Expression Of Interleukin-10 In Cantang Hybrid Grouper Infected With Viral Nervous Necrosis By Induction Of Protein Brachionus Sp.

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Abstract: This study examines the role of Brachionus sp protein on the interleukin-10 anti-inflammatory immune system in cantang grouper infected with Viral Nervous Necrosis (VNN). The purpose of this study was to determine the protein content of Brachionus sp. which has the potential as an antivirus and to find out the benefits of Brachionus sp. on the expression of Interleukin-10 as an indicator of increased fish body defense system against VNN. Testing of Brachionus protein by injection at doses of 35 µl, 105 µl and 170 µl /150 gram cantang grouper. The results showed that the lowest decrease was at a dose of 105 µl/150 gram cantang groupers.

Keywords : Interleukin-6, Cantang Grouper, Brachionus sp., Viral Nervous Necrosis

1 INTRODUCTION

Hybrid grouper is a new prima donna for fish cultivators. The advantage of hybrid grouper compared to other groupers is that it grows faster than other grouper seeds. The hybrid grouper which is the prima donna is the cantang grouper, the cantang grouper has the advantage that the growth can reach 724% compared to the tiger keraou which is only 295% [1]. In addition, hybrid grouper is a commodity that is in demand by the export market, especially for the China and Hong Kong markets [2]. In Indonesia, grouper fish has a market price of 230,000 per/kg. This high price has encouraged grouper farmers to start cultivating this fish, although they are faced with several challenges in the process. The biggest challenge in the cultivation of cantang grouper is the attack of diseases caused by parasites or intracellular viruses. One of them is the attack of Nervous Necrosis Virus (VNN) which causes the majority of grouper cultivation failures. This virus is capable of causing mass death at various stages of development of marine fish species, especially in the larval and seed stages, and is even capable of causing death up to 100% [3,4].Groupers infected with VNN have clinical symptoms including irregular swimming movements resulting from necrosis and vacuolation in the nervous system and retina, loss of appetite and changes in pigmentation [5].

Regulatory mechanisms of the immune system are very important for the successful fight against this VNN infection. One of the candidates that can be used in VNN management is protein the zooplankton type Brachionus sp. Brachionus sp. contains crude fat of 17.17%, crude protein of 63.53%, carbohydrates 7.74%, phospholipids 44%, EPA 41 g/kg, DHA 66 g/kg and abundant amino acids including glutamic acid (Glu) 62 g /kg dry weight; lysine (Lys) 41.2 g/kg dry weight; aspartic acid (Asp) 39.2 g/kg dry weight; and Leusina (Leu) 38.8 g/kg dry weight [6]. Protein can function as an immunomodulator and stimulate the mobilization of inflammatory cells so that it will increase the immune system response of fish that are resistant to disease. This is because when malnutrition occurs due to protein deficiency, it can cause a decrease in immunity in phagocytosis [7].In the research Brachionus spp. has a relatively high molecular weight of 16-56 kDa where this protein has an important role in immunological processes such as cell growth, apoptosis and differentiation and inhibits components of inflammatory pathways [8]. This is in line with the research of Brachionus sp. is one of the candidates for zooplankton that can be used as an infection control in infected fish. Based on the above review, Based on this description, the research on Brachionus sp. expected to increase the activity of the immune system by decreasing the pro-inflammatory activity of IL-10 as the first microcidal in grouper infected with VNN. The purpose of this study was to determine the protein content in Brachionus sp. which has the potential as an antiviral and to know the benefits of Brachionus sp. on the expression of Interleukin-10 as an indicator of increased fish body defense system against VNN.

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2 MATERIAL AND METHODS

2.1 Time and Place

This research was performed in the laboratory of Patology and Biochemistry, Faculty of medicine University Brawijaya, CV SAA grouper in Banyuwangi and dry laboratory of PSDKU Banyuwangi University Airlangga from November 2020-September2021. Cantang Grouper (7-10 cm length, and weighing ±15 gram) and Brachionus sp. from Situbondo brackish water cultivation center. The cantang grouper were in healthy condition and not infected by a disease such as Viral Nervous Necrosis. Fish positive VNN originated were collected from pond farmers around the Situbondo city.

2.2 Research Materials

The materials used in this study were Cantang Grouper 7-10 cm in size, Anti-mouse antibody IL-6, Heparin sodium, Freshwater and Marine, Protein extract Brachionus sp. VNN positive grouper, aquades, is aquades, sea water, 70% alcohol, Phosphate Buffer Saline/PBS (bio-Rad), separating gel, stacking gel, lower gel buffer, upper gel buffer, PBS solution, T-acryl, ddH2O , Tetra Methyl Diamine (TEMED)(bio-Rad), ammonium persulfate, Tris (hydroxymethyl), HCl (Merck) pH=8.8 and 6.5, detergent Sodium Dodecyl Sulphate (SDS), aquadest and Comassie Brilliant Blue dye. The equipment used in this study were aeration hose, aeration stone, 5 L

Erlenmeyer, 2L jar, aerator, heater, Bunsen lamp, centrifuge, section set, volume pipette, mask, hand gloves, microtube, 1 ml syringe, label paper, freezer. -90^{o}C, hot plate, magnetic stirrer, set of electrophoresis (SDS-PAGE), Aquarium 70x70x40, and Filter.

2.3 Research Design

The present study used completely randomized design (CRD) with five treatment K+ (fish +VNN),P1(fish + VNN + 35 µl protein Brachionus sp, P2 (fish+ VNN + 105 µl protein Brachionus sp.), P3 (fish+ VNN + 170 µl protein Brachionus sp.), P4 (Fish+ 35 µl Protein Brachionus sp.), P5 (Fish+ 105 µl Protein Brachionus sp.), P6 (Fish+ 170 µl Protein Brachionus sp.) and each treatment had three replicates.

2.4 Work Procedure

2.4.1 Cantang grouper acclimatization

The test fish used were cantang grouper from the Situbondo brackish water cultivation center. The cantang grouper used is 9-10 cm in size and weight 15 grams. Newly arrived fish will be acclimatized for 12 hours until the fish show aggressive movements. The feed given to the grouper was Otohime EP3® pellet (48 % protein) and trash fish. Feeding was carried out twice per day at 07.00 and 15.00 am WIB.

2.4.2 Brachionus sp. Protein Test. In Cantang Grouper

In-Vivo

In this study, the protein used in clinical trials was the protein that showed the highest hemagglutination of Brachionus sp. This clinical test used 6 cantang groupers with a size of 7-10 cm and a weight of ± 150 grams with a total of 120 fish used. This testing process is carried out by injection. The clinically tested grouper will be boosted on the 0, 7 and 21 days and tissue samples will be taken on the 28th day.

2.4.3 In-Vivo Viral Nervous Necrosis Test on Cantang Hybrid

Grouper

VNN infection in test fish was carried out orally, namely by chopping the fish according to the grouper's mouth opening and giving it twice a day. VNN infection was carried out on day 5 and day 10 with a feeding dose of 5 grams/head. Then observations were made on fish, this observation was aimed at seeing changes in fish behavior from normal to abnormal or starting to appear specific symptoms of fish that were attacked by VNN such as swimming that started irregularly.

2.4.4 Imunohistochemistry analysis

The preparation of immunohistochemical preparations was carried out by preparing organ tissues that had been exposed to immunogenics and fixation using 10% formalin solution. Then embedding is done using paraffin wax. After that, the tissue was cut using a microtome with a thickness of 4-5 M, then placed on glass slides for special immunohistochemical preparations. The slide preparations were then paraffinized by heating the slides at 60-80^{0}C. then dipped in xylol for ± 5 minutes. Then the slides were dehydrated by rinsing with absolute alcohol for 2 repetitions at a concentration of 90%, 80%, and 70% for ± 5 minutes, respectively. Then the slides were rinsed again with 20 m dionized water for 3 repetitions, each for 5 minutes. Then rinsed with distilled water and prepare the preparation in the refrigerator (overmight). The next stage is immunostaining using the Scytek kit. The slite preparation was rinsed with PBS pH 7.4 20 m 3 times for 5 minutes each. Then incubated with peroxidase blocking for image analysis for 4 minutes at room temperature. Then rinsed again with PBS 3 times. After that, it was incubated on a super block for 24 hours at 40C. Then rinsed again with PBS 3 times. Prepare primary antibodies (IL-6) dissolved in blotto solution in a ratio of 1:1000. After that, it was incubated with primary antibody which had been diluted in a blocking super block overnight at 4^{0}C. The slides were rinsed again with PBS pH 7.4 3 times for 5 minutes each. Then, the slides were incubated with the secondary antibody CRF Anti polyvalent Biotylated HRP for 1 hour at room temperature. The slides were rinsed again with PBS 3 times. Then the slides were incubated with the ultratek HRP enzyme for 40 minutes at room temperature. Then the slides were rinsed with distilled water until the PBS disappeared and dried from the remaining PBS that was still attached. After that, the slides were incubated using DAB chromagen for 20 minutes, then washed again with PBS pH 7.4 3 times for 5 minutes each. Then given a counterstain with homotoxylen major for 10 minutes and rinsed again with DH2O for 3 repetitions each for 5 minutes. After this, it is dried by aerating. When the slide is completely dry, the slide is covered with entelan. The next slide was observed with a microscope with a magnification of 400x, observing the CPI images using an Olympus digital camera.

2.4.6 Measured observed Parameters

The main parameters in this study were Interleukin-6 which was analyzed by IHC and SPSS 20.0 and observed with a digital binocular microscope, as well as proteins from Brachionus sp. analyzed by SDS-Page.

2.5 Data Analysis

Data were analyzed using analysis of Variance (ANOVA) to determine the effect of each treatment [8]., When the ANOVA test results were significantly different, the Duncan Test (DMRT) was performed to determine the difference among treatments.

2 Result and Discussion

2.1 Interleukin 10 expression

Interleukin-10 analysis was performed on brain targets. IL-10 expression in organs was carried out using the immunohistochemical method. IL-10 is an anti-inflammatory cytokine that maintains the balance of the immune response, allowing the clearance of infection while minimizing damage to the host [9]. The results of the analysis of IL-6 expression can be seen in the figure 1.

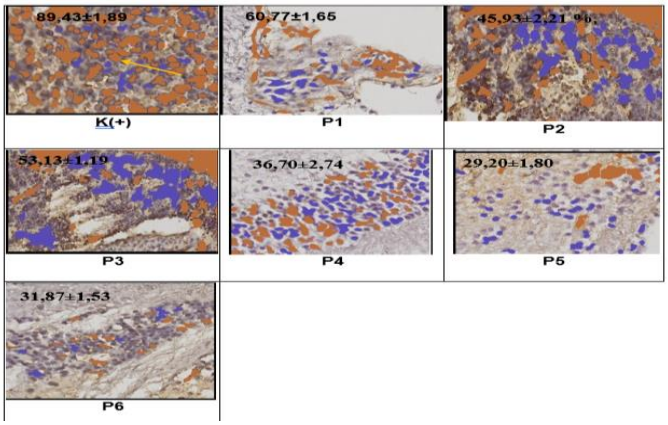

Fig 1. Immunohistochemical results were analyzed with image J of Interleukin-10 expression in grouper brains infected with VNN and treated with Brachionus sp.

Note: K+: Fish infected with VNN without Brachionus sp. protein, P1: VNN+35µL Brachionus sp. protein, P2: VNN+105µL Brachionus sp. protein, P3: VNN+170µL Brachionus sp. protein, P4: 35µL Brachionus sp., P5: 105µL Brachionus sp. protein, P6: 170µL Brachionus sp. protein Based on the immunohistochemical description above, further analysis was carried out using SPSS. The results of SPSS can be seen in Figure 2.

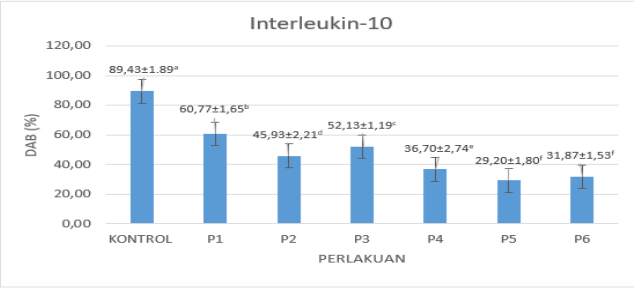

Fig 2. The results of immunohistochemistry tested by SPSS on il-10 expression in grouper infected with VNN with Brachionus sp. Different letters above the bar indicate a significant difference (P<0.05) based on Duncan's Multiple Range Test (DMRT). DAB is the expressed protein value from semi-qualitative calculations using image J.

Based on the analysis using SPSS, treatment with Brachionus sp. with different doses had a significant effect (P<0.05) on the immunological response of native interleukin 6. Interleukin-6 expression in K+ treatment was significantly different from P1 treatment by 60,77±1,65%, P2 by 45,93±2,21%, P3 by 52,13±1,19%, P4 by 36,70± 2,74%, P6 of 31,87±1,53% and the results of P5 treatment in the expression of interleukin-10 had the lowest value of 29,20±1,80%. This indicates that the protein Brachionus sp. can affect interleukin-10 levels in infected fish characterized by a decrease in interleukin-10 levels as an inflammatory response. Interleukin (IL)-10 a role in inhibiting the function of macrophage cells and dendritic cells by downregulating antigen presentation and inhibiting the production of cytokines, chemokines, nitric oxide, reactive oxygen species and costimulatory molecules. Where, IL-10 produced by T cells will inhibit the activation and production of Th1 cells. The ability of IL-10 to inhibit cytokine production by both T-cells and pleiotropic natural killer (NK) cells of IL-10 against B-cells and T-cells [10]. Decreased levels of IL-10 given protein treatment Brachionus sp. This is indicated because the protein that enters the body will be distributed to the blood plasma. Where when there is inflammation the protein in the plasma will bind to the antibody which will then bind to the protein produced by the virion thereby blocking the attachment of the virus and lysing the protein from the virion (opsonin) [11]. Moreover, the use of protein on infected tissue can reduce local inflammation and can activate immune cells [12].

4 Conclusion

From the research that has been done, it can be concluded that the process of giving the protein contained in Brachionus sp. can affect Viral Nervous Necrosis infection in grouper by reducing the infection process which is characterized by a decrease in interleukin-10.

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