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Profil darah ikan Nila *(Oreochromis niloticus)* pada pemberian pakan dengan fortifikasi tepung rumput laut *Gracilaria* sp.

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ABSTRACT

Gracilaria sp. seaweed is one of the marine plants that contain secondary metabolites that can be used as an immunostimulant in Nile tilapia (Oreochromis niloticus). Blood profile can be used as an evaluation material for the fish's physiological response. The aim of this study was to determine the effect of fortification of Gracilaria sp. seaweed powder on the blood profile of Nile tilapia (Oreochromis niloticus). The study used an experimental method with four treatments, namely, P1: control feed; P2: 4% fortification of Gracilaria sp. powder; P3: 8%; and P4: 12%, respectively. The research data was analyzed by linear regression for blood profiles and variance for absolute weight data, SR, and FCR. The results showed that the fortification of Gracilaria sp. seaweed in feed did not have a significant effect on hemoglobin levels, hematocrit, red blood cell count, and leukocytes. However, it had a significant effect on the increase in absolute weight, survival rate, and feed conversion rate. The fortification of Gracilaria sp. seaweed powder with a concentration of 8% or 80g/kg of feed was the best concentration that could affect the blood profile of Nile tilapia.

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1. Introduction

The Nile tilapia (Oreochromis niloticus) is a type of fish product that contributes significantly to the Indonesian economy due to its increasing production rate every year. In 2017, the production of Nile tilapia increased by 3.6%, reaching a total of 1.15 million tons compared to 1.14 million tons in 2016, based on data from the Indonesian Ministry of Marine Affairs and Fisheries (2018) (Wijayanti et al., 2019). This has a significant impact on the availability of feed for the fish.

Feed is an important factor that supports the cost-intensive process of fish farming. According to Janah et al. (2016), the availability of feed accounts for around 60% to 70% of the expenses in fish farming. Therefore, mistakes in choosing and giving feed can lead to stress and death in fish. Some factors that affect the quality of feed include the raw materials used, the protein and carbohydrate content, the presence of vitamins, the manufacturing process, and storage methods (Pratiwi et al., 2012).

In this study, feed formulation with the addition of Gracilaria sp. seaweed powder was used. Gracilaria sp. belongs to the Rhodophyceae or red algae family. It also contains agar and carrageenan as its main chemical components, which are very beneficial in various fields of life (Laode and Aslan, 1998). Furthermore, this type of seaweed contains organic compounds such as lipopolysaccharides and flavonoids, which can be used as immunostimulants to prevent stress in Nile tilapia, which is one of the early signs of fish disorders or diseases. This is in line with Rustikawati's research (2012) in Amanda et al. (2016), which found an effective increase in white blood cells in Nile tilapia helped by brown seaweed, and it is known that red seaweed also has similar secondary metabolite compounds as brown seaweed.

The blood profile or blood picture of fish can be used as an evaluation material for physiological responses that occur in fish. Physiological responses caused by the addition of Gracilaria sp. powder to Nile tilapia can be seen from changes in hemoglobin levels, red blood cells, hematocrit, and leukocytes. In abnormal or stress conditions, the number of red blood cells, hematocrit, and hemoglobin levels may decrease or increase, and usually, the number of leukocytes tends to increase (Royan et al., 2014). Therefore, research on the Blood Profile of Nile Tilapia (Oreochromis niloticus) in Feeding with Gracilaria sp. Seaweed Powder Fortification is necessary to determine the impact or physiological response of Nile tilapia to feed containing added Gracilaria sp. seaweed powder.

2. Material and methods

This research was conducted from April to June 2021 in the Aquaculture Laboratory, Department of Fisheries and Marine Science, Faculty of Agriculture, Mataram University. The containers used in this study had dimensions of L x W x H = 40 x 35 x 30 cm3. The method used in this study was an experimental method with a Completely Randomized Design consisting of 4 treatments and 2 replications. The treatment concentrations used in this study were as follows:

P1: Control (without the addition of Gracilaria sp. seaweed flour)

P2: Fortification with 4% Gracilaria sp. seaweed flour

P3: Fortification with 8% Gracilaria sp. seaweed flour

P4: Fortification with 12% Gracilaria sp. seaweed flour.

2.1 Procedure

Research containers consisting of 8 units with a water capacity of 25 liters were prepared. The test animals used were tilapia fingerlings obtained from the Lingsar Fish Seed Center with a weight of 15-20 grams. Prior to stocking, acclimatization and fasting of the fish were carried out to adjust to the new environment. Feeding was done twice a day, in the morning and afternoon, at 5% of the fish body weight. Blood samples were taken from the fish at the end of the study, including hemoglobin, hematocrit, erythrocytes, and leukocytes. Water quality management was performed by siphoning and changing water, as well as measuring water quality parameters such as temperature, dissolved oxygen (DO), and pH.*2.2 Parameters*

a) Hemoglobin

The hemoglobin level can be determined directly by looking at the number on the Hb-meter with the unit of G%.

b) Hematocrit

The hematocrit value can be determined as follows:

$$Hematocrit = \frac{length\ of\ red\ blood\ cell\ volume\ that\ settles}{total\ length\ of\ blood\ volume\ in\ the\ tube} \times 100\%$$

c) Total of erythrocytes

The total number of erythrocytes is calculated from 4 small squares and is determined using the following formula:

$$\sum erythrocytes = Mean\ of\ counted\ leukocyte\ cells \times \frac{dilution\ factor}{volume}$$

d) Total of Leukosit

The total number of leukocytes is counted in 4 small squares, and its amount is calculated according to the following formula:

$$\sum Leukosit = Mean\ of\ counted\ leukocyte\ cells \times \frac{dilution\ factor}{volume}$$

e) Absolute Weight Growth

The formula for calculating the absolute weight growth of Nile tilapia during maintenance is as follows:

Absolute Weight Growth (g) = Final Weight (g) - Initial Weight (g)

f) Survival Rate

The calculation of survival rate (SR) of tilapia fish can be done using the formula as proposed by Effendi et al. (2006) and Waryanti (2019), which is as follows:

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

g) Food Convertion Ratio

The feed conversion ratio (FCR) can be used to assess the quality of the feed given to the fish in relation to their growth. The formula for calculating the FCR is as follows:

$$FCR = \frac{F}{Wt - W0}$$

The water quality was measured 3 times during 45 days, specifically on day 0, 22, and 45 in the morning at 08.00..

2.3 Data Analysis

The obtained data on hemoglobin, hematocrit, erythrocyte, and leukocyte levels were analyzed using Simple Linear Regression method, while growth and survival data were statistically analyzed at a significant level of 5% with a 95% confidence interval using ANOVA analysis. If the data showed a significant effect, a further Duncan test was performed. Meanwhile, the water quality measurement data were analyzed descriptively.

3. Results and Discussion

3.1. Hemoglobin

The average hemoglobin level was highest in treatment P3 at 5.7 G% and the lowest average hemoglobin level was obtained in treatment P1 at 3.3 G%. Regression analysis showed that fortification with seaweed flour had a positive effect on hemoglobin levels, but the effect was not significant and the R2 value was 0.209%.

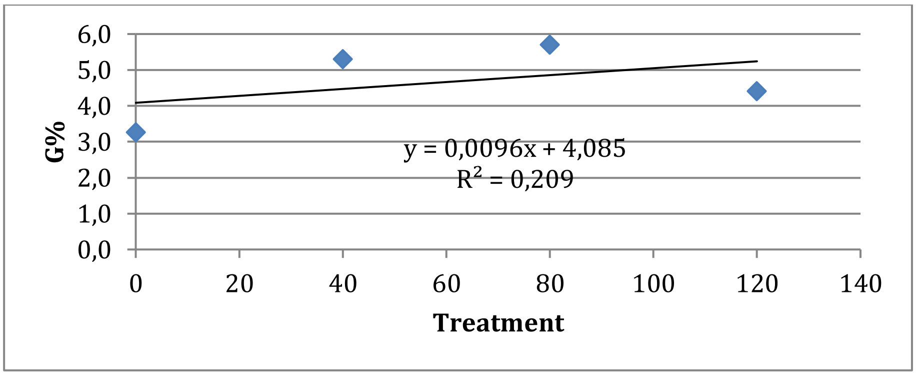

Figure 1. Hemoglobin Levels.

Hemoglobin has several functions, including transporting oxygen from the fish respiratory organ pumped by the heart to all cells and body parts, transporting nutrients into cells to become energy, and disposing of metabolic waste in the body (Royan et al., 2014). Based on the regression analysis (Figure 1), the pattern of adding Gracilaria sp. flour could increase hemoglobin levels with an R2 value of 0.209%. Sembirin (1995) in Setyowati & Astuti (2020) stated that the closer the R2 value is to 1, the better the data fit with the influence (Gracilaria sp. flour), and vice versa if the R2 value is closer to 0, the worse the data fit with the influence. According to Salasia et al. (2001) in Royan et al. (2014), 5.05-8.33 G% is the normal range of hemoglobin levels in tilapia fish. The highest increase in hemoglobin levels in tilapia fish occurred in treatments P3 and P2, which means that hemoglobin levels are still within the normal range, indicating that the fish's physiological condition is quite good and not in a state of stress or anemia. The increase in hemoglobin levels is thought to occur because tilapia fish receive additional nutrition in the form of minerals, namely iron (Fe), which is contained in Gracilaria sp. seaweed, which is very useful for producing hemoglobin and increasing the number of red blood cells in the body (Mutiara et al., 2021).

The low hemoglobin levels in treatment P4, despite receiving iron supplementation from seaweed, are thought to be due to stress (Pattipeilohy et al., 2020) caused by environmental changes during blood collection. Environmental changes in all treatments can trigger stress in fish, but in treatments P2 and P3, fish were still able to maintain their stability, as evidenced by normal hemoglobin levels in those treatments. This is consistent with the research of Kurniawan et al. (2019) in Fauziah et al. (2013), which showed a decrease in hemoglobin levels after tilapia fish were infected with bacterial infections. This decrease occurs because blood will undergo phagocytosis as a form of resistance to pathogenic bacteria. This statement can be supported by the high number of leukocytes in treatments P4 and P1, where leukocytes are non-specific indicators of the body's defense system in fish.

3.2. Hematocrit Level

The average hematocrit values in the study are presented in the form of a graph (Figure 2). The regression analysis results indicate that fortification with Gracilaria sp. can have a positive effect on hematocrit levels, but the effect is not significant and has an R2 value of 0.0514%. The highest value is found in treatment P2 at 22%, while the lowest value is in treatment P1 at 12%.

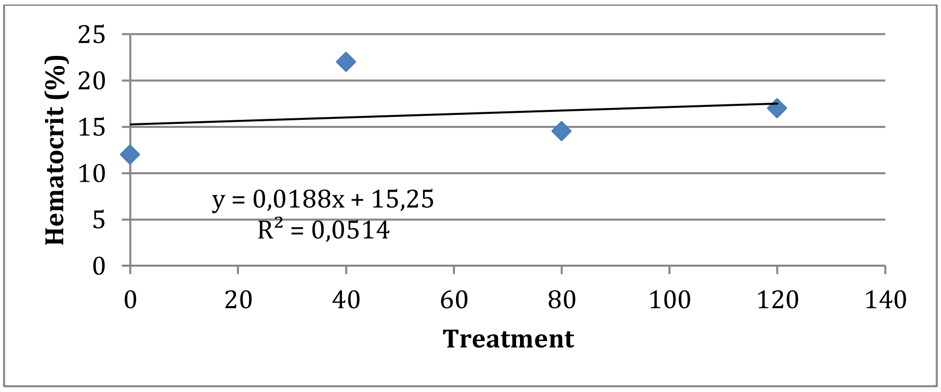

Figure 2. Hematocrit Level

Hematocrit is a measure of the amount of red blood cells in the blood (Hastuti and Subandiyono, 2010). According to Hardi (2011) in Utami et al. (2013), a hematocrit level of around 27.3%-37.8% is considered normal for tilapia fish. In this study, all treatment and control feeds, starting from P1, P3, P4, and P2, show values below the normal range of hematocrit, indicating that the physiological state of the fish is disturbed or under stress.

Dellman and Brown (1989) stated that a hematocrit level below the normal range indicates a lack of protein content in the feed and poor water quality. However, this statement contradicts the high protein content in the artificial feed used in this study, which ranges from 26.99-30.29%, and meets the proximate standards for tilapia fish feed for growth, where the minimum protein level is 25% (SNI 01-7242-2006 in Endraswari, 2021). Moreover, the water quality obtained during the study is good.

It is suspected that factors other than nutrition and water quality may have caused the low hematocrit levels in the tilapia fish in this study, such as changes in the environment during the blood sampling process, where the fish were taken out of their living media. Consistent with Hardi et al. (2011), stress can occur due to factors such as the environment, handling during the blood sampling process, and the fish's body resistance (Eviani et al., 2019). The form of feed also affects hematocrit levels, as the sinking feed used in this study made it difficult for tilapia fish to eat, resulting in reduced appetite. This is consistent with Campbell (2015) in Lestari et al. (2017), who found that low hematocrit levels can lead to decreased appetite in fish.

3.3. Total Erithrocyte

The graph of erythrocyte measurement showed the highest value in treatment P4 at 1,900,000 cells/mm^3 and the lowest in treatment P1 at 1,210,000sel/mm^3.

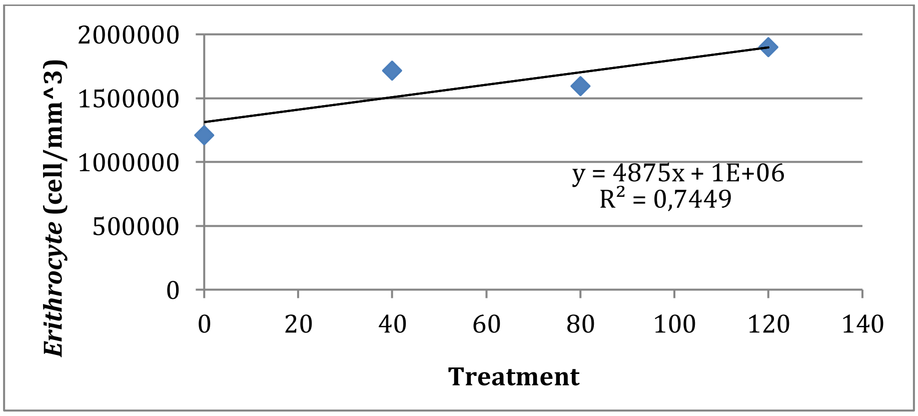

Figure 3. Total of Erithrocyte

Erythrocytes are a group of blood cells that dominate the blood (Yuni et al., 2019). In this study, the number of erythrocytes in tilapia fed with artificial feed showed an increase between the treatment and control groups. Based on the regression analysis results in Figure 3, a good pattern was observed between the addition of Gracilaria sp. powder to the number of erythrocytes, with an R2 value of 0.7571% and a positive effect, but the effect was not significant. Generally, the normal range of erythrocytes in teleostei fish is 1.05-3.0x106 cells/mm^3 (Royan et al., 2014). The results indicated that all treatments, including the control, were within the normal range of erythrocyte counts in fish. The number of erythrocytes can indicate the health status of the fish.

There are several factors that cause variations in erythrocyte counts, including age, gender, and environment (Oktavia, 2011 in Sarkiah et al., 2016). If we look at the factors that affect erythrocyte counts, all of these factors were the same in this study, and there was no difference between the treatment and control groups. Therefore, it is suspected that the factor that may influence and increase erythrocyte counts is the addition of seaweed powder, as seaweed contains amino acids that can play a role in erythrocyte formation. In line with Ma'ruf et al. (2013) in Azizah et al. (2015), amino acids in seaweed consist of L-Serine, L-Thereonin+L-Glycine, L-Alanine, L-Arginine, L-Valine, L-Leucine, and L-Lysine, where amino acids are essential components of proteins that function to synthesize erythrocytes. The normal number of erythrocytes in fish in this study avoided oxygen deficiency and stress in the fish. Excessive erythrocyte counts beyond normal limits indicate that the fish are under stress (Rahma et al., 2015).

3.4. Total of Leukocytes

The average value of leukocytes is presented in the form of a graph (Figure 4). Differences in high and low leukocyte values can be observed, with the highest value found in treatment P1 with a value of 267,250 cells/mm^3, while the lowest value was 91,750 cells/mm^3 in treatment P2.\

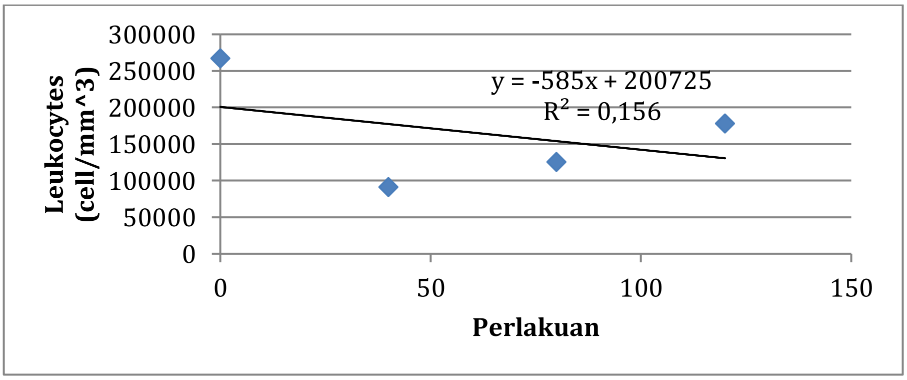

Figure 4. Leukoocytes level

The blood cells that function in maintaining the body's defense system or immune system are leukocytes. Through the immune response system, leukocytes help to clean and protect the body from foreign substances, including pathogen attacks (Royan et al., 2014). The number of leukocytes is often used as an indicator of non-specific immune defense system (Azhar, 2013) or as a guide to indicate if fish are experiencing health problems such as stress and disease.

Based on the regression analysis results (Figure 4), it was found that the pattern of adding Gracilaria sp. flour was able to increase the number of leukocytes with an R2 value of 0.156%, but it did not have a significant effect. The number of leukocytes in treatments P1 and P4 respectively had very high values, which were 267,250 cells/mm3 and 178,000 cells/mm3. These numbers indicate a disturbance in the fish's physiological condition. This is supported by Lagler's statement (1997) in Royan et al. (2014) that 20,000-150,000 cells/mm3 is the range of leukocyte count in normal fish. It is suspected that the high number of white blood cells in the blood is due to the fish experiencing stress and indications of being attacked by a disease. The increase in fish leukocyte production is one sign of defense that the fish's body is fighting against pathogens. In line with Zulfarina (1998) in Ayuzar (2003) in Amanda et al. (2016) that the increase in total leukocytes is an initial sign of fish experiencing infection, stress, or leukemia.

The leukocyte count calculation results in treatments P2 and P3 showed good values, which were 91,750 cells/mm3 and 125,500 cells/mm3, respectively. These numbers can be said that the fish are in good health because they are still within normal limits. This can prove that by adding Gracilaria sp. seaweed flour, it can control the increase in leukocyte count from tilapia fish according to the frequency given. In line with Amanda and Ayuzar's research (2016), it is proven that on the body of fish infected with Streptococcus iniae bacteria, given red seaweed powder can increase their immune system. The ability to increase leukocyte count in a controlled manner is due to the active compound content in Gracilaria sp. seaweed, such as polyphenols and flavonoids, which act as antimicrobials, antivirals, and immunostimulants and are easily soluble in water (Naiborhu, 2002 in Widiawati, 2018). High antioxidant activity is also possessed by polyphenolic compounds (Subagiyo, 2009 in Widiawati et al., 2018). Subagyo's research (2009) in Satyantini et al. (2016) found that polyphenols and flavonoids are needed to increase hemosites in shrimp so that they can support shrimp health.

In addition, Gracilaria sp. also has a high content of sulfated polysaccharides that act as immunostimulants. Sulfated polysaccharides such as carrageenan are abundant in the cell walls of seaweed such as red algae, and have various secondary metabolite compounds that are beneficial as anticoagulants, antioxidants, anticancer agents, and increase the activity of oxygen radical secretion and phagocytosis (Wijesekara et al., 2011 in Satyantini et al., 2016). Other studies also mentioned that polysaccharides extracted from algae and combined with vaccines were able to show promising results in increasing total white blood cell count (Indriasari et al., 2014).

3.5. Absolute weight

Based on the obtained results, the highest absolute weight gain of tilapia was observed in treatment P3 with a value of 168.80 grams, while the lowest was in treatment P1 with a value of 75.93 grams. The ANOVA analysis with a 95% confidence interval (α = 0.05) showed that the addition of Gracilaria sp. seaweed flour had a significant effect on the absolute weight gain of tilapia during the maintenance period. As the results obtained were significant, further analysis using Duncan's multiple range test was conducted. The absolute weight gain of tilapia in treatment P1 differed significantly from treatments P2, P3, and P4. Treatments P1, P2, and P4 did not differ significantly from each other.

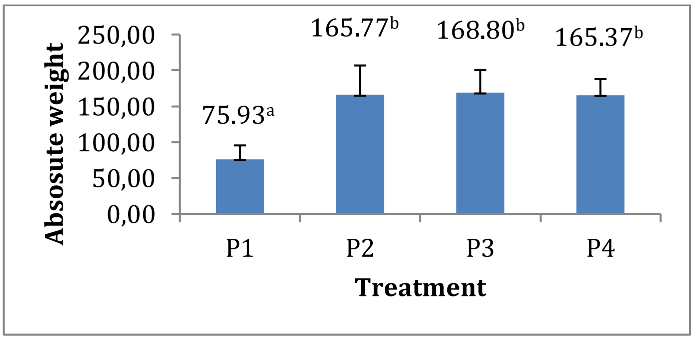

Figure 5. Absolute weight gain.

During the study, the growth of the weight of the tilapia fish increased over time in each treatment. Based on the results (Figure 5), it is shown that the absolute weight growth with the addition of seaweed flour has a positive effect between treatments and the control, but not significantly. The high values found in treatments P3, P2, and P4 are suspected to be due to the additional nutrients contained in the feed given. These nutrients are in the form of fats and carbohydrates that are utilized by the fish to optimize the protein in forming body tissues and increasing body biomass. Treatment P2 has the highest fat content, while P3 and P4 have high carbohydrate content. This mechanism is commonly known as the "protein-sparing effect", which means that carbohydrates and fats can balance the use of protein in metabolism and body maintenance so that the process does not only rely on protein and protein focus more on growth (Sanjayasari et al., 2010). In treatment P1, the value decreased, which is suspected to be due to the fish's inability to digest the feed properly, as there is no significant difference in terms of feed content between the control and the treatment. According to Marzuqi (2012), feed efficiency can indicate how much the fish can be utilized.

3.6. Survival Rate

Based on the results obtained, the highest survival rate of tilapia was found in treatments P2, P3, at 90%, while the lowest was in treatment P1, at 66.7%. Based on the analysis of variance with a confidence interval of 95% (α=0.05), it showed that the addition of Gracilaria sp. seaweed flour can significantly affect the survival rate of tilapia during the cultivation period. As the results obtained were significant, further analysis using Duncan was conducted. The survival rate of tilapia in treatment P1 differed significantly from treatments P2, P3, and P4. Treatments P1, P2, and P4 were not significantly different from each other.

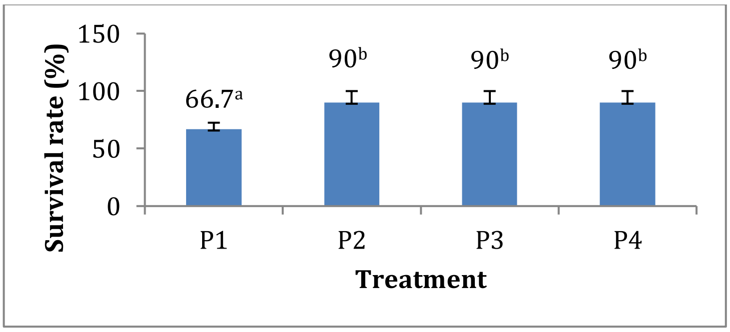

Figure 6. Survival rate

The survival rate is the number of fish that survived during the cultivation period until the end. Based on the research results, there were differences in the survival rates between the treatment and control groups. Fish mortality often occurs at the beginning of cultivation, which is suspected to be due to the fish adapting to their environment. This is in line with Fatimah's statement (1992) in Murjani (2011) that the survival of fish depends on the fish's adaptability to the environment, the fish's health status, stocking density, and water quality that supports growth. In treatment P1, the survival rate was lower compared to treatments P2, P3, and P4. Considering these results, it can be related to the fish's blood picture, where the number of leukocytes obtained during the study was very high in treatment P1 and low in hemoglobin, hematocrit, and erythrocyte counts.

3.7. Food Convertion Ratio (FCR)

Based on the results obtained, the highest FCR of tilapia was found in treatment P1, which was 5.9, followed by treatment P2 which was 3.2, treatment P4 which was 2.5, and the lowest was in treatment P3 which was 2.4. Based on the results of the ANOVA analysis with a 95% confidence interval (α=0.05), it was shown that the addition of Gracilaria sp. seaweed flour could significantly affect the FCR of tilapia during the rearing period. Since the obtained results were significant, further analysis was carried out using Duncan's multiple range test. The FCR of tilapia in treatment P1 was significantly different from treatments P2, P3, and P4. Meanwhile, there were no significant differences between treatments P1, P2, and P4.

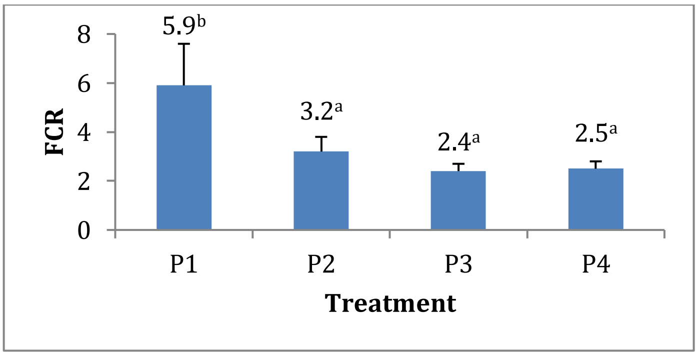

Figure 7. Feed convertion ratio

FCR is the ratio of the amount of feed given to the weight of fish produced. The average FCR values in this study ranged from 2.4 to 5.9 (Figure 7). The FCR range in this study between treatments and control was quite high. According to Shuforon et al. (2018), a good Food Conversion Ratio (FCR) value ranges from 0.8-1.6, which means that 1 kilogram of tilapia consumes 0.8-1.6 kg of feed. A low feed conversion ratio means good quality feed is being provided, while a high feed conversion ratio indicates poor quality feed. The lower the feed ratio value, the better the quality of the feed provided. This is in line with Susanti (2004) who stated that a high feed conversion ratio is influenced by poor quality feed.

3.8. Water quality

Table 1. Data of water quality

Parameters	Treatment	Scores	Reference of optimum condition
DO (mg/l)	P1	6,0-6,7	>3 (Tokah *et al.*, 2017)
	P2	5,9-6,8	
	P3	5,6-6,8	
	P4	5,6-6,8	
pH	P1	7,62-7,92	6,5-8,5 (Salsabila *et al.*, 2018)
	P2	7,82-8,05	
	P3	7,91-7,95	
	P4	7,68-7,84	
Temperature (^{0}C)	P1	26,6-28-6	25-32 (BSNI, 2009 *dalam* Tyen, 2016)
	P2	26,7-28,6	
	P3	26,5-28,6	
	P4	27,2-28,6	

Water quality is one of the parameters that must be maintained in the cultivation of Nile tilapia (Oreochromis niloticus). The measurement results of water quality data such as dissolved oxygen

(DO), pH, and temperature are presented in Table 1. The water quality during the study can be categorized as optimal.

The measurement results of dissolved oxygen (DO) in each treatment show values that are suitable for the survival of Nile tilapia, which is around 5.6-6.8 mg/L. According to Tokah et al. (2017), a dissolved oxygen value of >3 mg/L is optimal for the growth of Nile tilapia. According to Mudjiman (1984) in Utami (2014), efforts that can be made to maintain dissolved oxygen levels from becoming low include replacing old maintenance water with new water, maintaining water depth, preventing pollution due to waste, and using aeration in the aquarium.

The pH level in this study is good, with values ranging from 7.62-8.05 in each treatment. According to Salsabila et al. (2018), the optimal value to support the life of Nile tilapia is around 6.5-8.5. Maintaining pH is important in this study because it can affect the level of stress on fish and cause poor blood profiles in Nile tilapia. Acidic pH in the water medium can cause stress for fish, physiological disorders, susceptibility to disease, low productivity and growth, and even death (Syarifudin, 2016).

Based on the measurement results, the water temperature obtained during the study in each treatment ranged from 26.5-28.6°C. The temperature range obtained is still within the normal range for the survival of Nile tilapia. According to BSNI (2009) in Tyen (2016), it ranges from 25-32°C. According to Siegers et al. (2019), fish will die from cold at a temperature of 12°C. If the water temperature is >35°C, the fish will have difficulty breathing due to the high level of oxygen consumption and will also experience stress, and the formation of ammonia will occur more quickly due to the decrease in dissolved oxygen in the water.

4. Conclusion

Based on the research results, it can be concluded that the fortification of Gracilaria sp. seaweed flour in feed has a positive effect, but the effect is not significant on hemoglobin levels, hematocrit, red blood cell count, and leukocytes. However, fortification has a significant effect on absolute weight, survival rate, and feed conversion rate. The best concentration of Gracilaria sp. flour fortification is 8% or 80g/kg of feed, which can affect the blood profile of tilapia.

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