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Application of phytoimmunostimulant food to the hematology of common carp (*Cyprinus carpio*) preserved in brackish water

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Abstract
This research was conducted from October to December 2023 at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau. This study aimed to analyze the effect of adding guava leaves (*Psidium guajava*) on the hematology of common carp (*Cyprinus carpio*) reared in brackish water infected with *Aeromonas hydrophila*. The method used was an experiment with a Completely Randomized Design (CRD) of one level, the concentration of phytoimmunostimulant administration with five treatment levels and three replications, namely, P0 (commercial pellets), the addition of guava leaf flour dose P1 (1.0 g / 100 g feed), P2 (1.5 g / 100 g feed), P3 (2.0 g / 100 g feed), P4 (2.5 g / 100 g feed). Based on the study's results, it is known that adding guava leaf flour to common carp feed reared in brackish water media and challenged with *A. hydrophila* bacteria shows a significant effect ($P < 0.05$). The best dose was obtained in P2 (1.5 g/100 g feed) with the number of erythrocytes 1.54-1.63x10^6 cells/mm^3, hemoglobin 5.93-7.80 g/dL, and hematocrit 30.33-37.67.

1. INTRODUCTION

Common carp (*Cyprinus carpio*) is a type of freshwater fish favored by the public because it tastes good, has nutritional value, and is highly economical. It contains omega-3 and low fatty acids, so it can be used as a relatively cheap animal protein source to meet human nutritional needs. According to Saparinto (2010), Common carp is also safe for health because it can reduce increased cholesterol in the blood. Common carp are freshwater fish that live in media with a salinity level of 0 ppt. According to Nirmala et al. (2011), common carp can live in brackish water media with certain salinity levels (Nirmala et al., 2011).

The main problem in common carp cultivation is bacterial infections that cause decreased fish production. To overcome this problem, various antimicrobial compounds, such as antibiotics and other chemicals, are generally used. However, the application of antibiotics during fish production can cause other issues, namely antibiotic-resistant bacteria and food safety, because antibiotics are already widely stored in the fish's body. Therefore, alternative, more environmentally friendly treatments are needed and do not cause resistance effects on bacteria. One type of disease often found in cultivated organisms is a bacterial disease caused by *Aeromonas hydrophila*. This bacteria causes hemorrhagic septicemia, characterized by abdominal swelling, ulcers, lesions, skin abscesses, exophthalmia, and bleeding, especially in the gills and operculum. Attacks by these bacteria can decrease fish production by up to 80%.

The use of immunostimulants is an alternative to the use of antibiotics and chemicals. According to Bratawidjaja (2006); Effendi et al. (2022), The fish immune system can be improved by providing herbal plant materials that have many benefits, such as stimulating appetite and growth, stimulating the immune system, antimicrobial and antifungal activity, and anti-stress effects (Hai, 2015). Phytopharmaceuticals that can improve fish's immune system include guava leaves (*Psidium guajava*) (Effendi et al., 2023).

Guava leaves are an herbal plant with bioactive compounds that can increase the immune system of the common carp. These herbal plants contain bioactive compounds that can increase the immune system, including flavonoids, saponins, steroids, and alkaloids (Halimah et al., 2019). The content of secondary metabolite compounds, such as alkaloids, terpenoids, phenolics, polyphenols, quinones, lectins, and polypeptide compounds, are primarily alternatives to antibiotics, chemicals, vaccines, and other synthetic compounds.

The compounds in these plants are expected to increase the common carp immune system, as seen from the total leukocyte differentiation. Based on the background description above, the researcher is interested in researching the application of phytoimmunostimulant feed to the hematology of common carp kept in brackish water. This study analyzed the effect of adding guava leaves on the hematology and survival rate of common carp in brackish water infected with *A.hydrophila.*

2. RESEARCH METHODS

Time and Place

This research was conducted from October to December 2023 at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

Research method

The method used was an experimental method with a Completely Randomized Design (CRD) of one level, the concentration of phytoimmunostimulant administration with five levels of treatment. To minimize errors, each treatment was repeated 3 times. The treatment in this study refers to the research results of Effendi et al. (2023),the treatments are as follows: P0: Without adding guava leaves (control), P1: Addition of guava leaves at a dose of 1.0 g/100 g of feed, P2: Addition of guava leaves at a dose of 1.5 g/100 g of feed, P3: Addition of guava leaves at a dose of 2.0 g/100 g of feed, P4: Addition of guava leaves at a dose of 2.5 g/100 g of feed.

Procedures

Common carp maintenance using water with a salinity of 5 ppt and challenge testing using *A. hydrophila* was carried out in the Microbiology Laboratory of the Faculty of Fisheries and Marine, Universitas Riau, using a container with a diameter of 1 m with a water volume of 80 L. Each container was filled with fish with one fish/4L stocking density. The common carp used were 5.00±1.00 cm in size, weighing 4.00±1.00 g. The fish used were randomly selected based on their behavior, namely active swimming and no wounds or parasites. Fish maintenance was carried out for 60 days, and every 10 days, the length and weight were measured. Furthermore, a 14-day challenge test of *A. hydrophila* was carried out to see the effect of adding guava leaves on the immune response of common carp. The feed given was 5% of body weight. Hematological and physiological observations of the fish were carried out 3 (three) times at the beginning, day 60, and 14 days after the challenge test (Effendi et al., 2025; Effendi et al., 2023).

Measured Parameters

Red Blood Cells (Erythrocytes)

The red blood cells (erythrocytes) observed in this study included total erythrocytes, hemoglobin, and hematocrit. After the challenge test, erythrocyte cells were observed at the beginning of maintenance, day 60 and day 14.

3. RESULTS AND DISCUSSION

Red blood cells (erythrocytes) observed in this study included total erythrocytes, hemoglobin, and hematocrit. Erythrocyte cell observations were made at the beginning of maintenance, day 60, and day 14 of the post-challenge test. At the beginning of rearing, total erythrocyte cells were 1.22-1.28 x106 cells/mm3, hemoglobin was 5-5.2 g/dL, and hematocrit was 24-25%. Red blood cell observations on day 60 and post-challenge can be seen in Table 1.

Table 1. Red blood cells (Erythrocytes)

DJB treatment (g/100g feed)	Parameters		
	Total eritrosit (x10^6 cell/mm^3)	Hemoglobin (g/dL)	Hematocrit (%)
	60th Day		
P0 (kontrol)	1.35±0.23^a	5.33±0.12^a	25.67±0.58^a
P1 (1 g)	1.46±0.01^b	5.80±0.20^b	26.67±0.58^{ab}
P2 (1,5 g)	1.54±0.02^c	5.93±0.12^b	30.33±0.58^c
P3 (2 g)	1.49±0.01^b	5.73±0.12^b	28.00±1.00^b
P4 (2,5 g)	1.46±0.02^b	5.60±0.20^{bc}	27.67±1.53^{ab}
	Post-challenge (-75)		
P0 (kontrol)	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
P1 (1 g)	1.55±0.02^c	6.67±0.23^b	30.00±2.00^b
P2 (1,5 g)	1.63±0.02^d	7.80±0.20^c	37.67±0.58^c
P3 (2 g)	1.54±0.02^c	7.53±0.12^c	35.67±0.58^c
P4 (2,5 g)	1.50±0.01^b	6.47±0.12^b	30.33±1.53^b

Note: DJB: guava leaves; *Different superscripts in the same row indicate effects between treatments ($p<0.05$)

Based on Table 1 shows that common carp fed with guava leaf flour with different doses obtained total erythrocytes ranging from 1.35 - 1.63 x 10^6 cells/mm^3, hemoglobin ranging from 5.33-7.80 g/dL, and hematocrit 25.67-37.67%. The results of the Analysis of Variance (ANOVA) showed that feed containing guava leaf flour with different doses had a significant effect on the total erythrocytes, hemoglobin, and hematocrit of common carp ($p <0.05$). The results of the Student-Newman-Keuls showed the best total erythrocytes, hemoglobin, and hematocrit in P2 (1.5 g/100 g feed) for 60 days of maintenance. Namely, total erythrocytes of 1.54 x10^6 cells/mm^3, hemoglobin 5.93 g/dL, and hematocrit 30.33% differed significantly from other treatments, but P1, P3, and P4 were not significantly different.

It is suspected that the compounds contained in guava leaf flour are optimal for increasing erythrocyte, hemoglobin, and hematocrit levels. This shows that the content of secondary metabolite compounds in guava leaves, such as flavonoids, tannins, and saponins, can stimulate an increase in common carp erythrocyte cells. Flavonoids can increase the work of blood-producing organs (lymphomyeloids) so that blood production rises. Then, according to Effendi et al. (2023), the content of tannin, alkaloid, and saponin is responsible for the stability of the membrane cells against antigens that enter the fish's body. After the challenge test with A, the total erythrocytes of common carp fed with guava leaf flour were higher than those fed with commercial pellets without adding guava leaves (control). Hydrophila bacteria, there was an increase, namely the total erythrocytes ranged from 1.50-1.63 x10^6 cells/mm3, hemoglobin 6.47-7.80 g/dL, and hematocrit 30.00-37.67%.

Blood circulation and metabolism are essential, so larger fish have more erythrocytes than smaller ones. Post-challenge test at P0 could not be observed because mortality reached 100%. This shows that commercial pellet feed cannot provide optimal nutrition to increase fish immunity. Thus, adding guava leaf flour to fish feed is the best solution to improve the fish's immune system. This shows that giving guava leaves can increase the fish's immune response. During maintenance, the range of common carp erythrocyte cells is still within the normal range. Several studies have shown the same results for fish hematology. Namely, the total common carp erythrocytes range from 1.13-1.43x 10^6 cells/mm^3

(Mohammadi et al., 2020), 1.8-2.16 x10^6 cells/mm^3 (Sezgin & Aydin, 2021). Haemoglobin, 8.40-8.83 g/dL (Kesbic et al., 2020), and hematocrit ranged from 21.46-28.53% (Mohammadi et al., 2020), 30.33-39.67% (Effendi et al., 2023).

4. CONCLUSIONS

Based on the study's results, it is known that adding guava leaf flour to common carp feed reared in brackish water media and challenged with *A.hydrophila* bacteria shows a significant effect (P<0.05). The best dose was obtained in P2 (1.5 g/100 g feed) with the number of erythrocytes 1.54-1.63x10^6 cells/mm^3, hemoglobin 5.93-7.80 g/dL, and hematocrit 30.33-37.67%.

Further research is needed on using different salinity sources to determine its effect on the effectiveness and best dose of guava leaf flour in feeding to stimulate the growth and health of common carp. An economic study is needed on using phytoimmunostimulant feed in fish farming.

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