Haematological parameters of Catfish (Clarias sp.) fed by immunostimulant added with Cr+3- Yeast (Saccaromices cerevisiae) and Garlic

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Haematological parameters of Catfish (*Clarias* sp.) fed by immunostimulant added with Cr⁺³- Yeast (*Saccaromices cerevisiae*) and Garlic

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Abstract. A study on the effect of immunostimulant feed with additional Cr3+-Yeast (Saccharomyces cerevisiae) and Garlic on Haematological Parameters of Catfish (Clarias sp.) has been conducted. There were two types of immunostimulant, a commercial feed combined with Cr3+-Yeast and a feed in combination of commercial feed, Cr3+-yeast, and garlic. A 180 Catfish with an average of 8.79 g in weight and 11 cm in length were used in the study. Haematological parameters measured were haematocrit level, total leucocyte, phagocytic index, and leucocyte differential. Haematological parameters were observed at day 0, 7, 14 after immunostimulant application, and also one and 5th days after challenge test. Catfish fed with immunostimulant Cr3+-Yeast and garlic combination show higher but not significantly different (P>0.05) haematocrit level (20.5%) than those fed with Cr3+-Yeast added immunostimulant (20%). A significantly higher (P<0.05) total leucocyte on immunostimulant added with Cr3+-Yeast and Garlic (58.850 sel/mm3) than Cr3+-Yeast (41.075 sel/mm3) and only feed (23.650 sel/mm³). Phagocytic index were observed significantly higher (P<0.05) on Cr³+-Yeast-Garlic (22%) than Cr3+-Yeast (13%). Leucocyte differential were observed lower in monocyte (4%), higher in neutrophil (23%) and highest in lymphocyte (73%) on Cr3+-Yeast-Garlic combination compared to Cr3+-Yeast combination (14% monocyte, 70.5% lymphocyte and 15.5% neutrophil).

1. Introduction

Intensive catfish farming with high stocking densities often leads to MAS (Motile Aeromonas Septicamea) disease attacking catfish caused by bacterial infection Aeromonas hydrophila [1]. The ability of A. hydrophila to cause disease is quite high. The pathogenicity shown by LD₅₀ is quite varied, ranging from 104-106 cells/ml [2]. Prevention of MAS disease generally uses antibiotics, but the use of this material has a bad impact because it can cause residues in fish and will endanger the health of consumers if consumed [1] so other alternative materials such as the use of Cr^{+3} -yeast and garlic are needed.

Trivalent Chromium (Cr⁺³) is the most stable oxidation state and is thought to be the most important for the organism. As trivalent Chromium, the mineral is often bound to ligands that contain nitrogen, oxygen, or sulfur to form complex compounds Groff and Groper [3]. Cr⁺³ plays a role in the metabolic system of the fish's body, Cr⁺³ can increase the action of insulin through the essential Glucose Tolerance Factor (GTF) where Cr⁺³ will form a complex with insulin and receptors. According to NRC [3], animals whose impaired glucose tolerance shows GTF deficiency, and

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supplementation can increase glucose tolerance. Cr⁺³ can increase the deposition of body proteins for growth. Shiau and Lin [3] reported an increase in energy disposition, liver glycogen disposition and significant weight gain in tilapia caused by improved glucose utilization from rations containing 2 mg CrCl₃ / kg of feed. Also, Chromium-yeast can play a role in increasing the immunity response by increasing the activity of phagocytic cells, respiratory bursts and serum lysozyme [4]. Yeast generally contains microorganisms that carry out fermentation and culture media for these microorganisms. Microorganisms that exist in yeast generally consist of bacteria or fungi such as Saccharomyces, Lactobacillus, and Acetobacter [5]. Bread yeast (Saccharomyces cerevisiae) can increase the immunostimulant system and fish growth. The use of garlic as an additional immunostimulant material because garlic contains allicin which is an antibacterial compound.

Haematology is the study of blood cell components and functional abnormalities of these cells. Haematology is closely related to pathology, especially to obtain a picture of the health condition of fish whether in a healthy or sick condition. The blood will change in composition, especially if it is infected. Disturbances in the body of the fish are shown by changes in the blood picture, such as haematocrit values, haemoglobin concentrations, and blood cell counts. Espelid et al. [6] states that haematological changes in peripheral blood can be used as indicators of infection and stress conditions in fish; while Ellsaesser and Clem [7] examined the decrease in the number of lymphocytes that correlated with an increase in neutrophils after the catfish channel was injected with physiological doses of cortisol and then observed differences in the range of blood picture parameters in freshwater fish to determine their normal range so that it can be a guide in seeing the extent to which if a change occurred. Blood picture parameters studied were haematocrit, haemoglobin, total erythrocytes, total leukocytes, lymphocytes, monocytes and neutrophils. According to Bastiawan et al. [8] in fish affected by changes [8] the haematocrit value, Hb levels, the number of erythrocytes and the number of leukocytes. This study aims to determine the effect of Cr⁺³-yeast and garlic immunostimulant on catfish (*Clarias* sp.) through haematological parameters.

2. Materials and methods

The study was conducted at the Laboratory of the Center for Freshwater Aquaculture, Sukabumi, West Java. The materials used in this study were 180 catfish, with an average size of 11 cm and an average weight of 8.79 g, immunostimulants in the form of Cr⁺³-yeast-containing feg., garlic powder, *Aeromonas hydrophila* bacterial culture, bacterial culture *Staphylococcus aureus*, BHIA (Brain Heart Infusion Agar), BHIB (Brain Heart Infusion Broth), PBS (Phosphate Buffered Saline) solution, Na-Citrate 3.8% as anticoagulant, 70% alcohol, methanol, Giemsa, Turk's, clove oil, immersion, aqua dest, and crystoseal oils. The tools used during the study were haemocytometer, syringe, ose needle, petri dish, test tube, Erlenmeyer, micropipette, tube corning, microtube, microhaematocrit tube, centrifuge, incubator, spectrophotometer, microtiter plate, laminar flow, bunsen, object-glass, cover glass, microscope, autoclave, hot plate, spatula, magnetic stirrer, electric scale, digital scale, ruler. The cultivation containers used were as many as 6 aquariums, aer pressure and siphon hoses.

The method used was an experimental method using a Completely Randomized Design (CRD) with 3 treatments with 3 replications for each.

- A = Chromium-Yeast immunostimulant administration
- B = Chromium-Yeast immunostimulant administration and garlic powder
- C = without immunostimulant administration

Dosing in this treatment C with ordinary commercial pellets, a dose of Cr⁺³-yeast 2.5g per kg of feed, and B dose of Cr⁺³-yeast of 2.5 g and garlic powder 20 g/kg of feed. Giving immunostimulant is carried out for 14 days with time feeding 3 times a day and the dose of administration of ad libitum or as full. The 15th day was challenged by injecting *A. hydrophilla* into catfish (*Clarias* sp.). The challenge test was carried out by intraperitoneal injection of 0.1 ml/8.79 g with a concentration of bacteria. Blood analysis was carried out on the 0th day (before immunostimulant administration), 7th

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day (1 week after immunostimulant administration), 14th day (2 weeks after immunostimulant administration), the 16th day (one day after the challenge test), and the 19th day (4 days after the challenge test). Haematological parameters performed were the measurement of haematocrit levels, total number of leukocytes, differential leukocytes and recentage of phagocytic index.

After tested by Anderson Darling normality test, the data were normally distributed (P>>0.05). One-way ANOVA test alor with Tukey test for Post hoc test were used to find differences between data measured. Minitab ver. 16 was used for statistical analysis.

3. Results and discussion

3.1. Haematocrit levels

Haematocrit levels of normal catfish before being given by immunostimulants have a range between 20-22%. On the 14th day after immunostimulant administration, the highest haematocrit level was obtained in treatment B which was 23.5% where this value had increased 1.25% from the first week of immunostimulant administration. On the 15th day, the *Aeromonas hydrophilla* bacteria were tested on catfish (challenge test). The 16th day (one day after the challenge test) and the 19th day is the fourth day after the challenge test, the average graph of haematocrit levels shows almost the same pattern between all treatments, both treatment of test fish given immunostimulant and fish not given immunostimulant. The haematocrit levels between treatment A and B were not significantly different (More details can be seen in Figure 1)

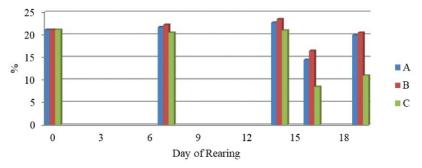


Figure 1. Haematocrit levels of *Clarias* sp. with treatment A(Cr³⁺-Yeast-Feed); B(Cr³⁺-Yeast-Garlic-Feed); and C (Feed)

On the 16th day (one day after the challenge test) the percentage of haematocrit levels decreased. According to Anderson [9], a decrease in the percentage of haematocrit levels indicates that fish get a pathogen infection. This is also reinforced by the opinion of Hedrick et al. [10] that the reduction in the percentage of haematocrit levels is caused by the number of infections. In addition to infection, decreased haematocrit levels are also triggered by the loss of appetite in catfish, decreased haematocrit levels can be used as a clue about the low content of feed protein, vitamin deficiency, or fish get infections so that appetite decreases [11]. An increase in haematocrit levels from the first day after the challenge test until the 3rd day after the challenge test showed that immunostimulants are able to improve the health status of fish. Brown [12] adds that body tissue cells depend on erythrocytes to obtain oxygen supply. With an increase in hematocrit levels indicates that the blood is in good condition and is able to bind oxygen well. According to Bond [13], the haematocrit value of teleost fish ranges between 20-30%. The increase in haematocrit levels again is a fish's immune response to the treatment of immunostimulants. However, there were not significantly different (between haematocrit on treatment A, B and C.

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3.2. Total leukocytes

Before giving immunostimulant (day 0) the average total leukocytes of normal catfish were 23,600 cells/mm³ with a range of total leukocyte values of 23,150 - 24,200 cells/mm³. The increase occurred every week of maintenance during immunostimulant administration in all treatments A, B and C. The highest total leukocytes obtained in treatment B was 71,075 cells/mm³ on day 14 (week 2) of immunostimulant administration. Decrease in average total leukocytes after a day after the *Aeromonas hydrophila* challenge test and seen an increase after the 3rd-day challenge test. The difference in average total leukocytes from each treatment before and after giving immunostimulant and post-challenge test can be seen in Figure 2.

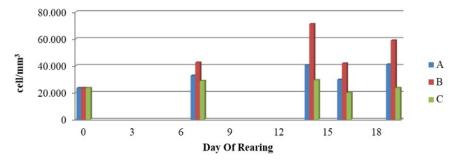


Figure 2. Average of Total Leucocyte (cells/mm³) of *Clarias* sp. with treatments; A. 2.5 g Cr³+Yeast/kg feed; B. 2.5 g Cr³+Yeast + 20 g Garlic powder/kg feed and C. regular feed without immunostimulant.

The total leukocytes in treatments A and B after being given immunostimulant until day 14 (week 2) experienced a higher increase than treatment C. Post-day test challenge *Aeromonas hydrophila* bacteria showed a decrease in total leukocytes in all treatments. These total leukocytes experienced an average decrease of 19,425 – 29,275 cells/mm³ of total leukocytes during the second week of maintenance in all treatments. Total leukocytes began to increase again on the 3rd day after infection where the highest value was shown in treatment B which was 58,850 cells/mm³ and the lowest value was shown in treatment C which was 23,650 cells / mm³.

The total number of leukocytes during immunostimulant administration tends to increase and peaks on the 14th day before the challenge test. The highest total number of leukocytes was shown in treatment B of 71,075 cells/mm3. The increase in the total number of leukocytes is due to the process of giving immunostimulants. This is related to the function of white blood cells as a defence tool. This condition shows that the administration of immunostimulants can increase cellular defence responses in the form of increased total leukocytes. Anderson (1992) in [14] stated that leukocytes are a component of blood cells that function as non-specific defences that will localize and eliminate pathogens.

A decrease in the number of leukocytes caused by leukocytes in the blood vessels is greatly reduced (decreased) because most leukocytes move towards infected tissues. A decrease in the number of leukocytes after the infection is caused by the active leukocytes coming out of the blood vessels into infected tissue [15]. This is a response of fish to recognize and recall the types of pathogens that entage.

Increasing the number of leukocytes indicates that the fish responded to the presence of foreign objects that enter the body. An increase in leukocyte cells is a reflection of the success of the fish's immune system in developing a cellular (non-specific) immune response as a trigger for an immune response [16]. Fish response to increase their endurance by increasing the number of leukocytes that have a function as a defence cell [17].

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3.3. Differential leukocytes

3.3.1. Cr^{+3} -Yeast Treatment

Leukocyte differential value taken was the average proportion of three types of leukocytes, namely lymphocytes, monocytes, and neutrophils. The profile of catfish blood profile is presented in Figure 3.

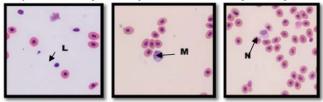


Figure 3. Lymphocyte (L), Monocyte (M) and Neutrophil (N) of Clarias sp.

Lymphocyte, Monocyte, and Neutrophil percentages were increased in each week on *Clarias* sp. fed with Cr+3-yeast-immunostimulant feed. Lymphocyte percentage on catfish blood has increased by 10% on the 14th day to become 73%, compared to the 0th day (before Cr⁺³-yeast-immunostimulant administration) where this value was the highest lymphocyte percentages. This body's defence mechanism occurs as an immune responses in catfish that triggered by immunostimulants. The percentage of monocytes has found relatively low, this occurred as a response to the haematological balance on leukocytes cell proportion, such as lymphocytes and neutrophils proportion. The percentage of lymphocytes and neutrophils increased after administrated by immunostimulants feed. An increase of lymphocyte cells was assumed that it activated by direct immunostimulant administration [17]. The number of neutrophils in the blood plasma was also shown a relatively low amount compared to the number of lymphocytes. The small number of neutrophil cells was assumed due to it difficulties to absorb dye, so it was very rarely found in fish [18]. The average percentage of lymphocytes, monocytes, and neutrophils from Cr⁺³-yeast treatment can be seen in Figure 4.

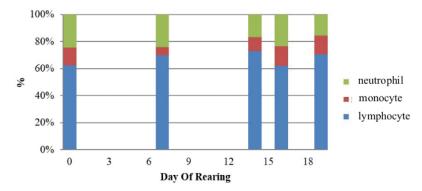
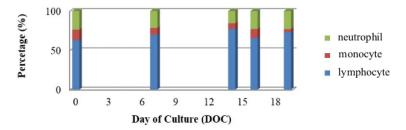


Figure 4. Lymphocyte, Monocyte and Neutrophil percentages of Catfish fed by 2.5g Cr⁺³-Yeast/kg feed (Treatment A).

Decreasing number of lymphocytes on the blood was observed on the day after the challenge test. It assumed due to the antibodies formed from lymphocyte cell migrated to infected area and attack the *Aeromonas hydrophila* as a resistance activity. The increased resistance activity caused a reduction in lymphocyte cells. This assumption was mentioned on the previous study, that the recognition of the

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intensity of infection by certain pathogens will trigger the need for white blood cells (lymphocytes) and an increase in these needs will result in a reduction in the body's agent providing immune cells, namely lymphocytes [19].



 $\label{eq:Figure 5.} \begin{tabular}{l}{\bf Figure 5. Lymphocyte} & , Monocyte and Neutrophil percentages of Catfish fed by $2.5g Cr^{+3}-Yeast+20g Garlic/kg feed (Treatment B)$\\ \end{tabular}$

A decrease of lymphocytes during the day after the challenge test due to an infection from the *Aeromonas hydrophila* bacteria. A decrease in the number of lymphocytes in the peripheral blood occurs because most lymphocytes are withdrawn from the circulatory system and compete into the tissue where inflammation is present [20].

The percentage of monocytes increased due to fish having *Aeromonas hydrophila* infection. Infection that enters the body will stimulate white blood cells to produce more monocytes. A similar statement to [21], that in the event of infection by a pathogen, the monocytes will move quickly leaving the infected blood vessels to do phagocytosis. Monocytes have the ability to penetrate capillary cell walls, then enter the tissue and differentiate into macrophages.

The increase in the number of neutrophils after the challenge test is the result of an immune mechanism that works in response to an infection in the body. This is related to the main function of neutrophils, namely the destruction of foreign material through the phagocytosis process, namely chemotaxis in which cells migrate to particles, adhesion of particles to cells, ingestion of particles by cells, and destruction of particles by the enzyme lysozyme in phagolysosomes [22].

3.3.2. Regular Pellet Treatment

The highest lymphocyte percentage is 68%, 3 days after the challenge test. The percentage of monocytes and neutrophils counterbalances the percentage of lymphocytes. The lymphocyte percentage increases during feeding and also decreases the day after the challenge test. Increasing the percentage of lymphocytes when feeding is not so optimal compared with the percentage of lymphocytes given catfish immunostimulants. The difference in the average percentage of lymphocytes, monocytes, and neutrophils from ordinary pellet feed treatment can be seen in Figure 6.

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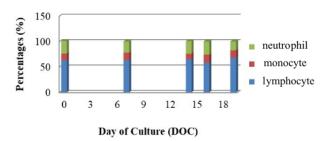


Figure 6. Lymphocyte, Monocyte and Neutrophil percentages of Catfish fed by regular feed (Treatment "C")

Based on Figure 6, the 16th day is the day after the challenge test and the 19th day is the third day after the challenge test, the treatment of ordinary pellet feed has a lower number of lymphocytes during maintenance, resulting in a lower survival rate. This is consistent with the opinion of Fujaya [12] that lymphocyte deficiency 10 reduce the concentration of antibodies and cause increased disease attacks. The percentage of the number of monocytes and neutrophils balances with the number of lymphocytes. The number of lymphocytes is not too high when compared to other treatments. The highest monocyte count is 16% on the day after the challenge test because at that time the number of lymphocytes decreased due to bacterial infection of *Aeromonas hydrophila*.

3.4. Phagocytosis index

Before giving immunostimulant (Day 0) the average percentage of the phagocytic index of normal catfish was 8.6% with the lowest range of 7% and the highest value of 10%. The increase that occurs every week during immunostimulant administration in all treatments A, B, and C. The average percentage of the highest phagocytosis index obtained in treatment B is 23.5% on the 14th day (week 2) immunostimulant administration where this value has a 3-fold increase compared to before giving immunostimulants. A decrease in the average percentage of the phagocytosis index occurred 12 or the day after the challenge test and was seen to increase again on the 3rd day after the challenge test. The difference in the average percentage of phagocytic index in catfish from each treatment before and after administration of immunostimulants and after the challenge test can be seen in Figure 7.

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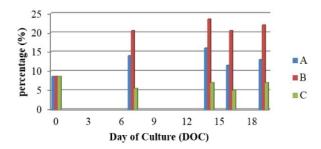


Figure 7. Phagocytic index percentages of Catfish with Treatment A $(2.5 \text{ g Cr}^{3+}\text{-Yeast/kg feed})$; B $(2.5 \text{ g Cr}^{3+}\text{-Yeast} + 20 \text{ g garlic/kg feed})$; and C (regular feed).

Based on Figure 7, the 16th day is the day after the challenge test and the 19th day is the third day after the challenge test, the test fish given immunostimulants appear to have increased phagocytosis index higher than treatment C. According to Tizard [22], phagocytic activity is the first defense of cellular response that is carried out by monocytes (macrophages) and granulocytes (neutrophils). Amrullah [23] added that increased fish immunity can be known and increased phagocyte cell activity from the blood.

3.5. Survival rate

The survival rate was observed after the challenge test, the observation was carried out for 5 days after the challenge test. Treatment C has the lowest survival value compared to all treatments. This is presumably because in C treatment the test fish only relied on their immune system due to the absence of additional Cr⁺³-yeast immunostimulants and garlic powder in the feed given for 14 days before infection causing the bacteria *A. hydrophila* to develop and cause death. In contrast to the treatment of the addition of Cr⁺³-yeast immunostimulant and garlic powder in treatment, B produced the highest survival rate. The survival of catfish can be seen in Figure 8.

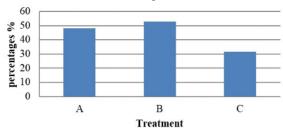


Figure 8. Average Survival Rate of Catfish with Treatment A (2.5 g Cr³⁺-Yeast/kg feed); B (2.5 g Cr³⁺-Yeast + 20 g garlic/kg feed); and C (regular feed).

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The high survival rate is thought to be the presence of yeast Chromium (Cr⁺³) capable of increasing the immune response by increasing the activity of phagocytic cells, respiratory bursts and serum lysozyme [4] and garlic powder containing allicyn which is an antibacterial compound.

4. Conclusion

The conclusion that can be drawn is the administration of Cr⁺³-yeast immunostimulant and garlic powder to catfish (*Clarias* sp.) Able to improve the fish's immune system better indicated by the percentage of haematocrit levels, the total number of leukocytes, the percentage of differential leukocytes, and the percentage of phagocytosis index. A good immunity response is also shown from the percentage survival rate of catfish which is 53%.

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