Hematology Response of Catfish (Clarias sp.) as an Indicator of Fish Health in Tuban Regency

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Abstract. Catfish farming offers strong potential for community economic growth. Catfish are resistant to various water conditions so that many people cultivate this fish. Just like other fish farming, the disease is the biggest obstacle in catfish farming. Changes in fish haematology in response to stress agents are indicators of the stress stage of fish, yielding useful information for curbing unfavourable conditions that can affect fish health. This study aimed to determine the haematological conditions of catfish in three different locations scattered in Tuban Regency as an indicator of fish health. The method used was descriptive by observing Erythrocytes, Leukocytes, Hemoglobin, and Hematocrit at 3 locations, namely in Tegalagung Village (a), Jenggolo Village (b), and Campurejo Village (c). The results of the erythrocyte at all locations were (a) 540,000 cells/mm³, (b) 1,980,000 cells/mm³, and (c) 1,690,000 cells/mm³. The results of the leukocyte count at all locations showed that (a) 301,000 cells/mm³, (b) 545,500 cells/mm³ and (c) 276,000 cells/mm³. Hemoglobin observations showed at the location (a) 16 g%, (b) 10.1 g% and (c) 10.8 g%. Hematocrit observation results showed at locations (a) 14%, (b) 13% and (c) 15%. Based on those results, it shows that catfish are in abnormal conditions. It is concluded that hematological parameters are outside the normal fish limits.

1. Introduction

Catfish (Clarias sp.) is one of the leading fishery commodities, mostly cultivated by Indonesian, especially in Tuban District. The increase in the price of catfish is an incentive for farmers to cultivate catfish. Catfish farmers sometimes do not pay attention to the health aspect. The health condition of catfish is visually difficult to determine because it often does not indicate to have a disease [1,2]

The freshwater aquaculture system, which has reached the intensification stage, is inseparable from the risk of disease [3]. Rustikawati, [4] says that the more intensive the fish culture, the higher the prevalence of disease infections. The disease infection is one of the factors that can reduce fish production. It arises because of an interaction between the host, pathogen, and the environment. The causes of disease can be divided into two groups, namely non-infection (stress, intoxication, nutritional deficiency) and infection (viruses, bacteria, fungi, worms and protozoa). Thus, other methods are needed to determine the health condition of catfish, apart from morphological observations, and clinical symptoms appeared from the outside. It is necessary to examine the haematological parameters of cultivated catfish [5].
Typical values of fish blood images are needed to determine the health status and help diagnose diseases in fish [6]. Therefore, blood smear (haematological) can be used as a severity indicator of a disorder. The examination of haematological parameters includes the examination of the hematocrit value, red blood cell, haemoglobin value, white blood cell count, and the observation of parasites in the blood. Diseased fish experienced changes in the hematocrit value, red blood cell, haemoglobin value, and white blood cell count. Blood tests (haematological) can be used as an indicator of disease severity. Haematological studies are essential criteria for diagnosis and determination of fish health.

2. Material and methods
The methodology used was descriptive exploratory presented descriptively through haematological observations of catfish (Clarias sp.) as an indicator of fish health. Fish samples were obtained from 3 locations in Tuban Regency: Tegalagung Village (a); Jenggolo Village (b); and Campurejo Village (c).

2.1. Calculation of Erythrocytes and Leukocytes
Blood sampling was done by rinsing the syringe with 3.8% Na Citrate as an anticoagulant. Blood samples were taken using a syringe in the 1 ml caudal vein. The blood was put into an Eppendorf tube for observing the blood image directly to measure erythrocytes and leucocytes.

The following formula can calculate the number of red blood cells (erythrocytes). According to Blaxhall and Daisley [7]:

\[ \Sigma \text{found erythrocytes} \times 10^4 \text{ sel/mm}^3 \] .......(1)

The number of white blood cells (leukocytes) can be calculated by the following formula. According to Blaxhall and Daisley [7]:

\[ \Sigma \text{found leukocytes} \times 50 \text{ sel/mm}^3 \] .......(2)

2.2. Haemoglobin
Hemoglobin levels were measured using the Sahli method. The principle of this method is to convert the hemoglobin in the blood into the form of hemotic acid by hydrochloric acid. Blood was extracted using a sahli pipette up to a scale of 20 mm³ and transferred into a hemoglobin tube containing 0.1 N HCL to a scale of 10 (yellow color), in it 3-5 minutes for hemoglobin to react with HCL to form hemotic acid. Then stir and add distilled water little by little until the color is the same as the standard color. The reading of the column scale is gram / 100 ml, which means the amount of hemoglobin in grams per 100 ml of blood. [8].

2.3. Hematocrit
The hematocrit value was examined using the microhematocrit method. The heparin microhematocrit is inserted into the blood sample until it fills approximately three quarters (3/4) of the capillary tube. Besides, one end of the capillary tube is blocked by sticking it with the wax plug. Then, it was centrifuged for 5 minutes using a microhematocrit centrifuge at a speed of 1,500 rpm. The hematocrit value is determined by measuring the percentage of erythrocyte volume from blood using a long measuring instrument (ruler) and expressed as a percentage (% Ht) [9].

3. Results and discussion
3.1. Erythrocyte and Leukocyte calculation results
The results of the mean calculation of the erythrocyte (Figure 1). This study revealed the results of the cultivation pond located in Tuban Regency: Tegalagung Village (a) amounted to 540,000 cells/mm3, Jenggolo Village (b) by 1,940,000 cells/mm3, and Campurejo Village by (c) 1,776,667 cells/mm3. Erythrocytes are the most numerous cells. In healthy catfish, the standard number of erythrocytes is 3.18 x 106 cells/mm³ [1]. This total value is still suitable for the range of healthy
catfish erythrocytes, and the results of this study are also not much different from previous studies by Lukistyowati et al., [10], explaining that the total erythrocytes for fish ranged from 1,000,000 - 3,000,000 cells/mm$^3$. Based on the results of erythrocyte calculations in catfish samples in Jenggolo Village and Campurejo Village, it showed that they were still in normal condition, but Tegalagung Village showed shallow results, below the normal range of erythrocyte levels in catfish. This indicated that the catfish in Tegalagung Village was not healthy. Red blood cells have an important role in immunity in fish. Based on research conducted by Yanuhar et al. [11] and Yuwanita et al. [12], the hemagglutinin test carried out on grouper blood cells with viral immunogenic proteins showed the results of the hemagglutinin sensitivity reaction up to 0.015625. This shows that red blood cells have an important role in the immune system in fish that are challenged with the virus.

Low erythrocyte levels indicate anaemia. Meanwhile, high levels indicate that the fish is under stress. Also, the low total erythrocytes will cause fish to be unable to take in large amounts of oxygen even though the availability of oxygen in the waters is sufficient. As a result, fish will experience anoxia (lack of oxygen) [13]. Erythrocytes are produced in the spleen and kidneys. Anaemia has an impact on inhibition of fish growth because the low number of erythrocytes causes a reduced food supply to cells, tissues, and organs so that the metabolic process of fish will be inhibited.

![Figure 1. Erythrocyte calculation results in catfish (Clarias sp.). (a) Tegalagung Village; (b) Jenggolo Village; (c) Campurejo Village](image_url)

**Figure 2** shows the total calculated leukocytes. The results showed that the total leucocytes in Tegalagung Village were 301,000 cells/mm$^3$, Jenggolo Village by 545,500 cells/mm$^3$, and Campurejo Village by 276,000 cells/mm$^3$. Based on the results obtained from these three locations, it exceeds the total range of leucocytes in healthy catfish, indicating that the fish are in an unhealthy state or stressful conditions. Leukocytes (white blood cells) have an oval or round shape, are colourless, and the fish blood per mm$^3$ ranges from 20,000-150,000 grains. Also, it is an active unit of the body's defence (immune) system [13]. Fish that experience stress caused by changes in environmental conditions and because of foreign bodies show a response to an increase in the number of leukocyte cells [14]. The increased production of white blood cell counts in cultured African catfish shows the body's resistance response to foreign substances that cause disease.
3.2. Haemoglobin Calculation Results
The results of the haemoglobin (Hb) calculation in catfish (Figure 3.) from the three research sites were: Tegalagung Village by 16 g%, Jenggolo Village by 10.1 g% and Campurejo Village by 10.8 g%. The research results indicated that the Hemoglobin value at three locations was still in the range of normal for catfish. Normal catfish (Clarias sp) haemoglobin concentrations range from 10 - 14 g% [15]. Hb functions to bind oxygen, which will then be used for catabolism to produce energy. The ability to bind oxygen in the blood depends on the amount of haemoglobin [1]. The low feed protein content will cause the haemoglobin content low too, which finally will cause fish to get an infection. The decrease in haemoglobin value indicates abnormalities in fish health.

3.3. Hematocrit Calculation Results
Based on the data in Figure 4, it can be seen that the hematocrit value of catfish from the three research locations is low. If the fish is affected by disease or their appetite decreases, the blood hematocrit content will be abnormal, and if the hematocrit value is low, the erythrocyte count is low. Hematocrit value of healthy catfish is 30.8 - 45.5% [1]. The results of the hematocrit calculation in this study were (a) Tegalagung Village by 14%; (b) Jenggolo Village by 13%; and (c) Campurejo Village by 15%. According to Bastiawan et al., (2001), if the fish is affected by disease or their appetite decreases, the blood hematocrit value will be abnormal, and if the hematocrit value is low, the erythrocyte count is low too. Normal hematocrit levels in Teleostei fish range between 20-30% and marine fish is around 42%, fish with anaemia have a minimum hematocrit percentage of 10% [16].
4. Conclusion
The overall results of the study indicate that based on the haematology observations of catfish (Clarias sp.) in three locations, Tegalagung Village, Jenggolo Village and Campurejo Village, Tuban Regency, the haematological description is outside the normal limits of catfish haematological content. It can be concluded that the catfish used are classified as fish with the disease. These results also support the statement that hematology can be used as an indicator of fish health in catfish aquaculture ponds.

5. References
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