Effectiveness of Adding Spirulina Meal in Improving the Color of Platy Fish (*Xiphophorus maculatus*)

ABSTRACT

This study was to determine the effectiveness of adding spirulina meal in increasing the color of platy fish and to determine the right dose of spirulina in feed on the intensity of the color of platy fish. The study was conducted in the maintenance activities at the Production and Reproduction Laboratory, Faculty of Agriculture, Mataram University and used the Completely Randomized Design (CRD) method, experiments, with 4 treatments and 3 repetitions. In P1 (100% control feed), P2 (99% feed + 1% spirulina meal), P3 (97% feed + 3% spirulina meal) and P4 (95% feed + 5% spirulina meal). The commercial feed used was in the form of powder mixed with spirulina meal according to the treatment. The fish used were platy fish seeds with a length of 1-2 cm. Observations of carotenoids on feed were carried out before feeding the test fish. The brightness level of the platy fish skin was measured on days 0, 15, 30 and 45, as many as 3 fish in each treatment using a colourimeter. The level of color brightness, lightness, redness, yellowness, hue and chroma based on the day and dose of ANOVA results showed that feeding with the addition of different spirulina meal had a significant effect ($P<0.05$) on the level of color brightness, the growth rate of platy fish, while the survival rate of platy fish had no significant effect. Conclusion this experimentadding 3% spirulina meal with a maintenance period of 30 days can improve the color of platy fishwith a Lightness (L*) value of 47.26, Redness (a*) of 24.63, Yellowness (b*) of 24.90, Hue of 61.77 and Chroma value of 44.77 with a carotenoid content of 15.56 µmol/l

Keywords: carotenoids, color brightness level, platy fish, spirulina meal,

1. INTRODUCTION

Platy fish are freshwater ornamental fish that have beautiful colors that vary in their bodies and fins and have a small body shape. This fish has a friendly and non-aggressive nature, making it very suitable for use as ornamental fish in aquascaping. The beauty of the color of platy fish is one of the consumer attractions, so farmers need to improve the color of platy ornamental fish(Amin et al., 2019). Efforts made by ornamental fish farmers to get bright colors in platy fish are to add pigment sources to the feed. the addition of dyes or pigments in feed (for ornamental fish) will help stimulate the dyes in the fish's body. One of the feed ingredients that can be used as a source of natural pigments is spirulina.

Spirulina contains phycocyanin, chlorophyll-a and carotene(Andriani, et al., (2018). Carotene is composed of xanthophyll (37%), β-carotene (28%) and zeaxanthin (17%)(Tongsiri et al., 2010). Spirulina meal can be used as a source of carotenoids which are components that form dyes that give red and yellow colors to ornamental fish Satyani and Sugito (1997) in(Rosida, 2018). According to(Malini et al., 2016)the β-carotene content in spirulina can increase the number of chromatophore cells so that the brightness of the color in ornamental fish can increase.

The addition of spirulina meal to increase the color intensity of ornamental fish has been carried out by several studies, including the results ofKoncara et al., (2014)research that 3% spirulina content has a more effective effect and produces brighter colors in guppy

fish. Likewise with the research ofNoviyanti et al., (2015)where the addition of 1.2 grams of spirulina meal gave the best effect on the color intensity of goldfish. While in the research of Amin et al., (2019)with a dose of spirulina meal 4% the highest dose to improve color quality in platy fish. The difference between this study and the previous one is in the different doses and using the colourimeter color parameter test.

Therefore, the importance of this study is to determine the effect of adding spirulina to feed on the color intensity of platy fish and to determine the right dose of spirulina in feed on the color intensity of platy fish.

2. METHODOLOGY

2.1 Time and Place

This research was conducted for 45 days from February 20 - April 5, 2024, maintenance activities at the Production and Reproduction Laboratory, Faculty of Agriculture, Mataram University, checking the quality of fish color at the Bioprocess Laboratory, Faculty of Food Technology and Agroindustry, Mataram University, Carotenoid content analysis was carried out at the Analytical Chemistry Laboratory, Faculty of Mathematics and Science, Mataram University.

2.2 Research Methods

This study was conducted using the Completely Randomized Design (CRD) method. The method used in this study was an experiment, using 4 treatments and 3 repetitions with a control dose (without spirulina), 1%, 3%, and 5%, spirulina meal, so that there were 12 experimental units.

P1 : Control feed (100%)
P2 : Feed (99%) and spirulinameal (1%)
P3 : Feed (97%) and spirulinameal (3%)
P4 : Feed (95%)and spirulina meal (5%)

2.3 Research Preparation

2.3.1 Feed Making

The feed used in this study used commercial feed with spirulina meal. The commercial feed used was in powder form mixed with spirulina meal according to the treatment and added 1% CMC then added 50 ml of water per 100 grams of feed and stirred until smooth(Diansyah, et al., 2019). then dried by drying in the sun until the feed is dry. Then the feed is ground according to the mouth opening of the test fish. Then a proximate test is carried out to determine the content of the feed.

2.3.2 Container Preparation

Preparation of the container begins with preparing a container in the form of a 30 liter container, a jar container, aeration hose, aeration stone and scoop soaked, then cleaned with running water and then dried. The clean container is filled with 15 liters of water or 50% of its volume, aeration is installed and left for 24 hours (Barus, 2014).

2.3.3 Test Fish

The fish used in this study were platy fish seeds with a length range of 1-2 cm. The density of fish stocking was 1 fish/l so that the number of test fish stocked was 10 fish per

container. The test fish used must be healthy by looking at the bright color and moving actively. The test fish were acclimatized in a temporary holding tank using aeration for approximately 3 days.

2.4 Maintenance Phase

Platy fish were kept for 45 days. The length and weight measurements of platy fish were carried out every 9 days to determine the growth rate, and for the survival of fish seeds, fish were counted at the beginning and end of the study for the total number of fish.

2.4.1 Feeding

The feed used during the study was fed according to the treatment. Feeding was done twice a day at 10:00 WITA and 15:00 WITA for each treatment. The amount of feed given was 5% of the average body weight of the fish (Barus, 2014).

2.4.2 Observation of Feed Carotenoids

Observation of carotenoids on the treatment feed was carried out before feeding the test fish. Observation of carotenoids in feed used spectrophotometry. The method of carotenoid analysis used the spectrophotometry method, namely the sample was added with 10 ml of technical acetone. Then homogenized at a speed of 1,500 rpm for 1 minute. The results were then filtered using filter paper and the volume (extract volume) was measured, then the absorbance was measured using wavelengths of 480, 645 and 663 nm. Furthermore, the value was entered into the formula to calculate the value of carotenoid content in the test fish (Hendry and Grime 1993 in Saputri (2017).

2.4.3 Improving Fish Color Quality with a Colourimeter

The skin brightness level of platy fish was measured on day 0 (before treatment), day 15, day 30 and day 45 (after treatment) for 3 fish in each treatment using a colourimeter. The colourimeter tool is turned on first, then the sensor is directed at the test material and then the measurement results will appear on the display screen.

2.5 Research Parameters

The parameters to be observed in this study are: testing of feed carotenoids, observing the color of test fish, specific growth and survival rate.

2.5.1 Calculation of Carotenoid Content in Feed

The calculation of carotenoid levels according to Hendry and Grime (1993) in Saputri (2017) is as follows:

$$Karotenoid(\mu molL) = \frac{(A480 + 0.114xA663 - 0.638xA645)xVx103}{112.5xW}$$

Information :

A480	: Absorbance at a wavelength of 480 nm
A645	: Absorbance at a wavelength of 645 nm
A663	: Absorbance at a wavelength of 663 nm
V	: Extract Volume (mL)
W	: Sample weight (g)

1µmol/L : 27.25 mg/L

2.5.2 Color Observation on Test Fish

The brightness level of the platy fish skin was measured on day 0 (before treatment), day 15, day 30 and day 45 (after treatment) as many as 3 fish in each treatment using a colotimeter (Minolta Meter CR-400). The measurement of the brightness of the platy fish was carried out by placing the sample directly under the sensor lens of the colotimeter tool, then the brightness value will be displayed on the monitor of the tool. The color brightness measurements tested include Lightness, Redness, Yellowness, Hue, and Chroma. The formula for finding Hue and Chroma according to(Sukarman, et al., 2018), is as follows:

Hue = arctan × (b*/a*)
Chorma = (a*2+ b*2)1/2

Information :
b* :*Yellowness*
a* :*Redness*

2.5.3 Specific Growth Rate Measurement

The daily growth rate, which is the percentage of the difference between the final weight and the initial weight divided by the length of maintenance time, is calculated using the formula ofRahmi *et al.*, (2017), namely:

$$SGR = \frac{Wt - Wo}{t}$$

Information:
SGR = specific growth rate (%/day),
W0 = initial weight of fish (g),
Wt = weight of fish at time t (g),
t = maintenance time (days).

2.5.4 Survival rate

Measurement of the survival of test fish can be calculated using the Effendi (2002) formula, namely:

$$SR = \frac{Nt}{N0} x\ 100\%$$

Information:
SR = Survival of test fish (%),
Nt = Number of test fish that died during the study (tails),
N0 = number of test fish at the start of the study) (tails).

2.6 Data analysis

SGR and SR test result dataThe results obtained were processed using Analysis of variance (ANOVA) and the results of the brightness test of the test fish were processed using Univariate Analysis of Variance (ANOVA) at a 95% confidence level using the SPSS program to determine the effect of each treatment . The results of the analysis that were

significantly different were further tested by Duncan. Meanwhile, the results of the carotenoid test data were analyzed descriptively.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Carotenoid Content in Feed

The results of the carotenoid test on feed with the addition of spirulina meal used in this study are in Table 1.

Table 1 Results of Feed Carotenoid Test

Feed Samples	Carotenoid levels (µmol/L)
P1 (Control)	5.57
P2	10.61
P3	15.56
P4	19.14

Information: Food Chemistry and Biochemistry Laboratory Test Results

3.1.2 Color Brightness Level

The results of the color brightness level test of platy fish during 45 days of maintenance when fed with the addition of different spirulina meal are shown based on the Lightness value (L*), namely white, the Redness value (a*), namely red , the Yellowness value (b*), namely yellow, the Hue value and the Chroma value.

a. Lightness (L*)

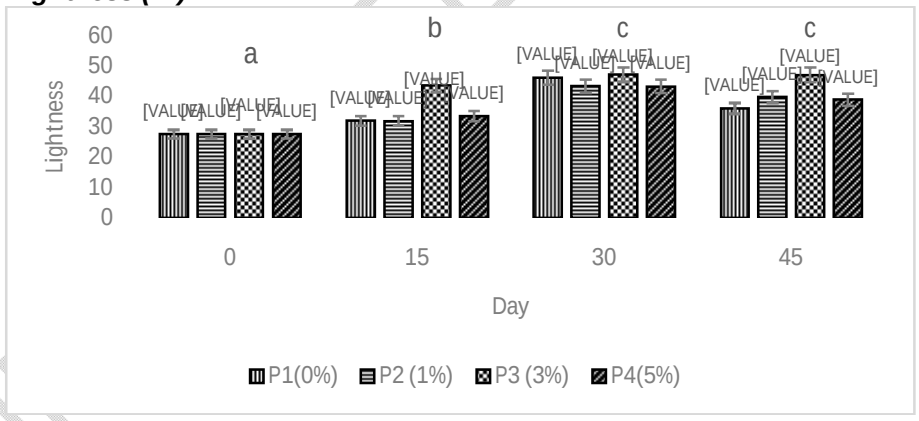

Figure 1 Lightness Value of Platy Fish (Xiphophorus maculatus) Based on Day

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect ($p<0.05$) on the Lightness value in platy fish based on the day. Based on the observation day, the Lightness (L*) results on day 0 showed different results with an increase in the Lightness (L*) value on the observations on days 15, 30 and 45, but showed the same results on days 30 and 45.

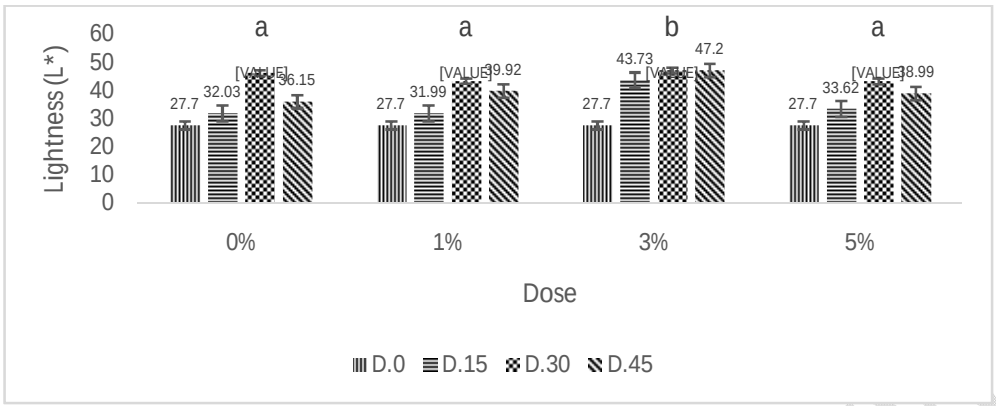

Figure 2 Lightness Value of Platy Fish (Xiphophorus maculatus) Based on Dose

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P<0.05) on the Lightness value of platy fish based on the dose. Based on the dose, the Lightness value of platy fish at a dose of 0% showed the same results as doses of 1% and 5%, but showed different results with a dose of 3%.

b. Redness (a*)

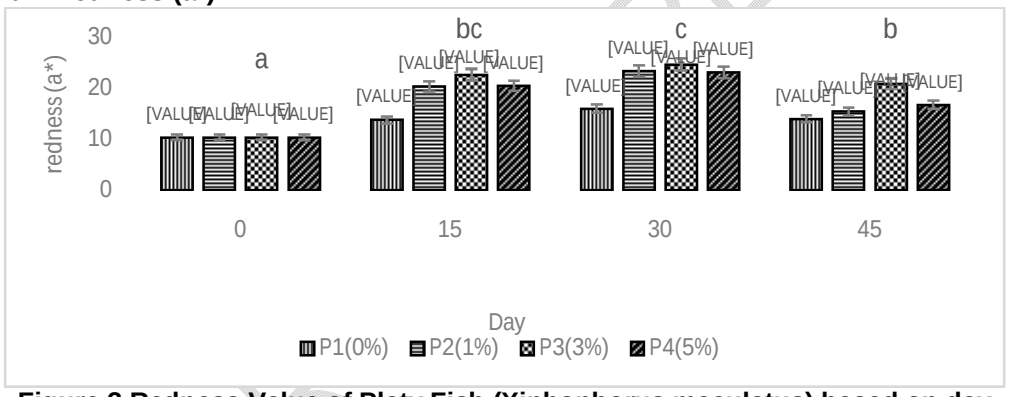

Figure 3 Redness Value of Platy Fish (Xiphophorus maculatus) based on day

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P<0.05) on the redness value in platy fish based on the observation day. So the Duncan test was conducted to determine the effect of adding spirulina meal to platy fish. The test results showed that the Redness value (a*) of platy fish on day 0 showed significantly different results with an increase in the Redness value (a*) on observations on days 15, 30 and 45, but day 30 showed different results on days 0 and 45.

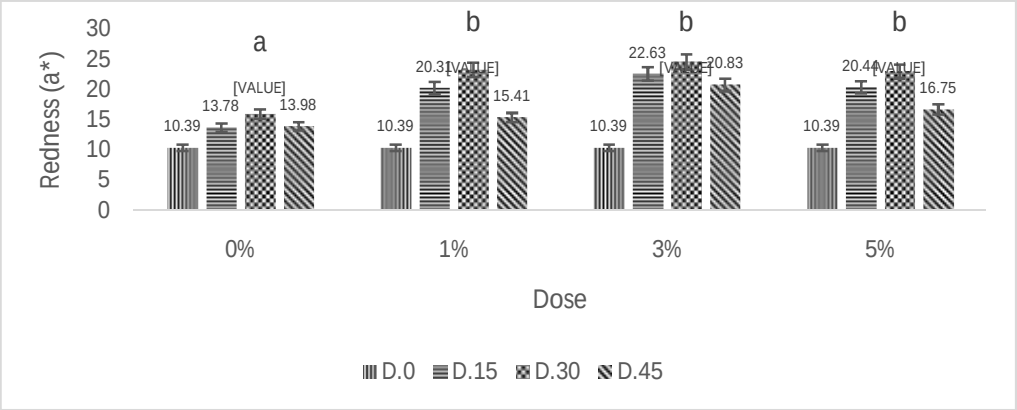

Figure 4 Redness value of Platy fish (Xiphophorus maculatus) based on dose

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P<0.05) on the Redness value (a*) in platy fish based on the dose. So the Duncan test was conducted to determine the effect of adding spirulina meal to platy fish. The Duncan test results showed that the Redness value (a*) of platy fish at a dose of 0% was significantly different from doses of 1%, 3% and 5%, but the dose of 3% was not significantly different from doses of 1% and 5%.

c. Yellowness (b*)

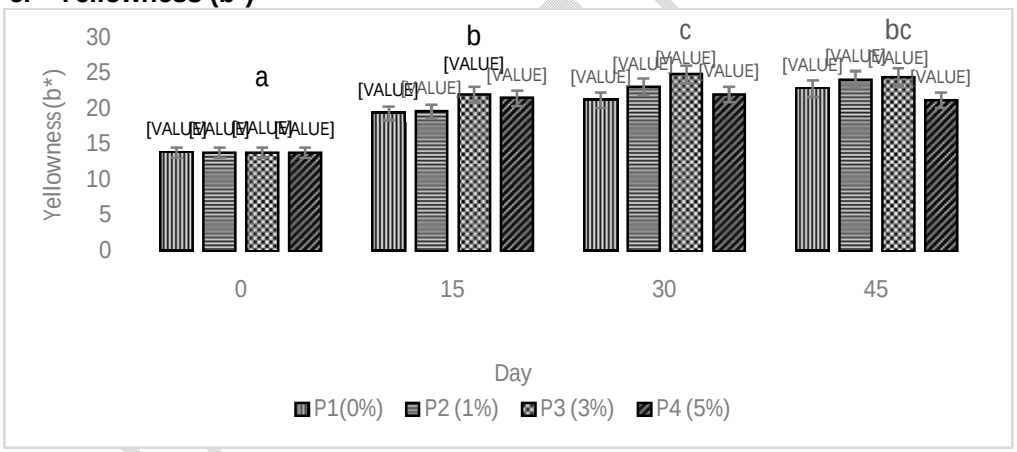

Figure 5 Yellowness Value of Platy Fish (Xiphophorus maculatus) Based on Day

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P<0.05) on the Yellowness value in platy fish based on the day. So the Duncan test was conducted to determine the effect of adding spirulina meal to platy fish. The test results showed that the Yellowness value (b*) of platy fish on day 0 was significantly different from the Yellowness value (b*) on observations on days 15, 30 and 45, but on observations on day 30 it was not significantly different from day 45.

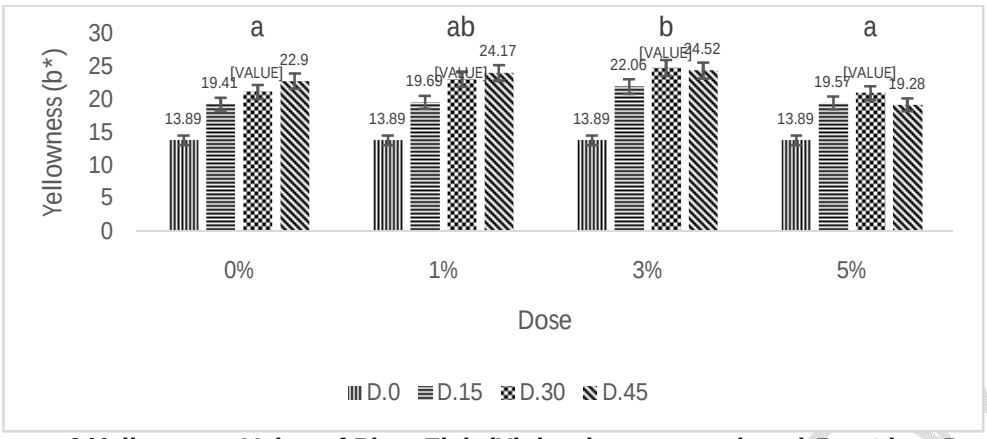

Figure 6 Yellowness Value of Platy Fish (Xiphophorus maculatus) Based on Dose

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P<0.05) on the Yellowness value (b*) in platy fish. So the Duncan test was conducted to determine the effect of adding spirulina meal to platy fish. The test results showed that the Yellowness value (b*) of platy fish at a dose of 0% was not significantly different from the doses of 1% and 5%, but was significantly different from the dose of 3%.

d. Hue

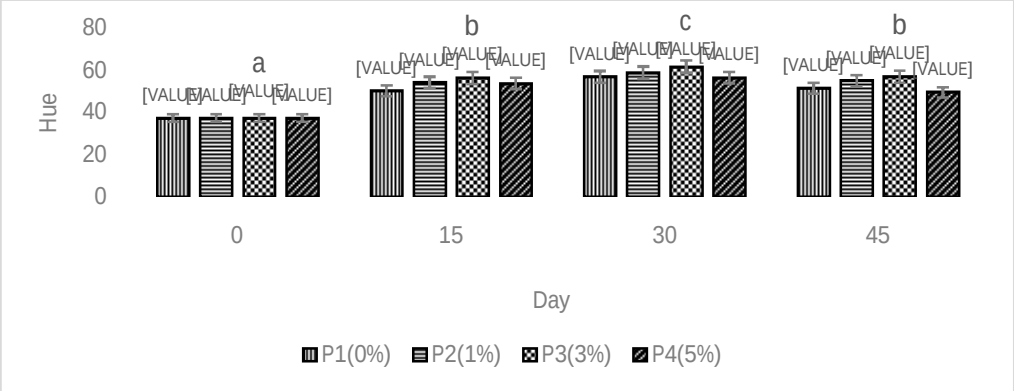

Figure 7 Hue Value of Platy Fish (Xiphophorus maculatus) Based on Day

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P<0.05) on the Hue value in platy fish based on the day. Therefore, the Duncan test was conducted to determine the effect of adding spirulina meal to platy fish. The Duncan test results showed that the Hue value of platy fish on day 0 was significantly different from days 15, 30 and 45, but day 15 was not significantly different from 45.

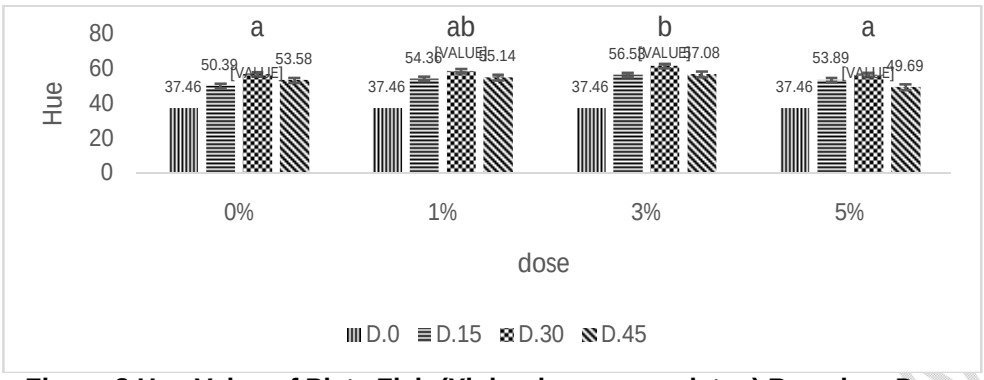

Figure 8 Hue Value of Platy Fish (Xiphophorus maculatus) Based on Dose

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P<0.05) on the Hue value in platy fish based on the dose. Therefore, the Duncan test was conducted to determine the effect of adding spirulina meal to platy fish. The Duncan test results showed that the Hue value of platy fish at a dose of 0% was not significantly different from the doses of 1% and 5% but was significantly different from the dose of 3%.

e. Chroma

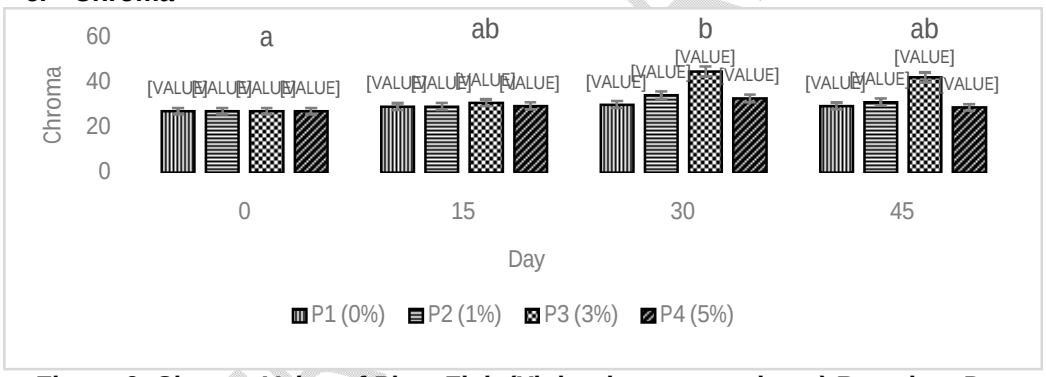

Figure 9. Chroma Value of Platy Fish (Xiphophorus maculatus) Based on Day

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P>0.05) on the Chroma value in platy fish based on the day. Therefore, the Duncan test was conducted to determine the effect of adding spirulina meal to platy fish. The Duncan test results showed that the Chroma value of platy fish on day 0 was not significantly different from days 15 and 45, but was significantly different from day 30. On day 15 it was not different from 30 and 45.

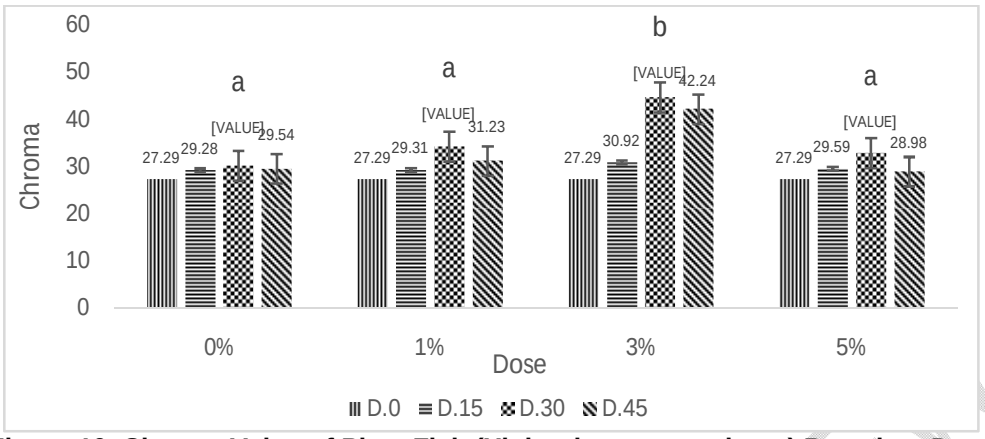

Figure 10. Chroma Value of Platy Fish (Xiphophorus maculatus) Based on Dose

The ANOVA results showed that the effect of adding spirulina meal to the feed had a significant effect (P<0.05) on the Chroma value in platy fish based on the dose. So the Duncan test was conducted to determine the effect of adding spirulina meal to platy fish. The Duncan test results showed that the Chroma value of platy fish at a dose of 0% was not significantly different from the doses of 1% and 5%, but was significantly different from the dose of 3%.

3.1.2 Specific Growth Rate of Platy Fish

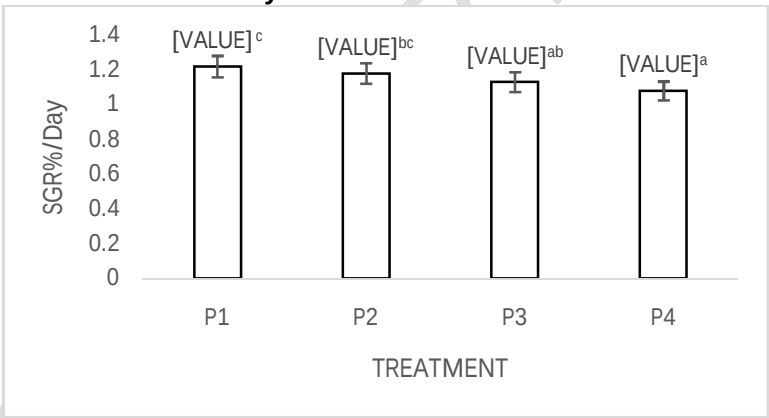

Figure 11 Specific Growth Rate of Platy Fish (Xiphophorus maculatus)

The ANOVA results showed that feeding with the addition of different spirulina meal had a significant effect (P<0.05) on the specific growth rate of platy fish. So that a further Duncan test was carried out. Based on the results of the Duncan test, it showed that the specific growth rate of platy fish in the control (P1) gave an absolute weight that was not significantly different from the P2 treatment (1%), but was significantly different from P3 (3%) and P4 (5%).

3.1.3 Survival Rate(SR)

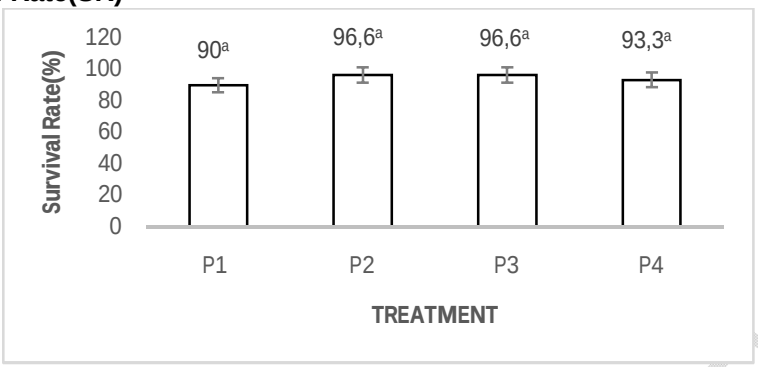

Figure 12 Survival Rate (SR) of Platy Fish*(Xiphophorus maculatus)*

The ANOVA results showed that feeding with different spirulina meal additions had no significant effect (P<0.05) on the survival behavior of platy fish. In P1(0%) the survival value was 90%, P2(1%) was 96.6%, in P3 (3%) was 96.6% while in P4(5%) was 93.3%.

2. DISCUSSION

The carotenoid levels in feed supplemented with spirulina meal increased except for the P1 (control) treatment value of 5.57 µmol/L without the addition of spirulina meal, this indicates that the control feed already has carotenoid levels in the feed. There was an increase, treatment P2 (1%) increased by 10.61 µmol/L. Treatment P3 (3%) increased by 15.56 µmol/L, while P4 (5%) increased by 19.14 µmol/L. This study shows that the addition of spirulina meal resulted in an increase in carotenoid levels of up to 5% in the feed. This is in accordance with the statement ofPutra et al., (2022)that increasing the carotenoid content in the feed given can increase carotenoids in fish.

The Lightness (L*) value is the level of brightness measured with a range of values 0 to 100 indicating dark to light colors. Therefore, the higher the L* value, the brighter the color tendency. The Lightness (L*) value of platy fish in this study increased in line with the addition of the spirulina meal dose, but there was a decrease in the P4 treatment with a dose of 5% spirulina meal. This shows that the addition of spirulina meal up to 3% (P3) to the feed is the optimum dose in increasing the color intensity of platy fish and can meet the carotenoid needs of the platy fish body. Simamora's statement (2019) that the best color brightness appearance in ornamental fish can be obtained by providing the right dose of color pigment sources, not excessive and not lacking. Therefore, the addition of spirulina meal was increased to 5% (P4) then there was a decrease in the Lightness (L*) value of platy fish. This is because excessive administration of carotenoids to fish cannot increase the intensity of fish color and can even reduce the quality of fish color and if the administration of carotenoid levels is reduced, it can affect the level of fish color intensity. It is known that the Lightness (L*) value at a dose of 3% with a maintenance period of 30 days increases the Lightness (L*) value compared to the control.

Redness(a*) indicates the color level from green to red with a range of values -80 to +100. Positive values indicate that the sample tends to show red and conversely negative values indicate that the sample shows green. Day 0 to 30 days the Redness value (a*) of platy fish tends to increase, but on day 45 there is a decrease. This is thought to be a physiological change caused by the activity of chromatophore pigment cell movement. In line with(Agustina *et al*., (2023)that on D-20 to D-40 shows that the color of the fish changes, or is less bright. Changes in pigmentation in fish from day 20 to 40 there is an increase and decrease in color due to the presence of Melanocyte Stimulating Hormone and Melatonin (MT). it is known that the Lightness value (L*) with a dose of 3% with a maintenance period

of 30 days increases the Lightness value (L*) compared to the control. the addition of spirulina meal for platy fish with a dose of 3% with a maintenance period of 30 days shows a high redness value (a*) for platy fish.

The Yellowness (b*) value ranges from -70 to +70 indicating from blue to yellow.). The Yellowness (b*) value in this study ranged from 19.41 to 24.9 so that the fish showed a yellow color. This is because the basic color of the platy fish is yellow. The Yellowness (b*) value in the platy fish before treatment was 13.89, this proves that the basic color of the platy fish is yellow to orange, so that there was an increase after the administration of spirulina meal. The Yellowness value showed an increase on days 15, 30 and 45, but the highest Yellowness value was on day 30 of 22.60. While the Yellowness (b*) value increased at a dose of 3% by 21.34. Based on observations of Yellowness (L*) it is known that the addition of spirulina meal on the 30th day with a dose of 3% showed the best results. According to(Nafsihi et al., (2016)that carotenoids form yellow, orange and red colors, while melanin mainly affects the formation of brown to black colors. Astaxanthin and xanthxanthin are two other types of carotene pigments that play a role in the formation of fish body color.

The Hue value in platy fish ranges from 50,390-61,770 which indicates that the platy fish is reddish yellow and the highest Hue value is in the P3 treatment (3%). According to (Sukarman & Hirnawati, 2018)that the value of 00 to 900 proves a color change from red to orange to yellow. The Hue value before treatment was 37,450, the platy fish showed a yellow color. In line with the statement ofNacing et al., (2021)that the Hue value range of 54 to 90 indicates a reddish yellow color. According to(Tasuib et al., (2022)stated that the lower the Hue value, the yellower the fish color becomes, conversely the higher the Hue value, the red-orange color of the fish. This is suspected to occur because platy fish tend to become reddish orange during the maintenance period. Based on observations during the maintenance period, it tends to be reddish orange, the highest value was obtained on the 30th day with a dose of 3%.

*Chroma*is the color concentration of the test material. According to Sukarma et al., (2017) in(Ayuningsih et al., 2024)that the higher the Chroma value, the more concentrated the color of an object. This study shows that the highest chroma value in the P3 (3%) treatment was 44.77. The Chroma value before treatment was 27.29, and there was an increase for 30 days, but on the 45th day there was a decrease due to the presence of Melanocyte Stimulating Hormone and Melatonin (MT). This is thought to be because the fish show color concentration. Based on Chroma observations, it is known that the addition of spirulina meal on the 30th day with a dose of 3% showed the best results. This is in accordance with the statement of(Ayuningsih et al., 2024)that the chroma value indicates the accumulation of carotenoids in pigment cells (chromatophores). The chroma value indicates the color concentration so that the higher the Hue value or type of color produced, the more concentrated the fish's body will produce.

The highest specific growth rate in treatment P1 (0%) was 1.22%/day. While the lowest specific growth rate was in treatment P4 (5%) was 1.08%/day. This study shows that the addition of spirulina meal to platy fish feed provided the lowest average growth rate in treatment P4 (5%), and the highest in treatment P1 (0%). It is suspected that platy fish utilize the spirulina meal content to improve color quality rather than the growth of platy fish. The low growth in the treatment given spirulina meal, in addition to fish using the spirulina meal content for color quality, is due to feed nutrition. In addition to protein, crude fiber and fat content are factors for the absolute weight growth of fish. This is in accordance with the research of(Rosida, 2018)where the provision of spirulina meal in feed has no effect on fish weight growth. According toAmin et al., (2019)ornamental fish that are fed carotenoid sources utilize the dye more to improve their body color.

The survival rate of platy fish in this study was very good ranging from 90 to 96.6%. This shows that the addition of spirulina meal to the feed is not harmful to the growth and survival of fish. Good and proper adaptation process so that fish can survive and grow in

controlled maintenance containers. the occurrence of fish deaths during maintenance is suspected due to stress experienced when taking data on length and weight, checking fish color, because fish must be removed from the container resulting in changes in environmental conditions. while fish experience stress when changing water and when siphoning. according toSari et al., (2014), fish survival can be influenced by biotic and abiotic factors. Biotic factors that influence are competitors, parasites, age, predation, population density, animal adaptability and human handling. while abiotic factors that influence include the physical and chemical properties of an aquatic environment.

4. CONCLUSION

Adding 3% spirulina meal with a maintenance period of 30 days can improve the color of platy fish.with a Lightness (L*) value of 47.26, Redness (a*) of 24.63, Yellowness (b*) of 24.90, Hue of 61.77 and Chroma value of 44.77. with a carotenoid content of 15.56 µmol/.

CONFLICT OF INTEREST

"The author has declared that there is no conflict of interest."

AUTHOR CONTRIBUTIONS

All authors designed the study, performed the statistical analysis, wrote the first draft of the manuscript, managed the study analysis, and managed the literature search. All authors read and approved the final manuscript."

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