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FEEDING *OF ULVA LACTUCA EXTRACT* AGAINST THE IMMUNE PARAMETERS AND SURVIVAL OF DUCK GROUPER FISH (*Cromileptes altivelis*)

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Abstract: High mortality in humpback grouper infected with *Vibrio alginolitycus* disease is a major problem in farming activities. The aim of this research was to examine the effect of oral administration of *Ulva lactuca* extract in feed to improve the non-specific immune system and survival of *Vibrio alginolitycus*. This research was carried out from July to December 2023 at the Tual State Fisheries Polytechnic Campus. The method used was a Completely Randomized Design (CRD) method with 3 treatments, namely treatment A Ulva extract 1 gr/kg feed, treatment B 2 gr/kg feed and treatment C 3 gr/kg feed, plus 2 treatments as controls, namely K(+) was not fed Ulva extract, was challenged with *Vibrio alginolyticus* bacteria 108 CFU/ml and K (-) was not fed Ulva extract, was not challenged but was injected with PBS. The results of the study showed that oral administration of *Ulva lactuca* extract containing antibacterial compounds was thought to be able to increase the non-specific body defense system of humpback grouper, characterized by increased levels of hemoglobin (Hb), total erythrocytes, total lymphocytes found in treatment C at a dose of 3 g/kg feed. and the best survival in treatments B and C was 80%.
Keywords: *Ulva lactuca*, Humpback Grouper, *Vibrio alginoliticus*

1. INTRODUCTION

Duck Grouper (*Cromileptes altivelis*) is one of the leading commodities in fisheries and has a high selling value so that the demand continues to increase every year. This allows the cultivation system to be carried out intensively, with a high chance of disease in fish. Fish diseases are one of the main problems that cause losses in the cultivation business. The large losses due to disease cases are quite high due to bacterial attacks. Vibrio *alginolyticus bacteria* include gram-negative bacteria. The mechanism of inhibition of macroalgae anibacterial compounds against the growth of *V. alginolyticus* bacteria is suspected to be due to damage to the peptidoglycan component in bacterial cells, so that the cell wall layer is not formed completely and causes cell death (Mazni et al., 2018). Many efforts have been made to overcome diseases in farmed fish, one of which is by using natural ingredients that contain antioxidants. Antioxidants are chemical compounds or components that in certain levels or amounts are able to inhibit or slow down damage due to oxidation processes and are needed to prevent free radical attacks (Rompas and Gasah, 2022). One of the marine plants that

has a high antioxidant content is macroalgae of the Ulva *lactuca type*. *Ulva* sp. is one of the types of macroalgae that are widely found in Indonesian waters. The waters of Tual City have many macroalgae of *the Ulva* sp. And the number is very abundant, because the benefits and content are not yet known in full by the public. *Ulva* sp. belongs to *father seaweed*, which is edible seaweed, so another name for *ulva* is seacress. The nutritional composition *of Ulva lactuca* is ash content 26.63%, moisture content 14.57%, *crude fiber* 3.12%, lipid 0.47%, protein 16.51% and carbohydrates 38.7% and the active compounds it contains, including: flavonoids, steroids, triterpenoids, saponins, alkaloids and phenol hydroquinone (Liswandari et al., 2018).

The results of phytochemical tests of 70% ethanol extract *of Ulva Lactuca* show the presence of alkanoids, saponins, flavonoids, triterpenoids and steroids that are effective for antioxidants and have a very strong power for antioxidant inhibition with an average IC50 value of 46.68 ppm, so that it can be used as a sustainable food source (Rompas and Gasah, 2022). In line with research conducted by Metungun, et al., (2023) that phytochemical studies of *Ulva lactuca seaweed* found active compounds suspected to be antibacterial, namely alkaloids, flavonoids, steroids, tannins and saponins. The antibacterial test of green algae (*Ulva* sp.) with solvents of diethyl ether, ethyl acetate, and ethanol in inhibiting the growth of *Staphylococcus aureus* bacteria obtained the most effective results in inhibiting the growth of *Staphylococcus aureus bacteria* was most effectively inhibited by 96% ethanol extract with a concentration of 750 ppm with an inhibition zone of 9.56 mm. The antibacterial activity of green algae extract (*Ulva* sp.) in inhibiting *Staphylococcus aureus bacteria* is due to the presence of secondary metabolite compounds including triterpenoids, flavonoids, and saponins (Liswandari et al., 2018).

From the research that has been carried out above, the formulation of the problem that will be studied is how the effect of giving *Ulva lactuca* extract with different doses in duck grouper fish feed is able to increase the specific immune system of duck grouper fish and survival against *V. alginolitycus bacteria*. The purpose of this study is to test the effect of administering the best dose of *Ulva* sp. extract in feed to increase the non-specific immune system and immunity to *V. alginolitycus*.

2. RESEARCH METHODS

Time and Place

This research will be carried out from July to December 2023 at the Laboratory of Fish Pests and Diseases of the Tual State Fisheries Polytechnic.

Research Preparation

Manufacture of *Ulva* sp.

This research process began with sample preparation. Ulva samples were taken from the waters of the city of Tual. All parts *of Ulva* are taken, then washed clean, air dried and mashed by blending and sifting. Then the extraction process is continued with the maceration method. By soaking *Ulva* powder with 70% ethanol solvent in a ratio of 1:4 (Rompas and Gasah, 2022), until completely submerged for ± 24 hours. Then filtered with filter paper, the residue is macerated again in the same way up to 3 times. The resulting maceration or filtrate extract, is held together and evaporated, to separate the solvents. then evaporated and concentrated using *a rotary evaporator* with a temperature of 400C until a concentrated sample of 70% ethanol extract was obtained. Next, pressing and drying is carried out using a tray until dry then ground and filtered/sifted using a fine sieve (100 mesh size) so that it becomes flour. *Weighing Ulva* flour as many doses as each dose. How to mix *ulva* sp. *extract.* In the feed, the flour is weighed according to the treatment dose, mixed into the pellet feed (standard) evenly, put a little splash of water so that the flour and pellet feed can be mixed after it is dried at room temperature. The feed is ready to use, then the rest is put in a plastic bag then stored in the refrigerator at 4oC until it is ready to be used. *Ulva* excast feed is ready to be used for further treatment.

Fish and Container Preparation

The fish used was 15-17 cm duck grouper size as many as 100 heads. Before use, the fish are first adapted in an outdor tub equipped with an aerator. During fish rearing they are given commercial feed 2 times a day. The containers used are in the form of a 60 x 30 x 40 cm2 aquarium with 15 aquariums and one aquarium with 5 fish. Before use, the aquarium is washed using detergent and sterilized with 100 ppm chlorine for 24 hours, clean until completely clean, add seawater and give it strong aeration before using the aquarium. The fish were adapted in the aquarium for two weeks before the feeding treatment of *Ulva* sp. extract.

V. alginolyticus *bacteria* were obtained from the Laboratory of Fish Pests and Diseases of the Tual State Fisheries Polytechnic. Before being used for testing, the bacteria were increased in virulence by re-injecting them into healthy live fish and then isolated again using an ose needle to the kidneys then cultured in TCBS media and incubated for 24 hours at room temperature. To obtain a pure culture, each bacterial colony that grows separately and has a different morphology is isolated back into an oblique TCBS medium and incubated at room temperature for 24 hours.

Research implementation

This study consisted of 5 treatments and each treatment was repeated 3 times. Fish per aquarium as many as 5 fish/aquarium. Treat the administration of *Ulva* extract in feed with the following dosage;

A. Administration of *Ulva* extract at a dose of 1 gr/kg of feed (Rompas and Gasah, 2022). tested for *V. alginolyticus*
B. Administration of *Ulva extract* with a dose of 2 gr/kg of feed (Rompas and Gasah, 2022). tested against *V. alginolyticus*
C. Administration of *Ulva* extract at a dose of 3 gr/kg of feed (Rompas and Gasah, 2022). tested for *V. alginolyticus*

K (+). Commercial pellet feeding without *Ulva* extract, tested for *V. alginolyticus* as a positive control

K (-). Commercial pellet feeding, PBS injected as negative control.

Feeding of fish was carried out twice a day with an FR (*feeding rate)* of 3% of biomass weight for four weeks after which a challenge test was carried out with *Vibrio alginolyticus* bacteria 108 CFU/head, negative control (K-) which was only injected with PBS. Blood sample measurements were carried out at weeks 0, 1, 2, 3 and 4. The immune parameters measured are: hemaglobin, total erythrocytes, total leukocytes and differential leukocytes. After four weeks of retention, a challenge test was carried out with *V. alginolyticus* bacteria 108 CFU/tail and observations were made on the level of fish behavior and the survival rate of fish until the 14th day after the challenge test.

Data Analysis

The design used in this study was a complete random design (RAL) consisting of 5 treatments and 3 replications. To find out the difference between each treatment on immune and survivor parameters, the diversity was analyzed using ANOVA. If there is a difference between treatments, Duncan will continue testing using SPSS.

3. RESULTS AND DISCUSSION

Immune Parameters

Hemoglobin level (g%)

The results of the study show that the observation of hemoglobin levels during maintenance can be presented in Table.1 below.

Table 1. Average Total Hemoglobin Level (g%) in Each Treatment

Treatment	Week 1						
	M0	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**
A	5.67 ±1.15a	7.5±0.50a	7.5±0.50a	7.17±0.29a	7.17±0.29a	3.83±0.29b	4.90±0.66bc
B	5.67±1.15a	7.17±0.50a	7.17±0.29a	7.17±0.29a	7.33±0.58a	4.83±0.58a	5.17±0.29abc
C	5.67±1.15a	6.83±1.04ab	7.17±0.76a	7.33±1.15a	7.67±0.58a	4.90±0.79a	5.83±0.29b
K (+)	5.67±1.15a	6.33±0.58b	6.5±0.50b	5.33±0.58b	5.33±0.58b	3.57±0.50b	4.33±0.29c
K (-)	5.67±1.15a	6.33±0.58b	6.5±0.50b	5.67±0.58b	5.33±0.58b	5.50±0.50ab	5.67±0.58ab

Table 1, shows that in the 1st to 4th week there was an increase in the amount of hemoglobin levels in treatment A, B and C when compared to K(+) and K(-). However, in week 5, there was a decrease in hemoglobin in all treatment of Vibrio *alginolyticus* bacterial infection 108 CFU/ml. The results of this study showed that a decrease in hemoglobin levels followed by a decrease in total erythrocytes in the blood was indicated by the occurrence of lysis in red blood cells when observed under a microscope indicating that the fish were in an unhealthy condition as seen from the symptoms caused, namely the response of fish to feed was very low, fish were not active in swimming and tended to be silent if there was noise around the aquarium the fish were seen strains and swimming not directed until it can hit the wall of the aquarium container. Physiologically, the level of hemoglobin in the blood of fish determines the level of resistance of the fish body in relation to its close relationship with the binding power of oxygen in the blood. These results are according to the research of Ollin et al., (2021) that a decrease in hemoglobin levels followed by a decrease in total erythrocytes in the blood is shown by the occurrence of lysis in red blood cells, allegedly due to the rupture of red blood cells due to a bacterial toxin in the blood called haemolysin. This toxin will lyse and destroy hemoglobin (Figure, 1990 *in* Seuk et al., 2021), but in the 6th week there was an increase in hemoglobin levels for all treatments, namely treatment A by 4.90±0.66bc g%, treatment B by 5.17±0.29abc g%, treatment C by 5.83±0.29bg% K (+) by 4.33±0.29c g% and K (-) by 5.67±0.58ab g%.

Based on the results of the Statistical Test, it was shown that the addition of different ulva extracts gave a significant difference to grouper hemoglobin during the maintenance period ($P<0.05$). Where treatment C produced the highest average immunity and produced a noticeable difference with the treatment K(+) and A but between treatment B, C and K(-) there was no real difference. According to Metungun, et al., 2023, the ulva lactuca *type seaweed* used was found to have active compounds that are suspected to be antibacterial, namely alkaloids, flavonoids, streroids, tannins and saponins. It is supported by Bontjura, 2015 that the steroid functions as an

antibacterial that can damage cell membranes so that they become brittle and lysis. Hemoglobin in the blood is strongly correlated with the number of erythrocytes because hemoglobin is part of erythrocytes that function as oxygen binders and transport oxygen and carbon dioxide in the blood (Ratna, 2018).

Total Erythrocytes (x 106 cells/mm3)

The results of the observation of total erythrocytes during the study can be seen in Table 2.

Table 2. Average Total Erythrocytes at All Treatments

Treatment	Week 1						
	M0	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**
A	1.07±0.29a	1.49±0.22ab	1.82±0.27a	1.8±0.09ab	1.81±0.18a	0.94±0.12cd	1.14±0.20bc
B	1.07±0.29a	1.64±0.49a	1.66±0.26ab	1.74±0.26a	1.87±0.15a	1.07±0.22c	1.12±0.18bc
C	1.07±0.29a	1.62±0.67a	1.66±0.19ab	2.05±0.66b	2.17±0.34b	1.46±0.20b	1.91±0.78a
K (+)	1.07±0.29a	1.18±0.16b	1.32±0.14b	1.75±0.08a	1.78±0.10a	0.75±0.15d	0.84±0.18c
K (-)	1.07±0.29a	1.18±0.16b	1.32±0.14b	1.75±0.08a	1.78±0.10a	1.85±0.07a	1.67±0.21ab

The results of the Statistical Test showed that the addition of different ulva extracts made a significant difference to the total erythrocytes of duck grouper during the maintenance period ($P<0.05$). Where treatment C produced the highest immune average of 1.91±0.78a and produced a marked difference with treatment K (+) 0.84±0.18c but between treatment A 1.14±0.20ab, B 1.12±0.18ab and K(-) 0.84±0.18c treatment there was no significant difference. This increase in total erythrocytes is suspected to be due to the feeding of ulva extract carried out daily during maintenance with the right dose, where the ulva extract given contains alkaloid compounds, flavonoids, streroids, tannins and saponins as described at the beginning. The main function of erythrocytes is to transport hemaglobin and play a role in transporting oxygen from the gills or lungs to the tissues, and contains a large amount of carbonic acid that functions to catalyze the reaction between carbon dioxide and water, so that the blood can transport carbon dioxide from the jaingal to the gills. (Sadikin, 2002).

In the post-infection K (+) treatment, there was a very drastic decrease in total erythrocytes. In line with the research of Matofani (2013). Pathogenic bacteria cause a decrease in oxygen levels in the blood and directly decrease the value of hemoglobin levels in fish blood. The low number of erythrocytes can be influenced by several factors including nutritional conditions, physical activity and microorganism infections (Ngaddi, 2013). The results of this study show that fish infected with bacteria show symptoms such as low feed response, fish become stressed and swim directionally so that they can hit the aquarium wall. Thus, the physiology of the fish will be disturbed, so erythrocytes

will not be able to perform their functions properly. In accordance with the statement of Ollin et al., (2021) that erythrocytes are blood components that play a role in supplying food and oxygen to the entire body, so that when an infection occurs, the metabolic process in the body will be disrupted because the body has to fight incoming diseases. To overcome the decrease in total erythrocytes, natural ingredients are needed that can improve the physiological condition of the fish through feeding ulva extract so that it can treat fish naturally.

Total Leukocytes (x103 cells/mm3)

The average observation of total leukocytes during the study can be seen in Table 3.

Table 3. Average Total Leukocytes in Each Treatment

Treatment	Week 1						
	M0	M1	M2	M3	M4	M5	M6
A	8.98±1.43a	10.13±0.63a	10.13±0.59ab	9.87±0.78a	10.48±1.37a	14.08±1.32ab	13.59±1.21ab
B	8.98±1.43a	10.97±1.95a	10.66±1.24ab	8.92±0.28b	10.00±0.54a	12.45±0.45b	11.63±1.17bc
C	8.98±1.43a	7.27±0.89bc	8.24±0.59bc	8.47±0.52b	9.7±0.46b	12.15±0.65b	10.71±0.52c
K (+)	8.98±1.43a	6.79±0.56c	7.06±1.67c	9.66±0.76a	11.22±1.17a	14.43±1.59a	14.86±0.59a
K (-)	8.98±1.43a	6.79±0.56c	7.06±1.67c	9.66±0.76a	11.22±1.17a	9.76±0.60c	11.29±1.49BC

Table 3, shows that the average total leukocytes in the treatment of feeding ulva lactuca extract are highly variable and tend to increase after post-infection with *Vibrio alginolyticus* bacteria. When viewed in the 5th week post-infection, there was an average increase in total leukocytes at all treatments. However, K (+) has the highest value of 14.43±1.59a x 103 cells/mm3. These results suggest that an increase in total leukocytes is in line with an increase in infections in groupers. In accordance with the clinical symptoms found in the body parts that have suffered physical damage, especially in the area of the injection site, inflammation/lumps appear, peeling skin, hemorage (bleeding) and there are ulcers on the body parts of the fish until they die in line with the survival rate obtained at (+) very low at 40%. Leukocytes are responsible for the body's immune system and are responsible for destroying objects that are considered foreign and harmful by the body such as bacteria.

A'yunin et al., (2020) explain that leukocytes are the first defense agents in the body when an infection occurs. When a foreign object enters, the body signals so that white blood cells will be produced in large quantities and directly directed to the location of the infection to provide defense for the body against the attack of the disease. The results of the Statistical Test, showed that the addition of different ulva extracts gave a significant difference to grouper leukocytes during the maintenance period ($P<0.05$). Where the K (+) treatment produced the highest average immunity and produced a noticeable difference with the C and K(-) treatments, but between the A, B and K(-

) treatments, there was no real difference. The results of the statistical test showed that in the 6th week there was a decrease in total leukocytes in treatment C when compared to K (+), it is suspected that the type of *ulva* added to the feed contains antibacterial compounds including Antibodies that act as the fish's body's defense system in paralyzing incoming pathogens (Sukenda et al. 2014). Flaphonid. Flavonoids are thought to function as antibacterial agents by forming extracellular proteins from complex compounds that are soluble, so they can damage bacterial cell membranes and subsequently release intracellular compounds. In addition, flavonoids also inhibit the energy system and bacterial recovery, as bacteria need energy to absorb various metabolites, as well as to carry out the biosynthesis of macromolecular compounds in cells (Ngosi, et al., 2009). Alkaloids are thought to damage cell membranes and bacterial cell proteins causing bacteria to die (Brooks et al., 2005).

Differential Leukocytes

Observations of differential leukocytes can be seen in table 4.

Table 4. Total Differential Leukocytes in Each Treatment

Blood Components (%)	Treatment	Week 1						
		M0	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**
Lymphocytes	A	76.4	75.01	76.66	75.5	81.5	69.3	69.83
	B	76.4	76.33	75.33	76.67	80.56	76.3	76.3
	C	76.4	75.64	76.7	76.5	81.67	79.33	78.33
	K (+)	76.4	76.67	75.33	70.67	76.8	68.67	68.67
	K (-)	76.4	74.67	74.34	75.67	76.3	75.67	75.67
Netrofil	A	10.3	9.33	9	12.33	7.3	14.2	14.8
	B	10.3	12	12	12	8.42	11.35	11.35
	C	10.3	11.86	12.64	12.33	7.33	9.17	10.17
	K (+)	10.3	9.5	12.37	13.33	9.33	15.33	15.33
	K (-)	10.3	12	12	11.66	9.5	11.66	11.66
Monocytes	A	12.2	13	13.3	12.17	10.2	15.5	14.37
	B	12.2	10.67	11	11.33	10.01	11.35	11.35
	C	12.2	11.5	10.66	11.17	11	10.5	10.5
	K (+)	12.2	12.5	12.3	14.67	12.87	14.67	14.67
	K (-)	12.2	12.33	11.33	12.67	9.2	12.67	12.67
Platelets	A	1.1	2.66	1.04	0	1	1	1
	B	1.1	1	1.67	0	1.01	1	1
	C	1.1	1	0	0	0	1	1
	K (+)	1.1	1.33	0	1.33	1	1.33	1.33
	K (-)	1.1	1	2.33	0	5	0	0

The results of Table 4 show that the blood components of lymphocytes, neutrophylls, monocytes and platelets have a very variable percentage of presence in each treatment, but the dominant ones are lymphocytes. In week 1 to week 4, there was an increase in lymphocyte percentage in all treatments, but in week 5 after *infection with V. alginolyticus bacteria,* there was a decrease in lymphocyte count for all treatments except K(-) not injected with bacteria so that it tended to be stable. Lymphocytes circulating in the blood and tissues originate from the thymus and peripheral lymphoid organs such as the kidneys and spleen. Damage to these producing organs will inhibit the formation of lymphocytes. Lack of lymphocytes can decrease the concentration of antibodies and can increase disease attacks (Fujaya, 2004). Sukenda et al., (2014) that antibodies act as the defense system of the fish's body in paralyzing incoming pathogens. In week 6, there was an increase in the percentage of lymphocyte count for treatment A, B, C and K (-) when compared to K (+). This shows that feeding Ulva extract contains antioxidants that are good for the formation of antibodies so that the fish's body's defense system is strong in the face of disease attacks. According to Baratawidjaja (2006), the increase in lymphocytes plays a considerable role in increasing the immune response or resistance of the fish body to infection. Lymphocytes are not phagocytic but play an important role in the formation of antibodies. In line with Harpeni's (2015) research, the increased lymphocyte presentation indicates that the fish's non-specific immune response is triggered to fight bacterial infections.

Netrophils have a highly variable percentage and tend to increase in the 5th week after infection with *Vibrio alginolyticus bacteria.* According to Baratawidjaja (2006), neutrophil cells are only in circulation for less than 48 hours before migrating and moving very quickly to the area of infection. Under normal conditions, neutrophil populations are stored for emergencies in the lymphoid tissue of the kidneys. When a stimulus occurs as a result of inflammation or inflammation, the cells will migrate into the bloodstream and then enter the inflammatory wound. Then pathogenic bacteria will be phagocytes by the cell and then included in phagosomes which contain enzymes acid hydrolase, myeloperoxidase and lysozyme which will lyse and digest pathogenic bacterial cells. The results of this study are supported by Iwama (1996) that when the initial attack of pathogenic bacteria occurs, the cells that first reach the area of infection are neutrophils. Neutrophils move faster compared to monocytes and can reach the area of infection within 2-4 hours. At this time, defense or phagocytic cells are dominated by neutrophils. However, after a few hours later (about 7-8 hours) monocytes dominate.

Monocytes are able to enter the tissue and differentiate into macrophages. The role of

monocytes is very important as the main phagocyte cell in destroying various pathogens that attack and also acts as antigen presenting cells (APCs) which function to present antigens to lymphocyte cells (Kresno, 2001; Kollner et al. 2002). Monocytes are cells that are more powerful at phagocytes, particles or antigens compared to neutrophils. Monocytes that differentiate into macrophages in tissues are even able to phagocyte large particles in large numbers up to 100 bacteria (Fujaya, 2004). Meanwhile, platelets have a very low percentage of presence when compared to neutrophils and monocytes. Platelets play an important role in the blood clotting process and also function to prevent the loss of body fluids in surface damage. Platelets are not common in the blood component under normal conditions, but if there is a response due to treatment, the number of platelets can increase sharply. Platelets have an important role in inflammation Decreased platelet count is accompanied by bleeding (Baratawidjaja 2006).

Survival of duck grouper fish during Research

Observations of the survival of duck grouper fish during rearing, can be presented in Figure 1.

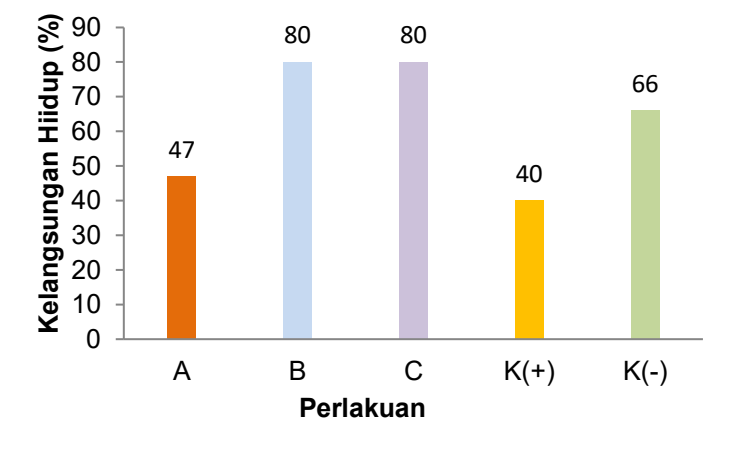

Figure 1. Survival of Duck Grouper Fish

Based on Figure 1, it shows that oral *feeding of Ulva* can affect the survival rate of duck grouper. When viewed in treatment A, the survival rate of duck grouper is 47%, treatment B is 80%, treatment C is 80%, K(+) treatment is 40% and K(-) is 66%. Based on the results of statistical tests, treatment B and C were the same, which was 80.00±0.00a%, in stark contrast to treatment K (-) of 66.67±23.09ab%, treatment A of 46.67±30.55ab%, and K (+) of 40.00±20.00b%. These results show that the treatment of feeding *ulva* extract with a certain dose has the best influence on the survival of duck grouper. This means that feeding *Ulva extract* containing antioxidant compounds is able to increase the production of duck grouper fish antibodies so that it can increase the non-specific immune response of triggered fish to fight bacterial infections. In line with research

conducted by Brooks et al., (2005) that alkaloids are suspected to damage the cell membranes and proteins of bacterial cells, causing bacteria to die. Flavonoids are thought to function as antibacterial agents by forming extracellular proteins from complex compounds that are soluble, so they can damage bacterial cell membranes and subsequently release intracellular compounds. In addition, flavonoids also inhibit the energy system and bacterial recovery, as bacteria need energy to absorb various metabolites, as well as to carry out the biosynthesis of macromolecular compounds in cells (Ngozi et al., 2009). Steroids are antibacterial that can damage cell membranes so that they become brittle and lysis (Bontjura et al., 2015).

4. CONCLUSIONS AND SUGGESTIONS

CONCLUSION

Oral feeding *of Ulva lactuca* extract containing antibacterial compounds is suspected to be able to improve the non-specific immune system of duck grouper fish characterized by increased hemoglobin (Hb) levels, total erythrocytes, total lymphocytes found in treatment C with a dose of 3 g/kg of feed and the best survival rate in treatment B and C is 80%.

SUGGESTION

Further research is needed on the frequency of feeding the best dose of ulva extract on the growth and survival of duck groupers.

5. ACKNOWLEDGMENTS

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