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Hematology and Physiology of Striped Catfish (*Pangasianodon hypophthalmus*) with the addition of guava leaf extract (*Psidium guajava* L)

Hematologi dan Fisiologi Ikan Patin (Pangasianodon hypophthalmus) dengan Penambahan Ekstrak Daun Jambu Biji (Psidium guajava L)

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Abstract

Striped catfish (*Pangasianodon hypophthalmus*) is a widely cultivated type of superior food fish. Intensive cultivation of catfish can cause disease. Feeding striped catfish with guava leaf extract is expected to reduce stress, which can be seen in striped catfish's hematological and physiological conditions. This research aims to obtain a dose of guava leaf extract *(Psidium guajava* L*)* to improve the health of striped catfish. The method used in the study was an experimental method that applied a one-factor, Completely Randomized Design (CRD) with five treatment levels. The treatment used was without adding guava leaf extract (P0); P1, P2, P3, and P4 with doses of 25, 50, 75, and 100 mL/kg feed, and all treatments were tested against *Aeromonas hydrophila*. The fish used in this research were 250 striped catfish seeds measuring 6-8 cm and kept for 45 days. Feeding with the addition of guava leaf extract at a dose of 75 mL/kg of feed had an influence on the hematological and physiological profile of post-challenge catfish, such as total erythrocytes of 2.40×10^6 cells/mm^3, hematocrit 35.66%, hemoglobin levels 9.61 g/dL, total leukocytes 10.54×10^4 cells/mm^3, blood glucose levels of 78.00 mg/dL and phagocytic activity 35.66%. Absolute weight growth 17.76 g, feed efficiency 41.07%.

Keywords: Striped Catfish, Hematological, Physiological

Abstrak

Ikan patin (*Pangasianodon hypophthalmus*) merupakan salah satu jenis ikan pangan unggulan yang banyak dibudidayakan. Budidaya patin secara intensif dapat menyebabkan penyakit. Memberi makan ikan patin dengan penambahan ekstrak daun jambu biji diharapkan dapat mengurangi stres, yang dapat dilihat pada kondisi hematologis dan fisiologis ikan patin. Penelitian ini bertujuan untuk mendapatkan dosis ekstrak daun jambu biji (*Psidium guajava* L) untuk meningkatkan kesehatan ikan patin. Metode yang digunakan dalam penelitian ini adalah metode eksperimental yang menerapkan Rancangan Acak Lengkap satu faktor dengan lima tingkat perlakuan. Perlakuan yang digunakan, yaitu tanpa penambahan ekstrak daun jambu biji (P0); P1, P2, P3, dan P4 dengan dosis 25, 50, 75, dan 100 mL/kg pakan, dan semua perlakuan diuji terhadap *Aeromonas hydrophila*. Ikan yang digunakan dalam penelitian ini adalah 250 biji lele berukuran 6-8 cm dan dipelihara selama 45 hari. Pemberian pakan dengan penambahan ekstrak daun jambu biji dengan dosis 75 mL/kg pakan berpengaruh pada profil hematologi dan fisiologis ikan patin pasca tantangan, seperti total eritrosit $2{,}40 \times 10^6$ sel/mm^3, nilai hematokrit 35,66%, kadar hemoglobin 9,61g/dL, total leukosit $10{,}54 \times 10^4$ sel/mm^3, kadar glukosa darah

78,00 mg/dL dan aktivitas fagosit 35,66%. Pertumbuhan berat absolut 17,76 g, efisiensi pakan 41,07%.

Kata kunci: Ikan Patin, Hematologi, Fisiologi

1. Introduction

Striped catfish *(Pangasianodon hypophthalmus)* is a widely cultivated type of superior food fish. Market demand for catfish consumption per capita tends to increase yearly, reaching 21.9% from 2014 to 2017, with a preference for fresh fish products of 76% (KKP, 2018). Due to the high market demand for catfish, catfish cultivation is carried out in brackish water. Catfish can grow and reproduce in all environmental conditions.

Intensive cultivation carries a disease risk, so it is necessary to improve fish health. If not handled properly, it will increase the spread of disease-causing microorganisms such as bacteria, one of which is *Aeromonas hydrophila*, which can cause the disease Motile Aeromonas Septicemia (MAS). *A. hydrophila* can generally attack internal organs such as the intestines and liver of fish. *A. hydrophila* attacks can kill fish seeds with a death rate reaching 80-100% within 1-2 weeks (Sari et al., 2018).

So far, fish farmers have used antibiotics and chemicals to prevent disease. However, the use of antibiotics and chemicals on fish poses a risk to environmental safety because uncontrolled use can increase the potential for bacterial resistance, affect the composition of plankton, and result in the death of nitrogen-managing bacteria. Efforts to improve fish health can be made by providing supplements from natural extracts added to the feed. The use of natural ingredients as supplements in feed has several advantages, namely being environmentally friendly and safe for consumers and the individual fish themselves (Zissalwa et al., 2020).

Several plants or natural ingredients are known to have active compounds that function as antibacterials, one of which is guava leaves. This material contains flavonoids, tannins, alkaloids, and saponins (Effendi et al., 2023; Kurniawan et al., 2024). According to Amelia et al. (2021), giving guava leaf extract as an immunostimulant with various concentrations given to goldfish had a real effect on leukocytes, erythrocytes, differential leukocytes, hemoglobin, relative protection level (RPS), phagocytic activity (AF), and phagocytosis index (IF) with a concentration of 250 ppm. Meanwhile, the hematocrit and survival parameters were 93.3%, which did not affect each treatment. Based on the description above, this research aims to obtain a dose of guava leaves (*P. guajava L*) for the hematology and physiology of striped catfish (*P. hypophthalmus*).

2. Material and Method

2.1. Time and Place

This research was carried out in December 2022 - February 2023. Fish rearing, hematological analysis, and fixation of histological organ samples were carried out at the Fish Parasite and Disease Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

2.2. Methods

The method used in the research was an experimental method that applied a one-factor, Completely Randomized Design (CRD) with five treatment levels. Three repetitions were done to reduce the error level, so 15 experimental units were needed. The feed given during maintenance is supplemented with guava leaf extract at a dose of 25, 50, 75, and 100 mL/kg of feed. Sampling was carried out three times, namely before the fish were treated, on the 30th day and the 45th day.

2.3. Procedures

2.3.1. Preparation of Containers

The containers used in this research were 15 aquariums measuring 40 x 30 x 30 cm^3. Before using the aquarium, wash it clean and fill it with water until it is complete, then add a potassium solution permanganate (KMnO4) and leave for 24 hours so that the aquarium is free from pathogenic microorganisms. After that, the aquarium is rinsed with water until clean and then dried. Once clean, each aquarium was filled with 20 L/container of 5 ppt salinity water and aerated.

2.3.2. Fish farming

The fish used 250 catfish seeds measuring 6-8 cm, and each aquarium was filled with ten fish. The striped catfish is first acclimatized in a fiber bath for 1 hour to reduce stress. Then, the fish were transferred to an aquarium containing water with a salinity of 5 ppt and adapted for 24 hours, then kept for 45 days and given test feed at satiation every 3x a day. Every 15 days, the body weight of the fish is measured to determine the development of the fish's body weight and the amount of feed given.

2.3.3. Making guava leaf extract

Fresh guava leaves were obtained from the fruit garden of Universitas Riau. The part of the leaf used is the fourth leaf from the top to the 10th leaf. The leaves are washed and dried in the sun. After drying, the leaves are separated from the middle bone and ground with a blender. Next, sift it with a sieve until you get a fine powder (simplistic). The extraction process is done by dissolving the simplicia of guava leaves and 96% ethanol. With a ratio of 1:4, it was soaked for 6 hours while stirring, then left for 1x24 hours. The extract was filtered with gauze and filter paper to obtain guava leaf extract in liquid form. The process was repeated two times with the same type and amount of solvent. Filtrates from three replicates were combined into one. Next, the extract is measured according to the required dose.

2.3.4. Provision A.hydrophila

The tools used, such as Petri dishes, test tubes, erlenmayers, and measuring cups, are sterilized first before being used in an autoclave at 121oC with a pressure of 1 atm for 15 minutes. *A. hydrophila* isolate was isolated from catfish, showing symptoms of bacterial infection. Bacterial virulence was increased by reinfection of the fish three times. Next, the bacteria were inoculated into Pseudomonas Aeromonas selective medium, namely GSP (Glutamate Starch Phenol), and then incubated at 27-28^{o}C for 18-24 hours. If bacteria grow on GSP media and the media turns yellow, it indicates that the bacteria growing are *A. hydrophila* according to the color and shape of the colony.

2.3.5. Challenge test

After 30 days of maintenance and being fed with the addition of guava leaf extract, the fish were challenged with *A. hydrophila* intramuscularly with a dose of 0.1 mL/fish and a density of 10^8 CFU/mL. Then, it was kept for 14 days, and during that time, the fish were still fed, and clinical symptoms were observed.

3. Result and Discussion

3.1. Total Erythrocytes

The number of erythrocytes was measured to see changes in the number of erythrocytes that occurred after feeding striped catfish containing guava leaf extract. The value of total catfish erythrocytes during the study can be seen in Table 1.

Tabel 1. Total erythrocytes of striped catfish

Treatment	Beginning	Day 30	Post-test challenge day 45
P0	1.57±0,08	1.76±0,03^a	1.23±0,01^a
P1	1.51±0,14	2.03±0,08^b	1.70±0,06^b
P2	1.54±0,05	2.14±0,08^{bc}	2.09±0.17^c
P3	1.55±0,11	2.37±0,03^d	2.40±0.11^d
P4	1.51±0,09	2.19±0,04^c	2.23±0.22^{cd}

Note: P0: Without adding guava leaf extract, P1: Adding guava leaf extract at a dose of 25 mL/kg feed, P2: Adding guava leaf extract at a dose of 50 mL/kg feed, P3: Adding guava leaf extract at a dose of 75 mL/kg feed, P4: Added guava leaf extract at a dose of 100 mL/kg feed and tested against *Aeromonas hydrophila*. Different superscript letters indicate significantly different (P<0.05)

Table 1 shows that the total erythrocytes of striped catfish at the start of rearing ranged from 1.51-1.57 x 10^6 cells/mm^3. After maintenance for 30 days, the number of catfish erythrocytes increased for all treatments, where the number of erythrocytes ranged from 1.76-2.37 x 10^6 cells/mm^3. This value is still considered normal. Lukistyowati et al. (2007) stated that the total erythrocytes of normal catfish ranged between 1.57-3.41 x 10^6 cells/mm^3.

The number of catfish erythrocytes increased after being fed feed containing guava leaf extract and kept for 30 days. This is caused by several factors, namely age, fish size, and feed nutrition. Factors influencing the number of erythrocytes are species, gender, age, feed nutrition, size, and physical activity (Emu, 2010). Syawal & Siregar (2011) stated that an increase in the number of erythrocytes in the blood shows that the oxygen content in the blood is increasing and that they are starting to adapt to the environment. After the challenge with *A. hydrophila* bacteria, the number of erythrocytes ranged between 1.23-2.40 x 10^6 cells/mm^3. Where the lowest total erythrocytes are at P0, namely1.23 x 10^6 cells/mm^3, the highest total erythrocytes at P3 was 2.40 x 10^6 cells/mm^3.

The increase in total erythrocytes in the P3 treatment was due to the flavonoid, alkaloid, tannin, and saponin compounds contained in guava leaf extract, which are active in improving the health of catfish. According to Wijaya (2015), flavonoids can increase the number of red blood cells because they can trigger the work of the blood-producing organs to increase blood production.

3.2. Hematocrit levels

Hematocrit is the percentage of erythrocyte volume in fish blood. This hematocrit level can be used to determine the impact of infection from bacteria, so it can be used to indicate the health condition of fish after being challenged (Anderson & Siwicki, 2019). The average hematocrit level of striped catfish during the study can be seen in Table 2.

Table 2. Calculation of hematocrit levels of striped catfish

Treatment	Beginning	Day 30	Post-test challenge day 45
P0	28.44 ± 0.38	30.16 ± 0.52[a]	29.66 ± 0.87[a]
P1	29.10 ± 0.37	31.17±0.53[b]	29.67 ± 0.87[a]
P2	28.22 ± 1.01	32.67±0.10[c]	30.90 ± 0.58[a]
P3	29.00 ± 0.88	35.62±0.72[d]	35.66±0.31[c]
P4	27.55 ± 0.97	32.82±0.48[c]	32.97±0.57[b]

Based on Table 2, hematocrit levels at the start of maintenance ranged from 27.55-29.10%. Sarjito & Alfabetian (2017) state that normal fish hematocrit levels range from 28-40%. The hematocrit level of catfish on the 30th day of rearing increased to 30.16-35.62%, where the highest hematocrit level was at P3, namely 35.62%, and the lowest at P0, namely 30.16%. An increase in the hematocrit value in the blood is always related to the rise in the total erythrocytes and hemoglobin levels in the blood.

The average hematocrit level of catfish after rearing and feeding with the addition of guava leaf extract for 30 days ranges from 30.16 - 35.62%, which is still within normal limits. According to Phu et al. (2016), the hematocrit value of catfish kept for 30 days ranges from 30.6-39.7% and is classified as good, and the fish can survive during maintenance. Suhermanto et al. (2013); Windarti et al. (2023) stated that the hematocrit value is influenced by several factors, including erythrocytes (number, size, shape, ratio of anticoagulant to blood, storage location, and homogeneity), environment, sex, species and age of fish.

Hematocrit levels after the challenge test with *A. hydrophila* bacteria in catfish increased in treatments P3 and P4, which were fed with the addition of high doses of guava leaf extract. The increase in hematocrit value was due to the presence of antibacterial ingredients in guava leaf extract, so a higher dose increased the hematocrit value due to the challenge test with *A. hydrophila* bacteria. Guava leaf extract, as an antibacterial ingredient, can inhibit and kill bacteria. Andeson & Siwicki (1993) explained that immunostimulants affect hematocrit values, although by a small percentage.

3.3. Hemoglobin levels

Hemoglobin functions to bind oxygen, which is used for the catabolism process so that energy is produced. Hemoglobin levels are in line with the number of erythrocytes. The higher the hemoglobin level, the higher the number of erythrocytes (Purwanto, 2006). Calculation of hemoglobin levels was carried out to show the hemoglobin that occurred after feeding with the addition of guava leaf extract. The results of calculating the average hemoglobin level of catfish during observation can be seen in Table 3.

Table 3. Calculation of hemoglobin levels in striped catfish

Treatment	Beginning	Day 30	Post-challenge test on day 45
P0	7.18 ± 0.09	8.10 ± 0.27[a]	6.11±0.20[a]
P1	7.29 ± 0.14	8.24 ± 0.14[a]	5.51±0.31[b]
P2	7.25 ± 0.01	8.76±0.21[b]	7.83±0.32[b]
P3	7.51 ± 0.40	9.41±0.31[c]	9.61±0.10[c]
P4	7.57±0.27	9.19±0.14[c]	9.25±0.17[c]

Based on the results of the analysis of variance (ANOVA), it showed that feeding with the addition of guava leaf extract had a significant effect ($P<0.05$) on the hemoglobin levels of catfish after rearing for 30 days. The average hemoglobin level of catfish after rearing by feeding with the addition of guava leaf extract for 30 days ranged from 8.10 to 9.41 g/dL, which is still within normal limits. According to Phu et al. (2016), the hemoglobin of catfish kept for 30 days ranged from 7.8-8.7 g/dL. According to Lavabetha et al. (2015), high hemoglobin levels can help store oxygen and carry out blood-buffering functions in fish. After being challenged with *A. hydrophila* bacteria, hemoglobin levels in P3 increased; this is thought to be due to the antibacterial activity of flavonoid, alkaloid, tannin, and saponin compounds in guava leaf extract so that the fish can form a body defense system against attacks by *A. hydrophila* bacteria. Karim et al. (2018) state that guava leaf extract contains secondary metabolites of flavonoids, alkaloids, tannins, and saponins.

3.4. Total Leukocytes

Total leukocyte measurements were carried out to see changes in total leukocytes that occurred at the beginning of maintenance, the 30th day of maintenance, and 14 days after the challenge test with *A. hydrophila* bacteria. The calculated value of total leukocytes for each treatment during the study can be seen in Table 4.

Table 4. Total leukocytes of striped catfish

Treatment	Beginning	Day 30	Post-test challenge day 45
P0	7.29±0,51	8.21±0,15[a]	8.56±0,41[a]
P1	7.14±0,21	8.54±0,48[ab]	9.14±0,71[a]
P2	7.20±0,20	8.77±0,58[ab]	9.32±0.30[a]
P3	7.20±0,13	9.67±0,16[bc]	10.54±0.53[b]
P4	7.09±0,35	9.17±0,19[c]	10.31±0.46[b]

The lowest total leukocytes are at P0, 8.21 x 10^4 cells/mm^3; the highest total erythrocytes are at P3, 9.67 x 10^4 cells/mm^3. According to Phu et al. (2016), the total leukocytes of catfish kept for 30 days ranged from 6.95-11.9x 10^4 cells/mm^3. After the *A. hydrophila* bacterial challenge test, the total number of leukocytes ranged between 8.56-10.54x 10^4 cells/mm^3. The highest total number of leukocytes after the challenge test was in treatment P3, and the lowest was in treatment P0.

Leukocytes function in the immune response. Especially if foreign substances or antigens enter the body, leukocytes make antibodies, which the immune system uses to stimulate, identify, and neutralize incoming foreign objects (antigens), such as bacteria. When an infection occurs, leukocytes are directed toward the site of infection to provide rapid defense against infectious genes. When fish are infected with bacteria, leukocytes increase (Fauzan et al., 2017; Kurniawan et al., 2020).

3.5. Phagocytic Activity

Phagocytic activity calculations were carried out to see the ability of leukocyte cells to consume foreign objects, especially pathogenic bacteria in catfish, at the beginning of rearing, on the 30th day of rearing, and after the challenge test with *A.hydrophila*. The results of observations of catfish phagocytosis activity during the study can be seen in Table 5.

Table 5. Observation results of phagocytic activity of striped catfish

Treatment	Beginning	Day 30	Post-test challenge day 45
P0	20.00±1.00	21.33±1.15[a]	22.00±3.00[a]
P1	22.00±1.73	24.00±2.00[b]	25.66±0.57[ab]
P2	20.00±1.00	26.00±2.64[bc]	27.00±3.60[ab]
P3	20.33±2.30	32.66±2.51[d]	35.66±2.08[c]
P4	21.00±1.00	28.66±1.52[c]	30.66±1.52[b]

Based on Table 5, the range of phagocytic activity at the beginning of maintenance ranged from 20.00-22.00%, and the range of phagocytic activity on day 30 of maintenance ranged from 21.33-32.66%. The lowest value was in treatment P0, 21.33%, and the highest was in treatment P3, 32.66%. This shows that feeding with the addition of guava leaf extract can increase the phagocytic activity of catfish leukocyte cells. According to Nuryati et al. (2010), phagocytes are the strongest and most important part of the body's defense system, which can immediately fight the invasion of microorganisms after crossing the body's surface and entering the body.

Phagocytic activity of catfish after challenge with *A. hydrophila* ranged from 22.00-35.66%, where the lowest was in the P0 treatment, namely 22.00%, and the highest was in the P3 treatment, 35.66%. This is thought to be because feeding with the addition of guava leaf extract can stimulate the activity of phagocyte cells. Phagocytosis activity can phagocytize foreign objects that will attack the fish's immune system.

3.6. Blood Glucose

Blood glucose measurements were carried out to see changes in blood glucose that occurred at the beginning of maintenance, the 30th day of maintenance, and 14 days after the challenge test with *A. hydrophila* bacteria. The calculated blood glucose values for each treatment during the study can be seen in Table 6.

Table 6. Results of blood glucose measurements of striped catfish

Treatment	Beginning	Day 30	Post-test challenge day 45
P0	37.33±0,57	43.66±1.52[a]	58.33±4.04[a]
P1	36.66±0,57	48.00±1.00[b]	64.66±2.51[ab]
P2	37.00±1.00	49.66±1.52[bc]	69.66±1.52[bc]
P3	37.00±1.00	57.33±2.51[d]	78.00±3.00[d]
P4	37.33±1.15	53.00±2.64[c]	75.00±5.00[cd]

Based on Table 6, it can be seen that catfish blood glucose at the start of rearing ranged from 36.66-37.33 mg/dL. Based on the results of the analysis of variance (ANOVA), it showed that feeding with the addition of guava leaf extract had a significant effect ($P < 0.05$) on catfish blood glucose after rearing for 30 days. The average blood glucose of catfish after rearing by feeding with the addition of guava leaf extract for 30 days ranged from 43.66 to 57.33 mg/dL. This value is still within normal limits. Following the opinion of Nasichah et al. (2016) stated that normal fish blood glucose levels range between 40-90 mg/dL, and according to Effendi et al. (2023), glucose levels in fish under normal conditions range from 41-150 mg/dL.

After the challenge test with *A. hydrophila* bacteria, blood glucose ranged between 58.33-78.00 mg/dL, where blood glucose was highest in treatment P3 while the lowest was in treatment P0. After being tested against the bacteria *A. hydrophila*, the fish's blood glucose continued to increase stress factors due to increase. Fish need much energy to adapt and fight stress when stressed. The high need for energy to maintain life will stimulate glucose mobilization into the blood (Costas et al. Syawal et al., 2011).

3.7. Absolute Weight Growth

Weight growth can indicate whether feeding with the addition of guava leaves affects striped catfish's absolute weight growth. Absolute weight growth data can be seen in Table 7.

Table 7. Absolute weight growth of striped catfish

Test	Treatment				
	P0	P1	P2	P3	P4
1	12.77	13,18	14.44	17.78	15.68
2	13.05	13.25	13.93	17.97	15.67
3	13.54	13.42	14.22	17.55	14.55
Amount	39.36	39.85	42.59	53.30	45.90
Average	13.12 ± 0.38^{a}	13.28 ± 0.12^{a}	14.19 ± 0.25^{b}	17.76 ± 0.21^{d}	15.30 ± 0.64^{c}

Based on Table 7, it is known that the absolute weight growth value of catfish is different in each treatment. It can be seen that the highest absolute weight growth of catfish during rearing is in treatment P3 at 17.76 g, followed by treatment P4 at 15.30 g, treatment P2 at 14 .19 g, treatment P1 at 13.28 g and treatment P0 was 13.12 g. Koesdarto in Iman et al. (2017) stated that increasing the absorption efficiency of food substances is necessary to meet life's needs and production, as indicated by weight growth. Samsudin in Iman et al. (2017) state that growth in fish weight can occur due to the allocation of energy from feed for growth after previously providing energy to maintain body condition and energy sources during maintenance.

3.8. Feed Efficiency

The results of calculating catfish feed efficiency during the research can be seen in Table 8.

Table 8. Feed Efficiency for striped catfish

Test	Treatment				
	P0	P1	P2	P3	P4
1	34.48	34.14	35.10	40.43	40.29
2	34.20	35.63	36.19	41.75	39.14
3	33.61	33.68	33.81	41.04	35.43
Amount	102.29	103.46	105.10	123.22	114.86
Average	34.10 ± 0.44a	34.49 ± 1.01a	35.03 ± 1.19a	41.07±0.66c	38.29±2.53b

Based on Table 8, it can be seen that feed efficiency during the research ranged from 34.10 – 41.07%. The highest feed efficiency value was found in treatment P3, while the lowest feed efficiency was found in treatment P0. The results of the Analysis of Variation test (ANOVA) showed that feeding with the addition of guava leaf extract to catfish had a significant effect on feed efficiency ($P<0.05$). The Student-Newman-Keuls further test showed that the P4 treatment significantly differed from the other treatments.

4. Conclusions

Feeding with the addition of guava leaf extract at a dose of 75 mL/kg of feed had an influence on the hematological and physiological profile of post-challenge catfish, such as total erythrocytes of 2.40 x 10^6 cells/mm^3, hematocrit 35.66%, hemoglobin 9.61g/dL, total leukocytes 10.54x 10^4 cells/mm^3, blood glucose levels of 78.00mg/dL and phagocytic activity 35.66%. Absolute weight growth 17.76 g, feed efficiency 41.07%.

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