Effect of addition of red spinach leaf extract (*Amaranthus tricolor* L.) in feed against the level of color brightness of sword platy fish (*Xiphophorus helleri*)

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Abstract. Color is important factor ornamental fish, especially sword platy fish, because can affect selling value. One factor influences color brightness is carotenoids. Carotenoids cannot synthesized by fish's body so must be supplied through feed provided. Red spinach is a natural ingredient that contains carotenoids. Aim of research was to determine effect of adding red spinach leaf extract to artificial feed to increase color brightness, growth and survival of *Xiphophorus helleri*. Research method used a completely randomized design with 4 treatments and 4 repetitions. Concentrations of red spinach leaf extract are 0%, 3%, 6% and 9%. Parameters observed were color brightness, absolute weight, absolute length, survival rate and water quality. Results showed that addition of red spinach leaf extract had a significant effect ($P<0.05$) on brightness of fish color but had no significant effect ($P>0.05$) on growth, weight, length and survival rate. Highest increase in color brightness and absolute weight growth was obtained in 9% treatment with an average color brightness score of 6.66, an absolute weight of 0.36 g. Highest absolute length growth occurred treatments C (6%) and D (9%) at 0.41 cm and highest survival rate was obtained treatments A, B and C with a survival percentage of 88%.

1 Introduction

Freshwater ornamental fish are one of the ornamental fish that have quite good opportunities for the economy of people in Indonesia. The attractiveness of ornamental fish can be measured from their brilliant color, physical shape and completeness, behavior, and health condition or stamina [1]. One ornamental fish that is very popular with the public is the sword platy fish. The sword platy fish (*Xiphophorus helleri*) is a freshwater ornamental fish that is widely cultivated by ornamental fish farmers because it has a unique tail (like a sword) and beautiful body color [2].

One of the problems faced by farmers is that the color of the fish's body can fade. This can be caused by several factors, such as light conditions, environmental stress, disease attacks and nutrients in the feed [3]. Pigmentation substances, such as carotenoids, are needed by the fish's body to increase the brightness of their body color. However, aquatic animals cannot synthesize carotenoids in their bodies, so they need to provide feed containing carotenoids which can be obtained from natural ingredients.

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Carotenoids are yellow, orange to red pigments that come from plants, animals, fungi, humans and bacteria [4]. One natural ingredient that contains carotenoids is red spinach (*Amaranthus tricolor L.*). Red spinach is very easy to find and also has a relatively cheap price [3]. The carotenoid content found in red spinach is the dye lutein (as the main component), zeaxanthin, violaxanthin, neoxanthin, and β-carotene [5]. One of the functions of the lutein pigment is as a pigment to produce a yellow color, but in the fish's body lutein is converted into astaxanthin as a source of pigment to form a red color [6].

The use of red spinach in ornamental fish feed to increase the brightness of fish color has been tested by several researchers. Stated that adding 3% red spinach flour to the feed was able to increase the brightness of the color of goldfish (*Carassius auratus*) [7]. Research by [3] on clown loach fish (*Chromobotia macracanthus Bleeker*) reported that feeding food containing 6% red spinach flour was able to increase the color pigmentation of clown loach fish. Furthermore, [5] reported that red spinach flour added to feed at a dose of 6% was able to increase the color intensity of albino Sumatran fish (*Punitigrus tetrazona*). However, so far research regarding increasing color brightness in sword platy fish with the addition of red spinach leaf extract has not been reported. Therefore, it is necessary to conduct research to examine the effect of red spinach leaf extract as a source of natural dye in increasing the brightness of the color of sword platy fish (*Xiphophorus helleri*).

2 Materials and Methods

2.1 Time and place

This research was carried out from January to February 2023 in the Fish Hatchery and Breeding Laboratory building, Faculty of Marine Affairs and Fisheries, Syiah Kuala University, Banda Aceh.

2.2 Research methods

The research method used in this study was an experimental method using a completely randomized design (CRD) with 4 treatments and 4 repetitions. The treatment in this experiment was the addition of red spinach leaf extract (*Amaranthus tricolor L.*) to commercial feed and to sword platy fish (*Xiphophorus helleri*). Determination of the dose of red spinach leaf extract in this study was a modification of research by [5]. The treatments used are:

1. Treatment A: Addition of 0% concentration of red spinach leaf extract
2. Treatment B: Addition of red spinach leaf extract with a concentration of 3%
3. Treatment C: Addition of red spinach leaf extract with a concentration of 6%
4. Treatment D: Addition of red spinach leaf extract with a concentration of 9%

2.3 Research procedure

2.3.1 Preparation of Research Containers

The containers used were 16 plastic jars measuring 10 liters. The prepared plastic jars are cleaned first and then dried, this is done to avoid the presence of disease pathogens such as bacteria and fungi. The maintenance containers are then installed with an aeration installation and filled with clean water up to 60% of the height of the container [1], before use the water is aerated for 24 hours [8].

2.3.2 Making Extracts

Making red spinach (*Amaranthus tricolor L.*) extract refers to research [9], namely that red spinach leaves are washed until clean, then dried indoors to avoid sunlight. The drying process is carried out for 8 days until the red spinach leaves are dry. The dried red spinach

leaves are then crushed using a blender until you get red spinach leaf powder (simplisia). The red spinach leaf simplicia powder was then taken to the Chemistry Laboratory, Faculty of Teacher Training and Education, Syiah Kuala University for extraction. Extraction is carried out by maceration using a solvent, namely 96% ethanol. Put 263 grams of red spinach leaf simplicia into a plastic jar and add 4.5 liters (until submerged) of 96% ethanol. The red spinach leaf simplicia is macerated for 3 days with regular stirring every morning and evening. The extract obtained was then filtered and the filter results obtained were then evaporated using a rotary evaporator at a temperature of 67ºC until a concentrated extract was obtained. The resulting red spinach leaf extract was put into a dark bottle and stored in the refrigerator at 4ºC.

2.3.3 Adding Extracts to Feed

The addition of red spinach leaf extract to feed was carried out by referring to research by [10], namely that red spinach leaf extract is measured according to a predetermined concentration and the extract is diluted using distilled water. After dilution, the extract is then sprayed evenly on the feed using a spray bottle. The feed that has been sprayed is then air-dried until dry, then the dried feed is put into labeled plastic bottles. Feed is stored in the refrigerator (refrigerator) until it is ready to be used.

2.3.4 Pisciculture

The test fish used in this research were sword platy fish (*Xiphophorus helleri*) which came from local farmers in Banda Aceh. The test fish used had an average size of 3.33 ± 0.16 cm. Before the fish are put into the test container, the fish are acclimatized for one hour to adjust the temperature to the rearing medium [11], then the fish are fasted for 24 hours with the aim of eliminating the influence of residual food in the fish's body [12]. Each container is filled with 6 fish. Fish are kept for one month (30 days). Measurements of fish length, weight and survival were carried out at the beginning and end of the study [13].

2.3.5 Feeding

The feeding of platy swordfish (*Xiphophorus helleri*) in this study was fed ad satiation (until the fish were full) with a feeding frequency of twice a day, namely at 09.00 and 16.00 WIB [14].

2.3.6 Siphoning

The water quality of the rearing media is a factor that has an important influence on fish survival and can indirectly influence the color of sword platy fish (*Xiphophorus helleri*). One way to maintain good water quality is through siphoning. Syphoning is carried out every 7 days by reducing the water by 20% of the height of the water volume in the maintenance container [15]. Siphoning is carried out with the aim of removing food and feces residues so that accumulation and rot does not occur in the rearing container.

3 Research Parameters

3.1 Identification of compounds using a Fourier Transform Infrared (FTIR) spectrophotometer

Identification of functional groups of compounds in red spinach leaf extract using FTIR. FTIR analysis was carried out at the Environmental Quality Testing Engineering Laboratory located in the Faculty of Engineering, Chemical Engineering Department, Syiah Kuala University. FTIR spectrophotometer is used to determine the functional group of an isolate. FTIR spectrophotometers can be used to analyze solid, liquid and gas samples. A small

amount of red spinach leaf extract was taken and analyzed using FTIR, then an FTIR spectrum was created in the wave number range of 4000 – 500 cm-[1].

3.2 Color brightness

Assessment of the color brightness of sword platy fish (*Xiphophorus helleri*) was carried out using a questionnaire on 11 respondents to assess the increase in color brightness of sword platy fish [16]. The selected respondents consisted of laboratory assistants and students who did not have visual impairments (color blindness and nearsightedness). Each respondent assessed the brightness level of the fish's color based on the code on the Toca Color Finder (TCF) paper which had been given a score of 1 – 9. Measuring body color started from the smallest value to the largest by comparing the color of the fish with the TCF paper. The color observations carried out only focused on one color that was close to the body color of the test fish, namely orange. The determinant of the color brightness level is that the higher the color assessment given, the brighter the color brightness level of the sword platy fish (Figure 1). Observations of the color quality of sword platy fish were carried out every 10 days for 30 days [8].

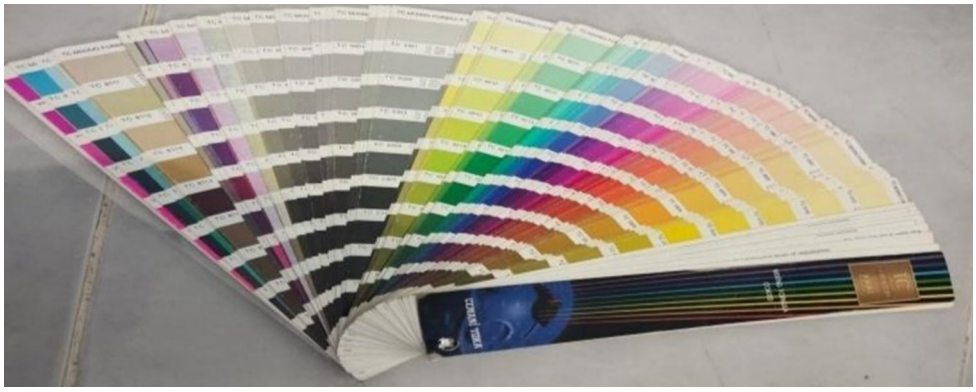


Fig. 1. Fish Color Measuring Tool

3.3 Absolute weight growth

Body weight measurements were carried out to determine the increase in fish weight during rearing [17]. The absolute weight growth of fish is calculated based on the [18] formula:

$$B = W_t - W_0 \quad (1)$$

Information:

B = Increase in absolute biomass of test fish (g)
W_t = Biomass of test fish at the end of the experiment (g)
W_0= Biomass of test fish at the start of the experiment (g)

3.4 Absolute length growth

Length growth is the amount of increase in the average length of fish during the rearing period. The increase in fish length can be calculated using the formula [19]:

$$P = P_t - P_0 \quad (1)$$

Information:

P = Fish length growth (cm)
P_t = Length of test fish at the end of the study (cm)

P_0 = Length of test fish at the start of the study (cm)

3.5 Survival rate

Survival rate is a comparison of the fish population at the end of rearing to the beginning of rearing expressed in percent (%). Measuring the degree of survival of test fish can be calculated using the formula of [20], namely:

$$SR = \frac{N_0 - N_t}{N_0} X\ 100\% \qquad (1)$$

Information :
SR = Survival of test fish (%)
N_t = Number of test fish that died during the study (tails)
N_0 = Number of test fish at the beginning of the study (tails)

3.6 Water quality

Observing water quality is a supporting factor for success in fish farming. The water quality observed is temperature, pH (degree of acidity) of water and dissolved oxygen (DO). Temperature is measured using a thermometer, pH is measured using a pH-meter and DO is measured using a DO meter. The water samples analyzed were taken from fish rearing containers. Water quality parameter measurements are carried out once a week [11].

3.7 Data analysis

Data obtained from the research results were analyzed using analysis of variance (ANOVA) with a confidence level of 95% to determine the effect of treatment on the observed parameters. If there are differences between treatments, further tests will be carried out. The further test used is determined based on the value of the Diversity Coefficient (KK). The Duncan test is used as a follow-up test if the KK value is large, namely at least 10%. The Least Significant Difference (BNT) test is used as a follow-up test if the KK value is moderate, namely in the range of 5-10%. The Honestly Significant Difference (BNJ) test is used as a follow-up test if the KK value is small, namely a maximum of 5% [21].

4 Results and Discussion

4.1 Identification of compounds using a Fourier Transform Infrared (FTIR) spectrophotometer

The results of analysis of red spinach leaf extract compounds using an FTIR spectrophotometer show an infrared spectrum which can be seen in Figure 2, while the analysis results showing the peak of carotenoid absorption from red spinach leaf extract at FTIR wave numbers can be seen in Table 1.

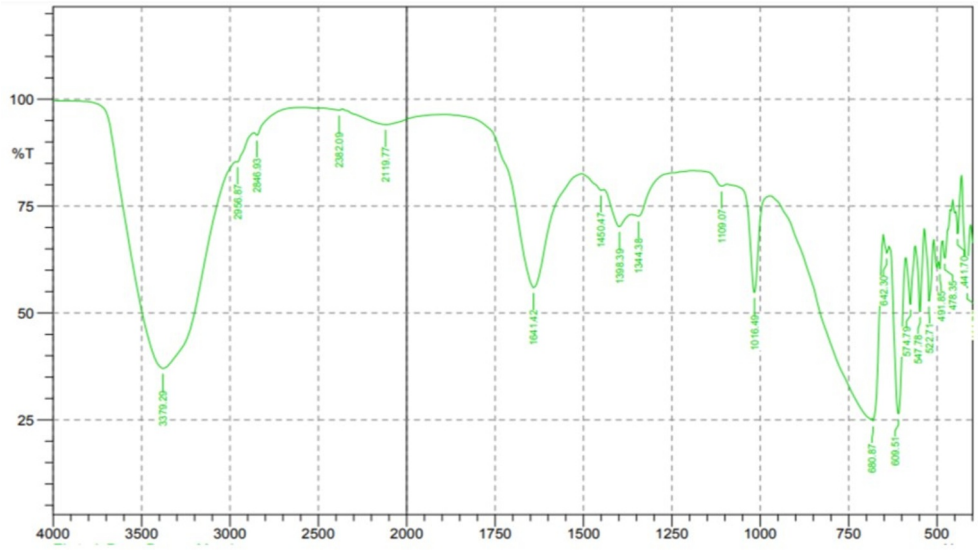


Fig. 2. FTIR Spectrum of Red Spinach Leaf Extract (*A. tricolor* L.)

Table 1. FTIR Spectrum Analysis Results

No	Wave Number (cm^{-1})		Functional groups
	Spectra	Literature	
1.	642.3 680.87	610 - 700	C-H bend alkene
2.	1344.38 1398.39 1450.47	1340 - 1470	C-H alkane
3.	1641.42	1600 – 1680	-C = C alkane
4.	2846.93 2956.87	2750 - 3000	C – H alkyl
5.	3379.29	3200 - 3500	-OH hydroxyl
6.	1016.49 1109.07	1000-1300	C-O

4.2 Color brightness, absolute weight growth, absolute length and survival rate

The results of the Analysis of Variance (ANOVA) test showed that the addition of red spinach leaf extract in the feed had a significant effect ($P<0.05$) on the color brightness level of sword platy fish (*Xiphophorus helleri*), but did not have a significant effect ($P>0.05$) on absolute weight, absolute length and survival rate of sword platy fish. Data on color brightness, absolute weight, absolute length and survival rate of sword platy fish can be seen in Table 2.

Table 2. Data on color brightness (score), absolute weight growth (g), absolute length (cm) and survival rate (%) of sword platy fish

Treatment	Parameter			
	Color Brightness (Skor)	Heavy Absolute (g)	Absolute Length (cm)	Survival rate (%)
A	3,28 ± 0,82[a]	0,17 ± 0,42[a]	0,30 ± 0,12[a]	87 ± 8,5[a]
B	4,44 ± 1,52[ab]	0,20 ± 0,12[a]	0,33 ± 0,23[a]	88 ± 15,84[a]
C	5,49 ± 0,52[bc]	0,25 ± 0,11[a]	0,41 ± 0,20[a]	88 ± 15,84[a]
D	6,66 ± 0,20[c]	0,36 ± 0,22[a]	0,41 ± 0,39[a]	83 ± 13,47[a]

Note: *Superscripts followed by different letters in the same column indicate significant differences.*

Observations of the color of sword platy fish kept for 30 days showed an increase in color brightness from each treatment during the study. Changes in increasing color brightness in sword platy fish from the beginning to the end of the study can be seen in Figure 3 and Figure 4.

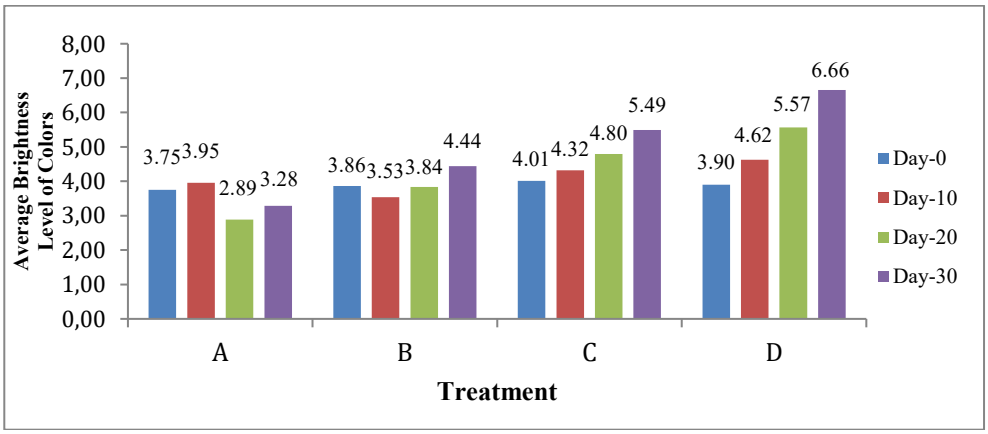


Fig. 3. Chart of Average Color Changes of Sword Platy Fish

Treatment	Before	After
A		
B		
C		

D		

Fig. 4. Sword Platy Fish Color Improvement

4.3 Water quality

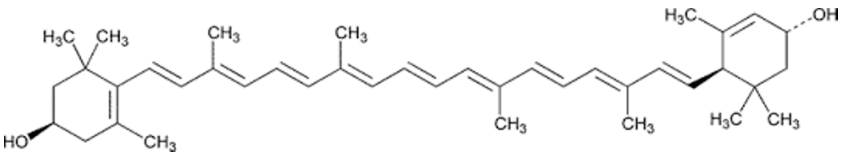
Based on the research that has been carried out, the results of water quality measurements, namely temperature, pH and dissolved oxygen (DO), are presented in Table 3.

Table 3. Data on the range of air quality parameters when rearing platy swordfish (*Xiphophorus helleri)*

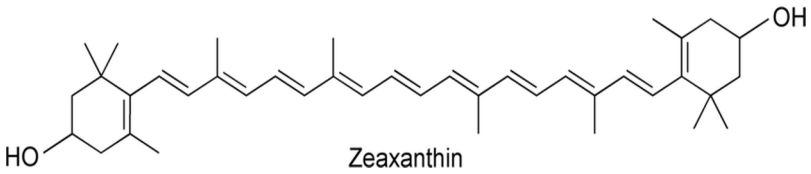
No	Parameter	Range	Optimum Value
1.	Temperature (ºC)	27,3-28,4	22-29 [2]
2.	pH	7,89-8,46	7-8,5 [22]
3.	*Dissolved oxygen* (DO) (mg/L)	3,30-3,45	>2 [2]

4.4 Discussion

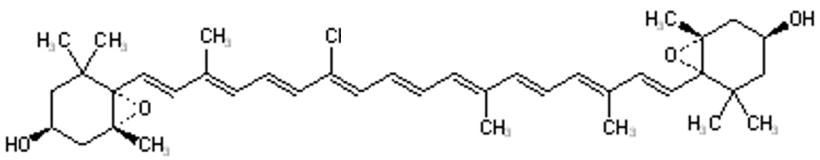
The vibration mode obtained from the results of Fourier Transform Infrared (FTIR) analysis shows the character of the bonds between atoms in compounds from red spinach leaf extract. The broad peak at 3379.29 cm^{-1} is the vibrational character of the –OH hydroxyl group [23]. Then, the absorption peaks are 2846.93 and 2956.87 cm^{-1} is the vibration of the C-H alkyl, then the C=C stretching of the alkene is seen at 1641.42 cm^{-1} [24]. The absorption peaks at 1344.38, 1398.39 and 1450.47 cm^{-1} are the C-H bending of alkanes, then the C-O stretching is seen at 1016.49 and 1109.07 cm^{-1}, then the peaks at 642.3 and 680.87 cm^{-1} are the C-H bending vibrational character of alkenes [25]. Based on the vibration results, it shows that all carotenoid components in red spinach leaf extract can be produced, namely the compounds lutein, zeaxanthin, violaxanthin, neoxanthin, and β-carotene. The chemical structure of the carotenoid compounds in red spinach leaf extract can be seen in Figure 5.



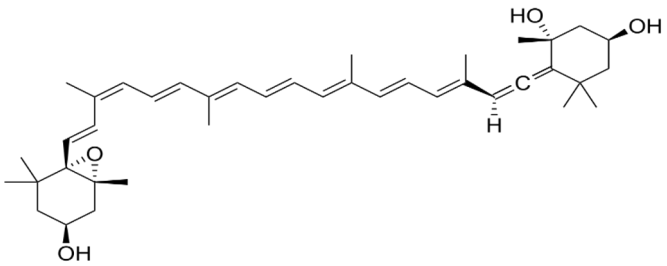
Lutein



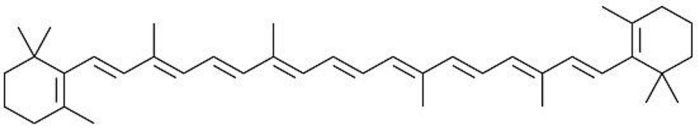
Zeaxanthin



Violaxanthin



Neoxanthin



β-Carotene

Fig. 5. Structure of Several Types of Carotenoids in Red Spinach Leaf Extract

Lutein is a yellow carotenoid pigment and belongs to the xanthophyll family [26]. Based on statements from [27], the main carotenoid component in red spinach (*Amaranthus tricolor L.*) is lutein. Lutein is the main source of pigmentation in freshwater fish. In the fish's body, lutein is converted into canthaxanthin and astaxanthin [28] so that lutein is a type of carotenoid that is used to improve the color quality of fish. The results of the 30 day study showed that the addition of red spinach leaf extract (Amaranthus tricolor L.) in the feed was able to improve the color quality of sword platy fish (*Xiphophorus helleri*) in all treatments compared to the control treatment. The color intensity of sword platy fish in treatments C (6%) and D (9%) had increased on the 10th day of observation, while treatment B (3%) had only increased on the 20th day and treatment A (0%) color quality tends to be unstable (Figure 3). The increase in color that occurs in the body of the sword platy fish is caused by the carotenoids provided through feed being able to be absorbed by the body of the sword platy fish.

Treatment D with a concentration of 9% red spinach leaf extract mixed into the feed showed the highest final color change with an average score of 6.66 on a scale of 9. Furthermore, treatment C with an extract concentration of 6% showed an average value of color brightness. of 5.49. Then, treatment B with an extract concentration of 3% showed an average color change value of 4.44. The lowest color change was in treatment A, namely commercial feed without the addition of red spinach leaf extract with an average color brightness score at the end of rearing of 3.28. The maximum increase in fish color brightness was obtained in treatment D, this was due to the carotenoid content at a concentration of 9% greater than the amount of carotenoids in the other treatments so that the increase in color brightness was more significant. This statement is in accordance with what was stated by [29][30][31] that the percentage of carotenoids mixed in the feed is directly proportional to the intensity of the color brightness of the fish. However, the addition of carotenoids to feed has a maximum limit, meaning that if carotenoids are added to feed in excess amounts, at a

certain point it will not provide a better color change and may even reduce the color value of the fish [32].

The increase in color which continued to increase in treatments B (3%), C (6%) and D (9%) until the 30th day of rearing is believed to be because sword platy fish still need carotenoid content in feed to improve the quality of their color, because of the concentration used in accordance with the ability of fish to synthesize carotenoids in red spinach leaf extract, so that fish metabolism and absorption work optimally. This is reinforced by the statement made by [33] who stated that, providing carotene levels that are appropriate to the fish's ability to synthesize carotene, can make the fish's absorption and metabolism work optimally. Based on the results of the analysis of variance (ANOVA), the brightness level of the color of the sword platy fish shows a significance value of 0.001, which is a value smaller than 0.05, so it can be concluded that the addition of red spinach leaf extract in the feed has a real influence on improving the color quality of the sword platy fish. Duncan's further test results showed that the addition of red spinach leaf extract at a concentration of 9% gave a higher color brightness score for sword platy fish. It is suspected that treatment D (9%) had a better effect on the color quality of sword platy fish because more red spinach leaf extract was added to the feed. This can be seen from the average color brightness score in treatment D (9%) of 6.66, which is the highest score among the other treatments (Figure 3).

The results of absolute weight analysis during the research showed that the addition of red spinach leaf extract to the feed did not have a significant effect on the absolute weight of sword platy fish ($P > 0.05$). Absolute weight measurements of sword platy fish were carried out at the beginning of rearing and at the end of rearing. During the research, sword platy fish experienced an increase in body weight with each treatment. Based on the data in Table 2, the highest average absolute weight growth of sword platy fish occurred in treatment D (9%) with an average value of 0.36 gr, followed by treatment C (6%) with an average absolute weight value of 0 .25 gr, then followed by treatment B (3%) with an average value of 0.20 gr, and the lowest absolute weight gain occurred in treatment A (control) with an average absolute weight value of 0.17 gr.

Although using the analysis of variance test, the treatment with the addition of red spinach leaf extract in the feed did not have a significant effect on the weight gain of sword platy fish, but biologically it can be seen that there were differences in weight gain at each treatment level. This is thought to be because the carotenoid content of red spinach leaf extract in treatments B, C and D stimulates the fish's appetite so that fish weight gain is better compared to the treatment without the addition of red spinach leaf extract in the feed. According to [6] carotenoids from fish meal contained in commercial feed as test feed can stimulate fish appetite but do not affect color changes.

The absolute length growth of sword platy fish in this study showed results that were not significantly different between treatments. The results of the analysis of variance (ANOVA) showed a significance value greater than 0.05, indicating that the addition of carotenoids to the feed did not have a significant effect on the length growth of the sword platy fish. Based on the data in Table 2, it can be seen that the highest absolute length increase occurred in treatments C (6%) and D (9%) of 0.41 cm, followed by treatment B (3%) 0.33 cm and the lowest absolute length increase in treatment A (control) was 0.3 cm.

The increase in length and weight of sword platy fish (*Xiphophorus helleri*) was not influenced by the carotenoids contained in red spinach leaf extract. These results are in accordance with the statement of [34][35][17][31] who stated that adding carotenoids to feed does not affect growth, because fish use these dyes more to improve the quality of their body color. The lowest increase in weight and length was in treatment A (control), namely 0.17 grams in weight and 0.3 cm in length. This is thought to be because treatment A does not meet the nutritional requirements of sword platy fish. This is supported by [36] who stated that increased fish growth has been obtained from food with added pigments, because

carotenoids are known to play a positive role in fish metabolism and can increase nutrient utilization which has a good impact on increasing growth. Based on the results of research conducted by [31] reported that the addition of red spinach flour to the feed resulted in higher absolute weight growth of koi fish compared to treatment without the addition of red spinach flour.

The addition of red spinach leaf extract in feed at various concentrations had no significant effect ($P > 0.05$) on the survival rate of sword platy fish. The highest average survival rate for sword platy fish was in treatments B (3%) and C (6%) with an average survival rate of 88%, followed by treatment A (control) with an average survival rate of 87% and treatment D (9%) had the lowest average survival value, namely 83%. [31], reported that the survival rate of koi fish (*Cyprinus carpio L.*) fed with the addition of red spinach flour at different concentrations (0%, 3%, 6% and 9%) did not have a significant effect on fish survival, where in this study the average survival rate for koi fish was between 90-100%.

The death of the fish at the start of the research was thought to be because during the research the fish were not used to measuring color, weight and length, which caused stress. This can be seen from the fish moving passively at the bottom of the container and not responding to the food given. Fish survival is more determined by optimal water quality as a cultivation medium for platy swordfish. The water quality in this study was considered quite good because it was still within optimal limits for cultivating platy swordfish. The water quality parameters measured in this study were temperature, pH and dissolved oxygen (DO). The temperature during the research ranged between 27.3-28.4ºC, this shows that the temperature in the maintenance container during the research was still in optimal conditions. According to [2], the optimum temperature for keeping platy swordfish ranges from 22-29ºC.

According to [37], temperature plays an important role in the continued growth of fish, fish metabolic activity is directly proportional to water temperature. The warmer the water, the more active the fish's metabolism will be, and vice versa. Temperature conditions have a significant influence on fish life. Low temperatures make fish lose their appetite and become more susceptible to disease. On the other hand, temperatures that are too high can cause respiratory problems for fish and even permanently damage their gills.

The results of pH measurements during the research showed that the pH in the rearing container was still within the tolerance limits of the fish, where the pH ranged between 7.89-8.46. This is supported by [29] statement which states that the optimal pH for keeping sword platy fish is in the range between 7-8.5. According to [38], low pH will reduce dissolved oxygen levels which will result in reduced oxygen consumption, increased respiratory activity, reduced appetite, stunted fish growth, increased susceptibility to bacterial and parasitic infections, and will even cause fish death.

Dissolved oxygen (DO) is the main factor that must be present so that fish can continue to survive. Dissolved oxygen is very important for fish to be able to breathe and maintain their metabolic cycles. The dissolved oxygen value during cultivation of sword platy fish ranges from 3.30-3.45 mg/L. This value is still within the optimum range for maintaining sword platy fish. According to [2], the optimal DO for rearing sword platy fish is >2 mg/L. This is in line with the statement made by [39] that dissolved oxygen levels <2 ml/L will result in a decrease in appetite and poor development in fish.

4 CONCLUSION

Based on the results and discussion, the following conclusions can be drawn, The addition of red spinach leaf extract (*A. tricolor L.*) to the feed had a significant effect on the color brightness of sword platy fish (*X. helleri*) where the best treatment was obtained by treatment D (9%) with the final color brightness score namely 6.66. The addition of red spinach leaf

extract (*A. tricolor L.*) to the feed had no significant effect on the growth in absolute weight, absolute length and survival of sword platy fish (*X. helleri*).

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