Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 160 (2021) 217-231 EISSN 2392-2192

# The Effect of Addition of Combination of Spirulina Flour and Marigold Flower Meal on Increasing Color Intensity of Swordtail Fish (*Xiphophorus helleri* Heckel, 1848)

**Ajeng Dyah Aprilia*, Wallim Lili, Sri Astuty, Roffi Grandiosa**

Faculty of Fisheries and Marine Sciences, Padjadjaran University,
Bandung – Sumedang KM.21 Jatinangor 45363, Indonesia

*E-mail address: ajeng17002@mail.unpad.ac.id

**ABSTRACT**

Feed greatly affect the growth and health of fish, can also serve to increase the color intensity value. Spirulina flour and Marigold flower meal contain beta carotene which plays an important role as a source of natural carotenoids. This research aims to obtain the optimal combination of Spirulina flour and Marigold flower meal in commercial feed that increases the highest color increase in swordtail fish. The method used was an experimental laboratory with a completely randomized design consisting of five treatments and three replications. The parameters observed included the intensity level of fish color, growth in absolute weight, survival rate and air quality. The results of the research showed that the optimum concentration to provide the highest increase in color intensity value in Swordtail fish were the addition of 4% Spirulina flour and 0.6% marigold flower meal in commercial feed. This concentration gave the highest increase in color intensity values, of 7.00 on the head and 8.37 on the tail, the highest absolute weight growth of 1.71g, survival rate of 100% and the value of water quality parameters, namely temperature 26°C, DO 4.33-5.87 mg/l and pH 7.27-7.90.

***Keywords*:** Swordtail fish, feed, Marigold flower meal, Spirulina flour, intensity color, beta caroten, *Xiphophorus helleri*

( Received 26 July 2021; Accepted 15 August 2021; Date of Publication 16 August 2021 )

## 1. INTRODUCTION

One of the most popular types of freshwater ornamental fish is the Swordtail fish (*Xiphophorus helleri*) since it is included in the 30 highest exports in sales [1, 21, 25, 27]. The Swordtail fish is widely cultivated because it is easy to maintain and breed and has a high tolerance for changes in air quality. The body shape of the Swordtail fish is very interesting, especially the male Swordtail fish which has an elongated back tail like a sword. The Swordtail fish has a very diverse color variant, namely red, orange and yellow, so it is often used as an ornamental fish [2].

Color is one of the main factors why ornamental fish are in demand by the public. Color quality in ornamental fish can be measured from the high and low chroma (color) contained in the fish's epidermal tissue. The number of pigment cells in fish skin can increase or decrease so that it can cause the color of the fish to change [3]. Factors that affect the number of pigment cells to decrease are environmental stress and the lack of color components in fish feed [4].

Carotenoids, which are the main pigments in the skin of ornamental fish, cannot be synthesized by the fish's own body, so a source of carotenoids is needed from outside the fish body [5]. Feeding containing color pigments can be given to fish cultivators to maintain the color of ornamental fish. In aquaculture, synthetic astaxanthin is a commonly used source of carotenoid synthesis. Synthetic astaxanthin has a relatively expensive price of around $1000/kg [6]. Therefore, there is a need for other natural alternative materials that can be optimized for astaxanthin at a price that is quite affordable for cultivators. Alternative ingredients that contain these carotenoids are spirulina and marigold flowers.

Spirulina is a microalgae that can be used to increase the brightness of fish color. The use of spirulina additives has been greatly studied to increase the color intensity of ornamental fish and lobsters. Spirulina has a high carotenoid content of 4.75 g/g, which is composed of ß-carotene (49.61 g/g), astaxanthin (6.61 g/g), lutein (0.06 g/g), cryptoxanthin (1.41 g/g) and zeaxanthin (1.25 g/g) [7].

Marigold is a plant that has a number of carotenoid compounds that play an important role in the formation of the color of ornamental fish, namely lutein [8]. Marigold flowers contain natural carotenoids of 389 mg/kg, while the petals contain carotenoids of 6,000-13,000 mg/kg [9]. Based on the advantages of spirulina and marigold flowers; therefore, in this study, it is necessary to increase the combination of spirulina flour and marigold flower meal in Swordtail fish feed. The right combination of these two sources of carotene is expected to increase the intensity of color better in Swordtail fish.

## 2. RESEARCH AND METHODS

### 2. 1. Time and Place of Research

This research was carried out at the Laboratory and Hatchery, Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Jatinangor. This research was done on January 2021 to March 2021.

### 2. 2. Materials and Methods

The tools and materials used in this research are 15 aquariums with a size of 40 × 20 × 30 $cm^3$, fiber tubs with a size of 60 × 40 × 40 $cm^3$, pumps, hoses, filter sponges, physical filters,

plastic hoses, digital scales, glass bowl, tray, spoon, plastic zip lock, thermometer, pH meter, Disolved Oxygen meter, Toca Color Finder, Swordtail fish (*Xiphophorus helleri*) measuring 2.5 – 3 cm, Carboxy Methyl Cellulose (CMC), spirulina flour, marigold flower meal, Matahari Sakti brand commercial feed (negative control) and Mutiara Tropical ornamental fish feed (positive control).

The method used in this research is a laboratory experimental method, using a Completely Randomized Design (CRD) consisting of five treatments and three replications. The treatment given was commercial feed that had been added to a mixture of spirulina flour and marigold flower meal, with the following combination or composition: (A) Commercial feed without the addition of spirulina flour and marigold flower meal (negative control), (B) Commercial feed with the addition of flour Spirulina 6% + marigold flower meal 0.3%, (C) Commercial feed with the addition of Spirulina flour 4% + marigold flower meal 0.6%, (D) Commercial feed with the addition of Spirulina flour 2% + marigold flower meal 0.9 %, (E) Special commercial feed for ornamental fish without the addition of spirulina flour and marigold flower meal (positive control).

Parameters observed in this research include the brightness of the head and tail color intensity, absolute weight growth, survival and water quality. Observations of color changes on the head and tail of the fish were observed every 8 days for 40 days. The parameter observed was an increase in the color value of the Swordtail fish as the main data by sampling three fish in each aquarium. The parameter tool used is Toca Color Finder, which is a tool to identify color specifications. The assessment starts from the smallest score of 1 to the largest score of 10 with color gradations from faded orange (TCF code 0615) to dark red (TCF code 1117). The color measurement of the test fish was carried out visually by comparing the original color of the fish in the Toca Color Finder and observed by three panelists who understand the color of ornamental fish and did not experience visual impairment (color blind and low vision).

Analysis of the data in this study used are the Kruskal–Wallis test for data on head and tail color observations of Swordtail fish and the Analysis Off Variance (ANOVA) test for data on weight gain and survival. Meanwhile, the water quality results were analyzed descriptively comparatively.

## 3. RESULT AND DISCUSSIONS

### 3. 1. Color Change of Swordtail Fish Head

Observation of color changes on the head of Swordtail fish during the study (40 days), showed an increase in color intensity in each treatment (Figure 1). In the treatment of feed that was added with a combination of Spirulina flour and Marigold flower meal (B, C and D) and positive control feed (Ornamental fish feed), an increase in the color intensity score occurred on day 8 to day 32, and on day 40 the color tends to be stable. While in the negative control (Fish feed), the increase in color intensity occurred on the 24$^{th}$ day to the 32$^{nd}$ day, and on the 40$^{th}$ day there was a slight decrease in color intensity.

Swordtail fish that were feed with a combination of Spirulina flour and Marigold flower meal added to a diet that resulted in an increase in the color intensity score on the head on the 8$^{th}$ day. The increase in color in the Swordtail fish in this study appeared faster, when compared to the results of research by Lili et al. [10] and Lili et al. [11], which uses only one type of carotenoid source.

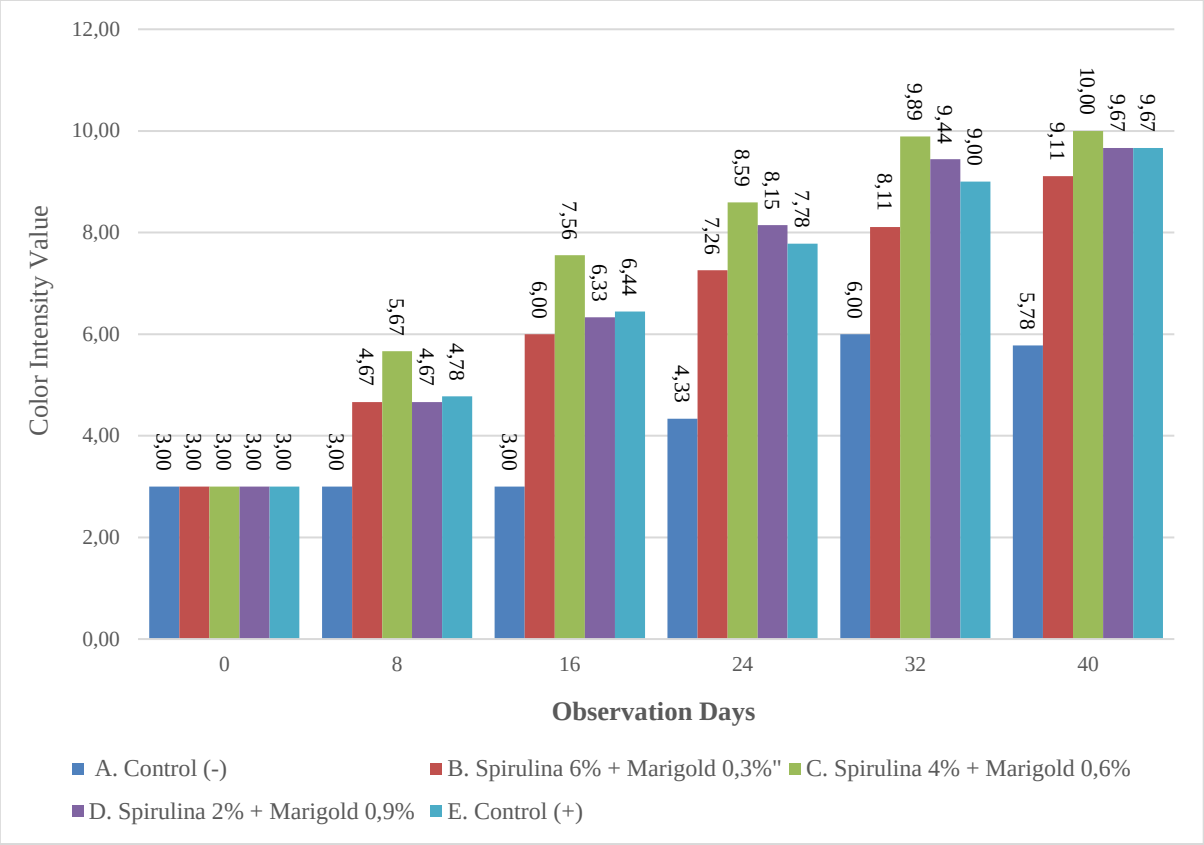


**Figure 1.** Increased Intensity of Swordtail Fish Head Color During Research at Each Treatment

In the treatment of negative control feed (feed without the addition of a combination of Spirulina flour and Marigold flower meal there was a slight increase on the 24$^{th}$ to 32$^{nd}$ day and on the 40$^{th}$ day there was a slight decrease in color intensity. There was an increase in color in the control feed treatment, it was possible that other carotene substances contained indirectly so that it affected color changes in fish. The carotene substance found in commercial feed is ß-carotene which is derived from fish meal. This is in line with the results of Ahlam's research (2019), the control feed treatment can experience an increase in color but the increase in color is not too high when compared to feed treatment with the addition of carotenoids [12].

The results of observing the color intensity of the head of the Swordtail fish on the 40$^{th}$ day or the end of the study were compared with the beginning of the research (Figure 2). It can be seen that the highest color intensity change was found in treatment C (4% Spirulina flour + Marigold flower meal 0.6%) with an average value of 10 and the lowest in treatment A (negative control) with an average value of 5.7. Wadge [13], suggested that the increase in color intensity in fish is influenced by several external and internal factors including blood capillary dilation, chromatophores, genetic and environmental factors, size, age of fish, sexual development, stress and composition of natural and artificial color enhancers and fish diet. Feeding with the right amount of carotene regularly will be directly proportional to the increase in the color intensity of the fish [14].

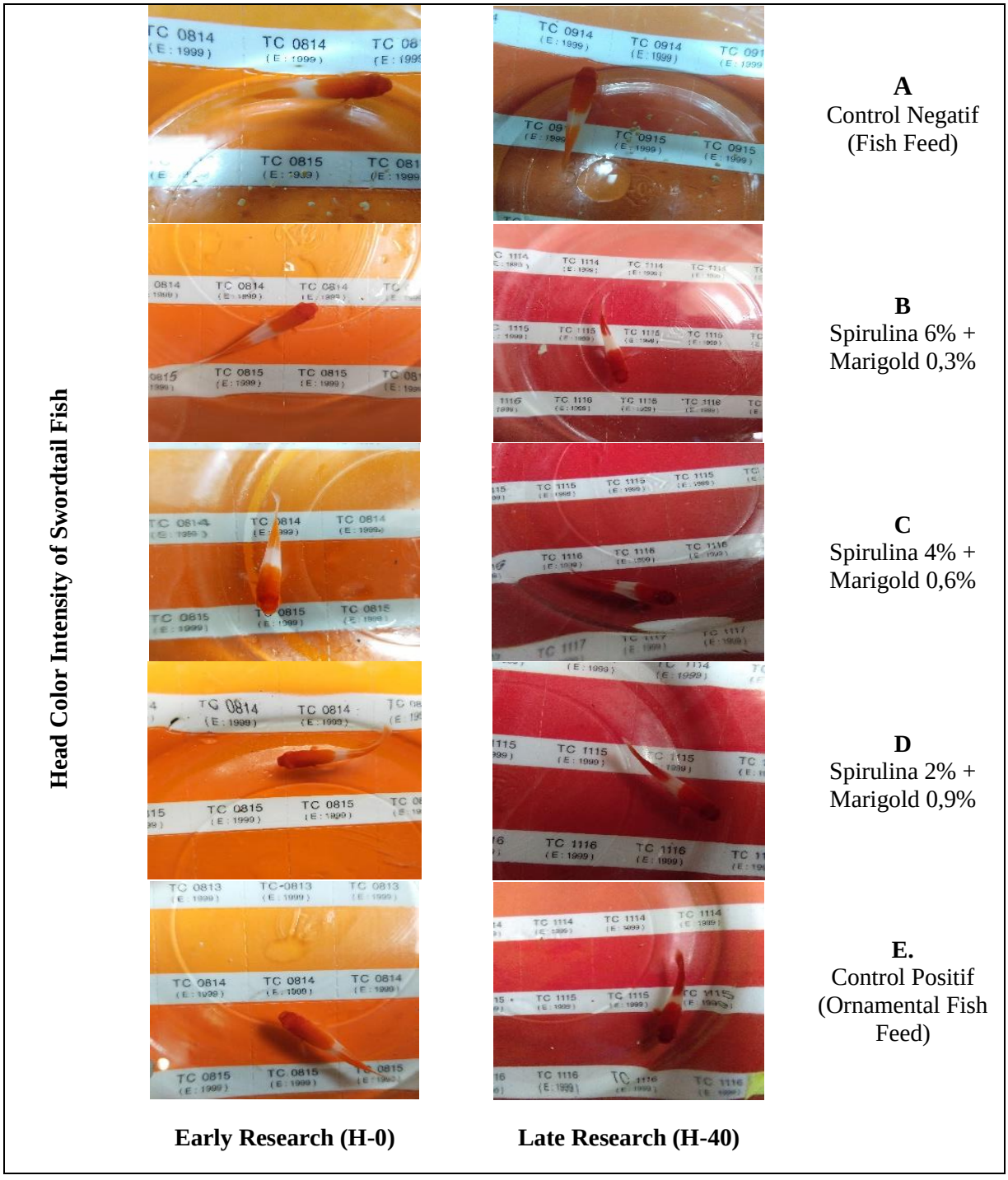


**Figure 2.** Figure Comparison of Head Color Intensity of Swordtail Fish in Early and Late Research.

The results of statistical analysis showed that feeding with different compositions of Spirulina flour and Marigold flower meal obtained significant differences in the color intensity of the head of Swordtail fish (Table 1).

**Table 1.** Average Color Intensity Value on the Head of the Swordtail Fish.

| Treatment | Increase in Color Intensity Value |
|---|---|
| A (Negative control, without Spirulina flour and Marigold flower meal) | $2.78^{a} \pm 0,64$ |
| B (Addition of Spirulina flour 6% + Marigold flower meal 0.3%) | $6.11^{b} \pm 0,97$ |
| C (Addition of 4% Spirulina flour + 0.6% Marigold flower meal) | $7.00^{c} \pm 0,00$ |
| D (Addition of 2% Spirulina flour + 0.9% Marigold flower meal) | $6.67^{bc} \pm 0,55$ |
| E (Positive control, special commercial feed for ornamental fish) | $6.67^{bc} \pm 0,48$ |

Note: Numbers followed by the same letter notation mean that there is no significant difference with a 95% confidence level

Based on the results of the Kruskal-Wallis test, it showed that there was a significant difference in treatment A (negative control without the addition of Spirulina flour and Marigold flower meal to all treatments added with Spirulina flour and Marigold flower meal and treatment E (positive control, special feed for ornamental fish without the addition of marigold flower meal).

The highest increas in color intensity on the head of the Swordtail fish with an average value of 7.00 was obtained in treatment C, which is commercial feed that has been added to a combination of 4% Spirulina flour + 0,6% Marigold flower meal. This high increas that the percentage composition of Spirulina flour and Marigold flower meal added to commercial feed was effectived in increase the color intensity of the head of Swordtail fish.

The color enhancement factor in fish is influenced by the absorption rate and optimal accumulation of a given amount of carotenoids and can regulate pigment cells in the appearance of color. The process of absorption of color supplements in the Swordtail fish body starts from the ß-carotene present in Spirulina flour and Marigold flower meal which is converted into 2 molecules of ritenol (Vitamin A) by the ß-carotene enzyme. Vitamin A in Spirulina flour and Marigold flower meal is broken down by fat which present in commercial feed. It will be converted by the fish digestive system into antioxidants, color in muscles and color in dermis cells as color pigments in color cells or chromatophores [15].

The ornamental fish business is not enough to just rely on efforts to stimulate the production of ornamental fish, but it needs to be accompanied by efficient steps regarding the appearance of beautiful colors [16].

### 3. 2. Color Change of Swordtail Fish Tail

Based on the results of research that has been carried out for 40 days, it shows that the addition of a combination of Spirulina flour and Marigold flower meal containing carotenoids in commercial feed can increase the color score of Swordtail fish tails. The increase in tail color intensity values began to be seen on the 8th day in treatments B, C, D which continued to increase until the 40th day. Whereas in treatment A (negative control) or without the addition of a combination of Spirulina flour and Marigold flower meal, the orange color of the Swordtail fish tail increased on the 24th day to the 32nd day then decreased until the 40th day or the end of the study (Figure 3).

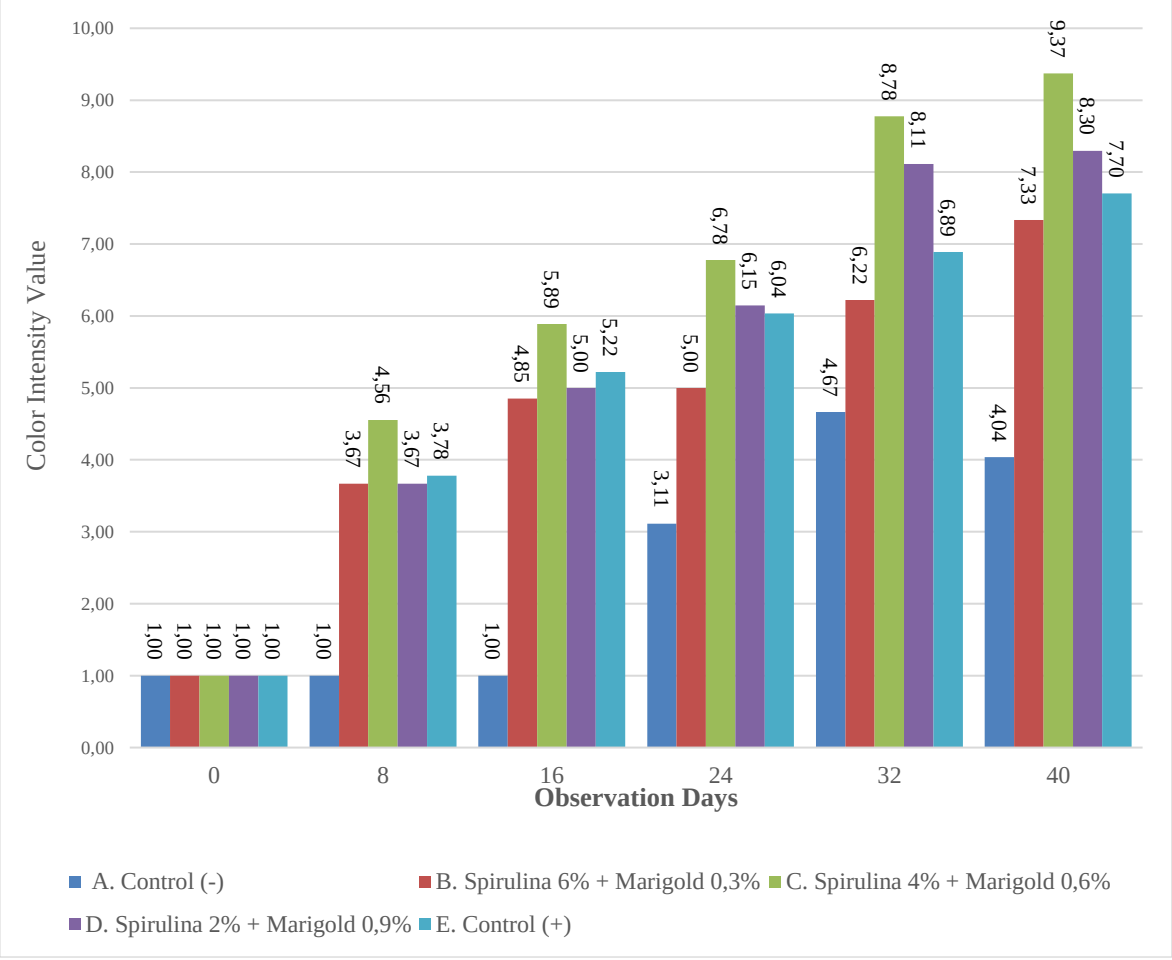


**Figure 3**.Increased Intensity of Swordtail Fish Tail Color During Research on Various Treatments

The increase in color intensity in the negative control treatment may occur due to the age factor. In immature fish, the content (accumulation) of astaxanthin is more in the flesh/muscle while in adult fish the astaxanthin content is more in the skin. As the age and body size of the fish increase, the color of the body will increase and become more visible [17].

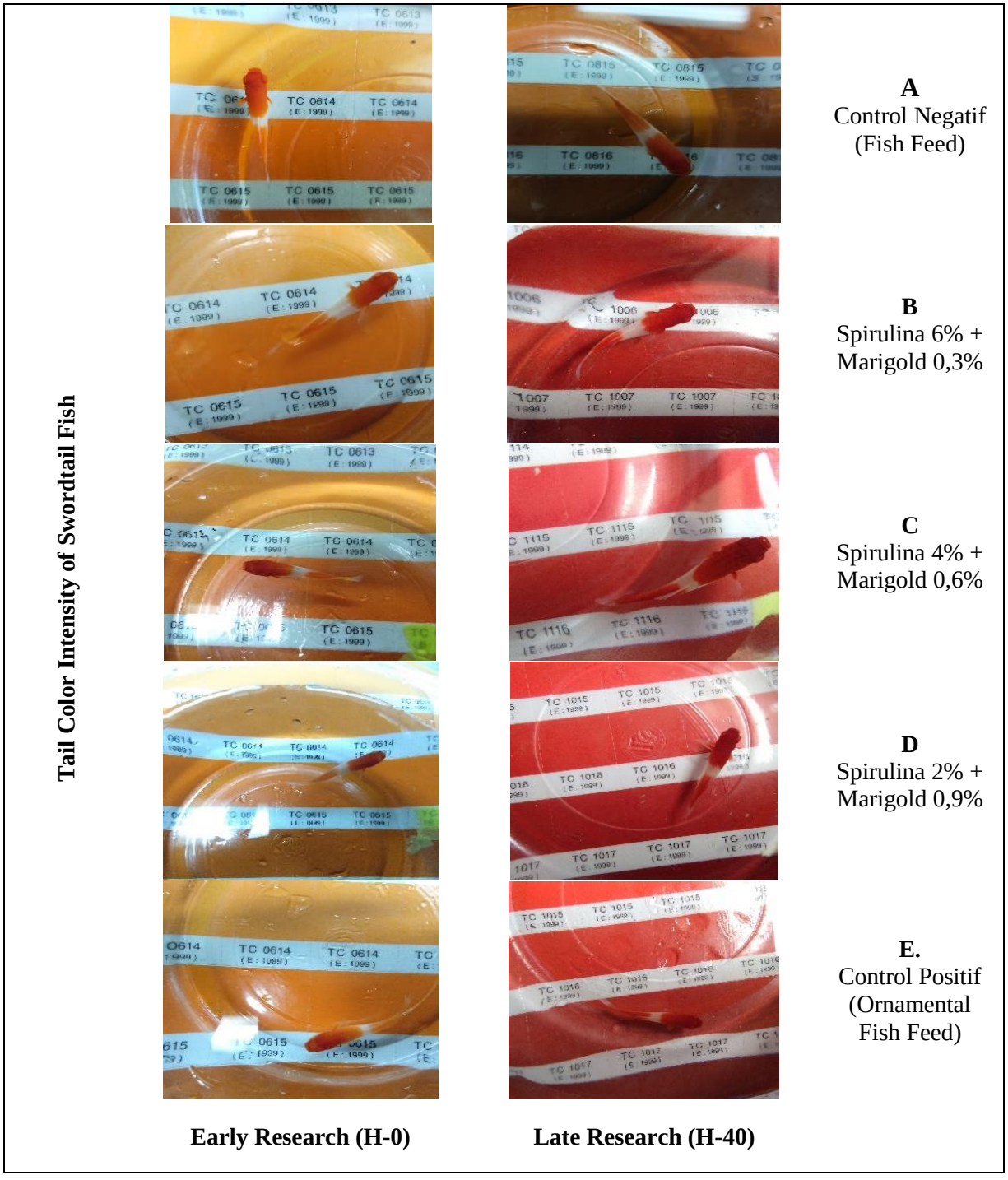


**Figure 4.** Figure Comparison of Tail Color Intensity of Swordtail Fish in Early and Late Research.

Visual observations until day 40 showed an increase in the intensity of the red color in each treatment added with a combination of Spirulina flour and Marigold flower meal (Figure 4). The highest color score was found in treatment C which is a commercial feed with the

addition of a combination of 4% spirulina flour and 0.6% marigold flower meal with an average score of 8.37.

The increase in color intensity in the negative control treatment may occur due to the age factor. In immature fish, the content (accumulation) of astaxanthin is more in the flesh/muscle while in adult fish the astaxanthin content is more in the skin. As the age and body size of the fish increase, the color of the body will increase and become more visible [17].

Visual observations until day 40 showed an increase in the intensity of the red color in each treatment added with a combination of Spirulina flour and Marigold flower meal (Figure 4). The highest color score was found in treatment C which is a commercial feed with the addition of a combination of 4% spirulina flour and 0.6% marigold flower meal with an average score of 8.37.

The research results of Gouveia et al. [18], showed that the use of color supplements in the form of artificial carotenoid ingredients mixed in commercial feeds would be faster and more certain in improving the color quality of fish.

The results of statistical analysis showed that feeding with different compositions of Spirulina flour and Marigold flower meal resulted in significant differences in the color intensity value of the tail of Swordtail fish (Table 2).

**Table 2.** Average Color Intensity Value on the Tail of the Swordtail Fish.

| Treatment | Increase in Color Intensity Value |
|---|---|
| A (Negative control, without Spirulina flour and Marigold flower meal | $3.04^{a} \pm 0,90$ |
| B (Addition of Spirulina flour 6% + Marigold flower meal 0.3%) | $6.33^{b} \pm 1,11$ |
| C (Addition of 4% Spirulina flour + 0.6% Marigold flower meal) | $8.37^{c} \pm 0,84$ |
| D (Addition of 2% Spirulina flour + 0.9% Marigold flower meal) | $7.30^{bc} \pm 0,87$ |
| E (Positive control, special commercial feed for ornamental fish) | $6.70^{b} \pm 0,54$ |

Note: Numbers followed by the same letter notation mean that there is no significant difference with 95% confidence level

Based on the results of the Kruskal-Wallis test, it was shown that the commercial feed treatment which was added with a combination of Spirulina flour and Marigold flower meal (B, C and D) as well as a positive control (ornamental fish feed), resulted in an increase in the color intensity value of the Swordtail fish tail which was significantly different from the treatment. The negative control (E) was without the addition of a combination of Spirulina flour and

Marigold flower meal (Table 2). The highest increase in color intensity of Swordtail fish tail occurred in treatment C, namely commercial feed added with a combination of 4% Spirulina flour and 0.6% Marigold flower meal and the lowest was in treatment A without the addition of a combination of Spirulina flour and Marigold flower meal. The high value of color intensity in treatment C is also predicted because there is a positive influence between the incorporation of carotenoids in Spirulina flour and Marigold flower meal, thus providing a maximum score and a faster change in color intensity, within the 8$^{th}$ day, an increase in color intensity has been seen. on the body of the Swordtail fish. Meanwhile, according to research by Lili et al. [19], the color intensity on the body of the Swordtail fish which was fed with additional carotenoids increased on the 20$^{th}$ day. The addition of a color enhancing source in the feed will encourage an increase in the color pigment in the fish body, or at least it would be able to maintain the color pigment in the body during the rearing period [20].

### 3. 3. Growth

Observation of absolute weight growth of Swordtail fish is a supporting parameter that is observed to determine the effect of adding a combination of Spirulina flour and Marigold flower meal to commercial feed on the growth of Swordtail fish. Growth is an increase in weight and volume over time. Fish growth is closely related to the nutrients contained in the food consumed by fish. The results of measuring the absolute weight growth of the Swordtail fish in this study showed different feeding treatments, resulting in different average values. The results of statistical analysis, obtained the average absolute weight of fish in the negative control treatment (A) which was significantly different from the absolute weight average in the addition of Spirulina flour and Marigold flower meal as well as in the Positive Control (Table 3).

**Table 3.** Absolute Weight Growth.

| Treatment | Average (Gram) |
|---|---|
| A (Negative control, without Spirulina flour and Marigold flower meal | 0.60 $^{a}$ ± 0,30 |
| B (Addition of Spirulina flour 6% + Marigold flower meal 0.3%) | 1.14 $^{b}$ ± 0,15 |
| C (Addition of 4% Spirulina flour + 0.6% Marigold flower meal) | 1.71 $^{c}$ ± 0,11 |
| D (Addition of 2% Spirulina flour + 0.9% Marigold flower meal) | 1.21 $^{b}$ ± 0,18 |
| E (Positive control, special commercial feed for ornamental fish) | 1.31 $^{b}$ ± 0,21 |

Note: Numbers followed by the same letter notation mean that there is no significant difference with 95% confidence level

Swordtail fish with the addition of a combination of Spirulina flour and Marigold flower meal had a greater weight growth than without the addition of a combination of Spirulina flour and Marigold flower meal (negative control). This result is in line with the opinion of Lili et al. [21] which feeds that were not added with carotenoids produced lower growth than those that were added with carotenoids. The increase in absolute weight of fish in feed containing carotenoids is due to another function of carotenoids, namely increasing immunity due to the antioxidant content, source of vitamin A and disease prevention [22].

### 3. 4. Survival Rate

Survival rate is the ratio between fish that live at the end of maintenance and the number of fish that exist at the beginning of rearing. In fish farming, mortality is a determinant of the success of the business. The results of the survival rate research of Swordtail fish showed that the addition of a combination of Spirulina flour and Marigold flower meal to commercial feed did not significantly affect the survival rate of Swordtail fish.

The results of the observation of the survival rate of the Swordtail fish during the study (40 days), obtained a survival rate of 100% for all treatments (Table 4). This is presumably because the carotene content in Spirulina flour and Marigold flower meal, apart from being a source of color pigments, also does not harm fish health.

**Table 4.** Survival of Swordtail Fish for 40 days.

| Treatment | Survival Rate (%) |
|---|---|
| A (Negative control, without Spirulina flour and Marigold flower meal | 100 |
| B (Addition of Spirulina flour 6% + Marigold flower meal 0.3%) | 100 |
| C (Addition of 4% Spirulina flour + 0.6% Marigold flower meal) | 100 |
| D (Addition of 2% Spirulina flour + 0.9% Marigold flower meal) | 100 |
| E (Positive control, special commercial feed for ornamental fish) | 100 |

Supporting factors that resulted in a high survival value in this research, supported by the feed given, stocking density settings and environmental conditions in the rearing media.

The addition of a combination of Spirulina flour and Marigold flower meal to the test fish can meet the nutritional needs and maintain their survival. Carotenoids naturally function as the basic ingredients of vitamin A, support thermoregulation or the process of regulating body

temperature, help the formation of egg yolks in the reproductive process, and affect fish health [23].

### 3. 5. Water Quality

Water quality parameters are one of the factors that affect the survival of the Swordtail fish. The role of water quality affects the metabolism and stress levels of fish so that it can affect the work of hormones associated with the increase and decrease in color quality [24]. The water quality parameters observed in the research were temperature, DO and pH. Water quality data collection in the research is carried out every 8 days. The results of observations of water quality parameters are presented in Table 5.

**Table 5.** Observation of Water Quality of Swordtail Fish.

| Parameter | Result | Reference (Tamaru et al. 2001) |
|---|---|---|
| Temperature (°C) | 26 | 22-29 |
| DO (ppm) | 4.33-5.87 | >2.00 |
| pH | 7.27-7.90 | 7.00-8.10 |

Temperature is an abiotic factor that affects the metabolism and growth of fish, including the Swordtail. The result of measuring the water temperature during the research, which is 26ºC, is still within safe limits. According to Tamaru et al. (2001) the optimum temperature for the maintenance of Swordtail ornamental fish ranges from 22-29 ºC.

Dissolved oxygen is an important element in metabolic processes which means the amount of oxygen in milligrams contained in one liter of water (ppm). The results of DO observations during the research showed that the average DO for each treatment during the research ranged from 4.33-5.87 ppm. According to Tamaru et al. (2001) that the optimal DO for the maintenance of Swordtail fish is in the range of >2 ppm.

The pH of water is a determinant of chemical processes in water. pH levels that are too high or too low lead fish to become stressed [25]. The results of the pH analysis during the research showed that the average of all treatments ranged from 7.27 to 7.90. The measurement results at the beginning of the research showed a pH value of 7.65 and then it increased and decreased but was still considered reasonable (not significant). This is due to the metabolic process in fish during the research. Ref. [26] Based on Tamaru et al. (2001) The optimal pH in the maintenance of Swordtail fish is in the range of 7.00-8.10.

## 4. CONCLUSION

Based on the results of research and discussion, it can be concluded that the optimum concentration to provide the highest increase in color intensity value in Swordtail fish were the addition of 4% Spirulina flour and 0.6% marigold flower meal in commercial feed. This

concentration gave the highest increase in color intensity values, of 7.00 on the head and 8.37 on the tail, the highest absolute weight growth of 1.71g, survival rate of 100% and the value of water quality parameters, namely temperature 26°C, DO 4.33-5.87 mg/l and pH 7.27-7.90.

## References


[1] Evers, H. G., Pinnegar, J. K., Taylor, M. I. Where are They All From? - Sources And Sustainability in the Ornamental Freshwater Fish Trade. *Journal of Fish Biology*, 94(6) (2019) 909-916

[2] Parawangsa, I. N., Rahardjo, M. F., and Simanjuntak, C. Reproduction Aspect of Green Swordtail, Xiphophorus helleri Heckel 1848 in Tamblingan Lake, Bali. *Jurnal Iktiologi Indonesia*, 10(1) (2020) 81-92

[3] Subamia, I., Meilisza, N., and Mara, K. Color Quality Improvement of Red Rainbow Fish (Glossolepis incisus, Weber 1970) Through Carotenoids Source Enrichment of Shrimp Head Meal in Feed. *Jurnal Iktiologi Indonesia*, 10(1) (2017) 1-9

[4] Daniel, N., Sivaramakrishnan, T., Subramaniyan, S., Faizullah, M. M., and Fernando, H. Application of Carotenoids on Coloration of Aquatic Animals. *International Journal of Fisheries and Aquatic Research*, 2(1) (2017) 1-7

[5] Gouveia, L., P. Rema., O. Pereira., dan J. Empis. Colouring Ornamental Fish (Cyprinus carpio and Carassius auratus) with Micro-Algal Biomass. *Journal Aquaculture Nutrition*, 9 (2003) 123–129.

[6] Panis, G., and Carreon, J. R. Commercial Astaxanthin Production Derivied by Green Alga Haematococcus pluvialis: A Microalgae Process Model and a Techno-economic Assessment All Through Production Line. *Alga Research*, 18 (2016) 175-190

[7] Hana, H., El-Baky A., Farouk K., El Baz, Gamal S., El-Baroty. Spirulina Species as a Source of Caretenoids and ß-Tocopherol and Its Anticarcinoma Factors. *Biotechnology*, 2 (3) (2003) 222-240

[8] Del Villar-Martinez, A. A., Orbe-Rogel, J. C., Vanegas-Espinoza, P. E., Quintero-Gutiérrez, A. G. And Lara-Floreset, M. The Effect of Marigold (Tagetes erecta) as Natural Carotnoid Source for the Pigmentation of Goldfish (Carassius auratus L.). *Research Jounal of Fisheries and Hydrobiology*, 8(2) (2013) 31-37

[9] Yanar, Y., H. Büyükçapar., M. Yanar., and M. Göcer. Effect of Carotenoids from Red Pepper and Marigold Flower on Pigmentation, SensoryProperties, and Fatty Acid Composition of Rainbow Trout. *Journal Food Chemistry*, 100 (1) (2007) 326-33

[10] Lili, W. Sunarto, B. J. Santoso, I. Zidni. Carrot Starch Addition Effect on Artificial Feed Towards Color Enhancement of Swordtail Fish (Xiphophorus helleri). *Global Scientific Journals*, 6 (8) (2018) 31-41

[11] Lili, W., Iskandar, R.M. Ramadhan, R. Grandiosa. The Effect of Addition Marigold-Meal to Artificial Feeds for Increasing Color Intensity of Koi Fish (Cyprinus carpio Linnaeus, 1758) Strain Kohaku. *World News of Natural Science*, 32 (2020) 49-60

[12] Ahlam, R.N., W. Lili, H. Hamdani, Rosidah, I. Bangkit. Effect of Adding Pumpkin Flour and Carrot Flour on Changes in Colour Intensity of Goldfish (Carrasius auratus L) Strain Oranda. *World News of Natural Sciences*, 26 (2019) 52-60

[13] Wagde, M.S., S.K.Sharma, B.K. Sharma, A.P. Shivani and N.R. Keer. Effect of Natural ß-Carotene From Carrot (*Daucus carota*) and Spinach (*Spinacia oleracea*) on Colouration of An Ornamental Fish Swordtail (Xiphophorus hellerii). *Journal of Entomology and Zoology Studies*, 6 (6) (2018) 699-705

[14] Woods, C. Growth and Survival of Jouvenile Seahorse Hippocampus Abdominalis Reared on Live, Frozen and Artificial Foods. *Aquaculture*, 220 (2003) 287-298

[15] Subamia, I., Meilisza, N., and Mara, K. Color Quality Improvement of Red Rainbow Fish (Glossolepis incisus, Weber 1907) Through Carotenoids Source Enrichment of Shrimp head Meal in Feed. *Jurnal Iktiologi Indonesia*, 10(1) (2017) 1-9.

[16] James, R. and Sampath, K. Effect of Meal Frequency on Growth and Reproduction in the Ornamental Red Swordtail (Xiphophorus helleri). *Isr. J. Aquacult-Bamid*, 55 (2003) 197-207

[17] Nurhadi, T., W. Lili, R. I. Pratama, K. Haetami. Effects of Astaxanthin and Canthaxanthin Addition to Ranchu Goldfish (*Carrasius auratus*) Diet Related to Rate of Color Quality Enhancement. *World News of Natural Sciences*, 24 (2019) 178-183.

[18] Gouveia, L., P. Rema., O. Pereira., dan J. Empis. Colouring Ornamental Fish (Cyprinus carpio and Carassius auratus) with Micro-Algal Biomass. *Journal Aquaculture Nutrition*, 9 (2003) 123-129

[19] Lili, W. Sunarto, B. J. Santoso, I. Zidni. Carrot Starch Addition Effect on Artificial Feed Towards Color Enhancement of Swordtail Fish (Xiphophorus helleri). *Global Scientific Journals*, 6 (8) (2018) 31-41

[20] Wayan, S., Nina M., and Karunia L.M. Improving The Color Quality of Rainbow Red Fish (Glossolepis incisus, Weber 1907) Through the Enrichment of Caratoid Shrimp Head Flour in Feed. *JurnalIktiologi Indonesia*. 10 (1) (2010) 1-9

[21] Lili, W. Sunarto, B. J. Santoso, I. Zidni. Carrot Starch Addition Effect on Artificial Feed Towards Color Enhancement of Swordtail Fish (Xiphophorus helleri). *Global Scientific Journals*, 6 (8) (2018) 31-41

[22] Maulana, M.R., M. Junaidi, B.D.H. Setyono. Combination of Pumpkin Flour (Cucurbita moschata D.) and Carrot Flour (Daucus carota L) on the Brightne Colr of Koi Fish (Cyprinus carpio). *Jurnal Biologi Tropis*, 21 (1) (2021) 81-88

[23] Amar, E.C., Kiron V., Satoh S., Okamoto N., and Watanabe T. Effect of Dietary b-carotene on the Immune Response of Rainbow Trout Oncorhynchus Mykiss. *Fisheries Sci*, 66 (6) (2000) 1068-1075

[24] Nurhadi, T., W. Lili, R. I. Pratama, K. Haetami. Effects of Astaxanthin and Canthaxanthin Addition to Ranchu Goldfish (*Carrasius auratus*) Diet Related to Rate of Color Quality Enhancement. *World News of Natural Sciences*, 24 (2019) 178-183

[25] Julia, R. O., Andriani, Y., Yuliadi, L. P. S., and Iskandar. The Effect of Addition of Butterfly Pea Leaf Meal (Clitoria ternatea) in Feed on the Quality of Color of Swordtail Fish Head (Xiphophorus helleri). *World News of Natural Sciences*, 26 (2019) 128-137.

[26] Tamaru, C. S., B. Cole, R. Bailey, C. Brown and H. Ako. A Manual for Commercial Production of the Swordtail, Xiphophorus helleri. United States Department of Agriculture, Center for Tropical and Subtropical Aquaculture, (2001) Publication Number 128. 36 pp

[27] Rafał Maciaszek, Dorota Marcinek, Maria Eberhardt, Sylwia Wilk, Alien freshwater fish, Xiphophorus interspecies hybrid (Poeciliidae) found in artificial lake in Warsaw, Central Poland. *World Scientific News* 132 (2019) 291-299