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Application of Immunostimulants from Caulerpa racemosa Extra ct to Improve Immune Response of Giant Gourami Fish (Osphronemous Gouramy) to Aeromonas hydrophila Infection

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ABSTRACT: Bacterial disease caused by Aeromonas hydrophila usually happens in giant gourami fish aquaculture. Preventive effort which can be done is application of immunostimulants. Seaweed (Caulerpa racemosa) is one of the natural immunostimulants which can be used to improve non-specific immune response of giant gourami so that it becomes more resistant to Aeromonas hydrophila bacterial infection. Method used was giant gourami's soaking in Caulerpa racemosa extract with dosages of 0.5 ppt, 1 ppt and 1.5 ppt. Then the fish were infected by Aeromonas hydrophila bacteria. The test parameters include total leukocytes, lymphocytes, monocytes, neutrophils, macrophages, and phagocytosis activities before and after the Aeromonas hydrophila infection. The results showed that the application of immunostimulants from Caulerpa racemosa extract can improve non-specific immune response of giant gourami fish by 5%. This research is expected to be a reference in future research to determine the application of immunostimulant from Caulerpa racemosa using other parameters.
Key words: Caulerpa racemosa, Immunostimulants, Non-Specific Immune Response, Giant Gourami

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INTRODUCTION

Giant gourami fish is one of the favored commodities of freshwater aquaculture in Indonesia. One common disease in giant gourami aquaculture is caused by Aeromonas hydrophila bacteria [1]. Aeromonas hydrophila infection is facultative anaerobic which attacks gills, kidneys, pancreas, spleen and even other organs. Symptoms are varied and depend on the fish stress factors, bacterial virulence and the fish resistance to the bacteria [2]. Preventive effort which can be done is application of chemicals and antibiotics. But the application of those can cause negative effects like improving the bacterial resistance, environmental pollution, and even to people who consume them [3]. Other effort which can be done is application of immunostimulants. One of natural immunostimulants which can be used is seaweed named Caulerpa racemosa. Based on previous researches, Caulerpa racemosa is known to possess active phenol compound which is able to improve non-specific immune response of the fish [4].

Therefore, application of Caulerpa racemosa extract is expected to improve non-specific immune response of giant gourami fish as protection from bacterial infection of Aeromonas hydrophila.

MATERIAL AND METHODS

Caulerpa racemosa Extract Preparation

Caulerpa racemosa is from Madura (East Java, Indonesia). 50 grams of dry Caulerpa racemosa is macerated with 200 ml ethanol for 2×24 hours. The result of the process is then evaporated using rotary evaporator so the extract of Caulerpa racemosa can be acquired.

Giant gourami fish preparation

The giant gourami used was from Pare (East Java, Indonesia). Giant gourami with length of 8-10 cm was acclimatized for 7 days. Then, it was contained in an aquarium with 10 fish for each treatment. Beforehand, the aquarium has also undergone treatments, by giving 5 ml of chlorine into 50 litres of water for three days. Then 5 ml Na-thiosulphate was added to neutralize the effect of chlorine.

Application of Caulerpa racemosa Immunostimulant

On Day 0, giant gourami fish was soaked into Caulerpa racemosa extract as the first booster with different dosages of 0.5 ppt, 1 ppt, 1.5 ppt for 36 hours. On Day 4, the same treatment was given as the second booster. On Day 5, observation was done to the fish blood and kidneys.

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Aeromonas hydrophila bacterial infection

On day 7, giant gourami fish which has been given the immunostimulants from Caulerpa racemosa is then infected by Aeromonas hydrophilaabout 108 cells/ml by soaking the fish for 29 hours. On Day 9, observation was done to the fish blood and kidneys.

Total Leukocytes Test

Total leukocyte test based on the method of Bijanti [5] was done by taking some fish blood which was already added with 0.5 µl coagulant using leukocyte pipette. Then it was diluted with 11 µl of Türk'ssolution and then is mixed until it is homogenous. First two drops of the mixture were tossed away the third drop was put on haemocytometer box and covered by glass cover. Observation then was done by using the microscope.

Leukocyte Differential Test

Leukocyte differential test was based on method of Bijanti [5] where the fish blood added by coagulant was dropped on the end of objecting glass. The blood then erased to form thin layer on the objecting glass then it was dried. When it was dry, objecting glass was fixated using methanol for 1-2 minutes. Staining with giemsa was done to the blood layer and then waited for ± 15 minutes. After that, it was rinsed with flowing water then it is dried. Observation was conducted with a microscope.

Total Macrophages Test

Total Macrophages test was based on method of Irianto and Austin [6] where the kidney of the fish was taken and weighed. RPMI 1640 (Sigma) was added with 1:10 ratio and then pulverized with a tissue grinder to acquire macrophages suspension. Then, the macrophages suspension was dropped onto groove and added with trypan blue. Observation was conducted with a microscope.

Phagocytosis Activity Test

Phagocytosis activity test was based on method of Irianto and Austin [6] where macrophages suspension was centrifuged at 1000 rpm of speed for five minutes to form precipitation pellet. Macrophages pellet is then dropped on the objecting glass and incubated in 260C temperature for 60 minutes. Then the objecting glass was washed with 100 µl RPMI 1640 (Sigma) to clean the cells which did not adhere. It was then added by Aeromonas hydrophila bacteria with 108 cells/ml density for about 250 µl and then incubated in 360C temperature for 60 minutes. Objecting glass was washed three times with RPMI 1640 (Sigma). Then it was fixated by absolute ethanol and left for 20-30 minutes. Then it was washed with flowing water. Objecting glass was observed with a light microscope and counted to determine ratio of cells which swallowed microbes.

Statistical analysis

The data were analyzed by one way analysis of variance (ANOVA) by SPSS 16.0 software. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Total Leukocytes

Leukocytes have an important role in the immune system to resist foreign particles. Based on the research, total leukocytes in the fish before and after the Aeromonas hydrophila infection can be seen in Figure 1.

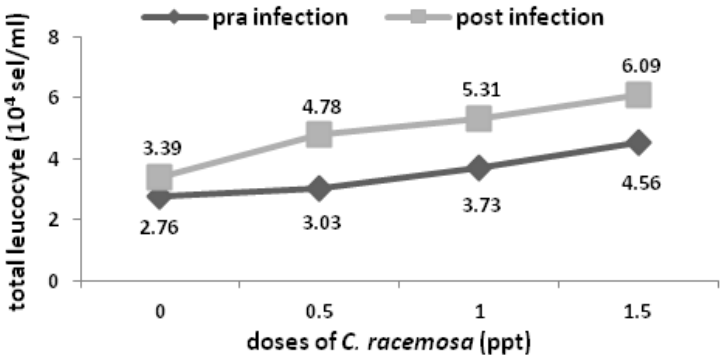

Figure 1. Total Leukocytes

In Figure 1, it is known that there was improvement on total leukocyte before the Aeromonas hydrophila infection (Sig = 0.004) and after the infection (Sig = 0.005) if it was compared to the control. It showed that the immune response and the reactive response were quite good. Total leukocytes number is improving along with the dosage addition and infection process duration. According to Fujaya's work [7], the infection caused

inflammatory process, leukocyte then reacted by improving their number and heading to the infected area to defend from foreign particles.

Lymphocytes

Lymphocytes are types of granular leukocytes which are not phagocytic but hold an important role in the antibody production. Based on the research on lymphocytes of the fish before and after Aeromonas hydrophila can be seen in Figure 2.

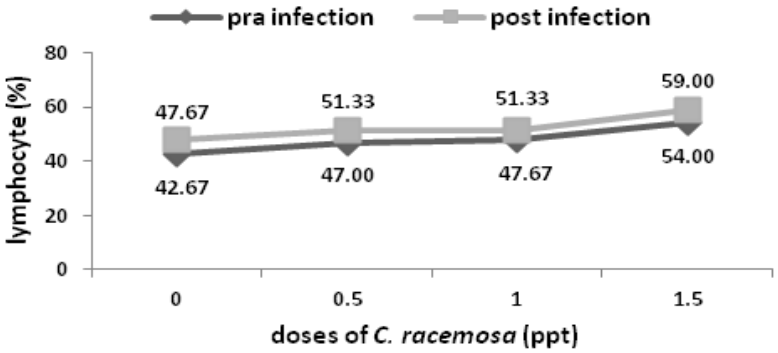

Figure 2. Limphocytes

In Figure 2, it is known that there was lymphocyte cells improvement before the infection of Aeromonas hydrophila (Sig = 0.003) and after the infection (Sig = 0.002) if it was compared to the control. Immunostimulants application and bacterial infection in the fish were considered as antigens. According to Moyle and Cech [8], lymphocyte cells improvement happened due to antigens stimulation which caused immune response. Lymphocytes work as antibodies producer which have important role in the immune system towards disease. The improvement of lymphocytes count caused the improvement of antibodies production 2-3 days after the antigen injection. High lymphocyte count is usually caused by bacterial infection in the fish.

Monocytes

Monocytes are type of a granular leukocytes with important roles in the process of phagocytosis and consume foreign particles which invade the body. Based on the research result monocyte count of the giant gourami before and after Aeromonas hydrophila infection can be seen in Figure 3.

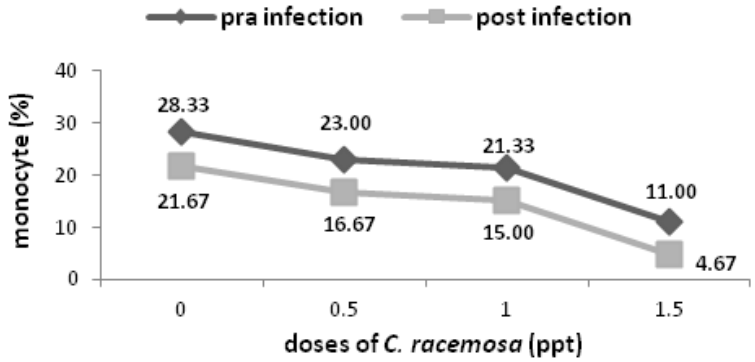

Figure 3. Monocytes

In Figure 3, it is known that there was decreasing monocyte cells before the Aeromonas hydrophila infection (Sig = 0.001) and after the infection (Sig = 0.001) if it was compared to the control. The decreasing monocyte cells was presumed due to monocyte cells were differentiated as macrophages and migrated to the infected tissue so the total monocytes in the blood became less at the time of infection. According to Selvaraj et al. [9], monocytes reside in the blood only for 24 hours. Monocytes are precursored macrophages which can be activated by antigen stimulation and are able to penetrate blood vessel wall swiftly to the infected tissue to perform phagocytosis activity.

Neutrophils

Neutrophils are type of granular leukocytes which are phagocytic for the first defense against infection. Based on the research results, neutrophil count of the giant gourami fish before and after the Aeromonas hydrophila infection can be seen in Figure 4.

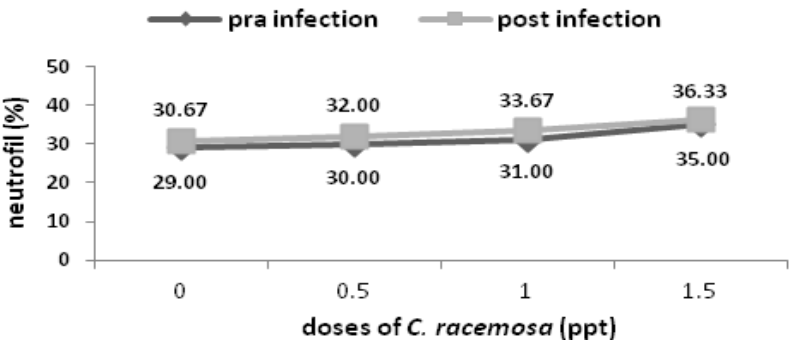

Figure 4. Neutrophils

In Figure 4, it is known that there was increasing neutrophil cells before the Aeromonas hydrophila infection (Sig = 0.037) and after the infection (Sig = 0.046) if it was compared to the control. The increasing neutrophil count indicated infection in the fish. According to Vadstein [10], neutrophils are the first defense system of non-specific immunity to fight pathogenic infection. The main function of neutrophils is destroying antigens by phagocytosis process including chemotaxis, adhesion, engulfment, and antigen destruction by lysosome enzymes in phagolysosomes.

Macrophages

Macrophages are monocyte cells which are matured and spread over the infected tissue. Macrophages function as phagocyte cells to foreign particles invading the body. Based on the research result, macrophage count of the giant gourami before and after Aeromonas hydrophila infection can be seen in Figure 5.

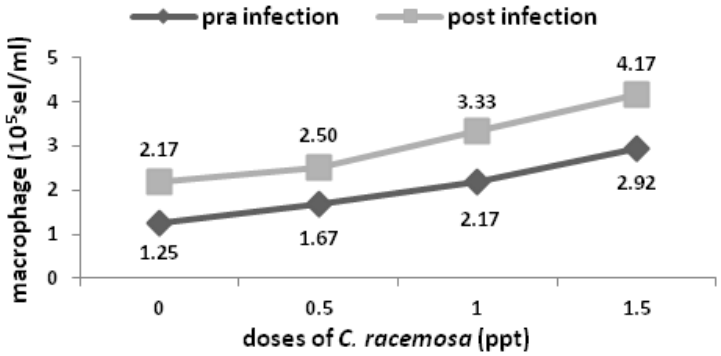

Figure 5. Macrophages

In Figure 5, it is known that there was increase in total macrophage count before Aeromonas hydrophila infection (Sig = 0.002) and after the infection (Sig = 0.001) if it was compared to the control. Immunostimulants application and infection degree can cause more production of macrophages. According to Secombes et al. [11], macrophages in the blood are named as monocytes and matured when they head for the infected area. Macrophages secreted interleukin-1 to stimulate T-cell proliferation and to form lymphokine which is assigned to pull macrophages to the infected area to perform phagocytosis activity.

Phagocytosis activity

Phagocytosis activity is a sequential process including adhesion, engulfment, degranulation, destruction and digestion of antigen. Based on the research result, phagocytosis activity value of the giant gourami before and after Aeromonas hydrophila infection can be seen in Figure 6.

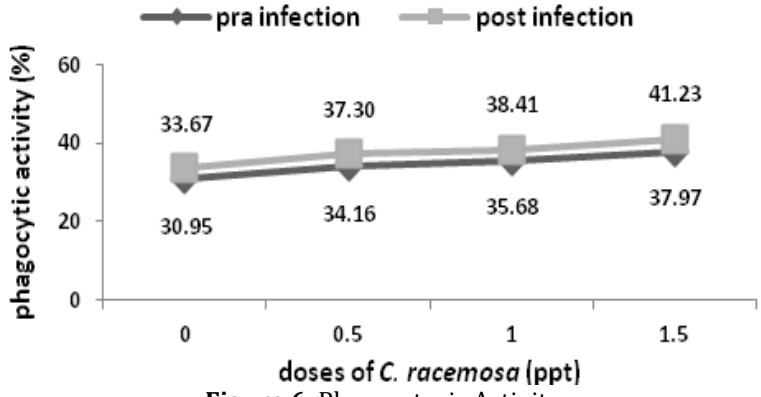

Figure 6. Phagocytosis Activity

In Figure 6, it is known that there was improvement in phagocytosis activity before Aeromonas hydrophila infection (Sig = 0.001) and after the infection (Sig = 0.001) if it was compared to the control. The higher dosage given, the more phagocytosis activity. It showed improvement on the giant gourami immunity. According to Brown [12], the improvement of immunity was noticed from the increasing phagocyte cells activity of hemocytes. Phagocyte cells function to perform phagocytosis to foreign particles invading the host body.

CONCLUSION

Application of immunostimulants from Caulerpa racemosa extract obviously affect to improve non-specific immune response by 5% through parameters including total leukocytes, lymphocytes, monocytes, neutrophils, macrophages, and phagocytosis activity of the giant gourami fish so it became more resistant to Aeromonas hydrophila bacterial infection.

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