Journal of Aquaculture and Fish Health Vol. 13(3) - September 2024
P-ISSN: 2301-7309 / E-ISSN: 2528-0864
doi : 10.20473/jafh.v13i3.30835

Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp.

Dwi Retna Kumalaningrum[1,3*], Uun Yanuhar[2], Mohammad Musa[2] and Nur Fauziyah Martiningsih[3]

[1]Program Study of Master Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Malang, East Java, 65144, Indonesia
[2]Department of Aquatic Resource Management, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Malang, East Java 65144, Indonesia
[3]Program Study of Aquaculture, Faculty of Health, Medicine and Life Sciences, Universitas Airlangga, Banyuwangi, East Java 68425, Indonesia

*Correspondence :
retnakumalaningrum@gmail.com

Received : 2021-10-20
Accepted : 2022-08-11

Keywords :
Interleukin-6, Cantang Grouper, Brachionus sp.

Abstract

This study examines the role of *Brachionus* sp protein on the interleukin-6 pro-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-6 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. In addition, *Brachionus* sp. contains 3 protein bands with molecular weights of 122.73 kDa, 75.49 kDa, and 13.77 kDa.

INTRODUCTION

Hybrid grouper is a new leading commodity for fish cultivators. The advantage of hybrid groupers over other groupers is that they grow faster than other grouper seeds. The prima donna of hybrid grouper is the Cantang grouper, it has the advantage of higher growth which can reach 724% compared to the Tiger grouper which is only 295% (Sutarmat and Yudha, 2013). In addition, hybrid grouper is a commodity in demand by the export market, especially for the China and Hong Kong markets (Sembiring *et al.,* 2014). In Indonesia, grouper's market price can reach 230,000 per/kg (KKP, 2021).

This promising market price has encouraged farmers to cultivate hybrid grouper, although there are several challenges in the cultivation 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 Nervous Necrosis Virus (VNN), which causes the majority of grouper cultivation failures. This virus is capable of causing mass death at various life stages of marine fish species, especially in the larval and seed stages, and it is even capable of causing death up to 100% (Kuo *et al*., 2011; Chen *et al*., 2014). 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

Cite this document as Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

changes in pigmentation (Yuwanita *et al*., 2013; Liu *et al*., 2015).

Regulatory mechanisms of the immune system are very important for the successful fight against VNN infection. One of the candidates that can be used in VNN management is protein the zooplankton *Brachionus* sp. *Brachionus* sp. contains 17.17% crude fat, 63.53% crude protein, 7.74% carbohydrates, 44% phospholipids, 41 g/kg EPA, 66 g/kg DHA and rich in amino acids such as 62 g /kg dry weight glutamic acid (Glu); 41.2 g/kg dry weight lysine (Lys); 39.2 g/kg dry weight aspartic acid (Asp); and 38.8 g/kg dry weight Leusine (Leu) (Lee *et al*., 2009; Hamre, 2015).

The protein can act 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. Because, when malnutrition occurs especially protein deficiency, it may lead to a decreasing immunity in phagocytosis (Baratawidjaja and Rengganis, 2018). Park *et al*. (2020) stated that *Brachionus* sp. 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. This is in line with the research of Yanuhar *et al*. (2019), *Brachionus* sp. is one of the candidates for zooplankton that can be used to control infection in fish.

The research on *Brachionus* sp. is expected to increase the activity of the immune system by decreasing the pro-inflammatory activity of IL-6 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-6 as an indicator of increased fish body defense system against VNN.

METHODOLOGY

Ethical Approval

The study was monitored and approved ethically by the Faculty of Fisheries and Marine Sciences; Brawijaya University (reference number 41/2020).

Place and Time

This research was conducted in the Laboratory of Pathology and Biochemistry, Faculty of Medicine Brawijaya University, CV SAA grouper in Banyuwangi, and Anatomy Laboratory of Airlangga University (Banyuwangi Campus) from November 2020 to September 2021. Cantang Grouper and *Brachionus* sp. was collected from Situbondo brackish water cultivation center.

Research Materials

The materials used in this study were Cantang Grouper (7-10 cm, ±15 g), Anti-mouse antibody IL-6, Heparin sodium, Freshwater and Marine, *Brachionus* sp., Whole Cell Protein *Brachionus* sp., Protein extract *Brachionus* sp. VNN positive grouper, aquadest, seawater, 70% alcohol, Phosphate Buffer Saline/PBS (bio-Rad), separating gel, stacking gel, lower gel buffer, upper gel buffer, PBS solution, T-acryl, ddH_2O, Tetra Methyl Diamine (TEMED)(bio-Rad), ammonium persulfate, Tris (hydroxymethyl), HCl (Merck) pH=8.8 and 6.5, detergent Sodium Dodecyl Sulphate (SDS), 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 °C, hot plate, magnetic stirrer, set of electrophoresis (SDS-PAGE), Aquarium 70x70x40, and Filter.

Research Design

The present study used completely randomized design (CRD) with five treatments such as; 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

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

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.

Work Procedure

Cantang Grouper Acclimatization

The test fish used were Cantang grouper from the Situbondo brackish water cultivation center. The Cantang grouper used is 7-10 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.

Culture of *Brachionus* sp.

Rotifers *Brachionus* sp. were cultured at the Situbondo brackish water cultivation center. *Brachionus* sp. These were cultured on media with a salinity of 30 ppt. Where the rotifer enrichment process is carried out every day and given strong aeration to help the oxygenation process. Harvesting of *Brachionus* sp. was performed at the 56th hour. *Brachionus* sp. transferred into the ependroph tube using a spatula spoon. Then *Brachionus* sp. is wrapped in aluminum foil and stored in the freezer at -80 °C until the *Brachionus* sample is used (Srivastava *et al*., 2006; Rumengan *et al*., 2012).

Preparation of Sample for Protein analysis

In the experiment, the rotifer sample was homogenized in phosphate buffer at pH 8.0 for 3 min at 4°C. Ingredient samples were homogenized in a Teflon stick homogenizer. After homogenization, all samples were centrifuged at 10000 g for 40 min at 4^{0}C. The supernatant containing the soluble protein fraction was sampled and stored at -80^{0}C until further analysis. The resulting pellet is a whole cell while the supernatant is crude protein *Brachionus* sp.

Protein Electrophoresis with SDS-Page

Protein analysis of *Brachionus* sp. using electrophoresis technique, namely SDS Page. The first step of the electrophoresis technique was to prepare the gel into a mold made of glass plates separated by a PVC spacer and made using “Glisseal” oil (Borer Chemic AG, Zuchwill/Switzerland). After filling the gel solution followed by coating using distilled water.

The SDS-PAGE was carried out on a 190 x 130 mm gel in a vertical well used for electrophoresis. The separating gel was polymerized from a 12.5% acrylamide solution; 0.344% bis-acrylamide; 3.5 mM SDS (Sodium Dodecyl Sulfate); 375 mM Tris-HC1 buffer; pH 8.8. Polymerization was initiated by adding 0.05 ml of 40% APDS and 0.025 ml of TEMED (tetra ethylene diamine) per 50 ml of the gel solution. The gel containing the substrate was added with 0.1 mg fibrinogen/ml separating gel solution and stirred for 45 minutes before polymerization. Separating gel is made of 4.38% acrylamide; 0.12% bis-acrylamide; 3.5 mM SDS in 125 mM Tris-HC1 pH 6.8. Polymerization was started by adding 0.025 m140% APDS and 0.01 ml TEMED per 10 ml gel solution.

The sample was dissolved in buffer S containing 80 mM SDS and 0.1 M DTT. The electrode buffer consisted of 192 mM glycine, 25 mM Tris, and 3.5 mM SDS. With a 2 mm gel electrophoresis (at 6 °C) starting with 30 mA, and increasing to 50 mA after 60 min, the current intensity was halved by 1 mm gel. Electrophoresis was stopped when Bromphenol Blue was about 1 cm from the bottom edge of the gel. The results of SDS-PAGE electrophoresis in the form of protein bands were determined to determine the molecular weight by calculating the Rf (Retardation Factor) value with the following formula (Rantam, 2003):

$$Rf = \frac{\text{the distance the protein moves from its starting point}}{\text{the distance the color moves from the starting place}}$$

Then the Rf value is entered in the linear regression equation with the formula:

$Y = a+bX$

Where :

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

Y = molecular weight
X = sample Rf value.

Electroelution and Protein Dialysis

Brachionus sp. proteins obtained from SDS-PAGE were identified and selected with the highest molecular weight. Protein bands are separated by the electroelution method using horizontal electrophoresis (Biorad). Protein pieces are inserted into the cellophane membrane and electrophoresed at 120 V, 400 mA for 120 minutes. Then the protein is dialyzed using PBS at pH 7.4 at 4^0C for 48 hours.

The liquid contained in the cellophane bag is taken and inserted in a microtube, then precipitated by incubation in a solution of acetone (1:1 v/v) overnight at 4 ºC. The protein and acetone solution were centrifuged at 12,000 rpm for 20 minutes at 4 ºC. Proteins that formed in the form of pellets. These pellets were then stretched and dissolved in 100 L tri HCl 0.5 M and pH 8.6. The protein concentration of *Brachionus* sp. is measured using a NannoDrop 1000 Spectrophotometer with an absorbance of 1 at 280 nm.

Immunohistochemistry Analysis

In this study, the immunohistochemical analysis method refers to Schacht and Kern (2015). The preparation of immunohistochemical analysis was carried out by preparing organ tissues that had been exposed to immunogenics. First, the organ was fixed using a 10% formalin solution. The embedding process was done using paraffin wax. After that, the tissue was cut using a microtome with a thickness of 4-5 μm and placed on glass slides for immunohistochemical analysis. The slides were paraffinized by heating in a hotplate at 60-80 ºC and dipped in xylol for ± 5 minutes. Then, the slides were dehydrated by rinsing with absolute alcohol twice (5 minutes each), 90% alcohol, 80% alcohol, and 70% alcohol for ± 5 minutes. Later, the slides were rinsed again with 20 Mol deionized water thrice (5 minutes each). Then, the slides were rinsed with distilled water and refrigerated overnight.

The next step is immunostaining using the Scytek Kit. The slides were rinsed with PBS pH 7.4 20 μm, 3 times for 5 minutes each, and incubated with peroxidase blocking for image analysis for 4 minutes at room temperature. Next, the slides were rinsed again with PBS thrice. After that, it was incubated on a superblock for 24 hours at 4 °C and rinsed again with PBS thrice. The primary antibodies (IL-6) dissolved in blotto solution with a ratio of 1:1000. After that, it was incubated with a primary antibody which had been diluted in a blocking super block overnight at 4 °C.

The slides were rinsed again with PBS pH 7.4 3 times for 5 minutes each. Subsequently, the slides were incubated with a secondary antibody CRF Anti polyvalent Biotylated HRP for 1 hour at room temperature and then rinsed again with PBS 3 times. In addition, 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 was removed and the remaining PBS was still attached. After that, the slides were incubated using DAB chromagen for 20 minutes and washed again with PBS pH 7.4 3 times for 5 minutes each and counterstained with homotoxylen major for 10 minutes then rinsed again with DH2O for 3 repetitions each for 5 minutes dan it is dried by aerating. When the slide is completely dry, the slide is covered with Entellan. The observation was conducted using a microscope with 400 x magnification, observing the CPI images using a digital camera.

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.

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

DOI : 10.20473/jafh.v13i3.30835

Data Analysis

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

RESULTS AND DISCUSSIONS

Fragment profile of *Brachionus* sp.

The fragment profile of *Brachionus* sp. was analyzed using the SDS-Page method. This method is one of the most widely used methods for detecting proteins. The results of protein analysis using the SDS-Page method can be seen in Figure 1.

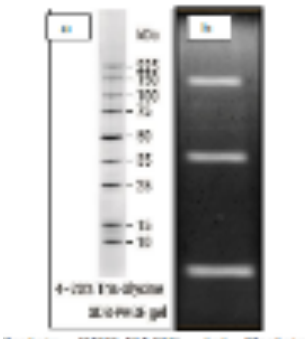

Figure 3. Protein profile of *Brachionus* sp. using SDS-PAGE method.
Note: (a) protein marker, (b) supernatant protein *Brachionus* sp.

The results of the above analysis show protein bands. These protein bands show the clearest and thickest color intensity so that they can be used as a reference to determine the molecular weight and protein profile contained in *Brachionus* sp. First, the calculation is carried out to find the linear band protein marker formula used. The calculated data on protein bands can be seen in Table 1 below:

Table 1. Calculation of molecular weight of Band Protein marker.

BM (Weight of Molecule)	Log BM (y)	A (mm)	B (mm)	Rf (x)
225	2.35	20	92	0.22
150	2.18	25	92	0.27
100	2.00	30	92	0.33
75	1.88	35	92	0.38
50	1.77	44	92	0.48
35	1.54	52	92	0.57
25	1.40	61	92	0.66
15	1.18	74	92	0.80
10	1.00	80	92	0.87

The results of the calculation of the molecular weight on the protein marker band above are used to see the pattern of the protein band by calculating the molecular weight (BM) for each Rf formula. then done by calculating the linear equation of the above results using Microsoft Excel. The calculation of linear graph calculations can be seen in Figure 2.

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

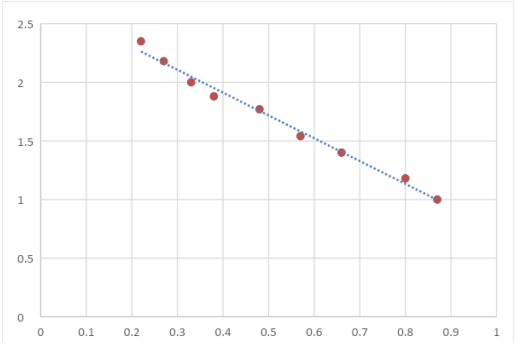

Figure 2. Linear graph of *Brachionus* sp.

The results of the graph above, the results of the linear equation in the marker protein band are y= -1.9472x+2.6909. The value of Y is the logarithm value of BM (molecular weight), and X is the value of Rf which is the result of dividing the distance between the movement of the protein band from the initial place and the distance of the color movement of the tracer from the starting place. After obtaining a linear formula, the molecular weight of *Brachionus* sp. protein is calculated. The results of these calculations can be seen in Table 2.

Table 2. Calculation of the molecular weight of *Brachionus* sp. protein bands.

A (mm)	B (mm)	Rf (x)	y= -1,9472x+ 2,6909	BM Sample
28	92	0.30	2.08	122.73
38	92	0.41	1.87	75.49
73	92	0.79	1.13	13.77

In the calculation of the molecular weight of the protein band above, it was found that the pigment fragment of *Brachionus* sp. contains 3 protein bands with molecular weights of 122.73 kDa, 75.49 kDa, and 13.77 kDa. Based on the visible molecular weight results, it can be indicated that the protein pigment fragments contained in Brachionus sp. namely the types of peridinin, apyrase, and threonine. According to Weiss *et al.* (2002), peridinin has two forms, one as a homodimer (short form) with a molecular weight ranging from 13-16 kDa and the other as a monomer (long form) with a molecular weight ranging from 30-35 kDa. This type of protein apyrase usually appears in the weight range between 65-86 kDa. While the type of threonine usually appears in the weight range between 119-125 kDa.

The protein content of *Brachionus* sp. can block the NF-κB pathway. This is because if the NF-kB pathway is not inhibited, it triggers severe inflammation in infected fish. Dong *et al*. (2017) stated that the administration of protein can inhibit the NF-kB pathway which activates pro-inflammatory cytokines and can send signals to activate anti-inflammatory cytokines to minimize severe inflammation. In addition, the protein apyrase contained can play a role in reducing cytokine storms such as decreased production of Il-6. According to Dixit *et al*. (2019), the use of apyrase protein in infected tissue can reduce local inflammation and activate immune cells.

Interleukin-6

Viral Nervous Necrosis infection in Cantang grouper causes immune interactions in infected organs, one of which is the expression of interleukin-6 due to inflammation. Interleukin-6 is a pleiotropic cytokine that plays a role in responding to tissue damage and infection (Tanaka *et al*., 2014). IL-6 expression can be seen using immunohistochemical methods. The results of the analysis of IL-6 expression can be seen in Figure 3. Based on the

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

immunohistochemical description, further analysis was carried out using SPSS. The results of SPSS can be seen in Figure 11.

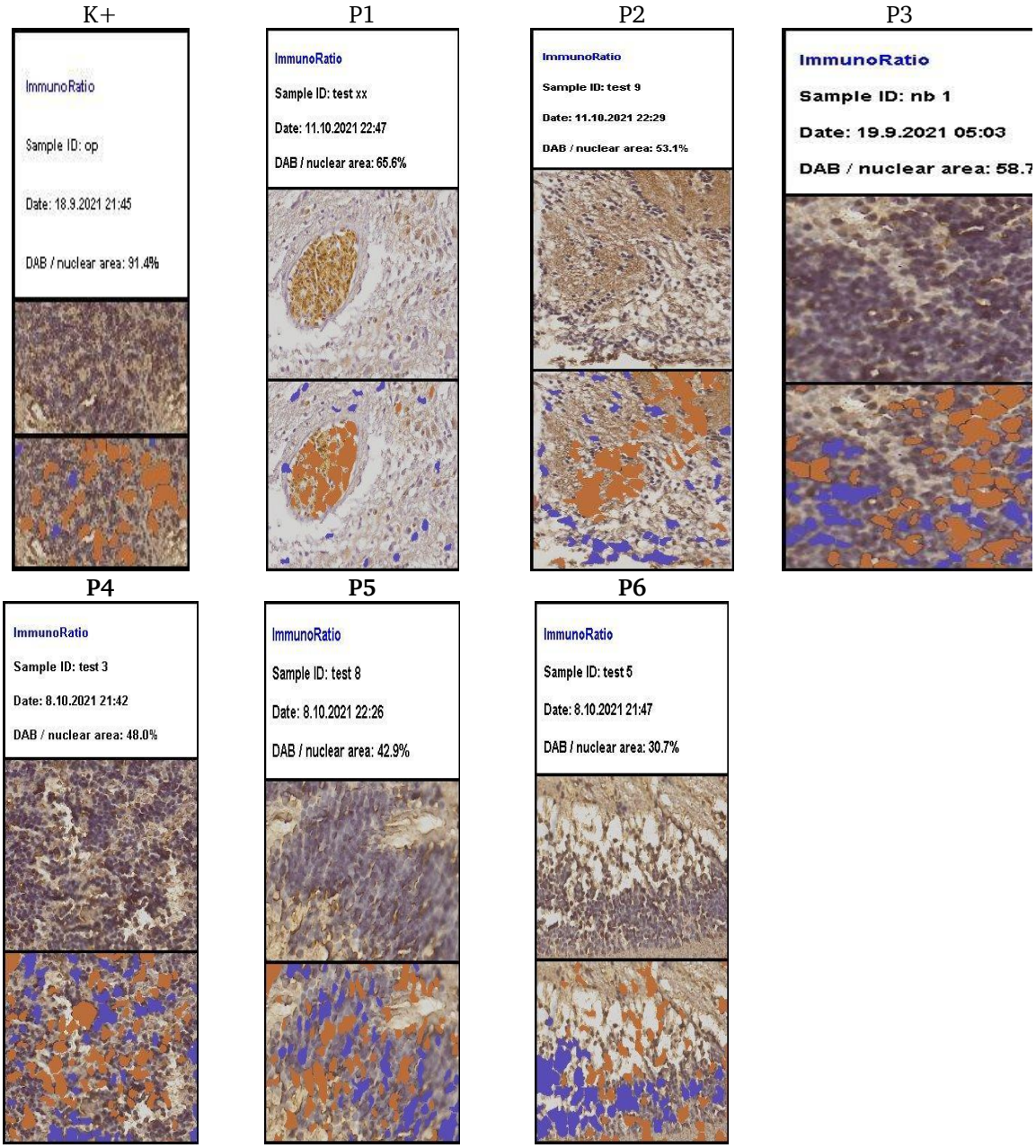

Figure 3. Immunohistochemical results were analyzed with Image J of Interleukin-6 expression in grouper brains infected with VNN and treated with *Brachionus* sp.

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. Remarks: brown color indicates the expression of IL-6.

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

DOI : 10.20473/jafh.v13i3.30835

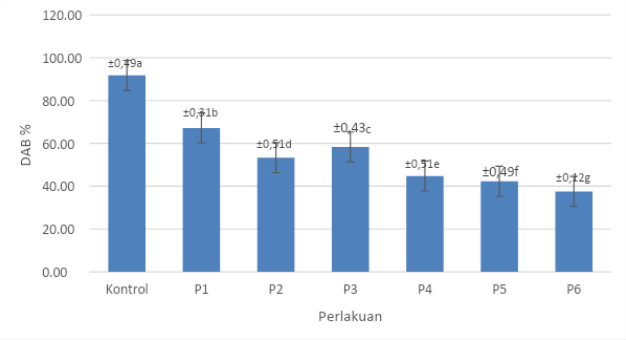

Figure 3. The results of immunohistochemistry tested by SPSS on il-6 expression in grouper infected with VNN with Brachionus sp. Different letters above the bar indicate a significant difference (P<0.05) based on Duncan's Multiple Range Test (DMRT). DAB is the expressed protein value from semi-qualitative calculations using image J.

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. Remarks: brown color indicates the expression of IL-6.

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 67.20±0.31%, P2 by 53.37±0.51%, P3 by 58.28±0.43%, P4 by 44.77± 0.51%, P5 of 42.33±0.49% and the results of P6 treatment in the expression of interleukin-6 had the lowest value of 37,57±0.21%. This indicates that the protein *Brachionus* sp. can affect interleukin-6 levels in infected fish characterized by a decrease in interleukin-6 levels as an inflammatory response.

Decreased levels of IL-6 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 to restrain the attachment of the virus and lyse the protein from the virion (opsonin). This is following the opinion of Rich *et al*. (2008), binding of virions by proteins that are in plasma or the body will inhibit the attachment of the virus in host cells by lysing virions (opsonins). In addition, the decreased condition after protein administration was thought to be caused by the protein being able to inhibit the increase in the number of IL-6 by limiting the synthesis of the IL-6 transcription gene. The transcriptional gene is Nf-Kb, where Nf-Kb plays a role in regulating inflammatory genes such as IL-6, COX-2, and TNF-α so that when Nf-Kb activation is inhibited it will eventually decrease pro-inflammatory genes (Supriono *et al*., 2018). However, we limited our observations during the study, so we did not test Nf-Kb levels.

CONCLUSION

It can be concluded that the process of giving the protein contained in *Brachionus* sp. can affect Viral Nervous Necrosis infection in groupers. The effect shown is to reduce the infectious process that was characterized by a decrease in interleukin-6.

CONFLICT OF INTEREST

There is no conflict of interest among the authors regarding the writing and publishing of this manuscript.

AUTHOR CONTRIBUTION

Dwi Retna Kumalaningrum conducted the experiment, analyzed the

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

data, drafted the initial manuscript, and approved it for publication. Uun Yanuhar reviewed the draft, contributed to the study design, and approved the final manuscript for publication. Mohammad Musa was involved in the study design, reviewed the draft manuscript, and approved it for publication. Nur Fauziyah Martiningsih also experimented with and analyzed the data.

ACKNOWLEDGMENT

We thank Brawijaya University and all parties who have aided in the completion of the research.

REFERENCES

Baratawidjaja, K.G. and Rengganis, I., 2018. Imunologi Dasar. Fakultas Kedokteran UI Press. Jakarta.

Chen, Y.M., Wang, T.Y. and Chen, T.Y., 2014. Immunity to Betanodavirus Infections of Marine Fish. *Development & Comparative Immunology*, *43*(2), pp.174-183. https://doi.org/10.1016/j.dci.2013.07.019

Dixit, A., Cheema, H., George, J., Iyer, S., Dudeja, V., Dawra, R. and Saluja, A.K., 2019. Extracellular release of ATP Promotes Systemic Inflammation During Acute Pancreatitis. *American Journal of Physiology- Gastrointestinal and Liver Physiology*, *317*(4), pp.G463-G475. https://doi.org/10.1152/ajpgi.00395.2018

Dong, Y.W., Jiang, W.D., Liu, Y., Wu, P., Jiang, J., Kuang, S.Y., Tang, L., Tang, W.N., Zhang, Y.A., Zhou, X.Q. and Feng, L., 2017. Threonine deficiency decreased intestinal immunity and aggravated inflammation associated with *NF-κB* and target of rapamycin signalling pathways in juvenile grass carp (*Ctenopharyngodon idella*) after infection with *Aeromonas hydrophila*. *British Journal of Nutrition*, *118*(2), pp.92-108. https://doi.org/10.1017/S0007114517001830

Hamre, K., 2015. Nutrient profiles of rotifers (*Brachionus* sp.) and rotifer diets from four different marine fish hatcheries. *Aquaculture*, 450, pp.136-142. https://doi.org/10.1016/j.aquaculture.2015.07.016

KKP, 2021. Total Produksi Ikan Kerapu di Jawa Timur. https://statistik.kkp.go.id/ [Accessed May 4, 2021].

Kuo, H.C., Wang, T.Y., Chen, P.P., Chen, Y.M., Chuang, H.C. and Chen, T.Y., 2011. Real-Time Quantitative PCR Assay for Monitoring of Nervous Necrosis Virus Infection in Grouper Aquaculture. *Journal of Clinical Microbiology*, *49*(3), pp.1090-1096. https://doi.org/10.1128/jcm.01016-10

Lee, J.K., Hong, S., Jeon, J.K., Kim, S.K. and Byun, H.G., 2009. Purification and Characterization of Angiotensin I Converting Enzyme Inhibitory Peptides Frim The Rotifer, *Brachionus rotundiformis*. *Bioresource Technology*, *100*(21), pp.5255-5259. https://doi.org/10.1016/j.biortech.2009.05.057

Liu, X.D., Huang, J.N., Weng, S.P., Hu, X.Q., Chen, W.J., Qin, Z.D., Dong, X.X., Liu, X.L., Zhou, Y., Asim, M., Wang. W.M., He, J.G. and Lin, L., 2015. Infections of nervous necrosis virus in wild and cage-reared marine fish from South China Sea with unexpected wide host ranges. *Journal of Fish Diseases*, *38*(6), pp.533-540. https://doi.org/10.1111/jfd.12265

Park, J.C., Kim, D.H., Lee, Y., Lee, M.C., Kim, T.K., Yim, J.H. and Lee, J.S., 2020. Genome-wide Identification and Structural Analysis of Heat Shock Protein Gene Families in The Marine Rotifer *Brachionus* spp.: Potential Application in Molecular Ecotoxicology. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *36*, 1000749.

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.

https://doi.org/10.1016/j.cbd.2020.100749

Rantam, F.A., 2003. Metode Imunologi. Surabaya: Airlangga University Press.

Rich, R.R., Fleisher, T.A., Shearer, W.T., Schroeder Jr, H.W., Frew, A.J. and Weyand, C.M., 2012. *Clinical immunology e-book: principles and practice*. Elsevier Health Sciences.

Rumengan, I.F.M., Budiyanto, Modaso, R., M., Dewanto, D. and Limbong, D., 2012. Mekanisasi Sitem Panen Pada Kultur Massal Rotifer, *Brachionus rotundiformis*. *Jurnal Riset Akuakultur*, *7*(1), pp.111-119. http://dx.doi.org/10.15578/jra.7.1.2012.111-119

Schacht, V. and Kern, J.S., 2015. Basics of Immunohistochemistry. *Journal of Investigative Dermatology*, *135*(3), pp.1-4. https://doi.org/10.1038/jid.2014.541

Sembiring, S.B.M., Hutapea, J.H., Muzaki, A., Wardana, I.K., Astuti, N.W.W. and Andamari, R., 2014. Reproductive Aspects of Cultured Humpback Grouper (*Cromileptes altivelis*) for Supporting Seed Production. *Indonesian Aquaculture Journal*, *9*(1), pp.1-8. http://dx.doi.org/10.15578/iaj.9.1.2014.1-8

Srivastava, A., Hamre, K., Stoss, J., Chakrabarti, R. and Tonheim, S.K., 2006. Protein content and amino acid composition of the live feed rotifer (*Brachionus plicatilis*): With emphasis on the water soluble fraction. *Aquaculture*, *254*(1-4), pp.534–543. https://doi.org/10.1016/j.aquaculture.2005.11.014

Supriono, Pratomo, B. and Praja, D.I., 2018. Pengaruh Kurkumin Terhadap Kadar Nf-Kb dan Derajat Fibrosis Hati pada Tikus Fibrosis Hati. *Jurnal Penyakit Dalam Indonesia*, *5*(4), pp.174-183. https://doi.org/10.7454/jpdi.v5i4.271

Sutarmat, T. and Yudha, H.T. 2013. Analisis Keragaan Pertumbuhan Kerapu Hibrida Hasil Hibridisasi Kerapu Macan (*Epinephelus fucoguttatus*) dengan Kerapu Kertang (*Epinephelus lanceolatus*) dan Kerapu Batik (*Epinephelus microdon*). *Jurnal Riset Akuakultur*, *8*(3), pp.363-372. http://dx.doi.org/10.15578/jra.8.3.2013.363-372

Tanaka, T., Narazaki, M. and Kishimoto, T., 2014. Il-6 in Inflammation, Immunity, and Disease. *Cold Spring Harbor Perspectives in Biology*, *6*(10), a016295. https://doi.org/10.1101/cshperspect.a016295

Weiss, E.L., Kurischko, C., Zhang, C., Shokat, K., Drubin, D.G. and Luca, F.C., 2002. The *Saccharomyces cerevisiae* Mob2p–Cbk1p kinase complex promotes polarized growth and acts with the mitotic exit network to facilitate daughter cell–specific localization of Ace2p transcription factor. *The Journal of Cell Biology*, *158*(5), pp.885-900. https://doi.org/10.1083%2Fjcb.200203094

Yanuhar, U., Musa, M., Junirahma, N.S., Caesar, N.R., Setiawan, F. and Sumsanto, M., 2019. The potential of *Brachionus* sp. for Koi fish (*Cyprinus carpio*) cultivation infected by *Myxobolus* sp. *AIP Conference Proceedings*, *2120*, 0800018. https://doi.org/10.1063/1.5115756

Yuwanita, R., Yanuhar, U. and Hardoko, 2013. Pathognomonic of Viral Nervous Necrotic (VNN) Virulence on Larvae of Humpack Grouper (*Cromileptes altivelis*). *Advances in Enviromental Biology*, *7*(6), pp.1074-1081. https://link.gale.com/apps/doc/A346926568/HRCA?u=anon~a7edf82&sid=googleScholar&xid=83fb6d80

Cite this document Kumalaningrum, D.R., Yanuhar, U., Musa, M. and Martiningsih, N.F., 2024. Immunological Response of Interleukin-6 on Cantang Hybrid Grouper (*Ephinephelus* sp.) By Induction Of Protein *Brachionus* sp. *Journal of Aquaculture and Fish Health*, *13*(3), pp.440-449.