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Substitution of Flour Sea Grapes (Caulerpa racemosa) Fermented on Tilapia (Oreochromis niloticus) Feed

Substitusi Tepung Anggur Laut (*Caulerpa racemosa*) Terfermentasi Pada Pakan Ikan Nila (*Oreochromis niloticus*)

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Abstract

This study evaluated the effect of substituting fermented seaweed flour (Caulerpa racemosa) in feed on the growth of tilapia (Oreochromis niloticus). The study was conducted over 50 days using a completely randomized design with four treatments (0%, 4%, 8%, and 12% fermented flour) and three replicates. The results showed that the addition of fermented flour influenced the absolute weight of the fish, but did not significantly affect the absolute length, specific growth rate, feed utilization efficiency, feed conversion ratio, and survival rate. The substitution of 4% fermented flour resulted in the best increase in absolute weight of 11.58 g/fish.

Keywords: Sea grapes, C. racemosa, Fermentation, Tilapia

Abstrak

Penelitian ini mengevaluasi pengaruh substitusi tepung anggur laut terfermentasi (*Caulerpa racemosa*) dalam pakan terhadap pertumbuhan ikan nila (*Oreochromis niloticus*). Penelitian dilakukan selama 50 hari menggunakan Rancangan Acak Lengkap dengan empat perlakuan (0%, 4%, 8%, dan 12% tepung fermentasi) dan tiga ulangan. Hasil menunjukkan bahwa penambahan tepung terfermentasi mempengaruhi berat mutlak ikan, namun tidak berpengaruh signifikan terhadap panjang mutlak, laju pertumbuhan spesifik, efisiensi pemanfaatan pakan, feed conversion ratio, dan survival rate. Substitusi tepung terfermentasi 4% menghasilkan peningkatan berat mutlak terbaik sebesar 11,58 g/ekor.

Kata Kunci : *Anggur laut, C. racemosa, Fermentasi, Ikan nila*

1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the most popular freshwater fish species in local, national, and even international markets. In addition, tilapia also has a high economic value so that it is in great demand in hatchery or enlargement activities compared to other freshwater. According to Shalsabila and Hari (2018), some of the advantages of tilapia are easy to breed, easy to maintain, and high adaptation to environmental changes. One of the important factors in fish farming activities is feed because it affects the growth and survival of fish.

Fish feed must have complete nutritional content such as: protein, fat, carbohydrates, vitamins and minerals. According to Amalia *et al.* (2013), the protein requirement for tilapia ranges from 25-35%. To achieve a balance of nutrients in the feed, proteins derived from plants (vegetable proteins) and animals should be used simultaneously. Several previous studies have shown that seaweed has been widely used as fish feed (Endraswari *et al.,* 2021; Irmadiati *et al.,* 2021 and Putri *et al.,* 2021;). One type of seaweed that can be utilized as a substitute ingredient in fish feed is sea grape (*Caulerpa racemosa*).

Sea grapes can be an alternative raw material to replace fish and soy flour, which has a fairly good nutritional content, namely 0.30% fat, 20% moisture content, 10.70% protein, 27.20% carbohydrates, 15.05% crude fiber (Nurjannah *et al*., 2018). The crude fiber content in sea grape flour is still said to be quite high, therefore to maximize the nutritional content in sea grape flour, a fermentation process is needed. The results of research by Gresela *et al.,* (2024) showed that gourami feed added with fermented sea grape flour can increase the absolute weight by 4.5 g. Furthermore, Alamsjah *et al., 2018*). Furthermore, Alamsjah *et al.* (2011) stated that the fermentation process aims to hydrolyze seaweed cells into the shortest nitrogen chains. One of the fermenters that can be used in the fermentation process is *Lactobacillus casei* and *Saccharomyces cerevisiae* and molasses. This study aims to analyze the extent of utilization of *C. racemosa* sea grape flour as a raw material in tilapia feed formulations.

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2. Materials and Methods

2.1. *Time and Place*

This research was conducted for 50 days at the Fish Production and Reproduction Laboratory of the Aquaculture Study Program, Faculty of Agriculture, Mataram University.

2.2. *Materials and Research Design*

The materials used were *C. racemosa* sea grape flour, *tilapia* fry (size 5 - 7 cm), fresh water, fish meal (62% protein), soybean flour (31% protein), corn flour (8% protein), corn oil, wheat flour, premix, soap, molasses, *L. casei* and *S. cerevisiae*. This study used a completely randomized design (CRD) consisting of 4 treatments (P1, control treatment/commercial feed; P2, 4% dose of *C. racemosa* flour; P3, 8% dose of *C. racemosa* flour and P4, 12% dose of *C. racemosa* flour 3 replicates so that 12 experimental units were obtained (Gresela *et al.*, 2024), 2024)

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2.3. *Preparation of Sea Grape (C. racemosa) Flour*

The sea grapes used are cleaned so that the remaining salt attached to the sea grapes disappears. After that, it is dried in the sun to dry, and ground using a blender until it becomes flour.

2.4. *Fermentation of Sea Grape Flour (C. racemosa)*

Sea grape flour as much as 100 g was mixed with *L. casei* and *S. cerevisiae* as much as 2 mL which had been mixed with 20 mL of molasses which served as a medium for bacterial growth. Furthermore, the sea grape flour was put into plastic, closed tightly and allowed to stand for 144 hours in order to provide opportunity for the fermentor to break the substrate during the fermentation process (Sofiana *et al.,* 2023). After 144 hours, steaming was carried out at a temperature of ± 80 ^{0} C for 2 minutes to inactivate microbes so that the fermentation process stopped (Aslamyah *et al.,* 2017).

2.5. *Feed Formulation*

Raw materials that have been deposited in the form of flour are stirred according to the dosage (Table 1). Stirring starts from a small amount of source material to a large amount. Then the well-mixed ingredients are steamed for 15 minutes. The steamed feed was molded using a feed grinder to form pellets

Table 1.
Feed Formulation in 1 kg Dosage

Feed Ingredients	Feed (g)			
	P0 (0%)	P1 (4%)	P2 (8%)	P3 (12%)
Fish meal	430	410	390	370
C. racemosa flour	0	40	80	120
Soy flour	300	280	260	240
Corn flour	130	130	130	130
Wheat flour	65	65	65	65
Fish oil	35	35	35	35
Corn oil	25	25	25	25
Premix	15	15	15	15
T O T A L	1000	1000	1000	1000

(Source: Endarswari *et al*., 2021)

The feed was then dried in the sun. The finished feed was then subjected to proximate test (Table 2).

Table 2.
Proximate Composition of Fish Feed

Treatment	Proximate Chemical Composition (% dry weight)				
	Protein	Fiber	Fat	Ash	Water
P1 (0%)	37,89	1,75	14,31	6,18	9,81
P2 (4%)	36,78	2,12	13,37	6,69	9,48
P3 (8%)	35,66	2,29	13,20	8,29	9,47
P4 (12%)	34,02	2,49	12,14	9,85	8,30

2.6. *Biological Test on Tilapia*

Feeding tests were conducted on tilapia fry reared in 70 cm x 20 cm containers for 50 days of rearing at a density of 10 fish per container. During the study, the test fish were fed 3% of the total fish weight 3 times a day. To maintain water quality during the study, daily watering was carried out as much as 20% of the total water volume. Tilapia weight measurements were taken every 10 days to determine the increase in weight and length of tilapia.

2.7. *Research Parameters*

The research parameters observed were absolute weight and absolute length (Mulqan *et al.,* 2017), Specific Growth Rate (Mulqan *et al.,* 2017), Feed Utilization Efficiency (Iskandar and Elrifadah, 2015), feed conversion ratio (Takrin and Ramli, 2019), survival (Takrin and Ramli, 2019) and water quality such as pH, temperature, and dissolved oxygen which were observed every ten days.

2.8. *Data Analysis*

Data were statistically analyzed using *Analysis of Variance* (ANOVA) at the 5% real level. Parameters that were significantly different were further analyzed with Duncan's test at the 5% real level. For water quality parameters, descriptive tests were used.

3. Results and Discussion

3.1. *Absolute Weight*

The results showed that the average absolute weight of tilapia was highest in the treatment of 4% concentration of *C. racemosa* flour (P2) at 11.58 ± 0.65 g/head. ANOVA test results showed that the provision of tilapia feed formulations with the addition of different concentrations of fermented *C. racemosa* flour had a significant effect ($p<0.05$) on the average absolute weight of tilapia. *Duncan*'s further test results showed that the treatment of adding 4% fermented *C. racemosa* sea grape flour concentration (P2) gave the highest average absolute weight of tilapia and was not significantly different from the control treatment (P1) and the treatment of adding 12% fermented C. racemosa sea grape flour concentration (P4), but significantly different from the treatment of adding 8% fermented C. *racemosa* sea grape flour concentration (P3) (Figure 1).

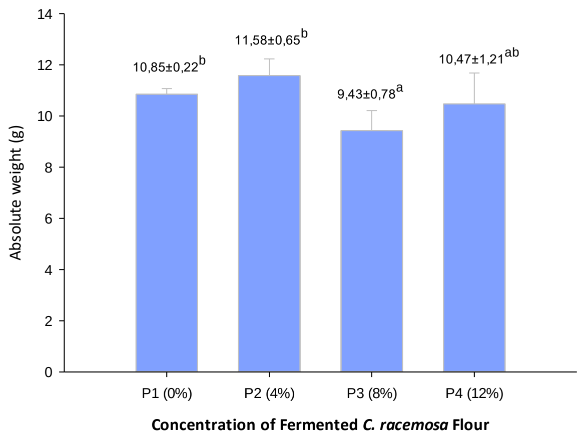

Figure 1. Average Absolute Weight of Tilapia (*O. niloticus*)

Fish growth is influenced by the quality of feed provided and the nutritional requirements of the fish. Some very important nutrients that must be available in fish feed include: protein, fat, carbohydrates, vitamins and minerals. Lack of one of these nutrients can reduce the growth rate of fish. Vice versa, if excess nutrients can cause the growth rate to be inhibited (Amalia *et al.,* 2018). The results of this study showed that all treatments of fermented C*. racemosa* sea grape flour substitution with different concentrations gave values of moisture content, protein content, fat content, crude fiber, and ash content that were still in accordance with SNI 01-7242-2006, namely maximum moisture content of 12%, minimum protein content of 25%, maximum crude fiber of 8%, maximum ash content of 15% and minimum fat content of 5% (Table 2).

The analysis showed that the addition of various concentrations of fermented C*. racemosa* sea grape flour to the feed only affected the average absolute weight of tilapia. The absolute weight parameter of tilapia showed that feeding with the addition of fermented *C. racemosa* sea grape flour up to a concentration of 4% (P2) could increase the growth of tilapia by 11.58 g/head. However, when the concentration of fermented *C. racemosa* sea grape flour was increased to 8% (P3) and 12% (P4), there was a decrease in the absolute weight of tilapia, even the growth value was lower when compared to the control treatment (P1). Although the addition of 4% fermented *C. racemosa* sea grape fishmeal (P2) gave the highest absolute weight of fish. However, it was not statistically significantly different from the control treatment (P1). This indicates that the addition of fermented *C. racemosa* sea grape flour in feed that can be tolerated by tilapia is only up to 4% concentration.

The addition of a better absolute weight average in the treatment of the addition of 4% fermented *C. racemosa* sea grape flour (P2) compared to the control treatment thought to be due to the mineral content in the *C. racemosa* sea grape flour used. As it is known that the mineral content of seaweed is very high because of its habitat in waters that are rich in various minerals. Tapotubun (2018), states that the high mineral content in seaweed is thought to come from the habitat of waters that have high salinity. Ma'ruf *et al.* (2013) stated that the mineral content of seaweed is influenced by the waters of its habitat and the processing process.

Macro and micro minerals are needed to support the body's metabolic system. Although minerals are needed in small amounts, their availability in feed is needed by fish in the growth process (Nugraha and Mikradullah, 2020). Mineral content in feed can affect the absorption and immunostimulant of fish. According to Rusyadi *et al.,* (2017) that the role of minerals is quite important because it is related to the osmoregulation system, the formation of bone structure and other hard tissues, ion regulation and acid-base balance. In addition, minerals can improve feed quality and growth rate. Meanwhile, when the treatment of fermented C. *racemosa* sea grape flour was increased in concentration to 8% (P3), and 12% (P4), it actually caused lower tilapia growth (Figures 1 and 2). This was thought to be because in both treatments there was an increase in fiber content due to the higher concentration of seaweed used (Table 2). The high fiber content of seaweed is due to the high carbohydrate content of seaweed (Agusman *et al*., 2014). The results of research by Lumbessy *et al.* (2020) showed that seaweed has a carbohydrate content of 47.36%.

Fiber content in feed can reduce the ability of fish to digest the nutrients in the feed. According to Amri (2007), high crude fiber content in fish feed will affect the digestibility and absorption of food substances in the fish digestive system. Furthermore, Rusmiati *et al.* (2017) stated that the presence of high crude fiber in the feed will accelerate the feed to pass through the intestines and have an impact on reducing the opportunity for the digestive tract to absorb other food substances contained in the feed, so that the absorbed feed becomes reduced, causing the low protein absorbed to be reduced and ultimately will cause low protein absorbed and can cause low fish growth.

3.2. *Absolute Length and Specific Growth Rate*

The highest absolute length of tilapia was found in the treatment of 4% fermented *C. racemosa* flour concentration (P2) at 2.90 ± 0.11 cm/head. ANOVA test results showed that the provision of feed formulations with the addition of different concentrations of fermented *C. racemosa* sea grape flour had no significant effect (p> 0.05) on the average absolute length of tilapia (Figure 2). While the highest average specific growth rate of tilapia was found in the treatment of 4% concentration of *C. racemosa* flour (P2) at 2.64 ± 0.15% / day. The ANOVA test results showed that the provision of feed formulations with the addition of different concentrations of fermented *C. racemosa* sea grape flour had no significant effect (p> 0.05) on the specific growth rate of tilapia (Figure 3).

The results of this study showed that all treatments of adding fermented *C. racemosa* sea grape flour to tilapia feed treatment gave the same effect as the control treatment on the parameters of length and specific growth rate of tilapia (Figures 2 and 3).

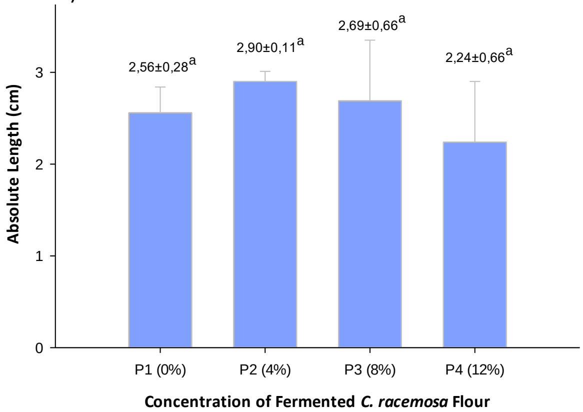

Figure 2: Average Absolute Length of Tilapia (*O. niloticus*)

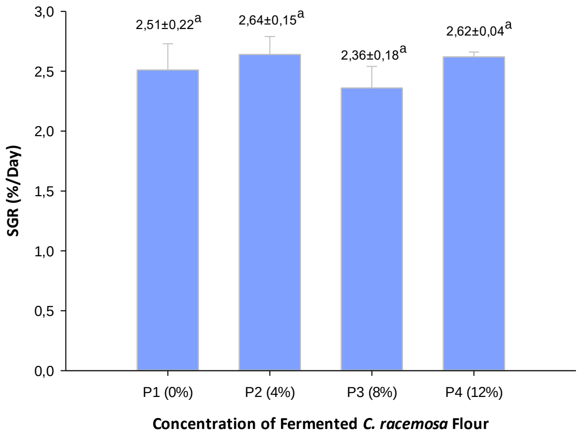

Figure 3. Average Specific Growth Rate of Tilapia (*O. niloticus)*

This shows that the test feed given fermented *C. racemosa* sea grape flour can also be utilized well by fish and can potentially be an alternative feed spike material in fish seed cultivation. The results of previous research by Endraswari *et al.,* (2021) with the addition of *Euchema cottoni* seaweed flour to tilapia feed showed that the specific growth rate of tilapia was 1.34-1.74% / day, lower than the specific growth rate of tilapia in the research conducted (2.36-2.64% / day). This is because the use of seaweed flour in previous studies was not fermented so that the fiber content in the feed was still high and could interfere with fish digestibility. According to Anggrek *et al.* (2020) fermented feed is more easily digested by fish compared to feed that is not fermented. This is because during the fermentation process there is a breakdown of complex compounds into simpler compounds, for example proteins are broken down into amino acids (Lumbessy, *et al*., 2020). This causes so that fish only need less energy to digest it and the excess energy can be used for growth.

3.3. *Efficiency of Feed Utilization (EPP) and Feed Conversion Ratio (FCR)*

The results showed that the average EPP of tilapia during the 50-day rearing period in the treatment of various concentrations of fermented C. *racemosa* sea grape flour in feed ranged from 76.8-95.8% (Figure 4). ANOVA test results showed that the provision of feed formulations with the addition of different concentrations of fermented C. *racemosa* sea grape flour had no significant effect (p>0.05) on the efficiency of fish feed utilization. Meanwhile, the results showed that the average FCR of tilapia during the 50-day rearing period in various concentrations of fermented *C. racemosa* sea grape flour in feed ranged from 1.05-1.31 (Figure 5). ANOVA test results showed that feeding formulations with the addition of different concentrations of fermented C. *racemosa* sea grape flour had no significant effect (p > 0.05) on fish FCR.

The analysis showed that the addition of various concentrations of *C. racemosa* sea grape flour fermented with a combination of molasses and *L. casei* and *S. cerevisiae* in the feed maintained for 50 days did not affect the feed utilization efficiency of the fish. The range of feed utilization efficiency (EPP) in the study was 76.8-95.8% (Figure 5). This range of EPP values is quite high and still classified as good. This indicates that the feed in all treatments can still be digested well by the fish so that the nutrients in the feed can be absorbed well by the fish. This high level of nutrient absorption will lead to high feed utilization values (Maulidin *et al.,* 2016)

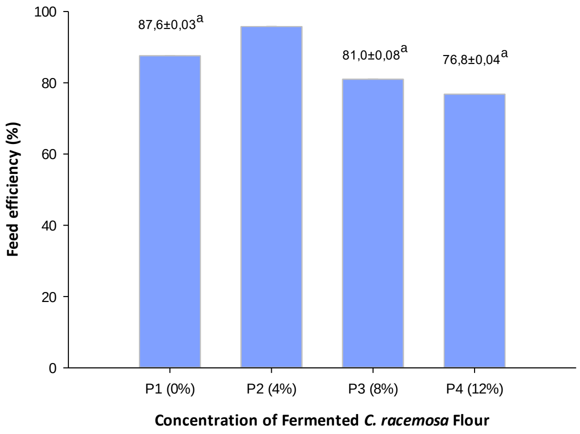

Figure 4. Average Feed Utilization Efficiency of Tilapia (*O. niloticus*)

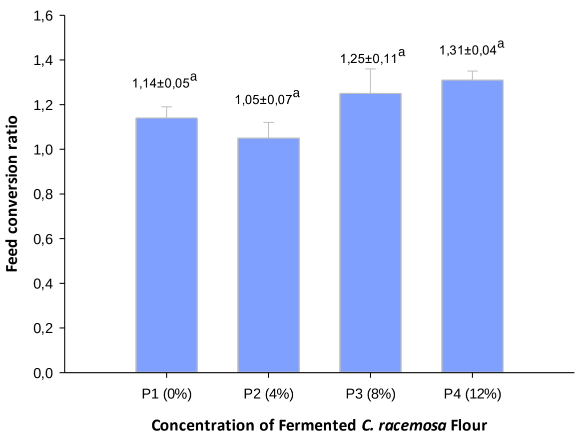

Figure 5. Average Feed Conversion Ratio of Tilapia (*O.niloticus*)

Mustofa *et al.,* (2019) stated that feed can be said to be good if the value of feeding efficiency is more than 50% or even close to 100%. Thus, the provision of *C. racemosa* sea grape flour fermented using molasses and *L. casei* and *S. cerevisiae* can be utilized properly by tilapia. This shows that all concentrations of *C. racemosa* sea grape flour in tilapia feed formulations do not interfere with the ability of fish to digest food and absorb feed nutrients, so that they can be utilized efficiently for tilapia growth. This is in accordance with the statement Sari *et al.* (2017) that feed efficiency is closely related to the fish's liking for the feed given, besides being influenced by the ability of fish to digest feed. An important factor affecting the high and low efficiency of feed is the source of nutrients and the amount of each component of the nutrient source in the feed.

The EPP value is also supported by the *Feed Conversion Ratio* (FCR) value. The higher the EPP value, the lower the FCR value. The FCR value in this study ranged from 1.05-1.31 (Figure 4.). The FCR value range is still in the good range. Darmawiyanti and Baidhowi. (2015) stated that the feed conversion value is still considered good if <3. The low FCR value indicates that the nutritional content of the feed can be utilized properly by the fish. According to Mudjiman (2004), the feed conversion ratio value is closely related to the quality of the feed, the lower the value, the higher the quality of the feed and the better the efficiency of the fish in utilizing the feed it consumes for growth, so that the body weight of the fish can increase because the feed can be optimally utilized by the fish body. Thus, the addition of fermented *C. racemosa* sea grape flour in this study can be a good alternative additional raw material in tilapia feed formulations. According to Laheng *et al.,* (2020) that the fermentation process can improve the utilization of feed nutrients for the better, where there is a role of *Lactobacillus casei* and *Saccharomyces cerevisiae* in the fermentation process of fish that can increase the work of enzymes as fermenters in feed.

3.4. *Survival Rate and Water Quality*

The results showed that the average survival rate of tilapia during the 50-day rearing period of various treatments of fermented *C. racemosa* sea grape flour concentration was highest in the treatment of 4% (P2), 8% (P3), and control treatment (P1) at 97 ± 0.06%. While the treatment of 12% C. *racemosa* flour concentration (P4) gave the lowest tilapia SR value of 90 ± 0.00%. The results of the ANOVA test showed that the provision of artificial feed formulations with the addition of different concentrations of cemented *C. racemosa* sea grape flour had no significant effect (p>0.05) on the survival rate of tilapia (Figure 6).

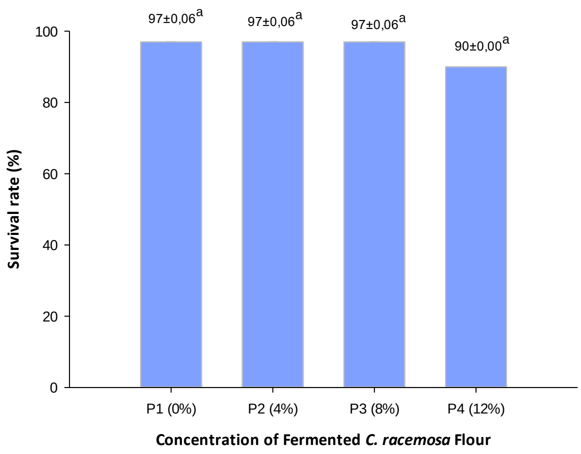

Figure 6. Average Survival Rate of Tilapia (*O. niloticus*)

The provision of *C. racemosa* flour fermented using molasses and *L. casei* and *S. cerevisiae* in tilapia feed does not affect the survival rate of tilapia with an average survival rate ranging from 90-97% (Figure 6). The range of *survival rate* (SR) is still classified as good. This is in accordance with the opinion of Simanullang (2017), that a survival rate of >50% is classified as good, survival of 30-50% is moderate and survival <30% is not good. The high survival rate of tilapia seeds is also supported by good water quality during maintenance. According to Siegers *et al.* (2019) that water quality which includes physical, chemical and biological factors is an important factor in fish farming and greatly affects survival, reproduction and growth.

Water quality parameters observed during the study were: temperature, *dissolved oxygen* (DO) and pH. Temperature observations during the study ranged from 27.4 - 29.3°C (Table 3).

Table 3.
Water Quality Parameters During Maintenance

Parameters	Concentration of Fermented C. *racemosa* sea grape flour			
	P1 (0%)	**P2 (4%)**	**P3 (8%)**	**P4 (12%)**
Temperature (°C)	28-30	27-29	27,9-29,3	28,1-29,2
pH	8,1-8,5	8,1-8,4	8,1-8,5	8,1-8,5
DO (mg/L)	3,8-5,8	4-5,9	3,8-5,2	4-6,5

Note: temperature 25-32 °C (Aliyas *et.al.,* 2016), pH 6-8.5 (Istiqomah *et al.,* 2018), DO >5-8.5 mg/L (Pramleonita, 2018)

The temperature range shows the optimal temperature for tilapia survival. According to Siegers *et al.,* (2019) water temperature or temperature greatly affects the metabolism and growth of organisms and affects the amount of feed consumed by aquatic organisms. The optimal temperature for tilapia growth is 25-30 °C. The temperature of the pond or water that can still be tolerated by tilapia is 15-37 °C

The DO value during maintenance ranged from 3-6 mg/L, where this DO value shows the optimal value for the growth and survival of tilapia. This is in accordance with the opinion of Siegers *et al.* (2019) that to increase fish productivity, the dissolved oxygen content in water should be maintained at a level of >5 mg/L, while if the dissolved oxygen content is <3 mg/L it can cause a decrease in fish growth rate.

The range of pH values obtained during tilapia rearing ranged from 8-8.5, where this value indicates that the pH value obtained is suitable for the growth and survival of tilapia. The optimal pH value for tilapia cultivation ranges from 6-8. This is in accordance with the statement of Siegers *et al.,* (2019) that fish growth will be inhibited if the pH value is not in accordance with the needs of fish.

4. Conclusion

The addition of fermented *C. racemosa* sea grape flour concentration can affect the absolute weight of tilapia, but does not affect the absolute length, specific growth rate, FCR value, feed utilization efficiency, and survival rate. The addition of C. *racemosa* sea grape flour fermented with *L. casei* and *S. cerevisiae* up to 4% concentration (P2) gave the best results because it could increase the absolute weight of tilapia by 11.58 g/head.

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