TIK-122 EFFECTIVENESS OF IRON EXTRACT ON THE HEMATOLOGY OF SNAKEHEAD FISH (CHANNA STRIATA) AFTER BACTERIAL INFECTION AEROMONAS HYDROPHILA

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EFFECTIVENESS OF IRON EXTRACT ON THE HEMATOLOGY OF SNAKEHEAD FISH (CHANNA STRIATA) AFTER BACTERIAL INFECTION AEROMONAS HYDROPHILA

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ABSTRACT
After being infected with the *A. hydrophyla* bacteria, snakehead fish made efforts to restore the condition by means of recovery. The content of secondary metabolites contained in kelakai leaves, namely alkaloid compounds, steroids, flavonoids and iron are believed to be blood booster supplements. This study aims to analyze the effectiveness of the iron content of kelakai extracts added to the feed on the hematological response of sodfish after *A. hydrophylla* bacterial infection. Recovery was carried out for 15 days with initial and final sampling. The treatments given for recovery were treatment A (Control), Treatment B (50ml / Kg Kelakai Extract), Treatment C (100ml / Kg Kelakai Extract), Treatment D (150ml / Kg Kelakai Extract). Parameters measured were iron, hemoglobin, hematocrit, MCHC and MCH. The results showed the best increase in hemoglobin and hematocrit in B (50 ml / Kg Kelakai Extract, while the best MCHC and MCH values were in C treatment (100 ml / Kg Kelakai Extract). Kelakai extract with iron content added to the feed was able to improve hematological use of snakehead fish during 15 days recovery after *A. hydrophylla* bacterial infection.

KEY WORDS
Kelakai, iron, hematological, snakehead fish.

Kelakai is a typical swampy plant that grows in South Kalimantan. Empirical studies of young kelakai leaves and stems are used by the Dayak people as a blood booster supplement, youth medicine, breast milk enhancer for breastfeeding mothers, high blood pressure medication, fever reliever and skin ailments such as itching and allergies (Maharani *et al*., 2005). Kelakai plants contain secondary metabolites in the form of leaf and stem samples, namely: water content of 8.56% and 7.28%, ash content of 10.37% and 9.19%, crude fiber content of 1.93% and 3.19%, protein content was 11.48% and 1.89%, fat content was 2.63% and 1.37%, the results of the analysis of Ca minerals were higher in leaves than stems, namely 182.07 mg per 100 g, as well as the highest Fe 291, 32 mg per 100 g. The highest phytochemical content of flavonoids, alkaloids and steroids was found in the stem, amounting to 3.010%, 3.817% and 2.583% (Maharani *et al*., 2005). The content of secondary metabolites contained in kelakai leaves, namely alkaloid compounds, steroids, flavonoids and iron (Anggraeni *et al*, 2015). Kelakai plants contain iron minerals of 291.316 mg / 100 g on the leaves and as large as 221.443 mg / 100 g on the stems. Iron content in the leaves of fresh kelakai plants was 3.285% or equivalent to 3285 mg / 100 g (Maharani *et al*, 2005). The addition of kelakai extract which contains iron into feed can contribute positively to the immunostimulant of snakehead fish (Norhayati *et al*, 2019).

Cultivation of snakehead fish (*Channa striata*) is currently growing, because the maintenance of this fish is relatively easy and very profitable. Cultivation there are still obstacles to the biological response which will later have hematological consequences. Hematological response is one of the most important factors that must be considered in the maintenance of snakehead fish (Sofian *et al*, 2019). The hematological condition of snakehead fish is low when there is stress, making it easier to get sick and even result in death. The average number of leukocytes is above the normal range (Hidayat *et al*., 2014).

Aeromonas hydrophyla is a type of bacteria that causes illness in fish. Bacterial diseases, especially MAS (Motile *Aeromonas Septicemia*) disease caused by *A. hydrophyla*

bacteria (Allan & Stevenson, 1981). Generally, this type of bacteria lives in fresh water which contains high organic matter. snakehead fish generally have an average hematocrit value of 24.40% in male fish and 23.25% in female fish. According to Ari (2019), the average number of erythrocytes in snakehead fish (242.1x104 cells / mm^3) is still in the normal range for both males and females. The average erythrocyte diameter in male snakehead fish (8.16 µm) was greater than that of female fish (7.69 µm). According to Hartika *et al*. (2014) the number of leukocytes in fish ranges from 20,000 150,000 cells per mm3 of blood.

Snakehead fish have an average Efforts to increase the iron content were carried out to accelerate the return of the hematological conditions to normal snakehead fish after being infected with *A. hydrophylla* bacteria. In order for the recovery process to run well, support is needed from various factors, such as environmental factors, water quality and nutrition in the feed given (Sarjito *et al*, 2012). The addition of iron in feed can affect the immune response of fish (Setiawati *et al*, 2007). This study aims to analyze the effectiveness of the iron content of kelakai extracts added to the feed to the hematological response of snakehead fish after *A. hydrophylla* bacterial infection.

MATERIALS AND METHODS OF RESEARCH

This research was conducted for 4 months from September to December 2020, covering the preparation, research implementation and reporting period, located at the Wet Laboratory, Banjarbaru.

The design used in this study was a completely randomized design (CRD) with 4 treatments and 3 replications, namely:

A. Commercial feed kelakai extract 0 ml/kg;
B. Commercial feed + addition of 50 ml/kg kelakai extract;
C. Commercial feed + addition of 100 ml/kg kelakai extract;
D. Commercial feed + addition of kelakai extract as much as 150 ml/kg.

Measurement of iron (Fe) using the AAS method (Years of Period 2003). Hematological measurements were carried out using a hematology analyzer. Hematology analyzer which functions for measurement and examination of blood cells in blood samples. The hematology analyzer has several advantages, namely time efficiency, sample volume, and accuracy of results. Checking with a hematology analyzer can be done quickly and only takes about 45 seconds. The blood sample used can use peripheral blood with a smaller amount of blood. The results issued by this tool usually go through quality control carried out by internal laboratories (Medonic, 2016). The parameters analyzed were hemoglobin, hematocrit, mean corpuscular hemoglobin concentration / mchc (average hemoglobin concentration for each blood cell and mean corpuscular hemoglobin / mch (average amount of hemoglobin in red blood cells (pg).

The parameter data observed in this study were analyzed descriptively and the results were presented in graphs and tables. Covariance analysis (ANCOVA) was applied to examine differences between treatments in terms of growth patterns. One-way ANOVA test was used to ascertain whether there was a difference in the length-weight measurement of snakehead fish reared in indoor containers between the four treatments. If there is, then the test (HSD) is applied. All tests were analyzed at the 0.05 significance level using the SPSS Version 26 software.

RESULTS OF STUDY

Snakehead fish before being given kelakai extract and prior to infection with A. hydrophila bacteria in Table 2. The most important result of this study is that the kelakai extract works well to respond to the hematological system in the body of snakehead fish after experiencing A. 3. In addition to meeting nutritional needs, kelakai also has the potential to be used as food (Rahmawati, *et al*. 2017). According to Sholihah *et al*. (2018), the addition of Kalakai also positively increases the iron content in baby porridge. In addition, Kalakai extract was shown to have a high average amount of antioxidant activity compared to vitamin

C (Bayliak *et al.* 2016), an inhibitory effect on Cd-induced glycation and fructation in vitro (Suhartono *et al.* 2016), as well as its properties. anti-metallotoxic (Biworo *et al.*, 2018).

The results of iron analysis using the AAS method in snakehead fish feed can be seen in Table 1 below.

Table 1 – Value of Analysis of Iron (Fe) for Snakehead Fish Feed

Parameter	Test Result (mg / kg)
A	136.640
B	94,320
C	88,640
D	101,420

Note:

A. Control
B. 50ml / Kg Kelakai Extract
C. 100ml / Kg Kelakai Extract
D. 150ml / Kg Kelakai Extract

Table 2 – The parameters observed for snakehead fish without giving kelakai extract before infection with A. Hydrophila bacteria

Parameter	Value			
	Control	B (50ml/Kg)	C (100ml/Kg)	D (150ml/Kg)
Hemoglobin (g/dL)	6.2	10.6	7.5	9.2
Hematocrit (%)	14.7	14.0	15.0	19.3
Mean Corpuscular Hemoglobin Concentration/MCHC (g/dL)	37. 5	35.1	36.1	35.0
Mean Corpuscular Hemoglobin /MCH (pg)	38.8	37.1	38.0	38.5

Table 3 – The observed parameters for snakehead fish that were given kelakai extract for 15 days after infection with A. hydrophila bacteria

Parameter	Perlakuan			
	Control	B (50ml/Kg)	C (100ml/Kg)	D (150ml/Kg)
Hemoglobin (g/dL)	11.7	14.8	9.5	10.2
Hematocrit (%)	27.3	31.0	17.7	21.3
Mean Corpuscular Hemoglobin Concentration/MCHC (g/dL)	38.4	38.8	40.0	36.2
Mean Corpuscular Hemoglobin /MCH (pg)	40.4	39.6	46.1	43.3

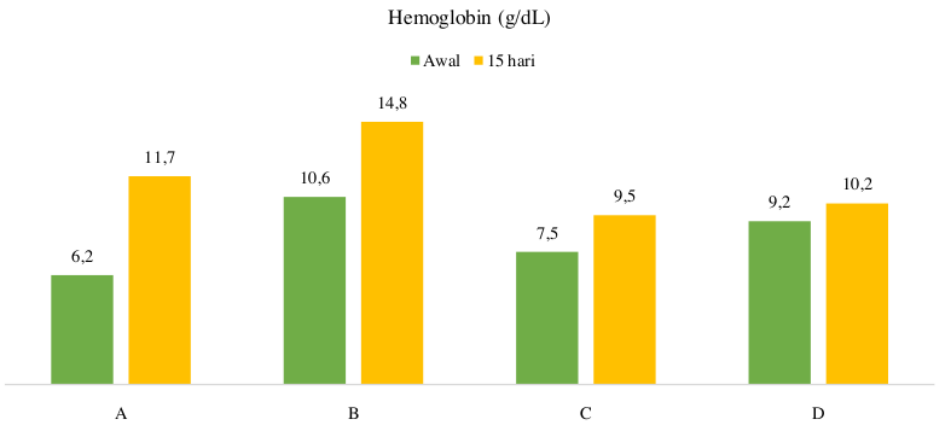

Figure 1 – Hemoglobin (g / dL) of snakehead Fish

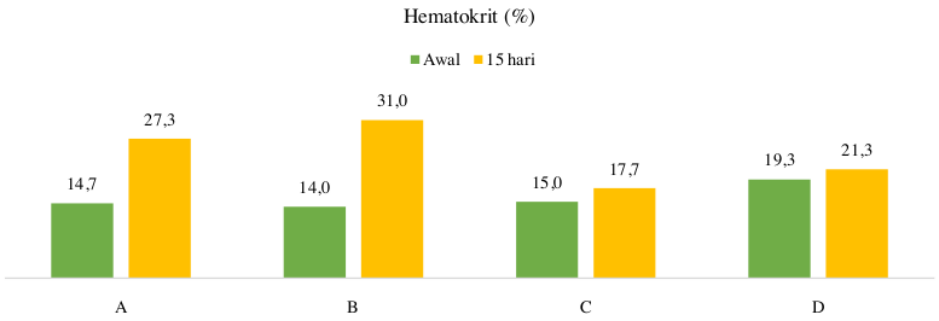

Figure 2 – Hematocrit (%) Snakehead Fish

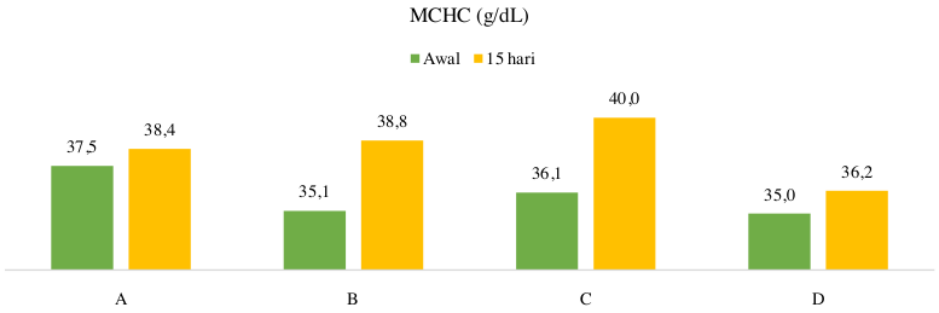

Figure 3 – Concentration of Mean Corpuscular Hemoglobin Concentration (g/dL) of snakehead Fish

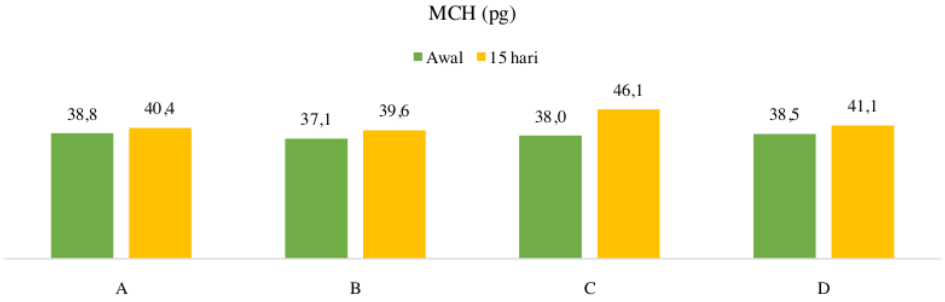

Figure 4 – Average MCH (pg) of snakehead Fish

DISCUSSION OF RESULTS

Snakehead fish is different at every sampling. During the research, 4 tails of snakehead fish were kept in *mesh container* with different feeding treatments. Treatment A (Control) the value of hemoglobin in fresh snakehead fish before infection of A. hydrophila battery was 6.2 g / dl, treatment B (50 ml / Kg kelakai extract) was 10.2g / dl, treatment C (100 ml / Kg kelakai extract) namely 7.5 g / dl and D (150 ml / Kg kelakai extract) which is 9.2 g / dl.

After infection, the snakehead fish was kept and fed for 15 days, the hemoglobin value experienced the highest increase in treatment B, namely 14.8 g / dl, then treatment A was

11.7 g / dl, followed by treatment D which was 10, 2 g / dl, and finally C treatment with an increase in hemoglobin that is 9.5 g / dl. The results of the analysis of diversity (ANOVA), showed Sig. (0.367)> 0.05. It means that H1 is rejected and H0 is accepted, it means that the kelakai extract that is given does not have a significant effect on the hemoglobin of snakehead fish.

The addition of kelakai extract to sodfish feed is effective for helping recovery after A. hydrophila bacterial infection. This condition is in accordance with the normal fish hemoglobin value between 10-12.3g / dl if it is below this value the fish is in an abnormal or stressful state (Siakpere, 2005). Physiologically, hemoglobin determines the level of endurance of the fish because it is closely related to the oxygen-holding capacity of the blood.

Based on data on iron content in feed, it shows that there is a difference in the response ability of fish to absorb iron. The absorption of iron by the snakehead fish has not been maximal. This condition can be seen from the value of blood hemoglobin where post-infection treatment, has increased compared to the time of infection. The availability of iron increased with the addition of the percentage of kelakai extract, it was seen that the hemoglobin value in treatment B increased from 6.2 g / dl to 14.8 g / dl, an increase of about 1.38%. This condition shows that the commercial feed given kelakai extract is effective in helping the recovery of fragrant snakehead fish after A. hydrophila bacterial infection. The effectiveness of adding kelakai extracts after A. hydrophila bacterial infection is mainly to increase hemoglobin in the presence of a high enough iron content.

Measurement of red blood cell count in blood (%) was done at baseline and 15 days after rearing snakehead fish was infected. The measurement data is presented in and in the following Figure.

The figure shows that the hazelnut fish hematocrit is different at each sampling. Treatment A (Control) the value of hematocrit in snakehead fish before infection of A. hydrophila battery was 14.7%, treatment B (50 ml / Kg kelakai extract) was 14.0%, treatment C (100 ml / Kg kelakai extract) namely 15.0% and D (150 ml / Kg kelakai extract) which is 19.3%.

After infection, the snakehead fish was kept and fed for 15 days, the hemoglobin value experienced the highest increase in treatment B, namely 31.0%, then treatment A was 27.3%, followed by treatment D which was 21.3%., and finally C treatment with an increase in hemoglobin, namely 17.7%.

All data during the study showed the highest hematocrit in treatment B (50ml / Kg Kelakai Extract), the average hematocrit concentration for each blood cell of snakehead fish from 14.0% to 31.0%, an increase of about 1.21%. The results of the analysis of diversity (ANOVA), showed Sig. (0, 564)> 0.05. It means that H1 is rejected and H0 is accepted, it means that the kelakai extract given does not have a significant effect on the hematocrit of snakehead fish.

According to Abdullah (2008) in Faizah (2013), freshwater fish are said to be healthy if their hematocrit levels range from 22-60%. A hematocrit concentration of <22% indicates that the fish have anemia and the possibility that the fish is experiencing pain or stress. The provision of feed containing extracts of kelakai to snakehead fish after A. hydrophila bacterial infection is effective in helping the recovery of fragrant snakehead fish and does not cause negative impacts, so it is suitable for use as a natural nutrient in feed, especially for increasing iron.

According to Guyton (1997) Hematocrit shows the percentage of solids (red blood cell levels, etc.) by the amount of blood fluid. The higher the hematocrit percentage, the thicker the blood concentration. This occurs because of the seepage (leakage) of fluid out of the blood vessels while the amount of solids remains, so the blood becomes thicker. A decrease in hematocrit occurs when the body experiences acute blood loss, sudden blood loss, for example when anemia, leukemia, chronic renal failure, malnutrition, deficiency of vitamins B and C. An increase in hematocrit above normal occurs in dehydration, severe diarrhea, eclampsia, the effects of surgery, and burns, and so on.

Measurement of the mean hemoglobin concentration for each blood cell (g/dL) was

carried out at baseline and 15 days of rearing after the snakehead fish was infected.

The picture shows that the MCHC of fragrant snakehead fish is different for each sampling. During the research, 4 tails of snakehead fish were kept in *mesh container* with different feeding treatments. Treatment A (Control), the MCHC value of the snakehead fish before infection with A. hydrophila battery was 37.5 g / dl, treatment B (50 ml / Kg kelakai extract) was 35.1g / dl, treatment C (100 ml / Kg kelakai extract) was 36.1 g / dl and D (150 ml / Kg kelakai extract) which is 35.0 g / dl.

After infection, the snakehead fish was kept and fed for 15 days, the MCHC value experienced the highest increase in treatment C, namely 40.0 g / dl, then treatment B was 38.8 g / dl, followed by treatment A which was 38.4 g / dl, and finally, treatment D with increased MCHC was 36.2 g / dl. All data during the study showed the highest MCHC in treatment C (100ml / Kg Kelakai Extract), the average MCHC of snakehead fish from 36.1 g / dl to 40.0 g / dl, increasing of about 0.10%. The results of the analysis of diversity (ANOVA), showed Sig. (0.709)> 0.05. It means that H_1 is rejected and H_0 is accepted, it means that the kelakai extract given does not have a significant effect on MCHC of snakehead fish.

MCHC is a calculation of the average concentration of hemoglobin in one red blood cell. (Laloan *et al.*, 2018). MCHC expresses the ratio of hemoglobin concentration to the volume of erythrocytes in whole blood. MCHC P. schlosseri results from this study were 29.80 ± 1.18 g / dL. MCH and MCHC values were higher in active fish than in less active fish (Goel et.al., 1981). According to Frandson (1992), the values of MCH and MCHC can be used in health examinations. An MCHC that is lower than the normal value is called hypochromic anemia, and MCHC that is higher than the normal value is called hyperchromic anemia.

A high MCHC value indicates a high hemoglobin level in each erythrocyte unit (Robbins, 1974), but the MCHC value of P. schlosseri is still lower than the MCHC value of frogs that have MCHC of 33.10 ± 0.14 g / dL (Omonona & Ekpenko, 2011) while the MCHC value of P. schlosseri was 29.79 ± 1.23 g / dL. Normal MCHC values in fish are generally 32-36 g / dl (Hrubec & Smith, 2000). MCHC values were higher in active fish than fish that were not very active (Goel *et al*., 1981 in Hidayaturrahmah *et al*.,, 2015). The treatment was given the extract of kelakai MCHC of snakehead fish, namely 36.0-40.0 g / dl. This condition means that the addition of kelakai extracts in the feed has a positive impact on MCHC of snakehead fish.

Measurement of mean hemoglobin in red blood cells (pg) was done at baseline and 15 days of rearing after snakehead fish infection. The measurement data is presented in the following Figure.

The picture shows that the MCH of fragrant snakehead fish is different for each sampling. During the research, 4 tails of snakehead fish were kept in *mesh container* with different feeding treatments. Treatment A (Control), the MCH value of the snakehead fish before infection with *A. hydrophila* battery was 38.8 pg, treatment B (50 ml / Kg kelakai extract) was 37.1, treatment C (100 ml / Kg kelakai extract) was 38.0pg and D (150 ml / Kg kelakai extract) which is 38.5pg.

After infection, the snakehead fish was kept and fed for 15 days, the MCH value experienced the highest increase in treatment C which was 46.1pg, then treatment D was 41.1, followed by treatment A which was 40.0, and finally treatment B with an increase in MCH of 39.6 pg. All data during the study showed the highest MCH in treatment C (100ml / Kg Kelakai Extract) the average MCH concentration of fragrant snakehead fish from 38.0 pg to 46.1 pg an increase of about 0.21%. The results of the analysis of diversity (ANOVA), showed Sig. (0.338)> 0.05. It means that H1 is rejected and H0 is accepted, it means that the kelakai extract given does not have a significant effect on MCH of snakehead fish.

MCH is a calculation of the average amount of hemoglobin in one red blood cell. MCH is the number of comparisons of hemoglobin levels with the number of red blood grains in units of pg (picogram) (Stockham & Scott 2008). MCH is the number of comparisons of hemoglobin levels with the number of red blood grains in units of pg (picogram) (Stockham & Scott 2008). Polizopoulou (2010) states that a high MCH value can indicate a regenerative anemia response caused by the erythrocyte hemolytic process.

The MCH value is influenced by the number of erythrocytes and the level of hemoglobin in the blood. The MCH value states how much hemoglobin is present in one erythrocyte cell. Erythrocytes that have high hemoglobin levels will also have high MCH values. The MCH value in this study ranged from 39.6 to 46.1 pg. It can be said that the MCH value in snakehead fish is within normal limits. Normal MCH values in fish are generally 30-100 pg / cell (Hrubec & Smith, 2000). This condition means that the addition of kelakai extract in the feed has a positive impact on MCH of snakehead fish.

CONCLUSION

Kelakai extract with iron content added to the feed was able to increase hematological effects of snakehead fish during the 15 days recovery after *A. hydrophila* bacterial infection. The best increase in hemoglobin and hematocrit was in B (50 ml / Kg Kelakai Extract, while the best for MCHC and MCH values was in C treatment (100 ml / Kg Kelakai Extract).

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