Blood Profile of Goldfish (*Cyprinus carpio*) Infected by *Pseudomonas Fluorescens* Through Giving Extraction of *Sargassum* sp.

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ABSTRACT

The Goldfish (*Cyprinus carpio*) is a commodity of freshwater fish that is favored by the community both for consumption and as ornamental fish. Cultivation of goldfish still has problems, namely disease attacks. Economically the problem of disease in fish is detrimental to cultivation, decreases production, quality of fish and can even cause mass death. The disease that attacks goldfish is the bacteria *Pseudomonas fluorescens*. Blood profiles can describe the health of the body of the fish, such as the number of erythrocytes, the number of leukocytes, hemoglobin levels and differential leukocytes. Seaweed is a multicellular algae containing immunologically active substances. One type of seaweed that can improve the body's immune system is *Sargassum* sp. The purpose of this study was to determine the blood profile of goldfish (*Cyprinus carpio*) infected by *Pseudomonas fluorescens* through the administration of *Sargassum* sp. This study used a Completely Randomized Design (CRD) consisting of 4 treatments and 3 replications to obtain 12 experimental units. Treatment A: infected with P. *fluorescens* without injection of extract of *Sargassum* sp., Treatment B: injection of 9% dose / fish with extract of *Sargassum* sp. and infected with P. *fluorescens*, treatment C: injection of 12% dose / fish with extract of *Sargassum* sp. and infected with P. *fluorescens* and treatment D: injection of 15% dose / fish with extract of *Sargassum* sp. and infected with P. *fluorescens*. Data from observations of white blood cells, red blood cells, leukocyte differentials and survival rates were tested using *Analysis of Variance* (ANOVA) and if significantly different then continued with the Duncan test at the 5% level. The results showed that all treatments had a significantly different effect ($p < 0.05$) on the number of leukocytes, number of lymphocyte cells and survival rates of goldfish, but did not have a significant effect on the number of erythrocytes, hemoglobin levels, number of monocyte cells and neutrophil cells . Giving extract of *Sargassum* sp. with a concentration of 15% is the best treatment compared to other treatments.

Keywords: Goldfish, bacteria, *sargassum* sp, leukocytes, survival.

1. INTRODUCTION

Goldfish (*Cyprinus carpio*) is one of the freshwater fish commodities that is very popular with the community both for consumption and as ornamental fish. The development of goldfish cultivation has been increasing lately, especially production carried out in the area of West Nusa Tenggara (NTB). This is due to the increasing demand for goldfish, both for

consumption and recreational fishing ponds. The development of goldfish cultivation can help improve people's living standards, can also expand employment and can improve community nutrition (Kholifah et al., 2012).

In the cultivation of goldfish still have problems, namely disease attacks. Economically the problem of disease in fish is detrimental to cultivation, decreases production, quality of fish and can even cause mass death. One of the diseases that attack goldfish is the bacterium *Pseudomonas fluorescens*. The bacteria Pseudomonas fluorescen includes in gram negative bacteria with a stem shape with a size of about 0.8 - 1.0 μm and are oxidative bacteria. Fish infected with the bacteria *Pseudomonas fluorescens* experience clinical symptoms of abnormalities such as protruding eyes (exoptalmia), blackened bodies, and the presence of red bruises or wounds on the surface of the body or fins sometimes even found fish experience bleeding (Hardi, 2012).

Blood profile can describe the health of the body of the fish, such as the number of erythrocytes, the number of leukocytes, hemoglobin levels and differential leukocytes. Increased immune system in fish can be seen from increasing parameters such as total erythrocytes (red blood cells), total leukocytes (white blood cells), hemoglobin levels, hematocrit levels, differential leukocytes and phagocytic indices (Puspasari, 2010).

Various ways have been done to overcome fish farming disease attacks, among others, by giving antibiotics. One such effort is to increase immunity (immunity) in fish from disease attacks. Immunostimulants play a role in activating non-specific defense mechanisms (cell mediated immunity) and specific immune responses. In addition immunostimulants increase resistance to disease by increasing specific defense mechanisms (Sakai, 1999).

Seaweed is a multicellular algae that contains immunologically active substances. One type of seaweed that can improve the body's immune system is *Sargassum* sp. According to Kastro et al., (2004) *Sargassum* sp. contains fucoidan compounds which are complex polysaccharides on brown algae cell walls and are the largest component capable of enhancing immunity by stimulating the production of immune cells, thus helping in fighting pathogenic bacteria. Besides that, Sargasum sp. contains iodine, tannin and phenol which functions to inhibit bacterial growth (Bachtiar et al., 2012). Polysaccharide and lipopolysaccharide compounds can be produced from the extraction process using water and

can increase fish resistance, as was done by Hou et al (2005) with Gracilariatenuistipitata hot water extract, Yeh et al (2006) and with *Sargassum*duplicatum extract.

Based on this, it is necessary to do a research on the blood profile of goldfish (*Cyprinus carpio*) infected by *Pseudomonas fluorescens* through the administration of *Sargassum* sp. Extract.

2. MATERIALS and METHOD

Time and place

This research was conducted on the 8th to 27th of September 2018, held at the Laboratory of Aquaculture, Aquaculture Study Program, University of Mataram.

Tools and materials

Tools and materials used in the research are glass aquariums, scales, aerators, syringes (syringes), eppendorf tubes, seser, petri dishes, drop pipettes, microscopes, small buckets, cameras, tissue boxes, hand tally counters, haemocytometers, syringes , ph meter, do meter, thermometer, goldfish (*Cyprinus carpio*), extract of *Sargassum* sp., bacteria p. *fluorescens*, aquades, artificial feed, fresh water, 10% edta, 0.1 nL solution, turk's solution, hayem's solution and giemsa dye.

Research Method

The research data collection method used is the experimental method. The research design used was a completely randomized design (CRD) consisting of 4 treatments and repeated 3 times to obtain 12 experimental units. Treatment A: Infected with P. *fluorescens* without injection of *Sargassum* sp. Extract. Treatment B: injection of 9% dose / fish with extract of *Sargassum* sp. and infected with P. *fluorescens* Treatment C: injection of 12% dose / fish with extract of *Sargassum* sp. and infected with P. *fluorescens* Treatment D: injection of 15% dose / fish with extract of *Sargassum* sp. and infected with P. *fluorescens*

Research procedure

1. **Tools and Materials Preparation**

1). Preparation of Test Fish and Maintenance Containers

The aquarium used in this study is 50x35x40 cm in size, serves as a place to accommodate and maintain goldfish during the study. Before use, the aquarium is cleaned first, then filled with fresh water. The goldfish used is purchased from the Fish Seed Hall with a size of 10-13 cm each and put into a maintenance container with a stocking density of 5 fishes / aquarium.

2). Making *Sargassum* sp. Extract and preparation of the bacterium *Pseudomonas fluorescens*

Extraction used in this study is by maceration. The powder is weighed as much as 50 grams and put in a 500 ml Erlenmeyer glass. Maseration is done with 90% ethanol solution as much as 100 ml. *Pseudomonas fluorescens* bacteria are grown on Agar (NA) Nutrient media. NA media that has been provided is sterilized using autoclave with a temperature of 1210 C for 24 hours. The density used is 1010 CFU / ml. According to Utami (2013) the density of these bacteria has been very susceptible to death, because the 4th day after infection with fish there are those who die. Giving *Sargassum* sp. Extract the fish is done by injection according to the dose of each treatment and the injection point is in the dorsal part of the fish's body. Injections are carried out in the first week and at the beginning of maintenance. Furthermore, fish infection was carried out in the second week by means of a fish maintenance container with 1 ml / aquarium of *Pseudomonas fluorescens*.

Parameter of research

The parameters used to test the results of this study are immune parameters (calculation of white blood cell count, red blood cell count, hemoglobin and differential levels of leukocytes, lymphocytes, monocytes, neutrophils), fish survival rates and water quality measurements.

1). Calculation of the number of white blood cells

The formula used to determine the total white blood cells according to Puspasari (2010) is:

$$\sum \text{SDP} = \text{Number of cells counted} \times \frac{1}{\text{large volume box}} \times \text{dilution factor}$$

$$= \frac{W1+W2+W3+W4}{4} \text{ x } 50 \text{ x } 22$$

$$= \ldots \text{ sel/mm}^3$$

2). Calculation of Total Red Blood Cells

$$\sum \text{SDM} = \text{ Number of cells counted x } \frac{1}{\text{volume kotak kecil}} \text{ x dilution factor}$$

$$= \frac{R1+ R2+ \ldots + R9+ R10}{10} \text{ x } \frac{1}{0{,}2 \times 0{,}2 \times 0{,}1 \text{ mm3}} \text{ x } 200$$

$$= \ldots \text{ sel/mm}^3$$

3). Measurement of Hemoglobin Levels

4). Leukocyte Differential Calculation

Differential leukocytes are calculated using the Rahma formula (2015).

$$\text{Percentage of Limfosit } = \frac{L}{100} x100\%$$

$$\text{Percentage of Monosit } = \frac{M}{100} x100\%$$

$$\text{Percentage of Neutrofil } = \frac{N}{100} x100\%$$

5). Survival Rate

Fish survival is calculated using the Effendie formula (1978) *in* Widiastuti (2009) as follows:

$$SR = \frac{Nt}{No} \times 100\%$$

6). Observation of clinical symptoms

Observation of fish abnormalities recorded clinical symptoms both during infection, during treatment, and during maintenance.

7). Water quality

Water quality data collection is supporting data which includes temperature, DO and pH. Data retrieval is done twice, namely in the first week of maintenance and the second week of maintenance.

Data analysis

Data from observations of white blood cells, differential leukocytes and survival rates were tested using *Analysis of Variance* (ANOVA) and if it was significantly different then continued with Duncan test at 5% real level, while water quality observation data were analyzed descriptively.

3. RESULT

Eritrocytes

The total observation of erythrocytes in the last observation showed that each treatment had increased. The results of the One-Way Annova test statistic analysis and continued with the Duncan test showed that there was no significant effect on each treatment. The results of the study can be seen in (figure 3).

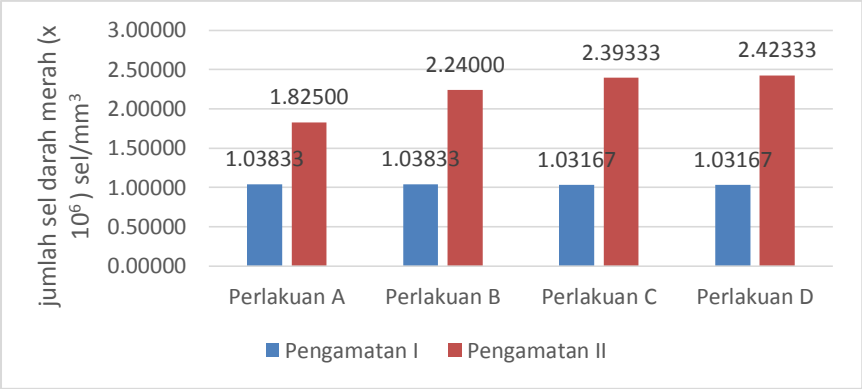

Figure 3. Graph of observation of goldfish red blood cells

Leukocyte

Observation of leukocyte counts is done twice, namely at the beginning and end of maintenance. The second observation was carried out to determine the increase in white blood cells or leukocytes, ie after the fish were given each treatment and infected with the bacterium *Pseudomonas fluorescens*. The results of the One-Way Annova test statistic analysis showed that the administration of *Sargassum* sp. and the infection of *Pseudomonas fluorescens* bacteria had a significant effect ($P < 0.05$) on leukocyte values in goldfish. After the One-Way Annova test was carried out, then continued with Duncan's advanced test analysis to find out whether there were differences between the treatment of leukocyte values (figure 4).

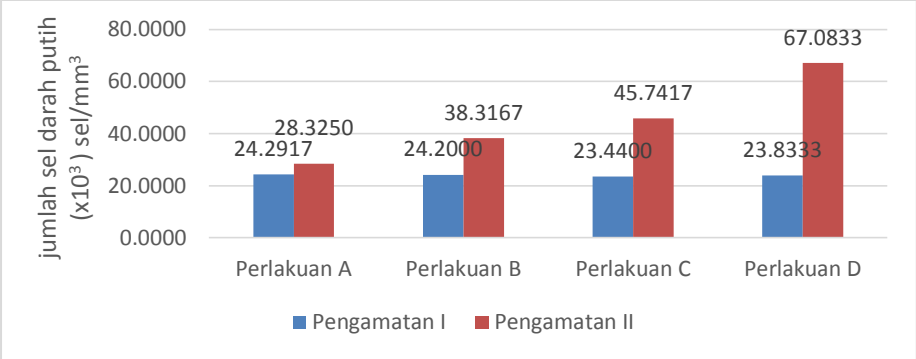

Figure 4. Graph of observation of total goldfish leukocytes

Hemoglobin

The One-Way Annova test results showed that all treatments had no significant effect. Figure 5. shows that each treatment experienced a decrease in the amount of hemoglobin. Treatment A (control) experienced the highest decrease compared to treatments B, C and D.

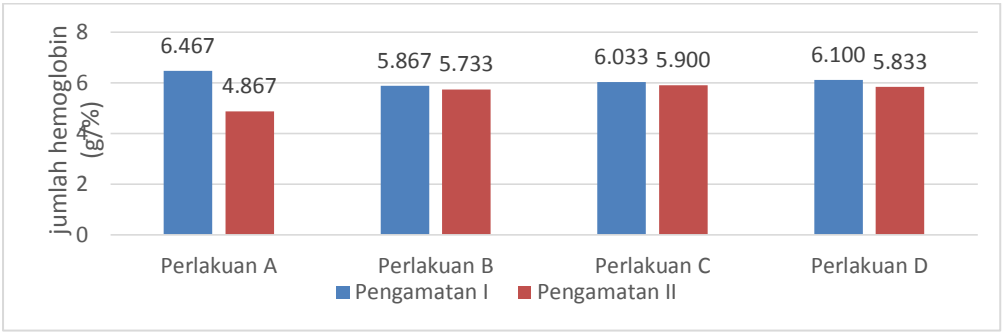

Figure 5. Graph of observation of blood hemoglobin

Differential leukocytes

Differential observation of leukocytes includes lymphocytes, monocytes, and neutrophils. Differential observation of leukocytes is carried out at the beginning of the maintenance and end of goldfish maintenance. From the results of the One-Way Annova test on differential leukocytes, there were significant differences in each treatment. Observation data can be seen in Table 3.

Table 3. Results of differential observations of leukocytes (lymphocytes, monocytes and neutrophils)

Observations	Treatments	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
Observation I (untreated and not infected with bacteria)	A	79.33	8.67	12.00
	B	80.33	8.00	11.67
	C	74.00	10.67	15.33
	D	77.67	9.33	13.00
Observation II (after treatment and infection with bacteria)	A	78.67^{a}	9.33^{a}	11.00^{a}
	B	84.33^{b}	5.00^{a}	10.67^{a}
	C	82.33^{b}	5.67^{a}	12.00^{a}
	D	85.00^{b}	4.00^{a}	11.00^{a}

Remarks: different letters indicate that there is a significant effect on each treatment

Survival Rate

The results of the One-Way Annova test statistical analysis showed that the administration of bacteria had a significant effect (P <0.05) on the Survival rate in goldfish. After the One-Way Annova test was carried out, followed by Duncan's advanced test analysis to find out whether there were differences between treatments. The value of the survival rate is shown in Figure 6:

Figure 6. Graph of the survival rate (SR) of goldfish life

Clinical Symptoms

In treatment A, fish experienced a lot of dead and infected with the bacteria P. *fluorescens*. The symptoms that occur in fish are fish begin to swim around, decreased appetite, not trending, some fish begin to weaken. Fish begin to be infected with the characteristics of the body color of the pale fish, swim around in circles, swim to the surface, fish fins begin to have bruises or bleeding, scales are released, bleeding in the operculum and there is freezing and bruising on the body.

Water quality

The water quality parameters observed were parameters of temperature, pH, and DO. Water quality from the beginning of infection, treatment and maintenance has no problems for fish, and water quality is normal. Water quality parameters can be seen in table 4 below:

Table 4. Water quality measurement data

Treatments	week I			week II		
	DO	pH	Temperature	DO	pH	Temperature
A	5,1-5,3	7,2-7,3	28,1-28,2	5,1-5,2	7,2-7,4	28,3-28,4
B	5,2	7,1-7,2	28,1-28,2	5,1-5,5	7,2-7,5	28,3-28,6
C	5,0-5,1	7,2-7,3	28,2	5,0-5,2	7,2-7,3	28,2-28,4
D	5,1-5,3	7,2-7,3	28,1-28,2	5,0-5,2	7,2-7,3	28,3-28,4

4. DISCUSSION

Erythrocytes

Observation of the number of erythrocytes or red blood cells during the study was carried out at the beginning of maintenance, before treatment was given and before infection with the bacterium *Pseudomonas fluorescens* and the second observation was carried out at the end of the study. Figure 3. shows that in each treatment the number of erythrocytes has increased after being injected with extracts of *Sargassum* sp. and infected with the bacterium *Pseudomonas fluorescens*. But there was no significant effect between treatments, so the results of this study showed that extract of *Sargassum* sp. does not affect the total number of goldfish erythrocytes. The results of red blood cells from all treatments ranged from 1,82500 x 106 - 2,42333 x 106 cells / mm3 and the number of erythrocytes is still in the normal range for fish red blood cells. In accordance with the statement of Hartika (2014) which explains that the normal range of fish erythrocytes in general is 20,000-3,000,000 cells / mm3. Red blood cells function as a distributor of food in the body and the distribution of oxygen in the body of the fish.

Leukocytes

Observation of leukocyte counts can be seen in Figure 4. Different letters in the diagram show significantly different results ($P < 0.05$). Giving extract of *Sargassum* sp. with different concentrations of goldfish tested challenged using *Pseudomonas fluorescens* bacteria significantly affected the number of leukocytes in goldfish. In treatment A was significantly different from treatment B, treatment B was significantly different from treatment C, treatment C was significantly different from treatment D. The results of

observation of total leukocytes at the end of maintenance showed that the results of each treatment had increased. Goldfish treated with *Sargassum* sp. Extract. experienced a higher increase than the control treatment or without the administration of *Sargassum* sp. extract. Based on Duncan's advanced test results, treatment D showed the best results with the highest number of leukocytes, namely 67.0833x103 cells / mm3 and significantly different from treatment C with a value of 45.7417 x103 cells / mm3, and treatment C was significantly different from treatment B with leukocyte counts 38.3167x103 cells / mm3. Furthermore, treatment B was significantly different from treatment A with the lowest number of leukocytes, which was only 28,3250 x103 cells / mm3. The number of leukocytes observed is still in the normal range. This is in accordance with the statement of Rastogi (1977) *in* Sani (2014) reporting that the number of fish leukocytes ranges from 20,000-150,000 cells / mm3 of blood.

The One-Way Annova test results showed all treatments were significantly different, because there was an increase in the number of leukocytes in each treatment, so that it could be stated that the addition of *Sargassum* sp. affect the number of white blood cells of goldfish. This is in accordance with the results of Raharjo (2014) study, that goldfish treated with the addition of *Sargassum* sp. has a very high increase in total leukocyte count compared to without *Sargassum* sp. or control treatment. Goldfish leukocytes increase because in extracts of *Sargassum* sp. contains iodine, tannin, phenol and fucoidan compounds. According to Castro et al., (2004) *Sargassum* sp. contains fucoidan compounds which are complex polysaccharides on brown algae cell walls and are the largest component capable of enhancing immunity by stimulating the production of immune cells, thus helping in fighting pathogenic bacteria. The mechanism of action of immunostimulants is when immunostimulants enter the body, immunostimulants stimulate macrophages to produce interleukins which will activate lymphocyte cells which then divide into T lymphocytes and B lymphocytes. Fucoidan compounds in *Sargassum* can stimulate the formation of immune cells in fish blood. The results of the Sani study (2014) stated that there was an increase in total leukocytes with the addition of commercial fukoidan in the main feed of tilapia, where the highest commercial fukoidan concentration (0.3%) produced the highest total leukocytes, which were 142.67 ± 58.97. This is also reinforced by the research of Ridlo and Rini (2009),

the highest total number of shrimp hemocytes was achieved by treatment with the addition of *Sargassum* sp. on the 12th day (1.127 x 107 cells / L ± 0.260).

Hemoglobin

Hemoglobin is a pigment in the blood that gives blood red. Hemoglobin is a protein in erythrocytes which is composed of colorless globin proteins produced in erythrocytes and the ability of blood to carry oxygen depends on Hb levels in the blood. The results of Figure 5. show that each treatment has a decreased amount of hemoglobin. Treatment A (control) experienced the highest decrease compared to treatments B, C and D. Hemoglobin levels from all treatments ranged from 4,867 - 5,900 g /%. But this level is not in accordance with what was stated by Figures (1985) in Raharjo (2014), stating that Hb levels of normal goldfish range from 10.3 to 13.5 Hb / 100 ml and healthy fish have higher hemoglobin levels than sick fish.

The One-Way Annova test results showed that all treatments had no significant effect. This shows that the administration of *Sargassum* sp. does not affect the hemoglobin level of goldfish. This is reinforced by Raharjo's research (2014), which states the hemoglobin level of goldfish given extract of *Sargassum* sp. that is equal to 7.51 Hb / 100 ml while the range of normal fish hemoglobin levels is between 10.3-13.5 Hb / 100 ml. According to Wedemeyer and Yasutake (1977) *in* Fauzan (2017) the normal hemoglobin level in fish ranges from 10 - 11.1 g / dl. In the lowest observation of fish hemoglobin due to fish experiencing stress due to being in a new environment and lack of dissolved oxygen due to transport from the Fish Seed Hall to the research site. While the decrease in hemoglobin levels in the second observation in each treatment caused fish in an unhealthy and sick condition caused by an attack from the bacterium *Pseudomonas fluorescens*. *Pseudomonas fluorescens* bacteria attack the body organs of goldfish and cause infections in the gills of fish so that the activity of taking dissolved oxygen is disrupted and the attack of these bacteria causes the working mechanism of the fish body to decrease so that the hemoglobin level decreases. Murwantoko (2013) states that P. *fluorescens* causes disease with symptoms such as bleeding and swelling which is limited to the injured area, loss of light pigment, tissue damage to its muscular. Healthy fish have higher hemoglobin levels compared to fish that are affected by the disease (Raharjo, 2014). In addition to attacks from pathogenic diseases, the hemoglobin level of fish has decreased because maintenance is carried out in

small containers and the availability of dissolved oxygen tends to decrease. This is confirmed by the statement of Subandiyono et al., (2010) *in* Raharjo (2014) that fish kept in media containing lower oxygen levels have lower hemoglobin levels

Differential leukocytes

The observation of lymphocyte counts in the last observation shows that each treatment has increased (table 3). The results of the One-Way Annova test statistical analysis showed that there were significant differences in each treatment. Treatment A had the lowest lymphocyte results, which was 78.67% and significantly different from treatment B 84.33%, treatment C 82.33% and treatment D had the highest lymphocyte count of 85.00%. The number of lymphocytes is still in the normal range, this statement is in accordance with Sani's statement (2014), the proportion of normal tilapia lymphocytes ranges from 68-86%. These results indicate that the treatment given extract of *Sargassum* sp. can increase lymphocyte counts higher than treatment without administration of *Sargassum* sp. extract. This statement is reinforced by Rustikawati (2012), that extract of *Sargassum* sp. can be as immunostimulant, which is able to stimulate an increase in the body's natural defense system, as evidenced by the increase in test fish lymphocyte levels.

Lymphocytes are the most dominant type of leukocytes in the leukocyte population in fish. Increased lymphocyte percentage is a reflection of the success of the fish immunity system in developing responses to cellular immunity (non-specific) as a trigger for an immune response. Basically lymphocyte cells consist of two populations of B cells and T cells. Cell B has the ability to transform into plasma cells, namely cells that produce antibodies. Whereas T cells play a role in the immunity of intermediate T cells (cytotoxic T cells) and control the immune response (suppressor T cells). According to Tizard (1987) in Rustikawati (2012), immunostimulants can increase T cell lymphocytes found in high blood circulation of animals which play an important role as celluloid immunity which is important for protecting the body from intracellular bacteria and viruses.

Activated lymphocytes will differentiate from cognitive cells that recognize antigens into effector cells that function to get rid of antigens into effector cells that function to get rid of antigens. After binding of antigens to lymphocyte cell antigen receptors, lymphocyte cells will divide and differentiate into effector cells and memory cells. Differential T-cytolytic cells have more cytoplasmic granules containing proteins that function to lyse the target. B

lymphocytes differentiate into plasma cells that produce antibodies. Lymphocytes formed by immunostimulants help in synthesizing antibodies and phagocytes of bacteria (Moyle and Cech, 2004 *in* Rustikawati, 2012).

The results of the study of monocyte cell counts ranged from 4.00 to 9.33%. This is in accordance with the results of a study by Hernawati (2013), the number of goldfish monocyte cells obtained was 8.4%. The results of the One-Way Annova test statistic analysis showed that the administration of *Sargassum* sp. and the infection of *Pseudomonas fluorescens* bacteria did not affect the number of goldfish monocytes. This is because the observation of monocyte cells is carried out too long after infection with the bacterium *Pseudomonas fluorescens* which is one week after infection. Monocyte cells will increase in number after a few hours or one day after infection because during an infection by a foreign body, the monocytes will move quickly leaving the vessels to the infected area to carry out phagocytic activity. Monocyte cells have the ability to kill various types of pathogenic agents, including bacteria and worm larvae (Moyle & Cech, 2004 *in* Mones 2008).

Monocytes act as macrophages and are often found in areas of inflammation or infection. Monocytes with local tissue macrophages will phagocytosis of tissue remnants and disease-causing agents. The percentage of monocytes in fish blood is around 0.1% of the total population of circulating leukocytes. This statement is supported by the statement Rustikawati (2012), that the number of lymphocyte cells is the most, then neutrophil cells and the least number is monocyte cells.

The results of observations of neutrophil cells ranged from 10.67 to 12.00%. Whereas according to Rustikawati (2012), the number of neutrophils of normal fish ranges from 20-25%. The results of the One-Way Annova test statistical analysis showed that there was no significant effect on each treatment of the number of neutrophils. This shows that the administration of *Sargassum* sp. and the infection of *Pseudomonas fluorescens* bacteria did not affect the number of goldfish neutrophils. This is the same as monocyte cells because observation of neutrophil cells is carried out too long after infection with the bacterium *Pseudomonas fluorescens*. When the infection occurs, the number of neutrophils will increase because the phagocytosis process occurs and a decrease in neutrophil cells occurs because the infection of pathogenic bacteria has begun to decrease, this is seen from the wound and infection in the fish body has decreased and the movement begins to return to

normal. This statement is supported by Rustikawati (2012), a decrease in the number of neutrophils due to reduced infection due to antigen attack activities. According to Tizard (1988) *in* Rahma (2015), this is related to the main function of neutrophils namely the destruction of foreign material through phagocytic processes namely chemotaxis where the cells migrate towards particles, laying particles on cells, ingesting particles by cells, and destruction of particles by lysozyme enzymes in phagolysosomes. So that without the stimulation of foreign matter in the form of bacteria, viruses, or neutrophil pathogens it will not show an increase in reaction. Increasing the number of neutrophil cells indicates an increase in the activity of collecting macrophages at the site of infection, so that macrophages will be easier to destroy foreign particles. One neutrophil can menfagosit 5 to 20 bacteria (Sani, 2014).

Survival rate (SR)

Survival was expressed as a presentation of the number of fish living at the end of the study divided by the number of fish at the beginning of the study. Effendi (1997) in Raharjo (2014) states that survival serves to calculate the presentation of live fish at the end of the study. The calculation of the Survival rate in the study was carried out after the challenge test for the administration of *Pseudomonas fluorescens*ya on the 7th day after maintenance.

Based on figure 6. describing different letters in the graph shows results that are significantly different (P <0.05). Giving extract of Sagassum sp. with different concentrations of goldfish tested challenged using *Pseudomonas fluorescens* bacteria significantly affected the gold survival rate. In treatment A (control) has the lowest SR results which is equal to 26.67% and significantly different from treatment B with SR 80.00%. While treatment B was not significantly different from treatment C and D. Treatment of C and D by giving extracts of *Sargassum* sp with a concentration of 12% and 15% had the best results with a value of SR 100%.

From Duncan's further analysis, it is known that the survival rate of goldfish treated with extracts of *Sargassum* sp. for an increase in the immune response tested by the *Pseudomonas fluorescens* bacterium it was significant (P <5). This is because of the administration of *Sargassum* sp. Extract. can increase the body's immune system against the attack of *Pseudomonas fluorescens*. Raharjo (2014) states the factors that influence the level of survival of an organism of water quality, abiotic, competition between species, lack of

feed, addition of population in the same environment, parasites and diseases, handling of humans, organism's age and adaptability to the environment.

The high SR goldfish treated with extracts of *Sargassum* sp. because of *Sargassum* sp. has good anti-bacterial properties. In accordance with Raharjo's statement (2014), the administration of *Sargassum* polycystum allows anti-bacterial fish to be higher than fish that were not given *Sargassum*polycystum. While the low SR in treatment A without the administration of *Sargassum* sp. caused by low natural immunity in the fish's body. This is reinforced by the statement Rustikawati (2012), the natural immunity of fish bodies that are not stimulated by stimulant material contained in extracts of *Sargassum* sp., Namely alginate, as a result the fish is unable to resist the pathogenic attack of the *Streptococcus* ineae.

Clinical symptoms

Clinical symptoms observed during infection with the bacterium *Pseudomonas fluorescens*. In general, the symptoms that occur in fish are fish begin to swim around, decreased appetite, no trending, some fish begin to weaken. Fish begin to be infected with the characteristics of the body color of the pale fish, swim around in circles, swim to the surface, fish fins begin to have bruises or bleeding, scales are released, bleeding in the operculum and there is freezing and bruising on the body. This was stated by Murwantoko (2013) P. *fluorescens* bacteria have been reported as disease-causing bacteria with symptoms including bleeding and swelling which is limited to the injured area, loss of light pigment, tissue damage to muscle.

In treatment A, fish experienced a lot of dead and infected with P. *fluorescens*. This is because the body's natural immune system is too low and is unable to fight off incoming pathogenic bacteria. The attack from these bacteria can make the fish unhealthy and until death in fish. According to Yulvizar (2014), many pathogenic bacteria that cause clinical infections and deaths in goldfish are Vibrio sp., Aeromonas sp., Pseudomonas sp., Streptococcus, Pasteurella sp., Mycobacterium sp. Aeromonas liquefaciens, Aeromonas hidrophila and *Pseudomonas fluorescens*.

Water quality

Water quality parameters during infection, treatment, and maintenance did not affect the goldfish. This can be seen in table 4. That is for the temperature parameters in the measurement in the first week of treatment A ranged from 28.1-28.2 ^{o}C, in the second week 28.3-28.4 ^{o}C and not much different from treatment B, C, and D. For DO measurements in the first week of all treatments ranged from 5.1 to 5.3 ppm while in the second week measurements ranged from 5.0 to 5.2 ppm. And for pH measurements in all treatments in the first week ranged from 7.1-7.3 and in the second week ranged from 7.2-7.5. In waters during infecting and maintaining goldfish, the water quality is still normal, and good. In accordance with the literature obtained, namely temperatures of 25-30 ^{o}C.

5. FINALITY

Conclusion

The conclusions that can be drawn from this study include the following: Giving extract of *Sargassum* sp. by injecting the body of goldfish does not have a significant effect on the number of red blood cells, hemoglobin and differential leukocytes (monocytes and neutrophils), but has a significant effect on leukocyte count, lymphocyte cell count and goldfish survival rate (SR) . The highest leukocyte count was in treatment D with a concentration of 15% extract of *Sargassum* sp. with the results of 67.0833 x103 cells / mm3 and treatment A (control) had the lowest yield of 28.350 x103 cells / mm3. The highest lymphocyte cells were treatment D, which was 85.00% and the lowest lymphocyte treatment was treatment A, 78.67%. The lowest survival rate is treatment A (control) 26.67% and significantly different from treatment B concentration of 9% with a result of 80%, while the highest SR results are treatment C concentration of 12% and D concentration of 15% is 100%. Treatment A (control) experienced the most clinical symptoms due to infection from the bacterium *Pseudomonas fluorescens* compared to treatments B, C and D.

Suggestion

The suggestions that can be given from the results of the research that have been done are as follows:

For goldfish breeders can use *Sargassum* sp. Extract. to prevent attacks from the bacterium *Pseudomonas fluorescens*.

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