Leukocyte Count and Differential Leukocyte Count of Carp (*Cyprinus carpio Linn*) after Infected by *Aeromonas salmonicida*

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Abstract: The hematology examination is to describe the health of fish. The aim of this research is to study leukocyte and differential leukocyte count in Carp (*Cyprinus carpio Linn*) after infected by *Aeromonas salmonicida.* P0 was a control group and waa not infected with *Aeromonas salmonicida*. P1, P2, and P3 group were infected by *Aeromonas salmonicida* through different doses; 105 cell/ml, 106 cell/ml, and 107 cell/ml. Three days after infection, blood samples were obtained from *Punctie Vena Caudal* which was deposited into EDTA tubes. Total number leukocyte and differential leukocyte count analyzed were carried out with the collected blood samples. Data were analyzed with ANOVA (Analysis of Variant) then followed by Duncan's multiple range test significance level of 5%. The results showed that there was significant increase ($p<0.05$) in the number of leukocytes compared with control (P0). There was significant increase ($p<0.05$) in the number of eosinophils, neutrophils, lymphocytes and monocytes. The number of basophils were not significant ($p<0.05$). Based on the results of research, it could be concluded that bacterial infections *Aeromonas salmonicida* in Carp cause changes in leukocyte count and differential leukocyte count. The change is an increase in the number of leukocytes count; while in the differential leukocyte count, there was an increase in eosinophils, neutrophils, lymphocytes and monocytes.

1 INTRODUCTION

Indonesia has abundant freshwater and is considerable potential for the cultivation of a wide variety of freshwater fish species (Cahyono, 2000). Freshwater fish have a relatively high protein. High content of protein and vitamins cause freshwater fish be easily cultivated and very helpful in nutrition fulfillment for the society. This is due to the fact that fish is an excellent source of protein, fat, and minerals (Ministry of Maritime Affairs and Fisheries, 2014).

Carp is considered as the most popular freshwater fish among the existing freshwater fish species (Supriatna, 2013). Various cultivation systems have been applied and prolonged to grow to obtain maximum goldfish production. One of the cultivation is by applying intensive cultivation system. However, the intensive Carp farming also has a negative impact, one of which is susceptible fish disease. One of the dangerous diseases is caused by *Aeromonas* bacterial infections; such as *Aeromonas salmonicida*. *Aeromonas salmonicida* is a cause of infectious diseases in salmonid fish that is furunkulosis disease (Nitimulyo et al., 1993). A number of reports indicate that there are also symptoms of bacterial infection *Aeromonas salmonicida* in cyprinid fish, namely *carp erytrodermatitis* disease (Irianto, 2005).

The presence of bacterial infections *Aeromonas salmonicida* characterized by changes in various clinical symptoms affects the blood picture of Carp. Blood picture is one indicator of infection (Nuryati et al., 2006). In the field of fisheries, hematological analysis can be applied as an early detection system to prevent mass death in fish cultivation (Noercholis et al., 2013).

Research on the number and counts of leukocyte of certain species can provide an overview of the environmental state of the fish, providing information about the health status and process of the occurrence of a disease in it. By analyzing the blood characteristics, a disease can be identified (Kumar and Ramulu, 2013). This is what encourages researchers to do research on the examination of and calculate the number and the type of Carp (*Cyprinus carpio Linn*) blood leucocytes after infected by *Aeromonas salmonicida*.

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2 MATERIALS AND METHODS

2.1 Place and Time of Research

This research was conducted in October - December 2017. The process of fish maintenance was done in the Laboratory of Veterinary Pathology, Department of Veterinary Pathology. The process of isolation and dilution of bacteria *Aeromonas salmonicida* was executed in the Laboratory of Bacteriology and Mycology, Department of Veterinary Microbiology. The process of taking blood, making blood smear, and examination of leukocyte count and differential leukocyte count was applied at Veterinary Clinical Pathology Laboratory, Departement of Veterinary Basic Medicine, Faculty of Veterinary Medicine, Airlangga University.

2.2 Material and Equipment of Research

The materials used in this research were Carp, pellet fish feed, water taps, Triple Soy Agar (TSA) Media, Triple Sugar Iron Agar (TSIA) Media, Sulfide Indol Motility (SIM) Media, Simon Citrate Agar (SCA) Media, Urea and MR-VP Media, physiologic NaCl, bacterial isolates *Aeromonas salmonicida*, EDTA solution, Dacies solution, 95% methanol, Giemsa dye and Giemsa buffer solution and oil emersion. The equipment used in this research were four aquariums containing 20 liters water, aerator machine, aerator hose, water purifier filter, zeolite stone, digital milligram scales, small fish net, pH meter, DO meter, 0.1 ml pipette with scale 0.001 ml, ose, petri dish, test tube, bunsen, petri dish, disposible syringe with needle, Improved Neubauer count chamber, Pasteur pipette, Blood Cell Counter, glass cover, light microscope, object glass, and glassware shelf.

2.3 Methods of Research

This study used Carp (*Cyprinus carpio Linn*) with length10-12 cm (age 6-9 weeks) and weight 21-23 g. Of 20 fish samples were randomized to four treatments, each treatment consisted of five replications. Carp adapted for one week.

2.4 Research Design

The experimental design used was Completely Randomized Design (RAL). The experiment was conducted with four treatments and five repetitions. Treatment under study:

P0: Negative control treatment group. The first aquarium was not infected by *Aeromonas salmonicida*
P1: Second aquarium was infected by *Aeromonas salmonicida* with a dose of 10^5 cells / ml P2: Third aquarium was infected by *Aeromonas salmonicida* with a dose of 10^6 cells / ml P3: Fourth aquarium was infected by *Aeromonas salmonicida* with dose 10^7 cells / ml

2.5 Data Processing

The data obtained in the form of the leukocyte count and differential leukocyte count were arranged in tabular form and then analyzed. Furthermore, a statistical test was performed using ANOVA (Analysis of Variant). If there was any difference between treatments proceed and Duncan Multiple Range Test, it would be a significance level of 5% to determine the best treatment. Data analysis was performed using SPSS 20 for Windows computer software.

3 RESULTS AND DISCUSSION

3.1 Leukocyte Count

The results of the total count of leukocytes in goldfish after given the treatment in detail can be seen in the following table:

Table 1: Mean and Standard Deviation of Carp Leukocyte After Infected by *Aeromonas salmonicida*.

Treatment	Leukocytes (cell/mm^3) (X ± SD)
P0 (without infection)	12400,00^a ± 1013,66
P1 (infection with dose 10^5 cell/ml)	19210,00^b ± 2278,54
P2 (infection with dose 10^6 cell/ml)	25990,00^c ± 3813,37
P3 (infection with dose 10^7 cell/ml)	45270,00^d ± 6986,65

Description: Different superscript letters in the same column show significantly different ($p<0,05$).

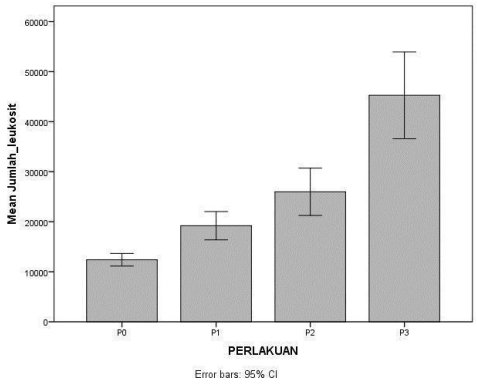

Figure 1: Graph of Leukocytes of Carp After Infected by *Aeromonas salmonicida*.

The results after analyzed by ANOVA assay showed that there was a significant difference in the number of leukocytes among treatments. After tested with Duncan Multiple Range Test, results illustrated an increase in the number of leukocytes were significantly different ($p < 0.05$). P0 was significantly different from P1, P2, and P3, while P1 was noticably different with P2 and P3.

Increased number of leukocyte cells in goldfish after treatment showed an immune response to the infection of *Aeromonas salmonicida*. This is in accordance with the opinion of Alamanda et al. (2007), which states that the increase in total leukocytes indicates a response of body resistance to disease-causing antigens. Increased total leukocytes showed an increase in body immunity characterized by increased activity of phagocyte cells that function to perform phagocytosis against foreign objects entering the fish body. Leukocyte systems and tissue cells of leukocytes work in two ways to prevent disease by damaging through the process of phagocytosis and forming antibodies (Suhermanto, 2013). Phagocytosis is the first step for mechanism of immune response, the next is the formation of specific antibody responses, whereas the increased phagocytic process suggests an increase in body immunity (Zainun, 2007).

3.2 Differential Leukocyte Count

The results of the leukocyte count in carp after treatment were given as follows:

Table 2: Mean and Standard Deviation of Differential Leukocyte of Carp after infected by *Aeromonas salmonicida.*

Treatment	Eosinophil (X± SD)	Basophil (X ± SD	Neutrophil (X ± SD)	Lymphocyte (X± SD)	Monocyte (X ± SD)
P0	934,50^{a} ± 88,21	499,70^{a}± 171,21	5121,00^{a} ± 736,72	3972,90^{a}±525,08	1871,90^{a}±154,13
P1	824,75^{a} ±157,38	529,36^{a}± 265,96	9947,30^{b}± 1557,88	5662,10^{b}±205,02	1967,30^{a}±349,46
P2	982,75^{a} ± 75,31	705,30^{a}± 191,70	15738,30^{c} ±312 0,51	6148,25^{b}±146,70	2215,40^{ab}± 285,90
P3	1634,80^{b}±582,15	849,45^{a}± 348,33	29415,00^{d} ±2743,15	7359,10^{c}±1536,28	2582,600^{b}± 415,20

Description: Different superscript letters in the same column show significantly different ($p < 0.05$).

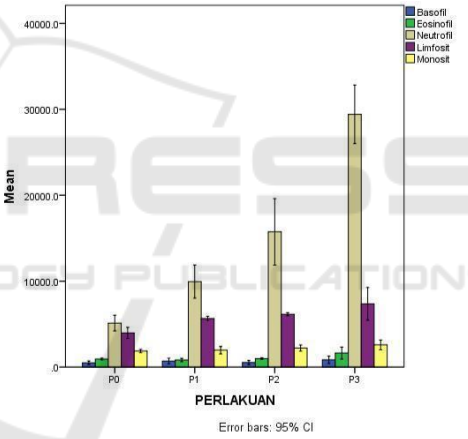

Figure 2. Graph of Differential Leukocytes of Carp After Infected by *Aeromonas salmonicida*.

The results after analyzed by ANOVA assay showed that there were significant differences in the number of neutrophils, eosinophils, lymphocytes and monocytes among treatments. After tested with Duncan Multiple Duncan Test, there was a noticable
increase of eosinophil, neutrophil, lymphocyte and monocyte counts ($p < 0.05$).

The highest increase of eosinophil is in P3 treatment. P0 was significantly different from P3, yet wasnot significantly different from P1 and
P2.

At basophil count, the results were not significantly different ($p < 0.05$) when compared with control (P0).

The highest neutrophil increase is in P3 treatment. P0 was significantly different from P1, P2, and P3, whereas P1 was significantly different from P2 and P3. P2 was significantly different from P0, P1, and P3.

The highest lymphocyte increase is in P3 treatment. P0 differed significantly with P1, P2 and P3, whereas P1 was not significantly different from P2 but significantly different from P3. P2 was significantly different from P0 and P3, yet was not significantly different from P1.

The increase in monocytes was highest in treatment P3. P0 was not significantly different from P1 and P2; this was in contrast with P3.

Eosinophils are the second major cell of the meiloid system. These cells are not as efficient as neutrophils in phagocytosis, yet have lysosomes and carry out a respiratory burst when precisely stimulated (Tizard, 1982). Increased eosinophils as an immune is a response to toxic and extracellular enzymes produced by Aeromonas. Pathogenic properties Aeromonas known as opportunistic pathogens in humans and fish, involving some extracellular enzymes, are reported to correlate with the mechanisms of infection and invasion of these bacteria (Rao et al., 1998).

Increased basophils occur due to the inflammatory process (inflammation), leukemia, and infective healing phases. Basophils are rarely found in the blood circulation of fish. Decreased basophils or basophenia may be caused by chemotherapy, in pregnancy, hyperthyroidism, radiation, in acute infection, and during treatment with glucocorticoids (Bijanti et al., 2010). The glucocorticoid hormone is one of the classes of the corticosteroid hormone. This indicates that the decrease in basophil count affects the production of corticosteroid hormones that play one of them as a suppressor of the immune response.

Neutrophils are the first cells to respond to infection by foreign bodies entering the fish body (Summers et al., 2010). To respond to bacterial infection, neutrophils leave the marginal group and enter the infection area and the thymus release its source of reserve resulting in increased granulopoiesis. The increase in granulopoiesis can be seen because there are many immature neutrophils that enter the blood circulation which is called a shift to the left. As the main function of neutrophils is phagocytosis (killing and digesting microorganisms), acute bacterial infections and trauma trigger neutrophil production. (Atmaja et al., 2016).

An increased number of lymphocytes can occur due to stressful fish (Sakai, 1999). Stress can cause non-specific immune response disorders, such as lymphocyte proliferation (increase in cell count and form changes into T cells and B cells). Lymphocytes are cells that function to produce antibodies or as effector cells in response to bound antigen macrophages. The circulating lymphocytes primarily originate from the thymus, some of which are relatively immature differentiated, multiply cells, are T lymphocytes. These, then then reenter the bloodstream. T cells are responsible for cellular immune reactions and have specific surface receptors to recognize foreign antigens. Other lymphocytes differentiate into B lymphocytes, by producing humoral antibodies in the bloodstream and binding specifically to foreign antigens causing phagocytosis, cell lysis, and killer cells (killer cells or K cells) of invading organisms. T cells and B cells morphologically can only be distinguished when activated by antigen (Tizard, 1982).

Increasing number of monocytes occurs because bacteria are foreign agents that must be eliminated so that monocytes will develop into macrophages to the place of infection to perform the process of phagocytosis. Inflammatory processes during tissue damage by infection or antigen-antibody reactions will increase monocyte production to two times more. The circulation of monocytes in the blood becomes shorter. Monocyte maturation which becomes macrophages happens more quickly and immediately leads to damaged tissue (Maftuch, 2007). The proportion of monocytes is very low in the leukocyte population, but may increase by about 38% in a short time if infection occurs (Andayani et al., 2008).

4 CONCLUSIONS

Based on the results of , it can be concluded that bacterial infections *Aeromonas salmonicida* in Carp cause changes in leukocyte count and differential leukocyte count. The change is an increase in the number of leukocytes count while in the differential leukocyte count, there is an increase in eosinophils, neutrophils, lymphocytes and monocytes.

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