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THE EFFECTIVENESS OF DIFFERENCES IN MAGGOT (HERMETIA ILLUNCES) FLOUR PERCENTAGE ON THE HEMATOLOGICAL SNAKEHEAD FISH (CHANNA STRIATA) POST BACTERIA INFECTION (AEROMONAS HYDROPHILA)

Dahlia Prayitno Devisa*, **Untung Bijaksana**, **Indira Fitriliyani**
Faculty Fisheries and Marine Science, University of Lambung Mangkurat,Indonesia
*E-mail: devisadprayitno02@gmail.com

ABSTRACT
The purpose of this study was to determine the percentage of fish meal with maggot flour that produced the best response to the hematological of snakehead fish (Channa striata) after *Aeromonas hydrophilla* bacterial infection. Rearing was carried out for 15 days with sampling at the beginning before and at the end after infection. The treatments were treatment A (100% fish meal), treatment B (75% fish meal + 25% maggot flour), treatment C (50% fish meal + 50% maggot flour), treatment D (25% fish meal + Maggot flour 75%). Parameters measured are hemoglobin, erythrocytes, leukocytes, and hematocrit. The research results showed The best increase in hemoglobin, hematocrit and leukocytes were in treatment B (75% fish meal + 25% maggot flour) and C (50% fish meal + 50% maggot flour), while the lowest erythrocyte level was in treatment D (25% fish meal + fish meal) Maggot 75%). The percentage of fish meal with maggot flour in feed was able to increase hemoglobin, erythrocytes, leukocytes, and hematocrit in snakehead fish (*Channa striata*) during the 15-day rearing period.

KEY WORDS
Fish meal, maggot flour, hematological, snakehead fish.

Maggot or larvae of the black soldier fly (Hermetia illicens) is an alternative feed that meets the requirements as a protein source. According to Handayani & Widodo (2010), protein sources are foods that have a protein value above 20%. Maggot has a fairly high crude protein content ranging from 30-45%, contains essential fatty acids (linoleic and linolenic) and 10 kinds of essential amino acids (Hadadi et al., 2009).

Snakehead fish (Channa striata Blkr) is a type with high economical value in swampy waters and the potential to be developed. According to Bijaksana (2006) Snakehead fish for the Banjar people is a "cultural" fish because some regional specialties are very dependent on Snakehead fish. It caused an increasing demand for Snakehead fish. Because of such a strong ontology, besides its function as one of the most effective internal wound healers with albumin content, it is appropriate for domestication efforts to be carried out (Bijaksana, 2010).

The biological response of cultured snakehead fish has obstacles that can affect its hematology. The hematological response is highly important to take into consideration in the raising of snakehead fish (Sofian et al, 2019). Stress causes l hematological condition lower, even the disease will attack and cause death easily (Hidayat et al., 2014).

Generally, the causes of infected fish are several factors, namely infectious diseases (parasites, bacteria, fungi, and viruses), non-infecting diseases (stress, intoxication, deficiency), MAS (Motile Aeromonas Septicaemia) caused by the bacterium Aeromonas hydrophyla is one of the bacterial diseases (Allan & Stevenson, 1981). Aeromonas hydrophyla is a bacterium that causes disease in freshwater fish whose waters contain 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/mm3) is still within the normal range for both males and females. The average diameter of erythrocytes in male snakehead fish (8.16 m) is larger than that of female fish (7.69 m), according to Hartika et al.

Fish have a high body defense condition against disease if their body condition is not disturbed and weakened by environmental conditions and the feed they consume is insufficient in nutrients (Mutakin, 2006). So the feed has an extra role in nourishing the fish's body and making it a source of energy in increasing body resistance, both for growth and reproduction. The nutritional content in fish feed must be sufficient, maggot has a high nutritional content such as protein, essential fatty acids and essential amino acids so that it is expected to increase the body's resistance of snakehead fish. This study aimed to analyze the hematological response of snakehead fish to a feed formulation containing fish meal and maggot flour.

METHODS OF RESEARCH

This research was carried out for 6 months starting from January to June 2021, covering the preparation period, research implementation and report. This research was conducted at the Wet Laboratory, Banjarbaru.

Research Implementation:

- Tools and materials preparation;
- Procurement of snakehead fish and bacterial isolation. Snakehead fish size: weight 18.17±0.76 grams and length13.42±0.7520-28 cm;
- Feed making by formulating feed;
- Rearing for 15 days;
- Sampling was carried out 2 times, at the beginning and the end of the research.

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

- Treatment A: 100% Fish Meal;
- Treatment B: Fish Flour 75% + Maggot Flour 25%;
- Treatment C: Fish Flour 50% + Maggot Flour 50%;
- Treatment D: Fish Meal 25% + Maggot Flour 75%.

Hematological measurements were carried out using a hematology analyzer A hematology analyzer was used for measurement and examination of blood cells in blood samples. The hematology analyzer has several advantages, such as time efficiency, sample volume, and accuracy of results. Examination 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 released by this tool usually have gone through quality control carried out by an internal laboratory (Medonic, 2016). Parameters analyzed in the form of hemoglobin, erythrocytes, leukocytes, and hematocrit.

Parameter data were analyzed descriptively. Results are provided in the graphs and tables. Analysis of covariance (ANOVA) was applied to examine differences between treatments in terms of growth patterns. One-way ANOVA test was used to determine whether there were hematological differences in snakehead fish kept in indoor containers between the four treatments. If there is, then the test (HSD) is applied. All tests were analyzed at a significance level of 0.05 using SPSS-26 software.

RESULTS AND DISCUSSION

The results of the Hematology analysis of Snakehead fish can be seen in Table 1.

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

Observation parameters	After Infection				
	0	A	B	C	D
Hemoglobin (g/dL)	2.0±0	4.1±3.5	4.8±2.5	6.1 ± 0.7	2.9±1.7
Erythrocytes (x10^6/µL) (cells/mm^3)	1.50±0	1.50±0.91	1.15±0.73	1.76±0.08	0.79±0.40
Leukocytes (x10^3/µL) (cells/mm^3)	4.3±0	69.7±108.8	62.6±105.4	111.2±96.4	1.1±1.9
Hematocrit (%)	13.9±0	10.6±7.9	12.8±6.3	17.7 ± 0.6	7.8±3.1

Note: 0 - No Treatment; A - 100% Fish Meal; B - Fish Flour 75% + Maggot Flour 25%; C - Fish Flour 50% + Maggot Flour 50%; D - Fish Meal 25% + Maggot Flour 75%.

Measurement calculation Hemoglobin (g/dL) was carried out at the beginning of the study and after infection *A. hydrophila* after 15 days of maintenance. The measurement data are provided in Figure 1.

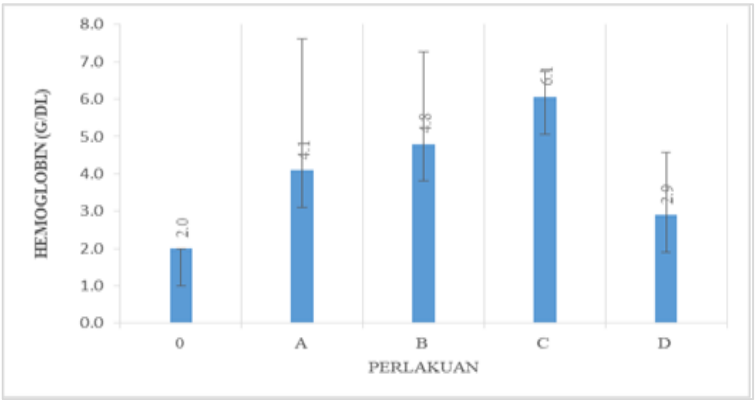

Figure 1 – Hemoglobin (g/dL) Snakehead Fish

Figure 1 shows the average hemoglobin level after infection with A. Hydrophila bacteria increased in all treatments A (4.1 g/dL), B (4.8 g/dL), C (6.1 g/dL) and D (2.9). g/dL). The hemoglobin level in this research was very low under the normal range as shown in Table 1. There are several possibilities regarding the hemoglobin level being under the normal range according to Royan et al. (2014) Low hemoglobin levels under the normal range indicate low protein content of the feed, poor water quality, and fish infection. According to Putra et al. (2015), hemoglobin levels after the first challenge test on nilem fish were 5.63 g%-6.77 g%. According to Fredi et al. (2017), the lowest hemoglobin level after the first challenge test in gouramy in his research was 5.6 g%. According to Hartika et al. (2014), hemoglobin levels with 1% probiotics in tilapia are 10.50 g%. Hastuti and Subandiyono (2015) reported that the hemoglobin level of catfish with biofloc raising at week 6 was 6.6 g%.

Measurement calculation Erythrocytes (x10^6/µL) (cell/mm^3) was carried out at the beginning of the research and after infection with *A. hydrophilla* after 15 days of rearing. The measurement data are provided in Figure 2.

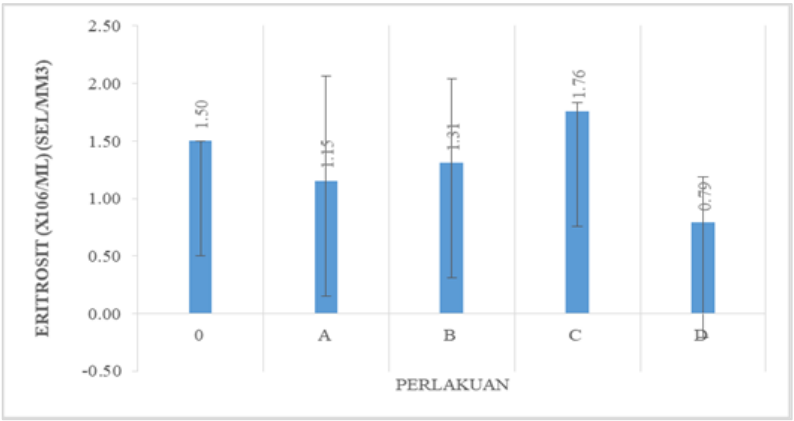

Figure 2 – Erythrocytes (x10^6/µL) (cell/mm^3) Snakehead Fish

The number of erythrocytes increased in treatment C (1.76 x 106 cells/mm^3), and decreased in treatment A (1.50 x 106 cells/mm^3), B (1.15 x 106 cells/mm^3), D (0.79 x 106 cells/mm^3) can be seen in Table 1. This decrease indicated the occurrence of anemia and the fish received infection as suggested by Salasia et al. (2001), the decrease in the number of erythrocytes in tilapia is influenced by *Aeromonas hydrophila* infection which affects the liver, spleen and spinal cord which function to form erythrocytes. This value in this study was still in the normal range except for treatment D which was below the normal range according

to what Irianto (2005) said that the normal number of erythrocytes in teleost fish was 1.05 x 106-3.00 x 106 cells/mm3. According to Fredi et al. (2017), the total number of erythrocytes after the first challenge test in gouramy was the lowest in his research, namely 1.71 x 10^6 cells/mm3. According to Hartika et al. (2014), the number of erythrocytes with 1% probiotics in tilapia was 3.14 x 10^5 cells/mm3. Rousdy and Wijayanti (2015) reported that the number of erythrocytes in carp with 1% humic acid was 1.27 x 10^6 cells/mm3.

Measurement Leukocytes concentration (x10^3/µL) (cell/mm^3) was carried out at the beginning of the research and after infection *A. hydrophila* after 15 days of rearing. The measurement data are provided in Figure 3.

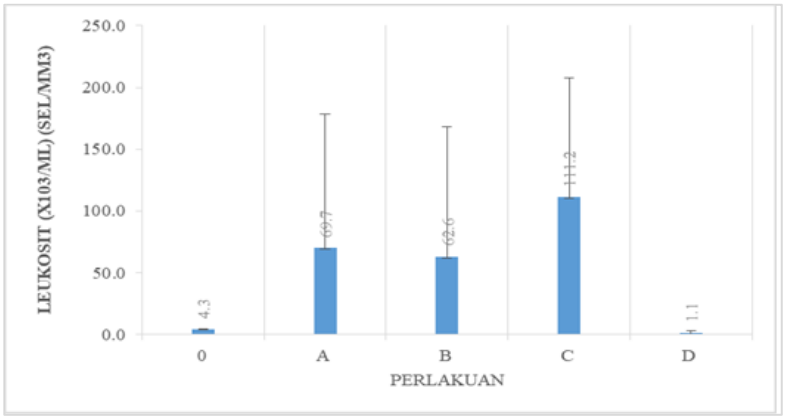

Figure 3 – Leukocytes (x10^3/µL) (cell/mm3) Snakehead Fish

Leukocytes are components of blood cells that act as the defense system of the fish body (Robert, 2012). According to Hartika et al. (2014), the number of leukocytes in fish ranges from 20,000 to 150,000 cells per mm^3 of blood. Snakehead fish has an average leukocyte count above the normal range. Adebayo et al. (2007) reported the number of leukocytes in fish Parachanna Obscura from the family Channidae around 4.01x10^3 cells/mm3. The high leukocyte count is thought to be due to stress on fish due to poor and polluted water quality. An increase in the number of leukocytes is called leukocytosis (Erika, 2008). The number of leukocytes is influenced by several factors, namely fish species, age, nutrition, and stress (Modra et al., 1998). The number of leukocytes and erythrocytes in snakehead fish has a negative correlation, namely the higher the number of erythrocytes, the lower the number of leukocytes. According to Adebayo et al. (2007), the number of erythrocytes and the number of leukocytes in P. Obscura fish were positively correlated. The difference in correlation may be due to differences in age, nutrition, and physical condition of the fish. Leukocytes are divided into two types based on the presence or absence of granules in the cell, namely granulocytes and agranulocytes. Granulocytes consist of neutrophils, eosinophils, and basophils while agranulocytes consist of monocytes and lymphocytes (Campbell & Ellis, 2013). According to Jain (1993), eosinophils and basophils play a role in parasitic infections and allergic responses and are associated with acute diseases. Therefore, in normal fish conditions or health conditions that are not too severe both are not found. The male snakehead fish had a larger leukocyte diameter (7.82 m) than the female fish (7.24 m). The number of erythrocytes and the number of leukocytes in P. Obscura fish were positively correlated. The difference in correlation may be due to differences in age, nutrition, and physical condition of the fish. Leukocytes are divided into two types based on the presence or absence of granules in the cell, namely granulocytes, and agranulocytes. Granulocytes consist of neutrophils, eosinophils, and basophils while agranulocytes consist of monocytes and lymphocytes (Campbell & Ellis, 2013). According to Jain (1993), eosinophils and basophils play a role in parasitic infections and allergic responses and are associated with acute diseases. Therefore, in normal fish conditions or health conditions that are not too severe both are not found. The male snakehead fish had a larger leukocyte diameter (7.82 m) than the female fish (7.24 m). the number of erythrocytes

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Measurement Hematocrit(%) was carried out at the beginning of the study and after infection *A. hydrophila*after 15 days of maintenance The measurement data are presented in and Figure 4.

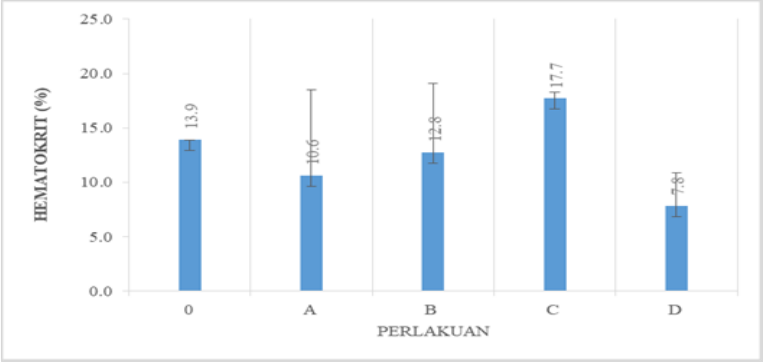

Figure 4 – Hematocrit (%) Snakehead Fish

Figure 4 shows the average number of hematocrit increased in treatment C (17.7%) and decreased in treatment A (10.6%), B (12.8), and D (7.8%). This shows that the hematocrit value in snakehead fish is under normal. Adebayo et al. (2007) reported that P. Obscura from the same family as snakehead fish (Channidae) had an average hematocrit value of 26.40% and an erythrocyte count of about 2,004x106 cells/mm^3. Hematocrit is a description of red blood cells percentage in the blood (Hastuti & Subandiyono, 2015). According to Salasia et al. (2001), the hematocrit value is directly related to the number of fish erythrocytes, which means that the hematocrit value will increase if the number of erythrocytes increases. The results obtained in this research also showed that the higher the hematocrit value, the higher the number of erythrocytes. Normal hematocrit values in Teleostei fish, especially freshwater fish, ranging from 22%-60% (Nabib & Pasaribu, 1989). Campbell (2015) revealed that hematocrit levels vary depending on nutritional factors, age, sex, body size, and spawning period. Hematocrit measurement can be used as a parameter to determine fish health, for example as an indication of stress. Stress in fish can occur due to several factors such as environmental factors, handling when taking blood (injection) or due to pathogen infection (Hardi et al., 2011). 1989). Campbell (2015) revealed that hematocrit levels vary depending on nutritional factors, age, sex, body size, and spawning period. Hematocrit measurement can be used as a parameter to determine fish health, for example as an indication of stress. Stress in fish can occur due to several factors such as environmental factors, handling when taking blood (injection) or due to infection with pathogens (Hardi et al., 2011).

CONCLUSION

The effectiveness of feed containing fish meal and maggot meal was able to improve the hematology of snakehead fish that had been reared for 15 days and then infected with A. hydrophila bacteria. There was an increase in histology in all test parameters, namely in Treatment C (50% Fish Meal + 50% Maggot Flour).

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