Hematological parameters of Catfish (*Clarias* sp) vaccinated by *Aeromonas hydrophila* with different application methods

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Abstract. The study aim was to find the effect of different vaccination application methods on hematological parameters of catfish (*Clarias* sp). A 10^7 *Aeromonas hydrophila* measured by 625 nm wave length of Spectrophotometer was applied on 25 catfish with average weight 15.83 g. The application methods were dipping (1 ml/10 l water) and intramuscular injection (0.1 ml/fish). Hematological parameter measured were hematocrit levels, total leukocytes and phagocytic index. Measurement were conduct on day 0, 21, 23 and 27 after vaccine application. The results showed that hematocrit level on fish with injection method were higher (21.02%) than dipping method (15,05%), total leukocytes on fish with dipping method were higher (3,29 x 10^4 cells/mm^3) than injection method (3,15 x 10^4 cells/mm^3) and phagocytic index on fish with injection were higher (25%) than dipping method (23%). The survival of catfish were also observed, and showed that catfish with injection method have higher survival rate (36%) than dipping method (34%). *Aeromonas hydrophila* vaccine applied with injection method have shown better immune response on catfish (*Clarias* sp) hematological parameters than one with dipping method.

1. Introduction

Catfish is one of the freshwater fishes which is most liked for it's delicious taste and savory flavor, besides, the price is relatively cheap compared to other fishes. In contrast, catfish cultivation often faces some obstacles, one of them is disease.

Disease attack in the fish pond is caused by the unbalance interaction between 3 main components in the cultivation pond, which are, fish, environmental conditions and disease-causing organisms. The disease which is found frequently in catfish is MAS (Motile Aeromonas Septicaemia), also called red spot disease caused by *Aeromonas hydrophila* [4]. This bacteria strongly affects freshwater aquaculture, especially catfish and frequently causes a high mortality rate disease (80 – 100%) in a short period of time (1 – 2 weeks).

Vaccinnation is one of many ways for prevent *Aeromonas hydrophila* bacteria to infect fish. According to Kordi & Ghufran [7], the vaccine is weakened or killed antigen, which is aimed at increasing the resistance (immunity) to a particular disease. The fish vaccination is expected to increase the fish immunity therefore not easily infected and become resistance to certain kind of diseases that depend on the vaccine types.

Hematology observation is often used to overview a fish health condition. It is closely related to pathology as blood is a medium in circulation system, which is funcionated to circulate essential nutrients throughout the body and carries the remaining metabolic and pathogenic results before reaching a dangerous concentration [10].

2. Methodology

2.1. Fish rearing

This research was conducted in March – May 2019, in the Disease Laboratory of Freshwater Fisheries Center (BBPBAT) Sukabumi, West Java. There were two treatments was used; dipping and injection (intra muscular). The catfish were fed 2 times per day with *Adlibitum.* The fish tanks siphoned 2 to 3 times per day to maintain the water quality. The observed parameters were; hematocrit level, total leukocytes and phagocytic index. The catfish reared in 60x40x40 cm size Aquarium. The catfishes were 14 weeks age with 9-11 cm in length and $\pm$ 15.83 gram of weight.

Each aquarium contains 25 catfishes. The fish were reared for 27 days. The blood was sampled 4 times, one day before the vaccine administrated on fish, 21st, 23rd, and 27th days after vaccination. The vaccine were used by using 1 ml/10 liters dose on dipping treatment for 25 fishes while the injection treatment used 0.1 ml vaccine for each fish. On the 22nd day, The challenge testing was done by using an *Aeromonas hydrophila* bacteria with 10^7 density number, 0.1 ml for each fish.

2.2. Hematocrit levels

Hematocrit is the comparison between blood cells volume and blood plasma. The blood was collected by using microhematocrit tube until it reach ¾ length of the tube. The tube sealed by *Crystoseal* for about 1 mm depth. After that the tube was centrifugated in 15 minutes (Anderson and Siwicki, 1993). The hematocrit value was determined by the length of the blood sediment in the base of the tube and the total lenght of blood in the tube. The hematocrit level was stated as % of solid blood cell volume. The hematocrit level was measured using the following formula [2] :

$$\text{Hematocrit level} = \frac{\text{blood sediment length volume}}{\text{total length of blood volume in the tubes}} \text{ x } 100\% \quad (1)$$

2.3. Total leukocytes

Leukocyte (white blood cells) is blood cell that play an important role in the immune system, which function is to remove the pathogen or antigen that infect fish. Infected fish will produce more leukocytes to phagocyte the bacteria and synthesize antibodies [10]. The total leukocytes were calculated in a few steps (Blaxhall and Daisley, 1973). Firstly, the blood was collected by using a WBC pipette contained a white bead until it reached 0.5 mm in scale. Then the Turk's solution was added until it reached 11 mm in scale. after that, the blood was stirred in the pipette for 3-5 minutes. Turk's solution was used to destroys the membrane of WBCs, RBCs and platelets within a blood sample, and stains the nuclei of the white blood cells. The first drip of blood solution in the pipette was not used for total leucocytes counting. The second drip were applied on the Haemocytometer type Neubauer, and covered with cover glass. The total leukocytes were counted by using microscope with 40x10 magnification. On 5 large squares of hemocytometer by using the following formula:

$$\textit{Total Leukocytes} = \textit{the total number of leukocytes x 50 cells/mm}^3 \quad (2)$$

2.4. Phagocytic Index

Phagocytic index measured according to phagocytic index by Anderson and Swicki [2]. A total of 50 µl blood was put into a microtiter plate, 50 µl of *Staphylococcus aureus* suspension was added in PBS (10^8 cells/ml), homogenized and incubated at room temperature in 20 minutes. After that, a total of 10 µl blood was prepared and dried. Then, fixed with methanol for 3 minutes and then dried. Furthermore, it was stained by Giemsa solution for 15 minutes, washed with aquades and let dried.

The number of cells indicate the phagocytic process, calculated from 100 cells of phagocyte observed under a microscope. Phagocytic index was measured by using formula:

$$Phagocytic\ index = \frac{The\ number\ of\ cells\ tha\ \ indicating\ the\ phagocytic\ process}{100} x\ 100\% \quad (3)$$

2.5. Survival Rate (SR)

The survival rate was observed on the 27th day after the challenge testing was conducted. The calculation of the survival rate was measured :

$$SR = \frac{Nt}{N0} x\ 100\% \quad (4)$$

Note:
SR = Survival Rate (%)
Nt = The number of on the final day of experiment
N0 = The number of fish on the beginning of experiment

2.6. Data Analysis

Anderson Darling test used to the normality of data. Where Mann-Whitney test used to analyze the differences between hematological parameter of vaccinated catfish with injection method and dipping method. The significant level of the statistical test was 95%.

3. Result and discussion

The result indicates that (Figure 1) the application of *Aeromonas hydrophila* vaccine on the catfish using dipping and injection methods have a different outcome on the blood description. Since the day 0 to the 27th day, the injection treatment generates the better blood description compared to the dipping treatment with the percentage 21,02%.

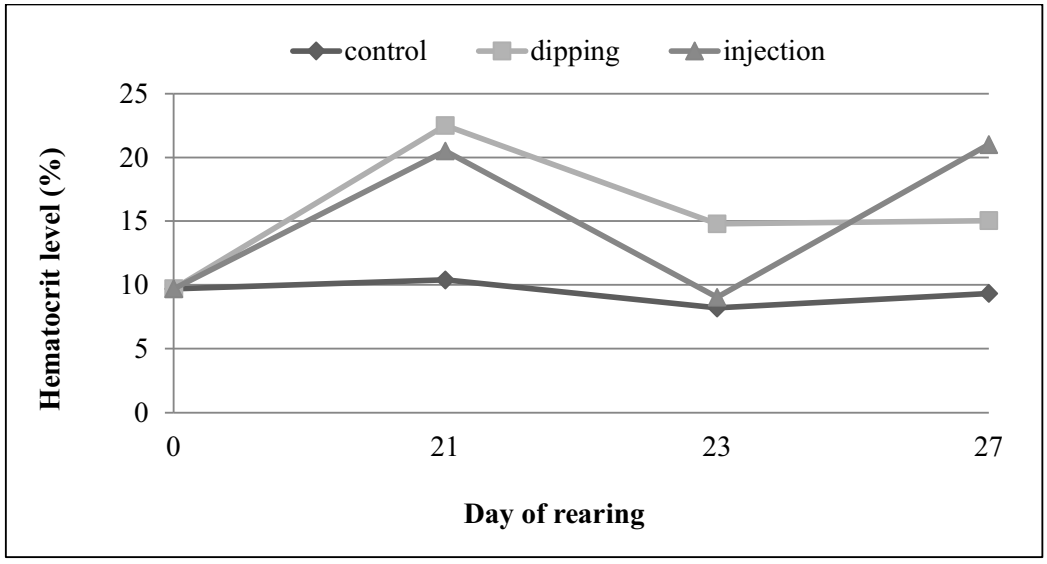

Figure 1. Hematocrit level in catfish with no treatment (control), dipping and injection vaccination methods.

The catfish average hematocrit level as shown in figure 1 has both increased 12,8% and 10,8% from 9,7% into 22,5% on 21st day after dipping method vaccination and into 20,5 after injection. On the 21st day after vaccination using dipping method, the highest hematocrit levels of catfish is gained 22.5% which is increased by 12.8%. The vaccination of catfish using dipping and injection method indicates that the hematocrit level of the catfish is increased during 21 days. This is in line with Santika, et.al [13] the enhancement of hematocrit levels of the vaccinated catfish tend to stimulate a response in the form of the enhancement levels of hematocrit to a certain range. The increase of hematocrit level indicates that the blood is in a good condition and able to bind the oxygen properly.

On the 23rd day after vaccination, the hematocrit levels of both dipping and injection vaccination treatments are decreased. It is caused by the infection of Aeromonas Hydrophila. This finding is in line with Anderson [3] that the decrease of the hematocrit level indicates that the fish is infected by pathogen. Furthermore, the decrease of hematocrit level after the challenge test is also caused by decreased-appetite as a consequence of the *Aeromonas hydrophila* infection. The decrease of hematocrite can be used as a clue to the lack of protein, vitamin deficiency, or infection of the fish as well as the decrease of the appetite.

On the 27th day, it is found that the hematocrit level increases back. The maximum value of hematocrit after the challenge test on day 27th is found in the vaccinated fish using injection method which reaches 21,02 %. The second increase of the hematocrit is as the second response toward the vaccination. This is in accordance with Santika, et al. [13] who stated that the increase of the hematocrit levels after the challenge test shows that the blood cells become the efector toward the increase of the specific fast response in adequate quantity to relieve the infection of Aeromonas hydrophila.

The result of the total leukocyte of the catfish before vaccination, after vaccination, and after challenge test can be seen in the Figure 2.

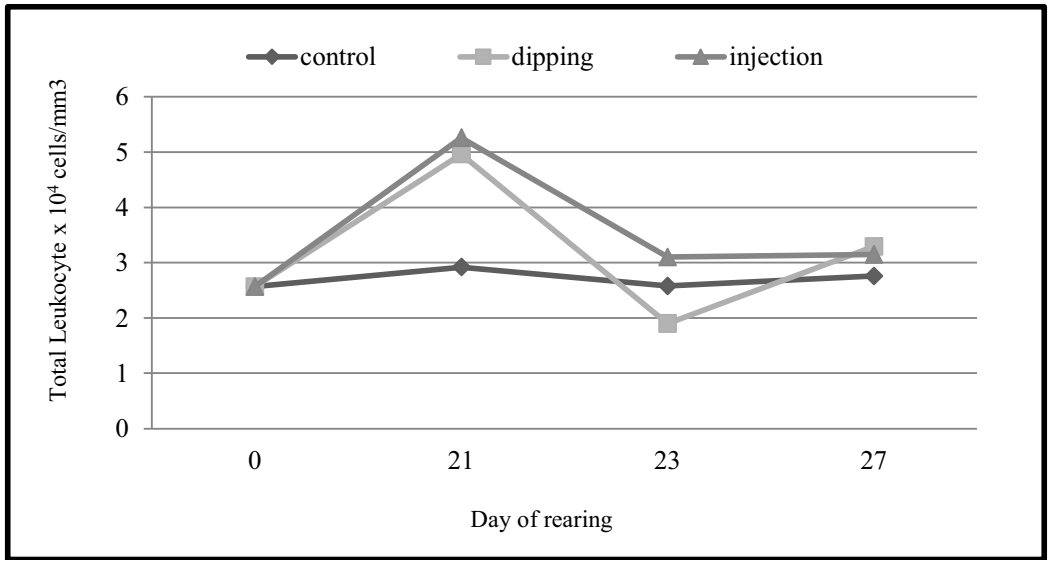

Figure 2. Total leukocytes on Catfish reared with no treatment, dipping and injection vaccination methods.

According to the Figure 2, it can be seen that before vaccination (day 0), the average total leukocytes in the normal catfish is 2,57 x 10^4 cells/mm^3. According to Putra [12], generally, the total leukocytes of fish is ranged from 2,00 x 10^4 - 15 x 10^4 cells/mm^3. The highest increase of total leukocytes during the 21 days vaccination is occured in the injection treatment vaccination, that is 5,26 x 10^4 cells/mm^3. This condition showed that the vaccine administration is immunogenic, so that, it

improves the defense response of the fish by increasing the total leukocytes. This finding is in line with Tizard [16] who stated that the immunogenic characters of the vaccine material at proper dose are able to increase the total leukocytes of the vaccinated fish resulting to the increased immune.

On the 23rd day, the number of leukocytes decreases for all treatments method. It is caused by the movement of the leukocytes from blood vessels to the cells which are infected by *Aeromonas hydrophila*. This finding is in line [11], who stated that the decrease of the total leukocytes after the challenge test is caused by the activation and the movement of the leukocytes from the blood vessels to the infected cells. This condition is also called as the fish response to recognize and recall the type of pathogens that infect the fish.

On the 27th day, all vaccinated fish treatment experienced an increase in the total number of leukocytes. The highest total leukocytes on the 27th day is found in the injection treatment, that is 3,15 x 10^4 cells/mm^3. This second increase indicates that the catfish is giving response toward the foreign substances that enter the fish body. According to Kresno [8], the increase of the total leukocytes is a reflection of the success of the immunity system. Suprayudi et al. [11] added that the kind of fish response to increase its immunity system is by increasing the number of leukocytes.
The results of phagocytosis index are shown in Figure 3.

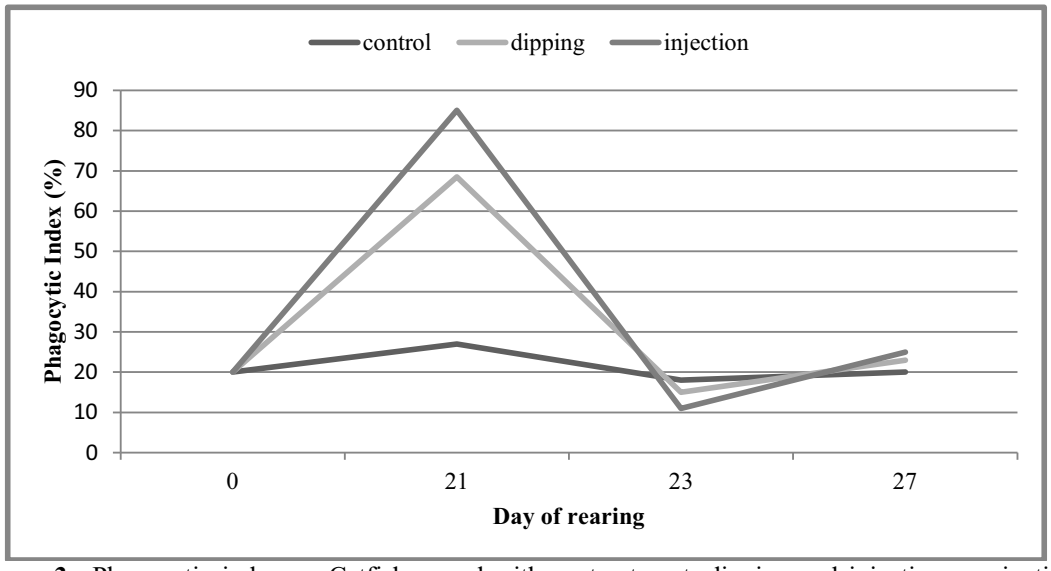

Figure 3. Phagocytic index on Catfish reared with no treatment, dipping and injection vaccination methods.

According to the Figure 3, on day 0 the average percentage of phagocytosis index is 20%. This is considered normal as the fish phagocytosis index is normally between 6.73 – 25% [6]. During the day 0 until 21st after vaccination, the phagocytosis index of all treatments increases. The highest increase is 65% on the injection treatment. The increase of the phagocytosis index shows that there is an enhancement on the fish immunity system. This is in accordance with Tizard (1998) who explained that the phagocytosis activity is the first defense of cellular response which is performed by monocytes (macrophages) and granulocytes (neutrophils). This is also in line with Amrullah (2004) who stated that the increase of immunity system can be seen from the increase of phagocytes cells activitiy on the blood.

On the 23rd day, the phagocytosis index of the dipping and injection treatments are decreased. This condition indicates that there is an infection of *Aeromonas hydrophila* and low production of antibodies.

On the 27th day, the infection of *Aeromonas hydrophila* begins to decline. This is showed by the increase of phagocytosis index of all treatments. The increase-decrease pattern of phagocytosis index is caused by the enhancement of the percentage on leukocyte especially on each limphocytes, monocytes, and neutrophils [1]. The highest phagocytosis index is 25%, found in the vaccination treatment using injection method. For injection treatment, there is an enhancement of phagocytosis index in the amount of 14%. It shows that giving vaccination can increase the catfish phagocytosis index after the challenge test by increasing the immunity components. The phagocytosis process helps the fish body to destroy the infection caused-bacteria in the fish body.
The catfish survival rate after vaccination and challenge test can be seen in the Figure 4.

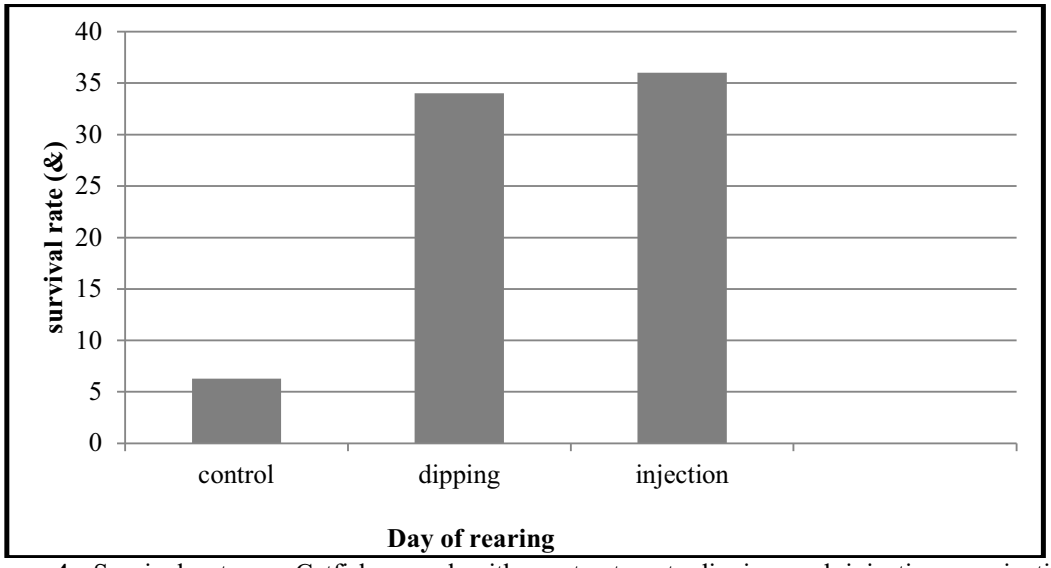

Figure 4. Survival rate on Catfish reared with no treatment, dipping and injection vaccination methods.

Based on the figure above, the survival rate after 27th day with injection method were higher than dipping and control. The survival rate with the injection method was 36%. It is caused by the vaccination using injection method allows the vaccine to enter the fish body 100%.

4. Conclusion

1. The result of vaccine administration using injection method showed a value of hematocrit 21.02%, total leukocytes of 3.15 x 104 cell/mm^3, and phagocytosis index of 25%.
2. On the other hand, the result of vaccine administration using dipping methods showed a value of hematocrit 15.05%, total leukocytes of 3.29 x 104 cells/mm3 and, and phagocytosis index of 23%.
3. After 7 days, the survival rate of catfish which is vaccinated using injection method is 36%, and using dipping method is 34%.

5. References

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