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The Effect of Adding *Sargassum* sp. Flour to Feed on the Brightness of the Color of Nemo fish (*Amphiprion frenatus*)

Pengaruh Penambahan Tepung *Sargassum* sp. Pada Pakan Terhadap Kecerahan Warna Ikan Nemo (*Amphiprion frenatus*)

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Sustanti Murtiningsih[a]*, Muhammad Junaidi[a] , Thoy Batun Citra Rahmadani[a]

[a] *Department of Aquaculture, Faculty of Agriculture, Mataram University, Jl. Majapahit 62, Gomong, Selaparang, Mataram City, West Nusa Tenggara*

Abstract

This study investigates how the addition of Sargassum sp. flour to feed affects the brightness of clownfish color. (Amphiprion frenatus). The experimental method used a completely randomized design (CRD) with five treatments and three replications, resulting in 15 experimental units. The treatments consisting of the addition of Sargassum sp. flour are treatment A (control), treatment B (10%), treatment C (20%), treatment D (30%), and treatment E (40%). Taking three clownfish randomly for each treatment to observe the color development and measure their length and weight every fifteen days on days 0, 15, 30, 45, and 60. Data from observations of color brightness, carotenoid content, and water quality were analyzed descriptively, while data on length and weight growth and survival were analyzed using variance analysis. The research results show that, based on the TCF paper method, Photoshop application, and carotenoid content, the addition of 40% Sargassum sp. flour to the clownfish feed has the greatest effect on the brightness of the clownfish color. There is no significant impact on the survival of clownfish. (Amphiprion frenatus).

Keywords: Color Brightness, Growth, Nemo Fish, Sargassum sp.

Abstrak

Studi ini menyelidiki bagaimana penambahan tepung Sargassum sp. pada pakan mempengaruhi kecerahan warna ikan nemo (Amphiprion frenatus). Metode eksperimental menggunakan pola rancangan acak lengkap (RAL) dengan lima perlakuan dan tiga kali ulangan, yang menghasilkan 15 unit percobaan. Perlakuan yang terdiri dari penambahan tepung Sargassum sp. adalah perlakuan A (kontrol), perlakuan B (10%), perlakuan C (20%), perlakuan D (30%) dan perlakuan E (40%). Mengambil tiga ikan nemo secara acak pada setiap perlakuan untuk melihat perkembangan warnanya dan mengukur panjang dan beratnya setiap lima belas hari sekali pada hari 0, 15, 30, 45, dan 60. Data hasil pengamatan kecerahan warna, kandungan karotenoid, dan kualitas air dengan analisis deskriptif, dan data pertumbuhan panjang dan berat serta kelangsungan hidup dianalisis dengan analisis varian. Hasil penelitian menunjukkan bahwa, berdasarkan metode kertas TCF, aplikasi Photoshop, dan kandungan karotenoid, penambahan 40% tepung Sargassum sp. ke dalam pakan ikan nemo memberikan pengaruh terbesar pada kecerahan warna ikan nemo. Tidak ada pengaruh signifikan terhadap kelangsungan hidup ikan nemo (Amphiprion frenatus).

Kata Kunci : *Ikan Nemo, Kecerahan Warna, Pertumbuhan, Sargassum* sp.

1. Introduction

Indonesia has many ornamental fish commodities, both freshwater and seawater ornamental fish. One type of seawater ornamental fish that has a large potential market value is nemo fish or clown fish, which is currently the most in demand in the global market. (Prasetyo, 2023). According to (Faturrahman *et al.*, 2020), the price of nemo fish with a size of 3-5 cm on the market currently ranges between IDR 5,000 and IDR 10,000 per fish. Nemo fish *(Amphiprion frenatus)* is one of the most common types of clowns, the easiest to find, and is a superior commodity of ornamental seawater fish that is very profitable, making this nemo fish a type of fish that has market value that has the potential to be developed through cultivation activities. Thus making this nemo fish is a type of fish that has a market value that has the potential to be developed through aquaculture activities.

Fish farming activities including nemo fish are usually inseparable from the problem of changing the color of the fish over time. According to Alfandi *et al.,* (2017) it can be seen that basically color becomes an indicator in ornamental fish, the brighter the color of ornamental fish, the more attractive it will be and also the higher the selling price. However, the obstacle that often occurs in ornamental fish farming, especially this type

* *Correspondence: Department of Aquaculture, Faculty of Agriculture, Mataram University, Jl. Majapahit 62, Gomong, Selaparang, Mataram City, West Nusa Tenggara*
e-mail: murtiningsihsustanti@gmail.com

of nemo fish, is a change in the brightness of its color. Therefore, color brightness is one of the things that must be considered when training this nemo fish. Color brightness in nemo fish can be improved through feeding carotenoid-enriched feed (Ningsi *et al.*, 2018).

Plant body parts, such as microalgae, contain many carotenoid compounds. Sargassum sp. is one of the brown algae from the Phaeophyta class. According to Astari *et al.* (2016), Sargassum sp. it is one type of carotene source that can be added to nemo fish feed to improve its color.

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This type of brown algae is rich in carotenoid content consisting of fukoxantin 20.95%, β-carotene 1.49% and also xanthophyll 8.46%, the advantage of *Sargassum* sp. compared to other carotenoid sources such as *Astaxanthin* and *Spirulina* where *Sargassum* sp. has a high content of carotenoid compounds in the form of fukoxantin (produces orange color) while in *Astaxanthin and Spirulina*, there is no fukoxantin content, from the presence of fukoxantin content in *Sargassum* sp. able to improve the color of fish scales (Nurusholah *et al*., 2014). In addition, *Sargassum* sp. is one of the alternative feed additives that contain a source of krotenoid pigments from easily available materials that are abundant on the coast of Indonesia, and also *Sargassum* sp. has a fairly affordable price compared to the type of material containing other carotenoid compounds such as *Astaxanthin* (carrots, pumpkin and other types) where this type of carotenoid material is still competing with human needs (*Astari et al*., 2016).Astari *et al*., 2016).

The addition of carotene sources in feed is needed to determine the effect of color changes in fish so that through the addition of carotene sources, the right handling efforts can be known for the formation of fish color (Sartikawati *et al*., 2020). This study investigates how the addition of Sargassum sp. flour to feed affects the color brightness of nemo fish *(Amphiprion frenatus).*

2. Materials and Methods

2.1. Time and Place

This study was conducted from May to July 2024 in Ekas Bay, Ekas Village, Jerowaru District, East Lombok Regency, West Nusa Tenggara Province. In West Sekotong Village, Sekotong District, West Lombok Regency, the Lombok Marine Aquaculture Center was where the nemo fish samples were taken. The Analytical Chemistry Laboratory at the Faculty of Mathematics and Natural Sciences, Mataram University measured the carotenoid content of the nemo fish samples.

2.2. Experimental Methods

An experimental method with a completely randomized design (CRD) pattern with five treatments and three replications was used in this study, resulting in fifteen experimental units, each with ten fish per unit, and five treatment doses. The addition of Sargassum meal to the feed was experimental.

2.3. Work Procedure

The research involved preparation and execution. The process is described as follows:

a. Container Preparation: In this study, the containers used were cages or nets of 15 pieces. The nets were made rectangular with a size of 1 x 1 x 1 meter and were installed in a randomized manner in floating net cages.
b. Fish Preparation and Adaptation Period: Nemo fish should be prepared at 4±0.3 cm in length and trained to acclimatize to the environment and feed type for 5-7 days.
c. Feed Preparation: During this study, the feed used was commercial pellets with 30% protein mixed with commercial Sargassum sp. flour, there were 5 treatments, namely treatment A, the feed used did not contain additional feed, treatment B contained 10% (100 gr/kg pellets) *Sargassum* sp. flour, treatment C contained 20% (200 gr/kg pellets) *Sargassum* sp. flour, treatment D contained 30% (300 gr/kg pellets) *Sargassum* sp. flour and treatment E contained 40% (400 gr/kg pellets) the mixing method used was the coating method. This feed coating method uses 1 kg of feed with the addition of *Sargassum* sp. flour according to the treatment dose, then added with 100 ml of water, 1 egg white is taken for 1 kg of feed, stirred until homogeneous, then dried using an oven. The dried feed is ready to be stored at room temperature before being given to the test fish,
d. Fish Maintenance: 10 fish per net were densely spread, with a size of 1 x 1 x 1 m. Then, the fish were fed according to the treatment.
e. Feeding is done through the at satiation method and is done twice daily: in the morning (08.00) and in the afternoon (16.00).
f. Sampling: Three nemo fish were randomly taken per treatment to observe their color development and measure their length and weight every fifteen days on days 0, 15, 30, 45, and 60.

2.4. Research Parameters

Water quality during rearing, survival rate, length and weight growth, carotenoid content, and color quality were all measured in this study. Andriani *et al*. (2018) used the Toca Color Finder (TCF) paper method with five panelists and the Adobe Photoshot CS4 method. The carotenoid content uses a spectrophotometer to determine the absorbance value at 480, 645 and 663 nm wavelengths. (Khymdeit *et al*., 2024; Rønsholdt & McLean, 2001).. Length and weight growth analysis were calculated using the formula by Banurea *et al*. (2021) and for fish life with the formula developed by Laheng *et al*. (2022).. Water quality at the study site was measured, including temperature and pH. Measurements were taken every 15 days during the study, on days 0, 15, 30, 45, and 60.

2.5. Data Analysis

To determine the effect of the research treatment, Analysis of Variation (ANOVA) will be used to analyze the data obtained from the research, such as absolute length growth and absolute weight survival rate, with a significant level of 0.05. While for color data, carotenoid content and water quality will be analyzed using descriptive analysis. Duncan's test is used to determine the location of significant data if the difference is real ($p < 0.05$).

3. Results and Discussion

3.1. Color Quality Improvement

The observation results presented are in the form of, color quality improvement including *Toca Colour Finder* (TCF) observation, *adobe photoshop* and measurement of carotenoid content.

a. *Toca Color Finder* (TCF)

The average value of observations using TCF paper according to panelists is based on Table 1 below.

Table 1.
TCF Observation Value According to 5 Panelists

Panelists	Initial	End				
	-	A	B	C	D	E
1	24	25	21	26	23	29
2	21	23	19	25	20	30
3	22	23	18	25	21	28
4	23	24	22	27	23	28
5	22	25	21	27	23	29

From the analysis, it is proven that adding *Sargassum* sp. flour to commercial feed for nemo fish (*Amphiprion frenatus*) with different doses visually using the *Toca Colour Finder* (TCF) at the beginning and end of maintenance shows a change in color, where the value is assessed by 5 panelists with the results obtained at H0 (initial) getting a range value from 5 panelists, namely 21-24, while for H60 (final) in treatment E gets the highest value from the assessment of 5 panelists with a range value of 28-30 compared to other treatments.

Observations using TCF paper are shown in Figure 1.

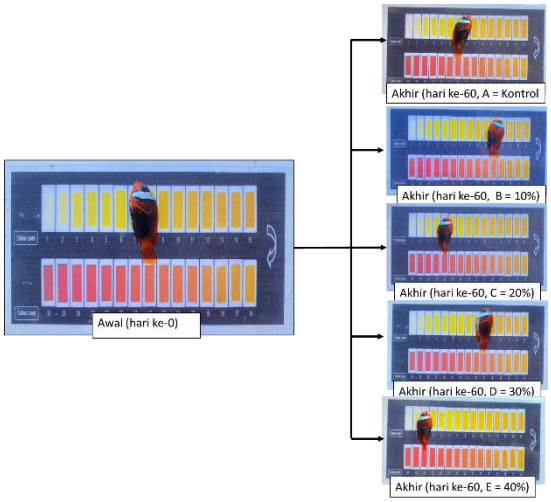

Figure 1. *Toca Color Finder*: Beginning, Day 0; End, Day 60 A. Control; B. 10% *Sargassum* sp.; C. 20% *Sargassum* sp.; D. 30% *Sargassum* sp.; E. 40% *Sargassum* sp.

Measurement of color intensity using TCF paper in this study, assessed by 5 panelists where it is known that treatment E has the highest color intensity measurement value, namely with a value range of 28-30. The high value is due to the additional effect of *Sargassum* sp. flour on food, which in *Sargassum* sp. there is a carotenoid content that can increase the color of the fish. This statement is the same as the research of Pratista *et al*., (2017) which states that *Sargassum* sp. contains carotenoids in the form of pigment compounds including chlorophyll a, b, β-carotene, violasantin, fukoxantin, pyrenoid and filakoid. From the presence of these contents, the fish will absorb the carotenoid content in the feed so that the carotenoid content will form a pigment that will increase the brightness of the color on the fish scales.

In addition, Sargassum sp. has a lot of nutritional content such as protein, carbohydrates, fat and several other types of nutritional content. The nutritional content in Sargassum sp. is not only to spur faster growth, but the nutritional content can be used to improve the color of fish to become brighter. The nutritional content in question such as protein can increase the color of the scales on the fish. According to Rizky et al., (2023) the mechanism of protein to improve color in fish begins with the protein contained in the feed being able to absorb and spread to the chromatophore of the fish then stimulates the hormone melanosite stimulanting hormone (MSH) which is responsible for the process of increasing pigmentation in fish. Similar to the statement Hafiz *et al.*, (2020) which states that the content of proper and high-quality nutrients can help growth to be better and brighten the color.

b. *Adobe Photoshop*

The results of the *adobe photoshop* test on the addition of *Sargassum* sp. flour to the color brightness of nemo fish (*Amphiprion frenatus*) were carried out at the beginning and end of the rearing time. Hue value measurements can be seen in Table 2.

Table 2.
Hue Value Measurement Using *Adobe Photoshop*

Day-	Treatment	Hue Value (º)
H0	-	22
	A (0%)	20
	B (10%)	26
H60	C (20%)	15
	D (30%)	24
	E (40%)	12

The adobe photoshop test results prove that the dose of Sargassum sp. flour added to the nemo fish feed is influenced by the brightness of the nemo fish color at the beginning and end of the study. Measurement of the hue value of nemo fish reared for 60 days showed that the best hue value was obtained in treatment E with the addition of Sargassum sp. flour in feed as much as 40%, which amounted to 12º.

Based on the results of the study, the brightness value of the fish can be determined using adobe photoshop application by looking at the hue value of the fish. The study showed that adding Sargassum sp. flour to nemo fish feed can increase the brightness of its color. In treatment E, the addition of 40% flour was the treatment with the lowest hue value, which is considered a good value. This is due to the content of carotenoid compounds in Sargassum sp. which can increase the number of chromatophore cells, improving the color performance of fish. According to Nurusholah *et al*. (2014) that in *Sargassum* sp. there is a carotenoid content such as beta carotene 1.49% and fucosanthin 20.95% where the content is able to increase the color of fish scales. In the hue value, it is known that the low hue value is a relatively good value, because basically, the quality of the fish color will be better with a lower hue value, and vice versa, the fish color will be more faded with a higher hue value. This is in accordance with the statement Rahman *et al.*, (2021) which states that fish with lower hue values tend to be reddish in color, and higher hue values tend to be faded yellow.

c. Carotenoid Content

Carotenoid content test on the color brightness of nemo fish *(Amphiprion frenatus)* by adding Sargassum sp. flour at various feed doses was carried out at the beginning and end of rearing time. Table 2 shows the measurement of carotenoid content.

Table 3.
Carotenoid Content Measurement Results

Treatment	Carotenoid Content (µmol/L)
A	261,86
B	198,22
C	298,26
D	208,11
E	470,26

The results of the measurement of the carotenoid content of nemo fish (*Amphiprion frenatus*) reared for 60 days showed that the highest value of carotenoid content was found in treatment E which amounted to 470.26 µmol/L.

Based on the results of the analysis of carotenoid content contained in nemo fish scales, treatment E with 40% addition of Sargassum sp. flour got the highest carotenoid content test value compared to other treatments with a value of 470.26 µmol/L. Since Sargassum sp. has the carotenoid compounds beta carotene, which gives a yellow-orange color, and fucosanthin, which gives an orange color, the addition of Sargassum sp. flour to fish feed helps improve the color of the fish. According to Astari *et al*. (2016)the color absorption process of Sargassum sp. is as follows: First, the color pigments present in the feed or carotene are absorbed directly, then move through the blood and finally stored in fatty tissue. The movement of pigment cells occurs by collecting and then will be absorbed and spread to the chromatophore contained in the epidermis, After that, the pigment granules will absorb the light completely, increasing the color of the fish scales.

3.2. Length and Weight Growth

a. Absolute Length

The results of research on nemo fish (Amphiprion frenatus) reared for 60 days with the treatment of the addition of Sargassum sp. flour proved that there was a significant difference in absolute length. The absolute length of fish in this study is presented in Figure 2 as follows. Fish that were given the addition of Sargassum sp. flour at a dose of 40% showed the highest absolute length of 1.59 cm, while fish that were not given the addition of Sargassum sp. flour showed the lowest absolute length of 1.11 cm.

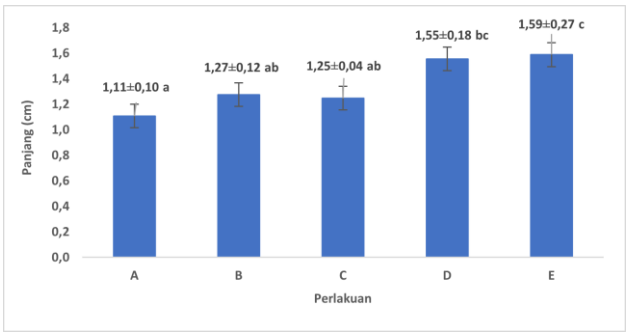

Figure 2. A. Control; B. *Sargassum* sp. 10% flour; C. *Sargassum* sp. 20% flour; D. *Sargassum* sp. 30% flour; E. *Sargassum* sp. 40% flour

Based on research, the addition of Sargassum sp. flour to feed can increase the absolute length growth of nemo fish *(Amphiprion frenatus)*. The absolute length growth performance of the treatment with the addition of Sargassum sp. flour tends to be better than the treatment without the addition of Sargassum sp. The results showed that treatment E, which added 40% Sargassum sp. flour, had the highest absolute length of 1.59 cm. The control treatment or treatment A, which did not add Sargassum sp. flour, had the lowest absolute length of 1.11 cm. Sargassum sp. is a type of seaweed that has many nutrients, including vitamins, proteins, minerals, carbohydrates, and other nutrients, which can accelerate fish growth. Ahriani & Syam, (2023) suggests, that Sargassum sp. can also be used as an additional feed for fish because it has many nutrients, including proteins, vitamins, carbohydrates, crude fiber, lipids, and minerals. It can increase fish appetite, improve energy utilization for growth.

b. Absolute Weight

The results of research on nemo fish *(Amphiprion frenatus)* reared for 60 days with the addition of Sargassum sp. flour showed a significant increase in absolute weight. Fish that were given a dose of 40% Sargassum sp. flour showed the highest absolute weight increase with 4.87 g, while fish that were not given this dose showed the lowest absolute weight increase with a value of 2.63 g. The absolute weight obtained during the 60-day rearing period is shown in Figure 3 below. The absolute weight obtained during maintenance can be seen in Figure 3 below.

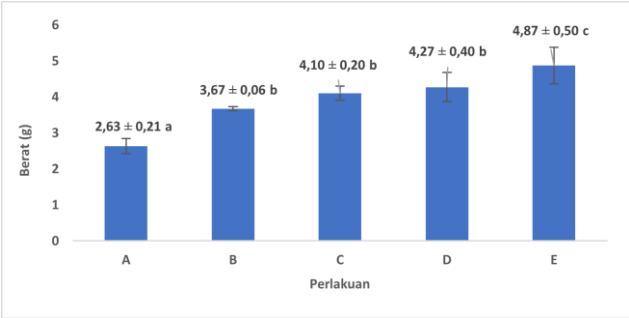

Figure 3. A. Control; B. *Sargassum* sp. flour 10%; C. *Sargassum* sp. flour 20%; D. *Sargassum* sp. flour 30%; E. *Sargassum* sp. flour 40%; C. *Sargassum* sp. flour 20%; D. *Sargassum* sp. flour 30%; E. *Sargassum* sp. flour 40%.

The results showed that the addition of Sargassum sp. flour to the feed can increase the absolute weight growth of nemo fish (Amphiprion frenatus). Treatment E, which added 40% Sargassum sp. flour, showed the highest absolute weight growth, with a larger body size than the treatment without the addition of Sargassum sp. flour. With the addition of 40% *Sargassum* sp. flour, treatment E produced the highest absolute weight growth of 4.87 g. Treatment A (control) produced the lowest absolute weight growth of 2.63 g. with the addition of 40% Sargassum sp. flour. Treatment A (control) produced the lowest absolute weight growth of 2.63g. The results showed that there were significant differences in the results of each treatment. The results during the study showed that the absolute weight of the fish increased as the dose given increased. This may be because the appetite of the fish increased along with the dose administered, which in turn caused the test fish to eat more feed. It is possible that the sufficient nutrient content of *Sargassum sp*., which met the nutritional requirements of the fish being reared, caused treatment E to produce high absolute weight growth values. This was compared to the control feed without the addition of Sargassum sp., but the addition of Sargassum sp. meal to the feed is thought to increase the number of fish eaten. According to Nugraha & Mikdarullah (2020), the content of the proximate analysis results of *Sargassum* sp. flour includes a protein content of 10.9%. Same with the statement Sahara *et al*., (2015) which suggests that feed added with Sargassum sp. flour can show an increase in absolute weight growth greater than feed that is not added with the flour. This is since Sargassum sp. flour contains growth-promoting compounds and essential

amino acids, both of which serve to improve feed nutrition, so that feed can be used for growth.

3.3. Survival Rate

The results of research on nemo fish (*Amphiprion frenatus*) reared for 60 days on the addition of *Sargassum* sp. flour with different doses, namely in treatment A using the level of 0% (control), treatment B with a dose of 10%, treatment C with a dose of 20%, in treatment D using a dose of 30% and in treatment E using a dose of 40% showed insignificant results on the level of survival. Based on the picture below:

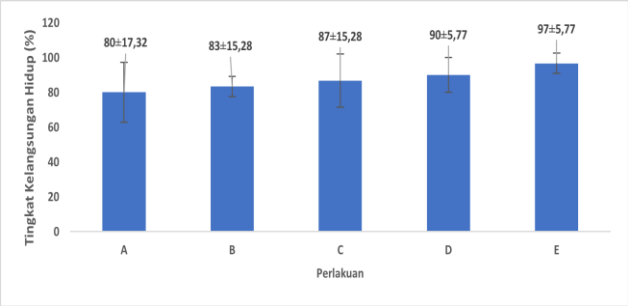

Figure 4. A. Control; B. *Sargassum* sp. flour 10%; C. *Sargassum* sp. flour 20%; D. *Sargassum* sp. flour 30%; E. *Sargassum* sp. flour 40%; C. *Sargassum* sp. flour 20%; D. *Sargassum* sp. flour 30%; E. *Sargassum* sp. flour 40%.

Based on the research that has been done, the survival rate (SR) value shows insignificant results (>0.05). Treatment E with the addition of flour *(Sargassum sp.* 40%) has the highest survival rate, while treatment A (control) has the lowest survival rate. The results of the survival rate obtained from all treatments are in the maximum category for nemo fish farming activities, where these results are obtained due to good initial preparation, starting from the acclimatization process of fish that is done well and controlling water quality regularly. Similar to what was stated by Hasanah *et al.*, (2020) which states that the survival value of clown fish obtained is in accordance with the value of clown fish production (SNI, 2012), which indicates that the survival of clown fish fry must be at least 75%.

3.4. Water Quality

The observation of water quality of nemo fish (*Amphiprion frenatus*) during the 60-day rearing period was carried out every 15 days. The water quality parameters measured during maintenance include temperature and pH. The results of temperature and pH measurements show that the value of the temperature range, and pH is still within optimal limits for the maintenance of nemo fish. Water quality measurements can be seen in Table 4.

Table 4.
Water quality during rearing

Parameters	Value Range	Literature
Temperature (℃)	27 - 27,9	27- 31
pH	7,13-7,18	7- 8,5

Source: Hasanah *et al.*, (2020)

The water quality, which was obtained in the rearing medium in this study, can be considered appropriate and optimal based on previous research on water quality for nemo fish *(Amphiprion frenatus).* In this study, water quality measurements, including temperature and pH, were taken every fifteen days. Temperature is one of the important components in aquaculture. The temperature measured during rearing ranged from 27.9°C to 27.9°C on average, which is the ideal temperature for nemo fish to live. This temperature is closely related to fish metabolism, respiration, and reproduction. Low or high temperatures, either below or above the ideal limit, can have an impact on fish diet and dissolved oxygen levels in the water. Low or high temperatures can also interfere with the growth of reared fish and may even cause death. However, acidity or pH is the level of acidity or alkalinity in the water or culture medium. During the study, the pH of the water ranged from 7.13-7.18, and this value is considered ideal for nemo fish rearing. Organisms will die if the water is too acidic or too alkaline, disrupting metabolism and respiration. This is in accordance with the opinion of Hasanah *et al.*, (2020), which states that the recommended water quality standards (SNI, 2012) for clownfish aquaculture show that the ideal water quality for nemo fish cultivation is a temperature of 27-31 degrees Celsius and a pH of 7-8.5.

4. Conclusion

The results showed that the addition of Sargassum sp. flour in feed with different doses affected the color brightness and growth of length and absolute weight of nemo fish *(Amphiprion frenatus).* The addition of *Sargassum sp*. flour to the feed also increased the color brightness of nemo fish. Treatment E, where Sargassum sp. flour was added at 40%, had the highest color brightness, growth, and survival rate.

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