The Effect Of Hermetia Illucens Larvae As Immunostimulant To Non-Specific Immune Response And Histopathology Of Gills Of Common Carp (Cyprinus Carpio) Challenged By Aeromonas Hydrophila Bacteria

Maftuch, Febi Nadhila Nurin

Abstract: This study examines Hermetia illucens Larvae as immunostimulant in common carp infected with A.hydrophila bacteria as seen from total leukocytes, differential leukocytes, total erythrocytes, hemoglobin, hematocrit, phagocytosis activity, and gill histopathology. Oral administration of immunostimulants ie with H. illucens larvae which has been mashed into powder is subsidized into commercial feed for 30 days in a controlled aquarium with a dose of H. illucens larvae A (4%), B (6%), C (8%) %), and D (10%). The results of this study indicate that at dose C (8%) which provides the best immune system improvement seen from the lowest total leukocytes, differential leukocyte results (lymphocytes, monocytes, neutrophils) indicate that at treatment C (8%) each has the lowest value, highest total erythrocytes, hemoglobin, and hematocrit. In gill histopathology test, it was found that common carp gill damage infected by A. hydrophila bacteria after being given immunostimulant H. illucens larvae for 30 days, namely edema, hypertrophy, and necrosis. The lowest mean score of scoring gills damage was also shown at dose C (8%)

Index Terms: C. carpio, A. hydrophila, H. illucens larvae, immunostimulant

1. INTRODUCTION

COmmon carp (C. carpio) is one type of freshwater fish that has a bright prospect to be cultivated and is one type of freshwater fish that has high economic value. Besides common carp is one of the leading commodities of freshwater fisheries because most of the Indonesian people are fond of these fish. The increasing demand for fishery products for domestic and export needs has now placed the fisheries sector in an important position. Carp production also plays an important role in improving the fisheries sector in Indonesia. Likewise, according to Wang et al., (2015) that intensive maintenance of carp in aquaculture produces environmental stress that leads to high susceptibility to various disease agents, such as viruses, bacteria, fungi and parasites. Therefore, various infectious diseases can result in economic losses in cultivation. Several cases of fish outbreaks that occurred in the past have caused no small losses. In 1980 there was an attack of A. hydrophila bacteria in common carp farming in Indonesia. In overcoming problems caused by pathogenic agent attacks on fish, fish farmers and entrepreneurs use a variety of chemicals and antibiotics in controlling the disease.

- *Febi Nadhila Nurin is a master degree at Faculty of Fisheries and Marine Science, University of Brawijaya, East JavaMalang, Indonesia.*
- *Corresponding Author: Maftuch is Professor at Faculty of Fisheries and Marine Science, University of Brawijaya,Indonesia. Email: maftuch@ub.ac.id*

But on the other hand the use of chemicals and antibiotics continuously with the dose/concentration that is not right, will cause new problems in the form of increased microorganism resistance to these materials. In addition, another problem is the danger posed to the surrounding environment, the fish concerned and humans who consume them(1). The use of antibiotics and chemotherapy for prophylaxis and treatment in intensive cultivation has been widely criticized for its negative effects (2) Therefore, the use of immunostimulants is being routinely introduced to fish farmers according to procedures as prophylactic measurements. So far this ingredient has not shown any negative side effects compared to antibiotics. Immunostimulants can increase resistance to infectious diseases by increasing non-specific defense mechanisms. The main components of the innate immune system are macrophages, monocytes, granulocytes, lysozyme, blood cells especially leukocytes. Immunostimulants are usually identified by their ability to activate leukocytes. The use of immunostimulants as dietary supplements can increase fish's innate defenses in resistance to pathogens (3). Therefore it is necessary to look for environmentally friendly measures against diseases to ensure the sustainability of the cultivation. One of the natural ingredients that is environmentally friendly and can be used to improve the fish's immune response is H. illucens larvae. One of the ingredients produced by H. illucens larvae to maintain its life has an antimicrobial effect.(4). Until now, H. illucens larvae therapy has been known as an effective method for the treatment of chronic, necrosis, and wound infections, as well as for healing burns in humans (5). In addition, this study shows not only the therapeutic effect of H. illucens larvae both "in vitro" and "in vivo" but also the various biological functions and effects of H. illucens larvae. The effect of active substances/compounds isolated from H. illucens larvae and potential pharmacological functions has not been reported in various bacteria, cells, and animals. In

a previous study, it was reported that H. illucens larvae extract contained hexanedioic acid which had antibacterial activity against gram-negative bacteria (6). Therefore in this study I want to find out how much influence H. illucens larvae has when it is made as an immunostimulant where in previous studies H. illucens larvae was used as an antibacterial, there is no research that uses H. illucens larvae as an immunostimulant in fish

2 MATERIAL AND METHOD

The research was conducted in October-December 2017. The implementation of the proximate test, conducted at the Processing of Fishery Products Laboratory, Faculty of Fisheries and Marine Science, UB, immune testing of common carp, conducted at the Disease and Health Fish Laboratory, UB.

H. illucens Larvae

H. illucens Larvae used in this research is the 6th instar H. illucens larvae purchased from a H. illucens larvae cultivator in Slipi, West Jakarta. H. illucens larvae cleaned and then preserved / turned off in the freezer, then H. illucens larvae is dried in the open and protected from sunlight to dry. The dried H. illucens larvae is then pulverized with a blender or grinding machine to obtain finer powder (7)

LD_{50} A. hydrophila

Bacteria of A. hydrophila were obtained from the Fish Quarantine Center of Class I Surabaya I in East Java with the results of the 2.726x109 cell / ml spectrophotometer. LD (Lethal Dose) test is carried out with bacterial density10^6 , 10^7, 10^8 sel/ml.

H. illucens larvae as Imunostimulan

H. illucens larvae simplisia is subsidized on mashed commercial feed. The dosage of H. illucens larvae substitution is determined from previous studies that conducted research using H. illucens larvae as feed substitution with the results of 8.6% of commercial feed which is the optimal administration with growth parameters (7), so that in this study using a dose of 4%, 6%, 8%, and 10% of commercial feed.

Common carp

The fish used in this study were ± 7-9 cm common carp purchased in Pasuruan, Malang, East Java. Common carp are kept in an aquarium (30x30x30 cm) with aeration system. Common carp reared for 30 days by giving immunostimulant feed 2 times a day as much as 5% of the weight of the fish.

Total of Leukocytes

The total leukocyte method is: taken blood of fish which has been added anticoagulant as much as 0.5 µl using a leukocyte pipette. Then diluted with turk solution in a leukocyte pipette up to 11 µl. Blood that has been mixed with turk solution is homogenized. The first two drops are removed from the mixture, the third drop is placed on a haemocytometer box then covered with a glass cover and observed using a light microscope by counting the total leukocytes in all leukocyte boxes. (8), the calculation of total leukocytes namely (9):

$$SDP = (A/N) \times (1/V) \times Fp$$
$$= (A/4) \times \{1 / (1x1x0.1)\} \times 20$$
$$= A / 0.4 \times 20$$
$$= A \times 50$$

Which is :
SDP = total of leukocytes
A = number of leukocytes counted
N = number of haemocytometer boxes observed
V = haemocytometer box volume observed
Fp = dilution factor

Total Erythrocytes

The amount of erythrocytes is calculated in a certain volume using a conversion factor. The calculation starts with filling the erythrocyte pipette ie blood is sucked up to the 0.5 mark line and diluent solution (hayem solution) to the 101 line. Removing the aspirator tube then homogenized 5-10 minutes to ensure proper mixing. Discard the first 3-4 drops, then place it in the counting booth. The filled calculating booth is placed on a microscope and the reading behavior is enlarged (40x)

$$\text{Erythrocytes} = (A/N) \times (1/V) \times Fp \quad (9)$$

A = ∑ cell counted
V = haemocytometer field volume
N = ∑ observed haemocytometer field
Fp = dilution factor

Hemoglobin

Measurement of hemoglobin (Hb) levels is done by the Sahli method which converts blood into hematin acid after the blood is added to HCl. First the blood is sucked with a sahli pipette up to a scale of 20 mm3 or on a scale of 0.02 ml, then the blood is transferred into a Hb-meter filled with 0.1 N HCl to a scale of 10, stirring and allowed to stand for 3-5 minutes. After that the distilled water is added to the blood and HCl color as the color of the standard solution in the Hb meter. The scale is read by looking at the surface of the liquid and is matched with the scale of the sahli tube seen on the gr% (yellow) pathway scale which means the amount of hemoglobin in grams per 100 ml of blood (10).

Hematocrit

Blood is sucked with a microhematocrit tube until it reaches ¾ of the tube. Then the end of the tube is closed with crytoseal as deep as 1 mm, so that the crytoseal plug is formed. Furthermore, the microhematocrit tube is centrifuged with a speed of 5000 rpm for 5 minutes with the position of the tubes having the same volume facing each other so that the centrifuge rotation is balanced. The value of hematocrit levels is determined by the percentage of the length of the blood that settles (a) and the total length of blood volume contained in the tube (b). So the level of hematocrit = (a / b) x 100% (10)

Activity of Phagocytosis

Fish blood samples that will be used to phagocytose bacteria are prepared in the following manner, blood samples with EDTA coagulant (1:10) are centrifuged at a speed of 3000 rpm for 3 minutes. The supernatant part is removed which is plasma using a micropipette. The middle part which is a bafiquat containing polymorphonuclear cells

(PMN) is taken with a 50 µl micropipette and placed in a microplate. 50 µl of fish blood is mixed with 50 µl of bacterial cell suspension and both are shaken slowly for 1 minute to ensure contact between bacteria and leukocyte cells. Mixture of fish blood with bacterial suspension is incubated at room temperature for 20 minutes. 5µl of the mixture is placed on an object glass to make thin blood film preparations. Furthermore, it is dried aerated at room temperature and after that it is fixed with methanol for 5 minutes and dried again. The preparations are stained with safranin 1% for 30 minutes, then rinsed with distilled water to clean. The number of bacterial cells ingested/phagocytes by fish leukocyte cells was observed using a 1000x magnification microscope (11). The function of macrophage phagocytosis is calculated by:

PA = (Σ macrophages that ingest bacteria/100 macrophages) ×100% (12)

Gills Histopathology

The preparation of gill histopathology preparations was carried out according to Susanto (2008) (13). Percentage of damage in each field of view is calculated based on the number of cells damaged by formula (14):

$$\text{Percentage of Damage} = \frac{\text{Total of cells damage}}{\text{Total of celsl analyzed}} \times 100\%$$

***Tabel 1**. Precentage scoring value (14).*

Scoring Value	Precentage of damage (%)
1	0-5%
2	6-25%
3	26-50%
4	>50%

Data Analysis

Data analysis was performed by analysis of variance ANOVA (SPSS version 16).

3 RESULT AND DISCUSSION

LD_{50} A. hydrophila Bacteria

LD_{50} test was carried out with a bacterial density of 10^6 cells/ml, 10^7 cells/ml, and 10^8 cells/ml, it was found that LD_{50} by infecting bacteria 10^7 cells/ml at the 8th hour of death was 50%. So that the density used for bacterial infection in the study is 10^7 cells/ml.

Total of Leukocytes

***Tabel 2.** Total leukocytes before being infected by the bacterium A. hydrophila*

Doses of H. illucens Larvae	Before Infected (10^4)	After Infected (10^4)
4%	$7,73±0.19^{ab}$	$15,87±0.07^{d}$
8%	$7,55±0.12^{a}$	$15,25±0.52^{d}$
6%	$7,86±0.06^{b}$	$13,10±0.02^{b}$
10%	$7,82±0.10^{ab}$	$14,25±0.01^{c}$
K+	$7,85±0.09^{b}$	$17,33±0.09^{a}$
K-	$7,83±0.07^{ab}$	$8,43±0.11^{e}$

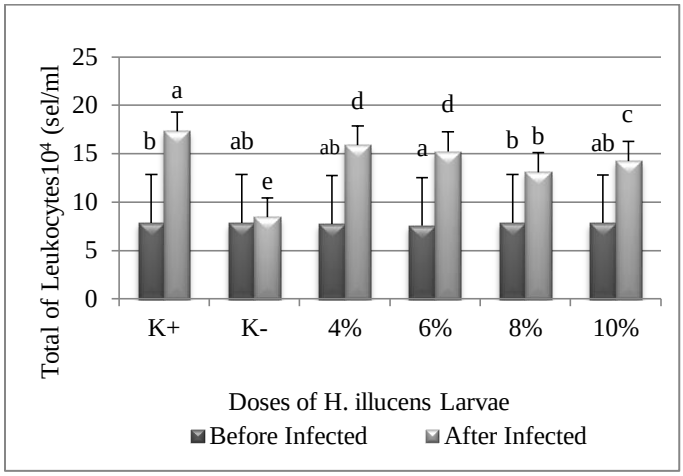


***Fig 1.** Graph of total leukocytes before and after bacterial infection of A. hydrophila*

In the picture above, it can be seen that the total leukocytes before being infected by the bacterium A. hydrophila showed no significant difference between treatments, this is because common carp have not been challenged with bacteria so that the total leukocytes are still stable between treatments. Leukocytes are blood cells that can form the immune system because it plays a role in fighting various infectious diseases and foreign bodies, so the more the number of leukocytes the more infectious diseases and foreign objects are resisted (15). Thus the absence of differences in the number of leukocytes means there is no difference in conditions (differences in resistance to foreign objects) on the body of the common carp. In the graph above it is also obtained that the total leukocytes after infection in all treatments have increased from before being infected that leukocyte fish Carassius auratus increased the number of leukocytes after being infected by A. hydrophyla (16). The total yield of leukocytes in all treatments is lower than the positive control and higher than the total yield of leukocytes in the negative control, it is because the immunostimulant given can work well, the specific purpose of giving immunostimulant is to obtain resistance to an certain infections, so that a high survival rate is obtained due to the immunological protection (17). In general, the benefits of immunostimulant include the increase in fish endurance, protection against certain infectious diseases, safety of aquaculture environment from the contamination of chemotherapeutic materials and consumer safety from antibiotic residues.

Diferential of Leukocytes

***Tabel 3.** Average of Lymphocytes*

Doses of H. illucens Larvae	Before Infected (%)	After Infected (%)
K+	$78.33±009^{a}$	$87.00±1.00^{b}$
K-	$72.00±0.11^{a}$	$75.67±0.57^{a}$
4%	$74.00±0.07^{a}$	$85.67±0.57^{b}$
8%	$75.67±0.52^{a}$	$83.33±1.52^{ab}$
6%	$75.33±0.33^{a}$	$81.67±0.57^{ab}$
10%	$75.00±0.57^{a}$	$82.33±0.57^{ab}$

***Tabel 4**. Average of Monocytes*

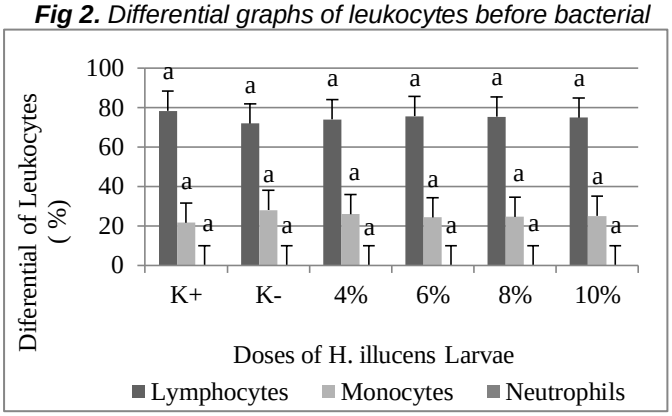
Doses of H. illucens Larvae	Before Infected (%)	After Infected (%)
K+	$21.67±2.64^{a}$	$13.00±0.22^{a}$

K-	28.00±1.52[a]	24.33±0.27[d]
4%	26.00±2.51[a]	14.33±0.47[ab]
8%	24.3±2.08[a]	16.67±0.90[bc]
6%	24.67±5.68[a]	18.33±0.30[b]
10%	21.67±2.08[a]	13.00±0.22[a]

***Tabel 5**. Average of Neutrophils*

Doses of H. illucens Larvae	Before Infected (%)	After Infected (%)
K+	0[a]	2.33±1.52[a]
K-	0[a]	0.00±2.51[a]
4%	0[a]	1.00±1.52[a]
8%	0[a]	0.67±0.57[a]
6%	0[a]	0.3±0.57[a]
10%	0[a]	0.33±0.57[a]

***Fig 2.** Differential graphs of leukocytes before bacterial infection of A. hydrophila*



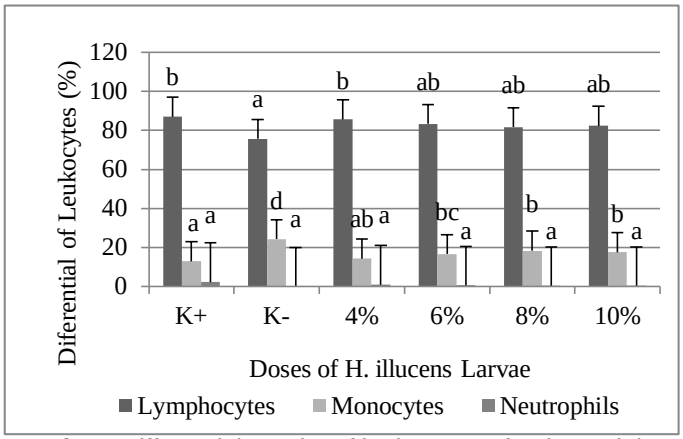


***Fig 3.** Differential graphs of leukocytes after bacterial infection of A. hydrophila*

From the table above we get the differential results of leukocytes where the number of lymphocytes, monocytes, and neutrophils is very different. Lymphocytes tend to be much higher compared to monocytes and neutrophils, even before infection in all treatments did not get neutrophils. The main function of leukocytes in general is the immune system, but there are different mechanisms in each leukocyte fraction. Changes in the number of leukocytes in the blood circulation can be interpreted as the emergence of agents of disease, inflammation, autoimmune diseases or allergic reactions. The percentage of heterophils will increase when there is a bacterial infectious disease in the body (18). Lymphocytes act to respond to antigens (foreign bodies) by forming antibodies and developing immunity (19). Therefore differential leukocyte results show different results between lymphocytes, monocytes, and neutrophils due to the onset of C. carpio disease so that these types of leukocytes work in accordance with their respective duties.

Total of Erythrocytes

***Tabel 6**. Total of Erythrocytes*

Doses of H. illucens Larvae	Before infected (10^5)	After Infected (10^5)
4%	2,74±0.11[a]	1,87±0.23[b]
6%	2,92±0.09[a]	2,08±0.19[b]
8%	2,79±0.55[a]	3,15±0.27[c]
10%	2,89±0.03[a]	2,16±0.06[b]
K+	3,11±0.41[a]	1,14±0.02[a]
K-	2,8±0.44[a]	3,37±0.21[c]

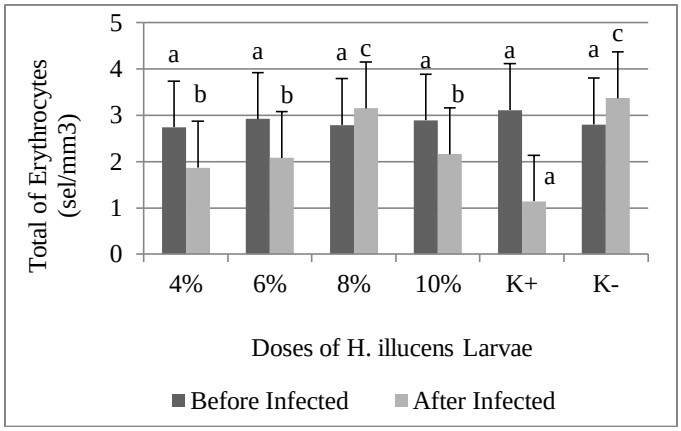


***Fig 4.** Total erythrocytes before and after bacterial infection of A. hydrophila*

From the table and graph above it can be seen that the total erythrocytes before bacterial infection of A. hydrophila are not significantly different between treatments, this is because before infection the condition of fish tends to be the same condition, but after infection with bacteria A. hydrophila shows that the highest erythrocytes in the treatment C (8%) is also close to the total negative erythrocyte control (K-), but the lowest total erythrocyte is in treatment A (4%) which is also closer to the total erythrocyte in the positive control (K +) treatment. This is because the dose of 8% immunostimulant given is able to fight bacteria so that the resulting leukocytes are only a few and make the total erythrocytes become more. The amount of erythrocyte content in the blood will increase along with the increase in the dose of infection in fish, this is due to the fish experiencing stress will have a higher erythocyte content.(20)

Hemoglobin and Hematocrite

***Tabel 7.** Precentage of Hemoglobin and Hematocrit*

Doses of H. illucens Larvae	Hemoglobin (gr%)	Hematocrit (%)
4%	3,3±1,15[a]	2,36±0,82[d]
6%	3,6±0.5[b]	3,18±0,91[b]
8%	4,06±2,13[bc]	4,72±0,07[bc]
10%	3,85±1,35[bc]	4,08±1,56[bc]
K+	2,4±1,25[a]	43,54±1,00[d]

K-	5,23±2,43[c]	4,86±0,04[c]

From the table above it can be seen that the results of the study indicate that A. hydrophila infection affects the hematocrit and hemoglobin values in fish blood, along with the levels of treatment of different infections. Data shows that all treatments infected with A. hydrophila have increased hematocrit, depending on the stress level of the fish. While the amount of hematocrit <30% is the depreciation of erythrocytes, therefore the results of the hematocrit test will correlate with the results of the erythrocyte test. The results showed that A. hydrophila infection affected the hematocrit and hemoglobin values in fish blood, along with the levels of treatment at different infection doses. The highest hemoglobin and hematocrit in treatment C (8%) which also approached the results of negative control and the lowest in treatment A (4%) which also approached the results of positive control. This shows that at low immunostimulant doses, bacteria will grow more and more especially in the gills, which results in lower hemoglobin in the blood. This is because the lower hemoglobin in fish, it means that the gills are disturbed, or damaged (20)

***Tabel 8**. Activity of Phagocytosis*

Doses of H. illucens Larvae	Before Infected Activity of Phagocytosis (%)	After Infected Activity of Phagocytosis (%)
4%	28,16±30,47[a]	38,41±2,08[d]
6%	26,80±15,11[a]	36,18±0,91[c]
8%	25,78±5,37[a]	29,52±1,00[b]
10%	26,80±1,64[a]	31,30±1,00[c]
K+	21,81±15,82[a]	43,54±1,00[d]
K-	21,89±13,14[a]	22,91±1,00[a]

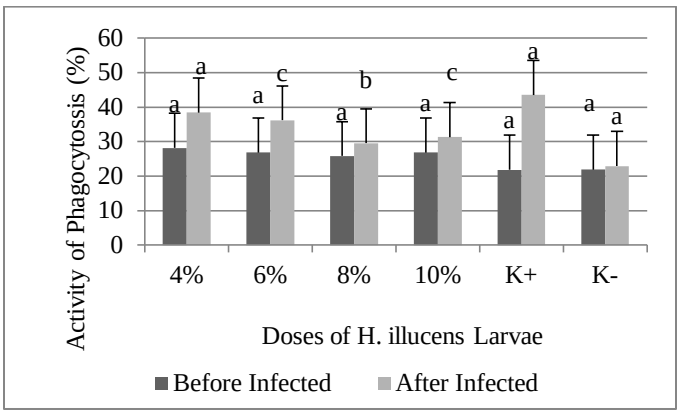


***Fig 5**. Activity of Phagocytosis*

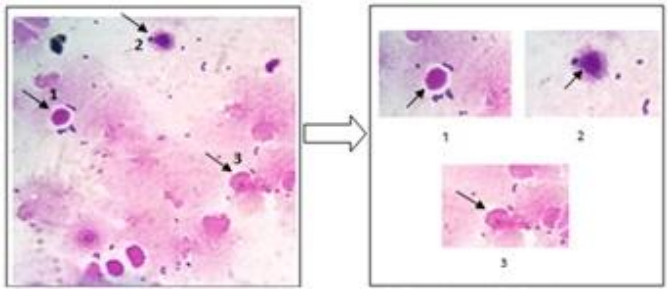


***Fig 6**. Phagocytic activity, 1. Sticking, 2. Ingestion, 3. Destruction*

From the picture above , the results of phagocytic activity before infected by A. hydrophila bacteria were not significantly different between treatments, but the results of phagocytic activity after being infected by A. hydrophila bacteria increased from before being infected, and the results of phagocytic activity in all treatments were lower compared to positive controls and more high compared with negative control, this is because there is a foreign object, namely A. hydrophila bacteria which is attacking the body of common carp so that phagocytosis activity occurs to ingest the incoming bacteria, in treatment C (8%) with b notation which is significantly different from treatment A, B, and D have the result of phagocytic activity which is the closest to negative control. It is suspected that at that dose the content of the compound contained in H. illucens larvae has an appropriate portion to inhibit incoming foreign objects, immunosuppressive material can affect the phagos system it through suppression of the myelopoiesis system will reduce the formation of oxidants to kill the incoming antigen (21) The increase in phagocytic activity caused by macrophages caused by direct interaction with activating agents such as microorganisms, can also be activated by lymphocyte products (lymphokines) which are stimulated by antigens/alkaloids active compounds (compounds that are also contained in H. illucens larvae (22). Once macrophage cells are activated, they will show their metabolite activity and increase in function, namely to phagocytosis and kill germs and process these germs. Phagocytosis is carried out by approaching foreign particles and releasing pseudopods in all directions around the particles (external chemical stimulation). Teleostei phagocyte cells have the ability to produce RNS and the process takes place through an induction process that produces nitrogen oxides (NO) and has the opportunity to produce nitrogen dioxide (NO2).), Nitrogen trioxide (NO3) and nitronium ions (NO) +2. In fish the killing mechanism in phagocytes through free oxygen in lysosomic vacuoles which can increase the permeability of bacterial cell membranes (12).Alkaloids and flavonoids play a role in increasing the immune response by increasing the activity of IL-2 (interleukin 2) and lymphocyte proliferation. Activated Th1 (T helper 1) cells will affect SMAF (Specific Macrofag Arming Factor), ie molecules including IFNγ (interferon gamma) that can activate macrophages. If there are antigens that enter the body, for example bacteria, then T lymphocytes and macrophages work together to kill the bacteria. Macrophages will phagocyte bacteria and T lymphocytes differentiate into CD4 + and CD8 +. CD4 + cells differentiate into Th1 which then produces IFNγ and TNFα cytokines and stimulates Natural Killer cells. CD8 + cells also produce IFN cytokines.

The cytokines will activate macrophages to produce compounds, one of which is nitric oxide which is useful to kill bacteria. This bacterium is able to induce cellular immune responses carried out by macrophages by the T-cell mediated immunity process (23)

Gills Hystophatology

Based on the results of the study, a picture of common carp gill tissue given H. illucens larvae immunostimulant for 30 days with different doses can be seen in the picture with a magnification of 400x. Where is the condition of common carp (C. carpio) gills after being infected by the bacterium A. hydrophila showing a different histopathological form in each preparation

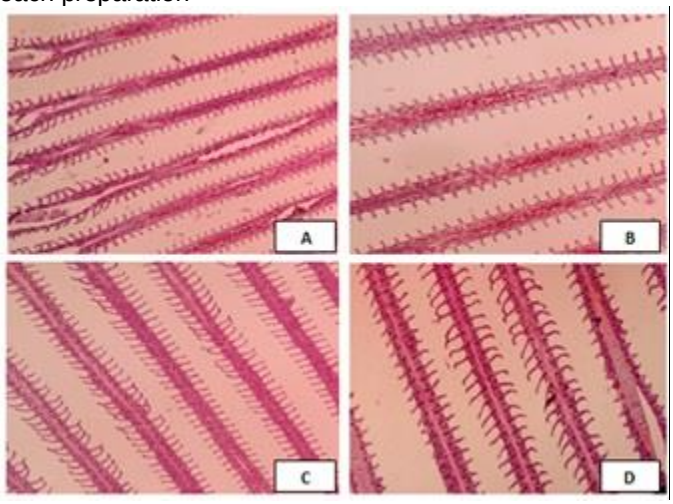


***Fig 7**. Histopalogy Overview of Common carp Gills (K-) negative control: (LP) Primary Lamella, (LS) secondary Lamella. (K +) positive control: ED (Edema), (N) Necrosis, (H) Hypertrophy. Figure A, B, C, D changes in the histopathological picture of carp gills in treatments A (4%), B (6%), C (8%), and D (10%).*

In gill histopathology test, it was found damage to carp gills's, namely edema, hypertrophy, and necrosis. Edema (swelling) is a part that is filled with fluid so that the part is enlarged and cannot function properly (24). Hypertrophy which continues to develop in lamella causes secondary lamella to join. Such an adaptation response to the lamella epithelium is a defense mechanism that can increase the distance between the blood and the external environment. These changes are a barrier to the entry of contaminants (25). Necrosis describes a condition where a decrease in tissue activity is characterized by the loss of several parts of the cell one by one from one tissue so that in a short time will die, histological necrosis is characterized by the appearance of boundaries cells and cell nuclei are unclear or even disappear (26). Each scoring average of gills damage can be seen in the following table:

***Tabel 9**. Results of common carp gill scoring infected by A. hydrophila bacteria*

Doses of H. illucens Larvae	Edema	Hipertrophy	Necrosis
4%	2.87 ± 0.41^{bc}	2.93 ± 0.64^{c}	2.8 ± 0.87^{bc}
6%	2.6 ± 0.40^{b}	2.73 ± 0.41^{bc}	2.67 ± 0.41^{bc}
8%	1.53 ± 0.20^{a}	1.67 ± 0.41^{ab}	1.4 ± 0.34^{a}
10%	1.73 ± 0.11^{a}	1.8 ± 0.40^{ab}	1.6 ± 0.34^{ab}
K+	3.53 ± 0.30^{c}	3.53 ± 0.30^{c}	3.53 ± 0.30^{c}
K-	1 ± 0.00^{a}	1 ± 0.00^{a}	1 ± 0.00^{a}

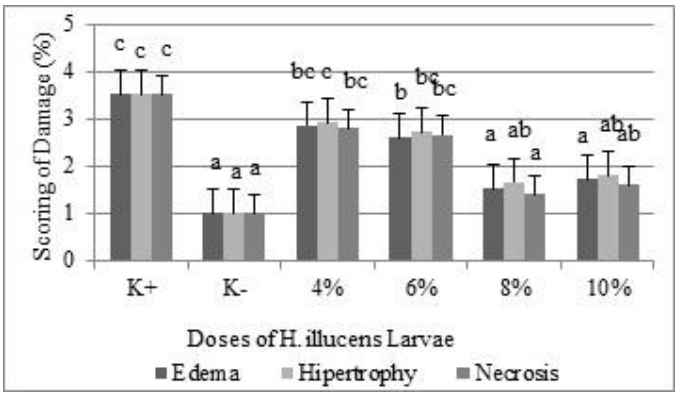


***Fig 8**. Scoring of Gills Damage C. Carp*

From the graph above shows that the treatment C (8%) has the lowest value of scoring gills damage that is edema damage, hipertrophy, and necrosis. Treatment C (8%) also showed an average value of scoring approaching positive control, but the results in treatment D (10%) were not significantly different except that they had higher damage than treatment C (8%). This is presumably because at this dose can produce the best immune response so that the damage to gills organs is smaller than other doses. Tissue recovery in these gills, is influenced by substitution of h. illucens larvae in commercial feed that has been given. Different doses will also affect the different tissue recovery rates indicated by the scoring value. The ability of immunostimulants to enhance the immune response and develop protection against pathogenic infections is influenced by the application dose. High immunostimulant doses can suppress defense mechanisms and low doses can be ineffective or insufficient to provide an immune response (27)). Based on the explanation and the results of the scoring above it can be concluded that the best dose that can reduce the level of damage to the gills tissue of carp is 8%.

4 CONCLUSION

The results of this study indicate that at dose C (8%) which provides the best immune system improvement seen from the lowest total leukocytes, differential leukocyte results (lymphocytes, monocytes, neutrophils) indicate that at treatment C (8%) each has the lowest value, highest total erythrocytes, hemoglobin, and hematocrit. In gill

histopathology test, it was found that common carp gill damage infected by A. hydrophila bacteria after being given immunostimulant H. illucens larvae for 30 days, namely edema, hypertrophy, and necrosis. The lowest mean score of scoring gills damage was also shown at dose C (8%). It is recommended to carp farmers to prevent the emergence of diseases caused by bacteria A. hydrophila by administering immungotimulant maggot mixed with commercial feed at a dose of 8% of the amount of feed given (per day)

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