6.6% and 6.9%, but P3 and P1 have different values ($p>0.05$), namely 6.3% and 5.9%, and The lowest hemoglobin value was found in K+ which was not different ($p>0.05$) from P1, namely with values of 5.7% and 5.9%.

Hemoglobin is an important indicator of blood plasma cells which play a role in the circulatory system. According to the statement of Safitri *et al.,* (2013), the hemoglobin content value in tilapia ranges from 5.05-8.33%. The highest hemoglobin value was found in K- because the A. hydrophila bacteria was not injected but NaCl was injected, so this caused the hemoglobin level to be high. According to Maulinia (2022) that fish hemoglobin levels will be high if the fish is healthy compared to fish that are infected with disease. In the P2 treatment which was given the addition of kawista leaf extract, it gave normal hemoglobin values, this was due to the flavonoid content which plays a role in fish blood. This is in accordance with the statement of Safitri *et al.,* (2013) that the increase in hemoglobin values in fish is due to the flavonoid content. Flavonoid activity can improve the working system of blood-producing organs, causing high blood production. Meanwhile, the K+ treatment had the lowest value because it was not given kawista leaf extract and was injected with A. hydrophila bacteria, which caused the fish to be stressed and have low hemoglobin values. According to Prasetio *et al.,* (2017) a decrease in hemoglobin values results in low metabolism and little energy. So this causes the fish to become weak, lack appetite and the fish appear to be on the surface of the water.

Hematokrit

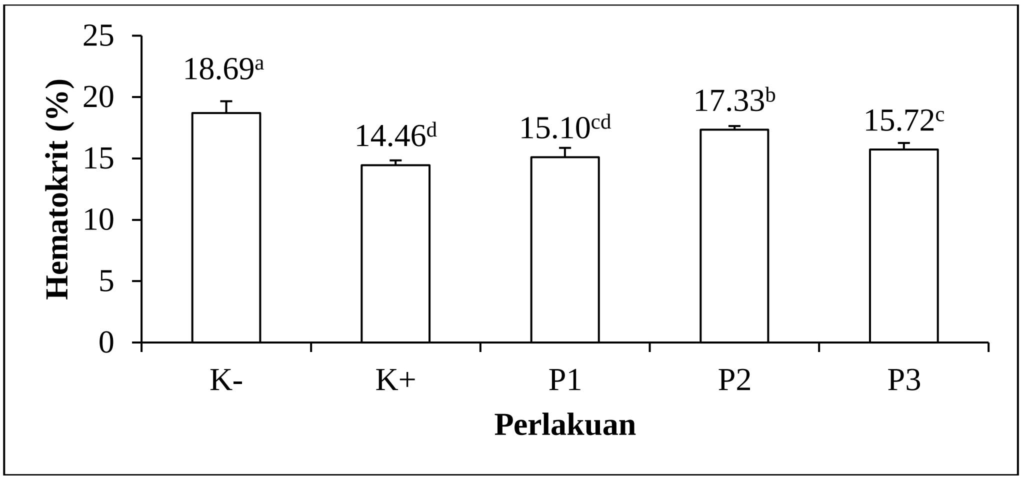

Figure 4. Average Tilapia Hematocrit Value

Figure 4 proves that the addition of kawista leaf extract to feed at different doses has an effect ($p<0.05$) on the hematocrit value injected with A. hydrophila bacteria. Treatment P2 had the highest hematocrit value which was different ($p<0.05$) from K-, namely with values of 17.33% and 18.69%, P1 and P3 had no different values ($p>0.05$) namely 15.10% and 15.72%, and the lowest hematocrit value was in K+ which is not different ($p>0.05$) from P1, namely 14.46% and 15.10%.

Based on the results of observations, hematocrit functions as a tool to determine the comparison between erythrocytes and blood plasma. Low hematocrit values are caused by fish experiencing stress and infections. According to Azhar's statement (2014), the low hematocrit value in the positive control and other treatments proves that the fish are experiencing stress, anemia and infection, which causes a decrease in hematocrit. According to Royan *et al.,* (2014) the increase in hematocrit levels is because the fish have adapted to the new environment, the fish have started to eat slowly like normal activities and have nutrients in their bodies..

Leukocyte differential

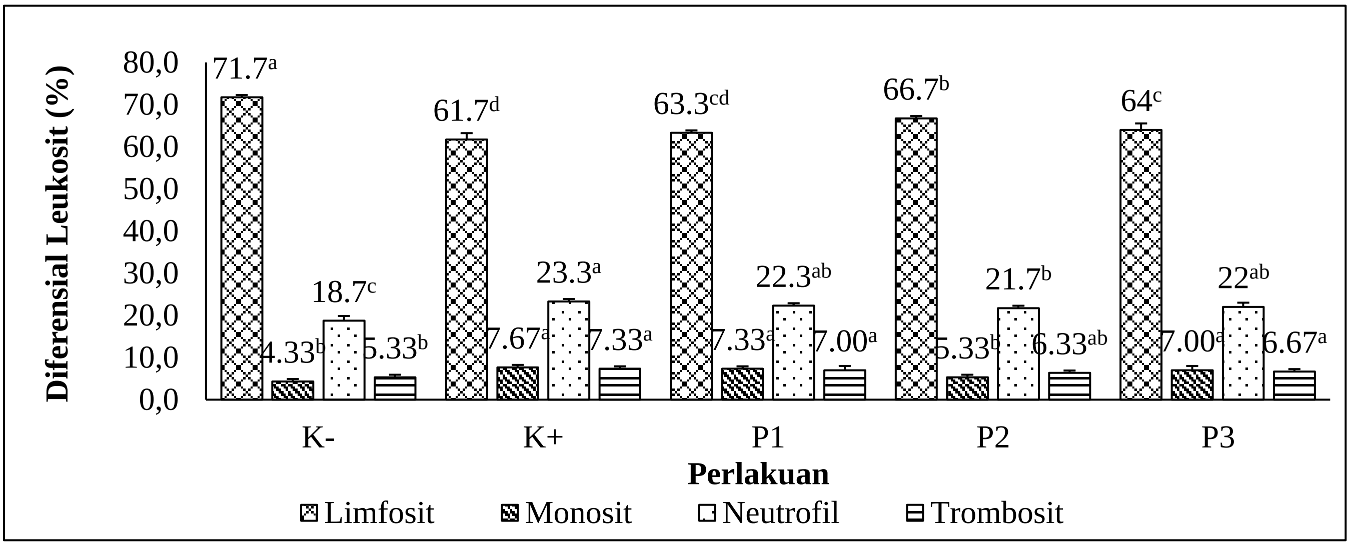

Figure 5. Average Differential Value of Tilapia Leukocytes

Note: Values with different superscripts in the same cell type show different results ($p<0.05$)

Based on the results of differential observations, leukocytes are divided into 4 cell parts, namely lymphocytes, monocytes, neutrophils and platelets. It can be seen in Figure 5 that the addition of kawista leaf extract to feed at different doses had an effect ($p<0.05$) on the cell values of lymphocytes, monocytes, neutrophils and platelets of tilapia injected with A. hydrophila bacteria. The highest value of lymphocytes was found in P2 which was different ($p<0.05$) from K-, namely 66.7% and 71.7%, followed by P3 and P1 which had values that were not different ($p>0.05$), namely with values respectively 64% and 63.3 %, while the lowest value of lymphocytes was found in treatment P1, namely 61.7%, which had a value that was not different ($p>0.05$) with K+, which was equal to 63.3%. Then the highest monocyte values are found in K+, P1, and P3 which have no different values ($p>0.05$), namely with values of 7.67, 7.33, and 7.00% respectively and the lowest values are found in K- which have no different values ($p>0.05$) with P2 namely 4.33% and 5.33%. Furthermore, the highest neutrophil values were found in K+, P1 and P2 which had values that were not different ($p>0.05$) with values respectively 23.3, 22.3 and 22%, however P2, P3 and P1 had neutrophil values that were not different ($p>0.05$) namely with values of 21.7, 22, and 22.3% respectively, and for K- it has different values ($p<0.05$) with all treatments, namely 18.7%. Next, the highest platelet value was found in K+, which had a value of 7.33% and was not different ($p>0.05$) from treatment P1, namely 7.00%, P3 and P2 had values that were not different ($p>0.05$), namely with values of 6.67% and 6.33%, whereas The lowest value for platelets is K-, namely 5.33%, which has a value that is not different ($p> 0.05$) with P2, namely 6.33%.

Observation of leukocyte differential is an important indicator that shows the performance of leukocyte cells in fish. Differential leukocytes are divided into 4 cell parts, namely lymphocytes, monocytes, neutrophils and platelets which function in responding to resistance to foreign particles in fish blood. Lymphocytes act as indicators in the body's defense which functions as a non-specific immune system that can protect the body from microbial attacks. The high value of lymphocytes proves that the administration of natural ingredients as immunostimulants can improve the body's defense system of fish attacked by A. Hydrophila bacteria. This is in accordance with Rustikawati's (2012) statement that the increase in lymphocyte cells is thought to be due to the presence of Sargassum extract which functions as an immunostimulant, so that it can stimulate the formation of a non-specific immune system. According to Azhar (2014) that the number of lymphocytes will decrease if there is a decrease in antibody concentration and high disease attacks.

Monocyte cells function to consume substances that enter the fish's body and provide warnings about disease attacks in leukocytes. The high percentage value of monocyte cells is thought to be due to the fish experiencing a bacterial infection. In accordance with the opinion of Utami *et al.,* (2013) that infections that enter the fish's body can increase white blood cells so that they produce a lot of monocytes. According to Hartika *et al.,* (2014) that the low value of monocytes is due to the fish being in a healthy condition, therefore monocyte cells are not needed to phagocyte because there is no bacterial infection that has entered so there is no stimulation from foreign objects to produce monocytes. Furthermore, Neutrophil Cells play an important role in the body's defense mechanisms. The increase in the number of neutrophils proves that there is an increase in macrophages that occur at the site of infection, making it easier for macrophages to be destroyed by foreign particles (Rustikawati, 2012). The low value of neutrophils is due to the phytochemical compounds contained in kawista leaves. According to the statement by Riswan *et al.,* (2021) that compounds in the form of flavonoids, phenols and alkaloids have an effect on neutrophil cells, so that these cells work actively in areas where wounds occur, and there will be fewer neutrophil cells in the blood circulation. Next, platelets function to help in the blood clotting process with the aim of preventing bleeding. Increasing platelet values can be used as an indicator in the wound healing process. The high platelet percentage value is thought to be because the fish is injured. In accordance with the opinion of Kurniawan *et al.,* (2020) that platelets have a function as wound closure, if platelets are high then the fish is experiencing wounds or bleeding. The decrease in the number of platelets is caused by the presence of metabolite compounds which can play a role in wound healing.

Phagocytic Activity

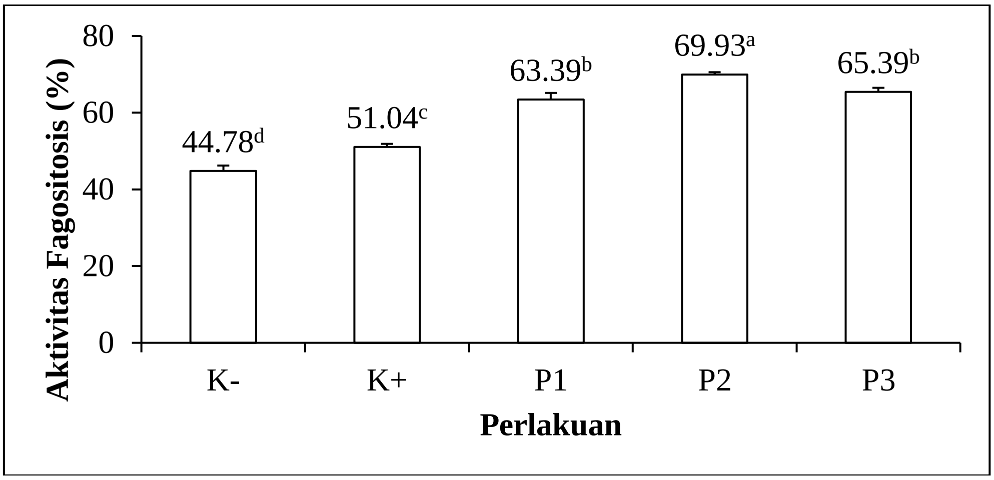

Figure 6. Average value of phagocytic activity for tilapia

Figure 6 shows that giving kawista leaf extract to feed at different doses had an influence ($p<0.05$) on the phagocytosis activity value injected with A. hydrophila bacteria. Where the highest phagocytic activity value was found in the P2 treatment which was different ($p<0.05$) with all treatments with a value of 69.93%, but in P3 and P1 the values were the same and not different ($p>0.05$), namely with a value of 65.39% respectively. and 63.39%, followed by K+ which has a different value ($p<0.05$) with all treatments, namely 51.04%, while K- has a phagocytic activity value the lowest was 44.78% and was different ($p<0.05$) with all treatments.

Based on the results of observations, phagocytic activity is an important indicator in controlling and destroying foreign particles that enter the fish's body. The best value is found in P2, while K+ and K- without treatment show low values. The increase in the value of phagocytic activity is due to the immune system providing resistance to pathogens that enter the fish's body. In accordance with the opinion of Sukenda et al., (2014) that the increase in phagocytic activity is due to the fish's immune system still providing resistance to bacterial infections, so that the value of leukocytes infected with bacteria will increase as an effort to defend the body. According to Mardiana et al., (2013), the low value of phagocytic activity in the control treatment or not given extract but given NaCl solution, proves that pathogenic and non-pathogenic bacteria can be phagocytosed by neutrophil cells, but pathogenic bacteria are phagocytosed in smaller numbers when compared. with non-pathogenic bacteria.

Total Bakteri Count

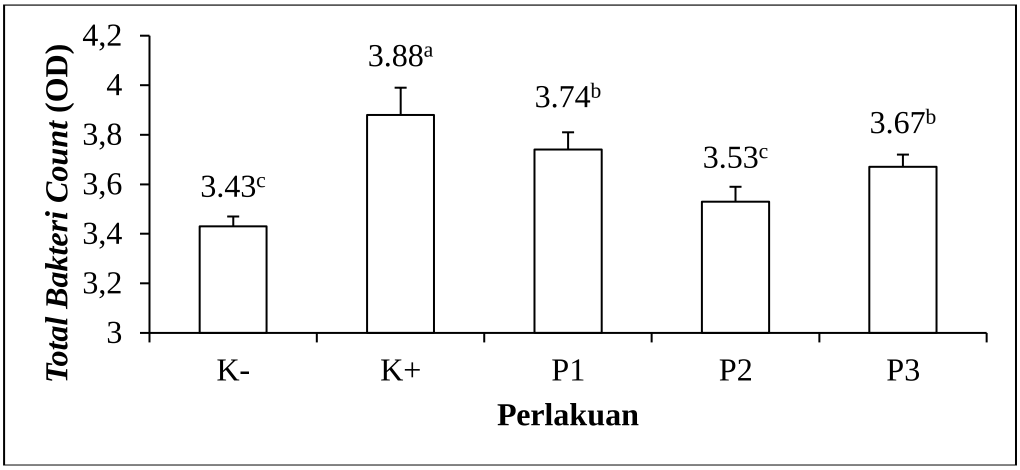

Figure 7. Average Value of Total Bacteria Count (TBC) for Tilapia

Figure 7 shows that the addition of kawista leaf extract to feed at different doses had an effect ($p<0.05$) on the total bacterial value injected with A. hydrophila bacteria. The highest total bacterial values were found in the different K+ treatments ($p<0.05$) with all treatments with an OD620 value of 3.88, however in treatments P1 and P3 the total bacterial values were not different ($p>0.05$) with respective OD620 values of 3.74 and 3.67, while the lowest value for total bacteria found in the P2 and K- treatments had the same value and was not different ($p>0.05$), namely OD620 3.53 and 3.43.

The results of observing the Total Bacterial Count (TBC) value are an indicator used to determine the level of bacterial density found in the intestines of tilapia fish and to determine the ability of kawista leaf extract as an immunostimulant system in inhibiting the growth of bacteria as pathogens. The highest value for total bacteria was found in K+ of all treatments, while the lowest value was found in treatment P2 which was no different from K-, this is thought to be because kawista leaves contain alkaloids, flavonoids and tannins which function as antibacterial activity. According to the statement of Junaidi et al., (2018) that the extract of Rizhophora apiculata mangrove leaves contains active ingredients in the form of alkoloids, flavonoids, tannins, saponins and terpenoids which play a role in inhibiting the growth of bacteria because they are antimicrobial.

Survival Rate

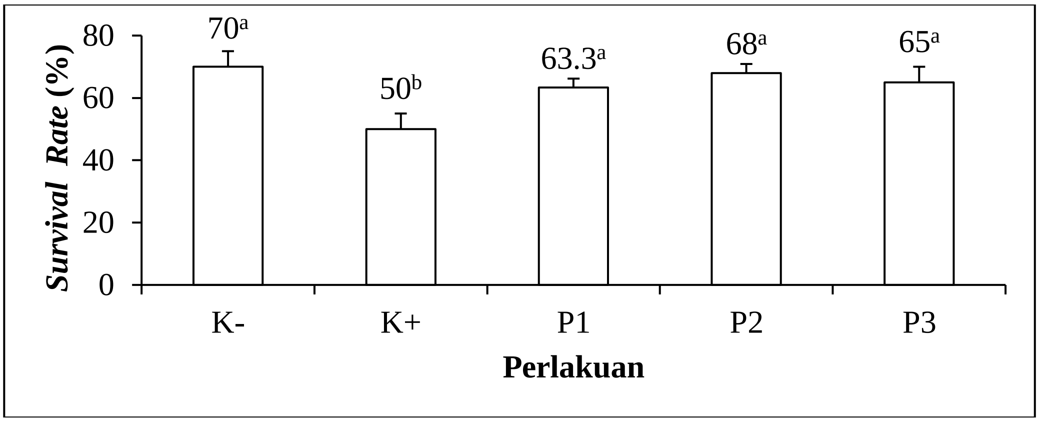

Figure 8. Average Survival Rate Value for Tilapia

The results obtained in Figure 8 show that giving kawista leaf extract to feed at different doses had an effect ($p<0.05$) on the survival rate of tilapia fish injected with A. hydrophila bacteria. The highest survival rate percentage values were found in K- and P2 which had values that were not different ($p>0.05$) with values of 70% and 68% respectively, while P3 and P1 had survival rate values that were not different ($p>0.05$). namely 65% and 63.3%, and the K+ treatment had the lowest survival rate value and was different ($p<0.05$) from all treatments, namely 50%.

Based on the results of observations, the survival rate value is one of the main parameters for determining the percentage value of success of a fish farm. The addition of kawista leaf extract affects the survival rate of fish. The highest survival rate value for tilapia was found in K-, namely 70% because in this treatment bacteria were not injected but NaCl was injected. The P2 treatment which was injected with bacteria had a value of 68%. This is thought to be because P2 has a high value due to the addition of kawista leaf extract to the feed which contains active compounds, one of which is flavonoids. According to Maryani *et al.,* (2021), the flavonoid content itself plays a role in inhibiting bacterial growth, inhibiting enteroxin production and stimulating the fish's immune system. According to Khaerani *et al.,* (2018) the survival value of fish given extracts tends to be higher compared to those without extracts, this is thought to be due to the presence of active compounds.

CONCLUSION

The use of kawista leaf extract at a dose of 1% in feed can improve the immune system of tilapia fish that are injected with A. hydrophila bacteria. The values obtained were total erythrocytes of 1.92x106 cells/mm3, total leukocytes 2.58x104 cells/mm3, differential leukocytes were divided into 4 parts, namely lymphocytes 66.7%, monocytes 5.33%, neutrophils 21.7%, and platelets 6.33%, hemoglobin 6.6%, hematocrit 17.33 %, activity phagocystosis 69.93%, and Total Bacterial Count (TBC) of OD620 3.53 and survival rate of 68%.

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