Effect of Spirulina Flour Addition in Feed on the Increase of Colour Brightness Nemo Fish (*Premnasbiaculeatus*)

ABSTRACT

The nemo fish (*Premnasbiaculeatus*) is a type of marine ornamental fish in Indonesia that is in high demand among the general public. The popularity of this fish can be attributed to several factors, one of which is the brightness of its coloration.One of the plants that can be employed as a color enhancer for ornamental fish is spirulina. Spirulina is a microalgae that has been demonstrated to enhance the brightness of fish coloration.Therefore, it is necessary to research on the effect of adding spirulina flour to feed on increasing the brightness of the color of nemo fish (*Premnasbiaculeatus*).The method used in this research is an experimental method with a completely randomized design. This treatment was carried out with 5 treatments and 3 repetitions, namely: P0 : 0%/kg, P1 : 2%/kg, P2 : 4%/kg, P3 : 6%/kg and P4 : 8%/kg. The 5 treatments were repeated 3 times, resulting in 15 experimental units.The results of the researches carried out, it can be concluded that the addition of spirulina flour to feed on the brightness of the color of nemo fish (*Premnasbiaculeatus*) is proven to increase the brightness of the color of nemo fish and the best treatment is p3 with a dose of 6%. The addition of spirulina flour to feed on the growth of length and absolute weight of nemo fish (*Premnasbiaculeatus*) has a significant effect, while the survival rate has no significant effect.

Keywords: Fish, Spirulina,Colour, Growth

1. INTRODUCTION

The nemo fish (*Premnasbiaculeatus*) is a type of marine ornamental fish in Indonesia that is in high demand among the general public. The popularity of this fish can be attributed to several factors, one of which is the brightness of its coloration [1]. Color in ornamental fish is very important, because the color of nemo fish determines the value of selling price and consumer enthusiasm for nemo fish [2] The price of nemo fish with a size of 3-5 cm on the market currently ranges from IDR 5,000 to IDR 10,000 per tail [3]. The vibrant and diverse hues observed in ornamental fish are aesthetically appealing. These colors are the result of the presence of chromatophores (pigment cells) within the epidermal layer, which possess the capacity to adapt to their surrounding environment and the number and location of chromatophore movements. Carotenoids are yellow, orange, and reddish-orange pigments that are dissolved in lipids, including a collection of hydrocarbons called carotenes and their oxygenated derivatives, xanthophyll [4].

Dietary carotenoids play a significant role in regulating fish coloration, as fish, like other animals, are unable to synthesize these compounds de novo. Consequently, the coloration of fish skin is highly dependent on the dietary intake of carotenoids. Following ingestion, fish modify digestive carotenoids and store them in the integument and other tissues [5]. One of the plants that can be employed as a color enhancer for ornamental fish is spirulina. Spirulina is a microalgae that has been demonstrated to enhance the brightness of fish coloration. The carotenoid content in commercially available spirulina ranges from 3.5 to 5.7 g/kg and can be used as a source of carotenoids [6]. The beta-carotene in spirulina gets taken in by the fish and used by the body to brighten the pigment cells [7].

Research on the addition of spirulina flour has previously been conducted by (Saputra*et al*., 2023) which can brighten the color of hickey (*Betta splendens*) with a dose of spirulina flour addition of 1.5%.Therefore, it is necessary to conduct research on the effect of adding spirulina flour to feed on increasing the brightness of the color of nemo fish (*Premnasbiaculeatus*).

2. MATERIALS AND METHODS

2.1 Research Methods

ThisresearchwascarriedoutinMay–July2024 at the Ekas By Floating Net Cage, EkasVillage,JerowaruDistrict,EastLombokRegency, West Nusa Tenggara Province, Indonesia.The method used in this research is an experimental method with a completely randomized design. This treatment was carried out with 5 treatments and 3 repetitions, namely: P0 :0%/kg, P1 : 2%/kg, P2 : 4%/kg, P3 : 6%/kg and P4 : 8%/kg. The 5 treatments were repeated 3 times, resulting in 15 experimental units. Each experimental unit was then arranged in one floating net cage unit.

2.2Research Procedure

Preparation of research carried out such as preparation of tools, materials, and biota as well as making feed. The tools used in this research were nets sewn into boxes measuring 1 x 1 cm, the biota used were 250 nemo fish with a length of 5 ± 3 cm. The depth used in this reserch is 1 meter. The nemo fish used were obtained from the Sekotong Marine Aquaculture Center (BPBL).

Initially, these marine biota were familiarized with their new environment by placing them in a container containing seawater for 15 minutes. This helped to maintain the temperature of the water in the bag to match that of seawater. Then, the plastic packaging is opened to allow the biota to acclimatize. This acclimatization period lasts about 5-7 days.

Once the requisite tools and materials had been prepared, the next step was to stock the Nemo fish. This was achieved by placing the fish into each net. The density employed was 10 nemo fish per net.Measurement of color brightness using the TCF method by five panelists, and checking the carotenoid content and taking pictures for Adobe photoshop were carried out at the beginning and end of the research. Then, length and weight of Nemo fish were recorded at two-week intervals. Furthermore, water quality observations were made at the research site, including temperature and pH measurements at 15-day intervals: 0 days, 15 days, 30 days, 45 days, and 60 days.

2.3 Data Collection

The primary parameters examined in this investigation were color characteristics (toca color finder, carotenoid content, adobe photoshop analysis), growth (absolute length and weight growth), survival rate, and data analysis.The parameters observed in this study were color quality improvement, carotenoid content, length and weight growth, survival rate and also water quality measurements during the rearing period. Color brightness using Toca Color Finder (TCF) paper method with 5 panelists [8] and method with Adobe photoshot CS4 software. Carotenoid content using a spectrophotometer to determine the absorbance value at 480, 645 and 663 nm wavelengths [9]. Length and weight growth analysis were calculated and fish survival rate with the formula by[10] and water quality data by way of description.

Data Analysis

Data obtained from the results of this research such as absolute length growth, absolute weight, and survival rate of nemo fish will be analyzed using Analysis of Variance (ANOVA) at a significant level of

0.05, if the results obtained are significantly different (p<0.05), then the Duncan test and homogeneity test are carried out to obtain the location of the significance of the data obtained. Meanwhile, data on color brightness and water quality were presented descriptively.

3. RESULTS AND DISCUSSION

3.1 Results

Colour parameters

Tocacolour finder (TCF)

Based on the results of observations of the color brightness of nemo fish (*Premnasbiaculeatus*) visually using TCF assessed by 5 panelists conducted at the beginning and end of the rearing time can be seen in Table 1 and Figure 1:

Tabel 1. Assessment Results by Panelists

Panelists	Beginning	End				
	Control	P0	P1	P2	P3	P4
1.	21	24	25	27	28	27
2.	20	23	26	27	30	28
3.	20	24	26	27	29	28
4.	21	24	25	26	30	27
5.	20	22	26	27	30	28

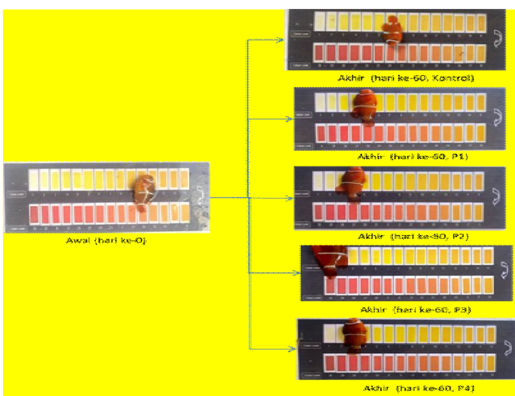

Figure 1. Colour of Nemo Fish before and after the effect of adding Spirulina Flour to the feed.

The beginning of the research, all treatments used the control treatment with an average value obtained from panelists of 20-21. At the end of the research, observations were made using 5 different research samples, with the highest average value of the final toca color finder found in the P3 treatment with a color level of 28-30 and the lowest final toca color finder value found in P0 with a color level value of 22-24

Carotenoid content

The test results of carotenoid levels in the addition of spirulina flour to the brightness of the color of nemo fish (*Premnasbiaculeatus*) carried out at the beginning and end of the maintenance time can be seen in table 2 :

Tabel 2. Results of carotenoid content

Treatment	Carotenoid content (µmol/L)
P0	197.7
P1	212.39
P2	339.69
P3	452.38
P4	354.93

The results of the carotenoid content test show that the provision of spirulina flour in nemo fish commercial feed with different doses of nemo fish color brightness makes a difference at the beginning and end of the research. Where at H0 which uses the control treatment, the carotenoid content value is 197.7 µmol/L, then at H60 shows an increase in carotenoid content test results with the highest treatment being P3 treatment of 452.38 µmol/L, then P4 of 354.93 µmol/L, P2 339.69 µmol/L, and the lowest treatment P1 of 212.39 µmol/L.

Adobe photoshop

The results of the adobe photoshop test on the addition of spirulina flour to the brightness of the color of nemo fish (*Premnasbiaculeatus*) carried out at the beginning and end of the maintenance time can be seen in table 3 :

Tabel 3. Results of hue value

Day to-	Treatment	hue value (°)
Beginning	-	33
End	P0	30
	P1	26
	P2	23
	P3	18
	P4	20

The adobe photoshop test results show that at H0 which uses the Control treatment, the hue value is 33. At H60, the hue value with the highest treatment is P0 treatment of 30, then P1 of 26, P2 of 23, P4 of 20 and the lowest treatment P3 of 18.

Growth parameters

Absolute length growth

Based on the observation of Absolute Length Growth of nemo fish (*Premnasbiaculeatus*) can be seen in Figure 2:

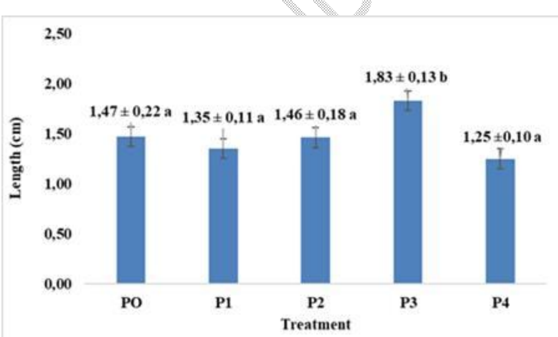

Figure 2. Absolute Length Growth of Nemo Fish

ANOVA test results showed that the provision of spirulina flour commercial feed for nemo fish with different doses had a significant effect ($p < 0.05$) on the average absolute length of nemo fish. Duncan's further test results showed that the spirulina flour addition treatment P0 treatment was not significantly different from the P1, P2 and P4 treatments but significantly different from the P3 treatment. This test shows the value of absolute length growth with the highest value found in the P3 treatment container which amounted to 1.83 cm, followed by P0 treatment of 1.47 cm, P2 treatment of 1.46 cm, P1 treatment of 1.35 cm and P4 treatment of 1.25 cm.

Absolute weight growth

Based on the results of observations and measurements of the weight of nemo fish (*Premnasbiaculeatus*) reared for 60 days. Absolute weight growth of nemo fish (*Premnasbiaculeatus*) can be seen in Figure 3:

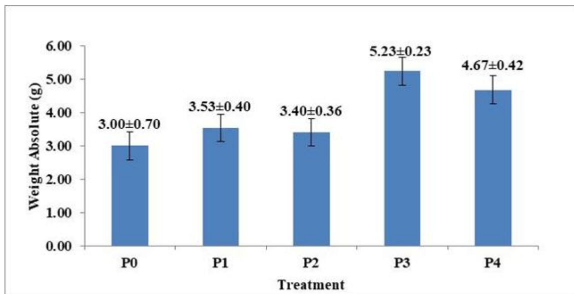

Figure 3. Absolute Weight Growth of Nemo Fish

ANOVA test results showed that the provision of spirulina flour commercial feed nemo fish with different doses had a significant effect ($p < 0.05$) on the average absolute weight of nemo fish. The results of Duncan's further test showed that the treatment of spirulina flour addition of 6% (P3) gave the highest average absolute weight of nemo fish. P0 treatment was not significantly different from P1 and P2 treatments but significantly different from P3 and P4 treatments. showed the value of absolute weight growth with the highest value found in the P3 treatment container which amounted to 5.23 g, followed by P4 treatment of 4.67 g, P1 treatment of 3.53 g, and P0 treatment of 3.00 g. The highest value was found in P3 treatment with spirulina flour addition of 6% (P3). The highest value was found in the P3 treatment with 6% spirulina flour and the lowest value was found in the P0 treatment without spirulina flour.

Survival rate

Based on the results of observations and measurements of the weight of nemo fish (*Premnasbiaculeatus*) reared for 60 days The survival rate is presented in Figure 4 :

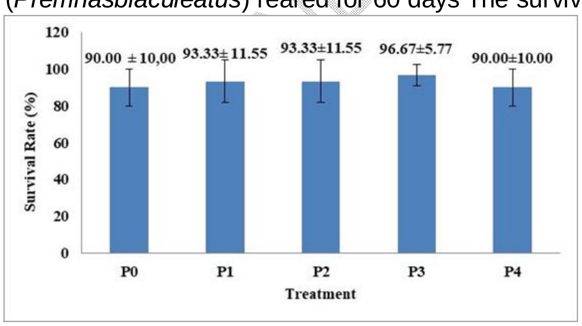

Figure 4. Survival rate of Nemo Fish

The results of the ANOVA test showed that the effect of the addition of spirulina flour in feed on the brightness of color in nemo fish with different concentrations had no significant effect ($p > 0.05$) on the SR of nemo fish.The survival rate of nemo fish in this research was P0 treatment of 90%, P1 treatment of 93.33%, P2 treatment of 93.33%, P3 treatment of 96.67%, and the value of the survival rate in the P4 treatment of 90.00%.

Water quality

In nemo fish (*Premnasbiaculeatus*) rearing activities, water quality parameters observed during the research include: temperature and pH (degree of acidity). From the results of observations of water quality showed optimal results. The results of observations of water quality parameters can be seen in Table 4 as follows:

Table 4. Results of Water Quality Measurements During Maintenance

Parameters	Range	Literature
Suhu (°C)	27,1-27,7	25-30 [9]
pH	7,12-7,17	7,5-8,4 [10]

3.2 Discussion

Colour parameters

Tocacolour finder (TCF)

The results of this quantification display 30 color rankings starting from the lowest color to the highest color. The nemo balong fish itself is generally red, so the color scale that is suitable for TCF starts on the red-orange color scale from a scale of 15-30. During the acclimatization period, all treatments used the control treatment and the initial average value obtained by the panelists on TCF was 20-21. At the end of the research, observations were made using 5 different research samples, with the highest average final toca color finder value found in the P3 treatment with a color level of 28-30 and the lowest final toca color finder value found in P0 with a color level value of 22-24.The use of 6% spirulina dose in P3 treatment experienced a higher color increase than all treatments. The high absorption of carotenoids in P3 is due to the sufficiency of carotenoid substances derived from spirulina as a source of carotenoids that are in accordance with the needs of fish, this is in line with the statement of spirulina [11]that spirulina contains carotenoids that can increase the color intensity of fish. The treatment with the lowest TCF score was the control treatment or without the addition of spirulina flour. This is due to the lack of carotenoids contained in commercial feed and the absence of additional carotenoid sources.Chromatophore cells do not increase when the feed consumed by fish does not have carotenoid content.The second highest treatment was P4, which involved a spirulina dose of 8%. At this higher dose, the color of the fish appeared to be more faded than in the P3 treatment, which involved a spirulina dose of 6%. This indicates that the degree of color brightness in fish is not solely contingent on the dosage administered, but rather on the fish's intrinsic carotenoid requirements. An increase in dosage may lead to the fish's inability to effectively process its carotenoids, potentially exceeding its metabolic capacity and resulting in the wasteful expenditure of these nutrients. This could, in turn, contribute to the decline of carotenoid cells within the fish body.at higher doses, excess carotenoids will not be digested by the fish body but will be excreted through the feces [12].

Carotenoid content

The best treatment was P3 with a carotenoid content value of 452.38 μmol/L. The high color change is due to the addition of spirulina flour which contains color pigments such as Beta-carotene, zeaxanthin and xanthophyll.Spirulina sp is a blue green algae that is rich in protein, vitamins, minerals and other nutrients. Spirulina contains a carotene source composed of xanthophyll (37%), Beta-carotene (28%) and zeaxanthin (17%) [13]. Carotenoids derived from spirulina will be absorbed and spread to the chromatophore of the fish and stimulate the hormone melanosite stimulating hormone (MSH) which is responsible for the pigmentation process of fish. The high protein content in spirulina is also able to increase cell division activity so as to stimulate the production of MSH hormone so as to increase the movement of chromatophore pigment granules[14]. Furthermore, the pigment granules will absorb light perfectly resulting in an increase in the color of the fish scales. The treatment of the lowest carotenoid value is found in the P0 treatment, this is because in the P0 treatment there is no addition of spirulina flour to the feed, so that the lack of carotene content synthesized by the fish body, chromatophore cells do not increase when the feed consumed by fish does not have carotenoid content.

Adobe photoshop

Hue is a combination of basic colors including red, green, blue in adobe photoshop so that the calculation of hue can represent the color expression of the fish [15]. During the acclimatization period, the initial fish object was selected, and a hue value score of 33° was obtained. At the conclusion of the research, the measurement results indicated that the hue value with the highest score was observed in the P0 treatment, with a value of 30°, while the lowest hue value score was noted in the P3 treatment, with a value of 18°. The discrepancy in hue values may be attributed to the influence of feed digested by the fish body [16]. The hue value is susceptible to alterations due to the type of feed and the interaction between feed type and consumption time. A lower hue value indicates a greater concentration of red hue in the object image, whereas a higher hue value indicates a reduction in red hue concentration and a corresponding increase in paleness, that a hue value approaching zero signifies the reddish hue of the fish, whereas a higher hue value indicates a yellowish hue[17].

Growth parameters

Absolute length growth

The highest absolute length obtained by P3 treatment with a dose of 6%/kg of feed, which amounted to 1.83 cm, is thought to be due to the adequate protein and fish nutrition provided by the addition of spirulina flour. spirulina possesses a protein content of up 55-70% [18]. The low value of absolute length growth in the P4 treatment is thought to be due to the fish being unable to utilize the feed properly, despite the fact that P4 has the highest treatment dose. It is suspected that the fish use energy from the feed much more than is absorbed for metabolic processes alone, exceeding the limits of fish needs to such an extent that energy reserves for long growth are reduced, the growth of fish body length is attributed to the utilization of protein in the digestive process of the feed provided [19].

Absolute weight growth

The highest value was found in the P3 treatment with 6% spirulina flour and the lowest value was found in the P0 treatment without spirulina flour. The high absolute weight growth in P3 proves that feed added with Spirulina flour can affect the increase in fish growth. This is because spirulina flour as a food source rich in nutrients such as protein that can stimulate fish growth and at these doses is in accordance with the needs of the fish body. Spirulina has B12, and contains quite complete amino acids[20]The low growth results in P0 are thought to be because the feed consumed by fish is only used for the performance or metabolic processes of the body and only a little for growth, compared to the treatment of the addition of spirulina flour which contains high protein the availability of protein in the feed will affect fish growth, because protein is a nutrient that is needed by fish for growth [21].

Survival rate

The results of the research indicate that the highest survival rate (SR) value is associated with the P3 treatment, while the lowest survival rate value is observed in the P0 and P4 treatments. The nemo fish survival rate values for each treatment in this research are as follows: P0 (90%), P1 (93.33%), P2 (93.33%), P3 (96.67%), and P4 (90.00%). The survival rate value obtained is still within the optimal range for nemo fish farming. The survival rate of nemo fish farming is at least 75% [22].These results were obtained with meticulous preparation of the containers utilized, a comprehensive fish acclimatization process, and an optimal cultivation environment. The survival of fish is also influenced by their nutritional intake. During the maintenance phase, the results demonstrate that the feed provided is within an adequate range for maintaining the quality of fish life .A high survival rate indicates that the quality and quantity of feed are optimal for meeting the growth and nutritional needs of fish [23].

Water quality

The water temperature during the research exhibited a range of 27.1-27.7°C, which aligns with the optimal range for the growth of nemo fish. This temperature range is conducive to nemo fish cultivation, with optimal growth occurring between 25-30°C [24]. This stable and optimal water temperature provides an environment conducive to the metabolic processes and growth of Nemo fish. Temperature is one of the most significant abiotic factors influencing the activity of aquatic biota. Excessive or insufficient temperatures can impede fish activity [25]An increase in temperature can result in a disruption of fish health, leading to a weakening of the body, a loss of body mass, and the emergence of abnormal behaviors. Conversely, low temperatures can also have a detrimental impact on fish, causing depression and a reduction in respiratory and cardiac activity, which can ultimately result in fish fainting.

pH or degree of acidity is an acidic or alkaline condition of a sea or cultivation media. Based on the results of the research, the pH value of water ranged from 7.12 to 7.17. The pH value is still in the optimal range for nemo fish. The optimal pH for clown fish growth is pH 7.2-8.3 or pH 8-8.3 [26]. A water body that has a low pH can result in decreased growth activity. low pH values can reduce growth rates. Low pH can also cause fish to be weak and more easily infected with disease [27].

4. CONCLUSION

Based on the results of the researches carried out, it can be concluded that the addition of spirulina flour to feed on the brightness of the color of nemo fish (*Premnasbiaculeatus*) is proven to increase the brightness of the color of nemo fish and the best treatment is p3 with a dose of 6%. The addition of spirulina flour to feed on the growth of length and absolute weight of nemo fish (*Premnasbiaculeatus*) has a significant effect, while the survival rate has no significant effect.

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