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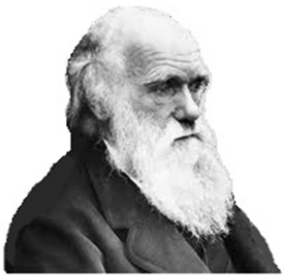


World News of Natural Sciences

An International Scientific Journal

WNOFNS 26 (2019) 128-137 EISSN 2543-5426

The Effect of Addition of Butterfly Pea Leaf Meal (*Clitoria ternatea*) in Feed on the Quality of Color of Swordtail Fish Head (*Xiphophorus helleri*)

Rintan Octaviana Julia*, Yuli Andriani, Lintang P. S. Yuliadi, Iskandar

Faculty of Fisheries and Marine Sciences, Padjadjaran University,
Jatinangor 45363, West Java, Indonesia

*E-mail address: rintanoj@gmail.com

ABSTRACT

The aim of this study was to determine the effect of butterfly pea leaf as a source of carotenoids on the quality of the color of the heads of swordtail fish. The study was conducted at the Aquaculture Laboratory, Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University, from March to April 2019. The research method used an experimental approach with Completely Randomized Design (CRD) consisting of 4 treatments and 3 replications. Those treatments are 0%, 1%, 6% and 12% of butterfly pea leaf meal. The parameters observed included changes in head color quality, absolute weight growth, absolute length growth and water quality. Color observation data were analyzed using Kruskal-Wallis analysis if there were differences between treatments. Multiple comparisons were performed with the Z test with a confidence level of 95%. Data on the length of growth rates and absolute weights were analyzed using analysis of variance via the F test. Here, 95% was set as the confidence level. If there were differences between treatments, this was followed by Duncan test with a confidence level of 95%. The results showed that the addition of 6% butterfly pea leaf meal in commercial feed was able to improve the best color on the part of the swordtail fish head. This saw an average color score 9.56 and color value of 82.2. Addition of carotenoids in feed, however, did not significantly influence the growth of absolute weight or length and water quality.

***Keywords*:** Butterfly pea, Carotenoids, Color quality, Feed, Swordtail fish, *Clitoria ternatea, Xiphophorus helleri*

(Received 20 July 2019; Accepted 08 August 2019; Date of Publication 10 August 2019)

1. INTRODUCTION

Swordtail fish (*Xiphophorus helleri*) is one type of freshwater ornamental fish that is popular today. Swordtail can be grouped into various types based on variations in color on the body and fins. Swordtail fish, especially males, have their own characteristics which are gonopodium or anal fins that are elongated. The aesthetic value possessed by ornamental fish can increase the economic value that is beneficial for farmers.

Color in fish is caused by the presence of pigment cells or chromatophores found in the dermis layer on the scales, outside and below the scales. One way to improve the color quality of ornamental fish is by providing nutritional intake containing color pigments in feed. Feeds containing carotenoids play a role in the formation of fish body colors.

Deposition of carotenoids in tissues and the presence of chromatophores containing pigments cause the appearance of colors in ornamental fish. The main component forming this red and yellow pigment is the carotenoid pigment. The addition of color enhancing sources in fish feed will result in an increase in color pigments in the body of the fish, at least the fish is able to maintain the color pigment on the body during the maintenance period. Natural carotenoids other than those in animals also exist in plants, one of the plants that contain carotenoids is the butterfly pea plant (*Clitoria ternatea*).

The leaf of the butterfly pea plant have the benefit of being able to be used as a source of protein for the production of leaf concentrate proteins. This plant can also be used as animal feed. This plant also has potential as a good feed because it has high nutritional value and is also very popular with livestock. In addition to the high protein content, butterfly pea plants can also be used as a source of carotene, where the carotene content reaches 587 mg/Kg of dry matter. The carotene content has the potential to improve the color quality of ornamental fish, so this research is needed.

2. MATERIALS AND METHODS

The research was conducted in March - April 2019 at the Aquaculture Laboratory, Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Indonesia

The tools used are 40×25×25 cm^3 aquarium, glass beaker, blender, DO meter, tube filter, label paper, millimeter block, tray, pH meter, transparant plastic pipe, plastic ziplock, water pump, meal filter, spoon, fish spoon, thermometer, digital scales, and Toca Color Finder (TCF). The ingredients used are kohaku type Swordtail fish measuring ±3 cm 120, Carboxy Methyl Cellulose (CMC), Butterfly pea leaf meal, and commercial feed.

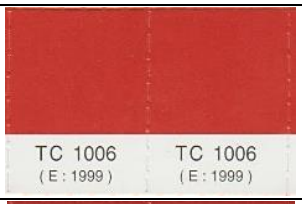
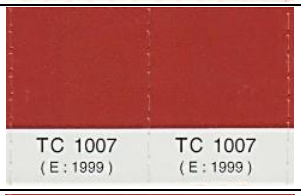
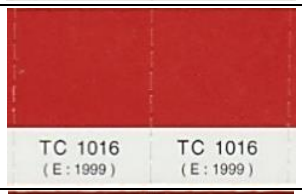
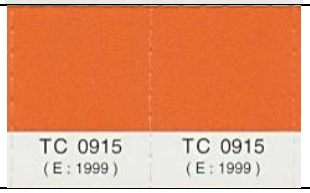
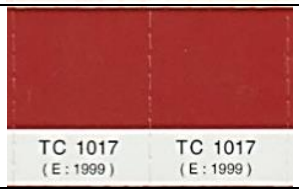
The method used in this research is the experimental method with Completely Randomized Design (CRD). There are four types of treatment with three replications in each treatment, including:

Treatment A = Without adding butterfly pea leaf meal (Control)
Treatment B = Feed contains 1% butterfly pea leaf meal
Treatment C = Feed contains 6% butterfly pea leaf meal
Treatment D = Feed contains 12% butterfly pea leaf meal

The research lasted 40 days with the treatment of feeding in the form of pellets with the addition of different butterfly pea leaf meal. Feed is given as much as 3% of the fish biomass

with a frequency of three times a day at 08.00, 13.00 and 16.00 WIB. The primary data measured in this research is the quality of the color of the fish's head that is done visually by using standard values obtained from TCF (Table 1) and supporting data observed included weight growth, lenght growth and water quality. Observation is carried out every 10th days during research.

Table 1. TCF Color Code

No.	TCF Picture	Note	No.	TCF Picture	Note
1.		Score 1 TCF Code 0615	6.		Score 6 TCF Code 1006
2.		Score 2 TCF Code 0715	7.		Score 7 TCF Code 1007
3.		Score 3 TCF Code 0816	8.		Score 8 TCF Code 1008
4.		Score 4 TCF Code 0906	9.		Score 9 TCF Code 1016
5.		Score 5 TCF Code 0915	10.		Score 10 TCF Code 1017

3. RESULT AND DISCUSSION

3. 1. Color change rate

The addition of butterfly pea leaf meal to feed had an effect on increasing the color of the swordtail fish head with the initial red color and an average color score of 4 for all treatments, treatment A increased on the 30th day with an average score of 4.22 and treatment B occurred an increase on the 20th day with an average value of 4.22, while treatment C and D was faster, namely an increase on the 10th day with an average score of 4.33 and 4.22. The increase in the average color score on the sword plate fish head is shown in Figure 1.

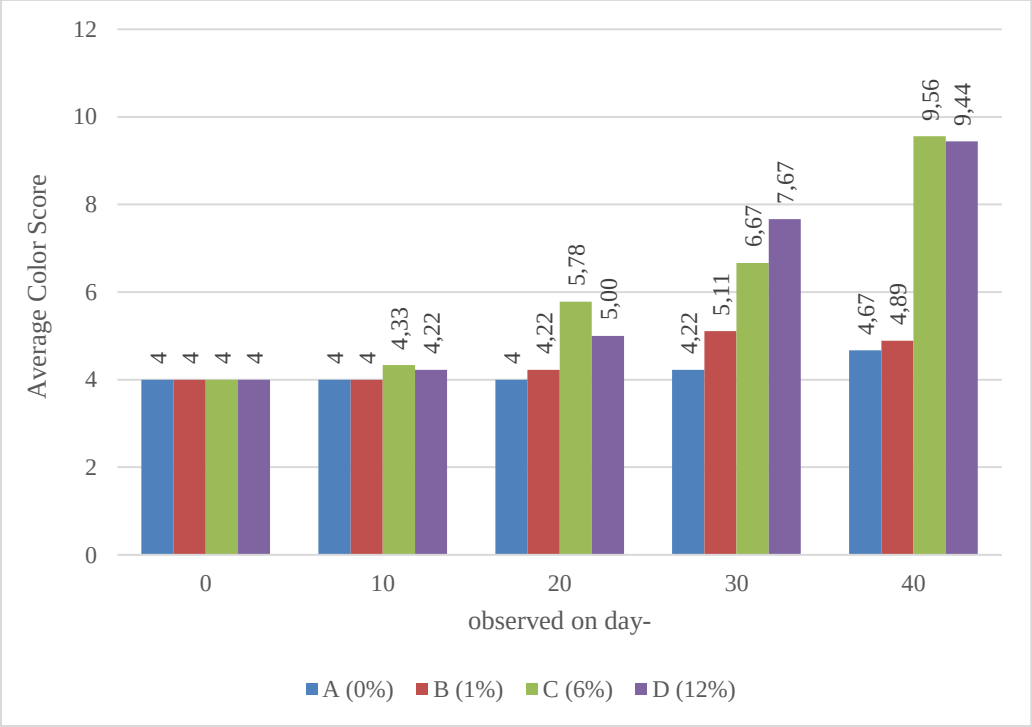
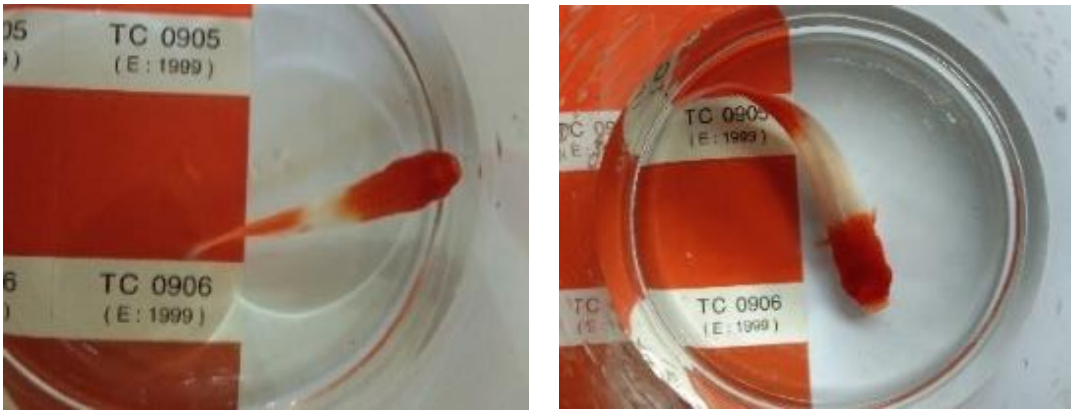


Figure 1. Average Color Score Increase on Swordtail Fish Heads

Treatment A has an initial reddish-orange color with the TCF code 0906 and starts to increase on the 30th day with an average score of 4.22 and continues to increase on the 40th day in red with an average score of 4.67 or with TCF code 1006. Color enhancement in treatment A is not due to carotenoids but by internal factors. Reported that 0% of shrimp head meal treatment continued to increase on the 20th day, both for the abdomen, tail fin and rainbow fish's back, this was caused by age factors. The color enhancement in treatment A is shown in Figure 2.



(a) on day 0 (b) On the 40th day

Figure 2. Comparison of Color of Swordtail Fish Head Treatment A

Treatment B has a reddish-orange base color TCF code 0906 which increases on the 20th day. On the 30th day to the 40th day there was a decrease in color from an average color score of 5.11 to 4.89, presumably this color reduction was caused by stress on fish. Chromatophore pigment cells scattered in the epidermal layer cause the pigment cell particles to absorb light perfectly, so that the color intensity of the fish's body becomes brighter and clearer, while the chromatophore pigment cells that gather near the nucleus cause a decrease in body color fish so that the body color of the fish looks dark and faded. The color enhancement in treatment B is shown in Figure 3.

(a) On day 0 (b) On the 40th day

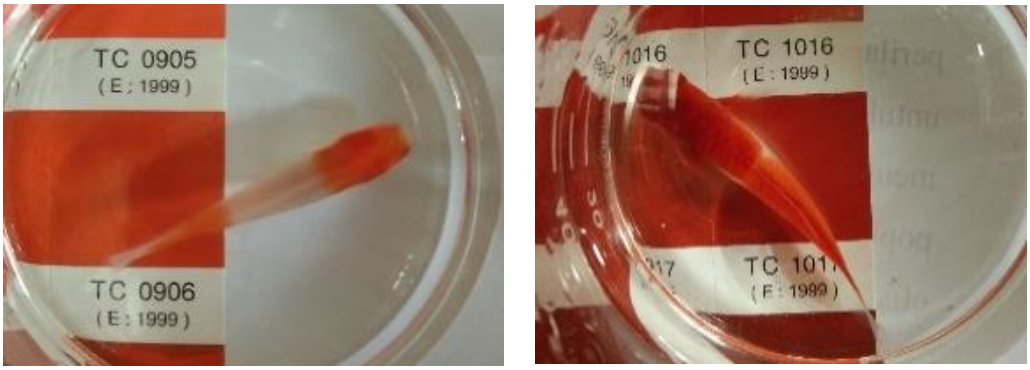
Figure 3. Comparison of Color of Swordtail Fish Head Treatment B

Treatment C has a reddish orange base color, which is TCF code 0906. Color enhancement occurs on day 10 with an average score of 4.33, still increasing until the 40th day with an average score of 9.56 or with a TCF code 1017 with deep red color. The final score on the part of the treatment of swordtail fish head was higher than that of treatments A, B, and D. The increase in the color of the initial and final heads of the swordtail fish in treatment C is shown in Figure 4.

(a) On day 0 (b) On the 40th day

Figure 4. Comparison of Color of Swordtail Fish Head Treatment C

Treatment D has a basic color like other treatments, namely reddish orange. The head of the 20th day has an average score of 5, lower than treatment C, which is 5.78. On the 30th day it increased to 7.67 higher than treatment C which was 6.67. On the 40th day with a final score of 9.44, it was lower than the final treatment C score of 9.56. The final result of increasing color D treatment is solid red with the TCF code 1017. The increase in the color of the beginning and end of the swordtail fish head in treatment D is presented in Figure 5.



(a) On day 0 (b) On the 40th day

Figure 5. Comparison of Color of Swordatail Fish Head Treatment D

Based on the results of the Kruskal-Wallis Test for the head section showed that there were significant differences and continued with the Z test (Table 2).

Table 2. Color Value on the Swordtail Fish Head.

Treatments	Head Color Value
A (Without addition)	$26^{a} \pm 0{,}290$
B (1% Addition)	$29^{a} \pm 0{,}521$
C (6% Addition)	$82{,}167^{b} \pm 2, 230$
D (12% Addition)	$80{,}833^{b} \pm 2{,}386$

Note: The value followed by the same letter is not significantly different based on the Z test at the 95% confidence level.

The highest color value of the head section was 82.167 in treatment C with the addition of 6% butterfly pea leaf meal. The lowest value is 26 in the control treatment or without the addition of butterfly pea leaf meal. It is suspected that the increase in color on the Swordtail fish head is still increasing with increasing maintenance time and age of fish, because fish have the ability to absorb different carotenoids. Reported that the addition of carotenoid sources in the form of shrimp head meal still had an increase in color on the rainbow kurumoi fish until

the 56th day and was thought to still be able to increase. The best treatment for increasing the color of the swordtail fish head is treatment C with the addition of 6% butterfly pea leaf meal in feed and occurs on the 40th day.

Carotenoids that enter the body of the fish are synthesized into certain color pigment, like other food subtances, the pigment will be carried along with the bloodstream and then the pigment is deposited on the chromatophores found in the dermis. The process of forming colors chemically in the body of a fish is fat-soluble carotenoids that are digested in the intestine by pancreatic lipase enzymes and bile salts. The pancreatic lipase enzyme will hydrolyze triglycerides to monoglycerides and fatty acids. Bile salts function as fat emulsifiers so that small fat particles are formed called micelle containing fatty acids, monoglycerides and cholesterol. Carotenoids in the cytoplasm of the mucosal cells of the small intestine are broken down into retinol and then absorbed by the intestinal wall along with absorbing fatty acids in passive diffusion and combined with micelles then gather to form bubbles and then absorbed through the lymphatic channels. Next micelle with retinol enters the bloodstream and is transported to the liver, in the liver retinol joins with palmitic acid and is stored in retinyl-palmitate form. If needed by body cells, retinyl palmitate will be bound by Retinol Binding Protein (PPR) which is synthesized in the liver. Then it is transferred to another protein, to be transported to tissue cells. Thus carotenoids can be absorbed in the body.

The use of different carotenoid sources but in the same fish is the source of carotenoids derived from marigold flour. The addition of 12 mg / g marigold flour in feed formulation is the most effective dose to improve the quality of the color of swordtail fish with the highest color intensity value of 4,983 using M-TCF. Giving a value or weighting on M-TCF color paper starts from the smallest score of 1, 2, 3 to the biggest score of 30 with gradations of color from young orange to deep red. So if compared with butterfly pea leaf comparable to treatment B or 1% butterfly pea leaf meal in feed with an average color score value on the 40th day that is 1.89 for the tail section and a score of 4.89 for the head section. The difference in scores obtained is also influenced by different weighting on TCF color paper.

States that the termination of carotenoid feeding and replacing it with basal feed has an influence on the resilience of fish color quality. Decreasing fish color quality began to occur on the 3rd day and decreased slowly until the 7th day after the cessation of carotenoid feed. So with this feeding with a mixture of telang leaf flour is highly recommended to maintain the quality of the color of the fish, especially swordtail fish.

3. 2. Growth in fish weight and length

Data on absolute weight and length growth for 40 research days, the results were not too significant for each treatment. The results of observations of absolute weight and length growth are presented in Table 3.

The results of the variance analysis state that Fcount <Ftable so that there can be no significant difference, it means that commercial feed with the addition of butterfly pea leaf meal has no significant effect on the growth of weights and lengths of swordtail fish, because when compared to the control treatment, the growth is not significantly different.

The highest growth of the average weight is 0.39 grams in treatment B and the lowest average weight growth is 0.29 in treatment D. The highest growth average length is 0.71 cm in treatment A and the lowest average length increase is 0.63 cm in treatment D. The growth is influenced by the nutrient content in the feed consumed by fish. Changes in growth are directly proportional, because every increase in weight increases the length of the fish body.

Table 3. Absolute Weight and Length Growth

Treatment	Increase Average	
	Weight (gram)	**Length (cm)**
A (Without addition)	$0,33^{a} \pm 0,083$	$0,71^{a} \pm 0,064$
B (1% Addition)	$0,39^{a} \pm 0,090$	$0,69^{a} \pm 0,172$
C (6% Addition)	$0,33^{a} \pm 0,053$	$0,67^{a} \pm 0,145$
D (12% Addition)	$0,29^{a} \pm 0,033$	$0,63^{a} \pm 0,031$

Note: The value followed by the same letter is not significantly different based on the Duncan test at the 95% confidence level.

Regarding the role of carotenoids in fish growth there are differences of opinion. There are several studies that report a positive influence or there is an effect of carotenoids on growth, while on the other hand there are those that suggest no negative influence or influence is found. The carotenoid derived from butterfly pea leaf meal did not have a significant effect, presumably because swordtail fish used more color substances, namely carotenoids to increase their body color. That feed added with carotenoids did not affect the growth of rainbow kurumoi fish. Found no significant difference in the growth of red porgy (Pagrus pagrus) fish fed carotenoids. Supported also by his research on that absolute weight gain in the treatment added with pumpkin meal and shrimp head meal with the treatment of control did not differ significantly between the treatments of goldfish. So that it can be concluded that butterfly pea leaf meal serves as a source of pigmentation rather than nutritional sources. Which states that marigold petals function better as carotenoid sources than nutritional sources, and if given continuously to fish *Xiphophorus helleri* will increase pigmentation. Which reported the addition of carotenoid sources in the form of astaksantin 260 mg kg^{-1} in feed was the best for the growth of rainbow kurumoi fish. The addition of carotenoid sources derived from rebon shrimp flour had a significant effect on the growth of goldfish.

3. 3. Water Quality

Other supporting parameters that must be observed are water quality, because water quality is very influential on cultivation. Based on the observations during the research, it was obtained a temperature value of 21.8-26.3 ºC. The lowest temperature value obtained during the research was due to the time when data collection was too early so that the temperature was also low. The temperature for swordtail fish ranges from 26.25 - 27.5 ºC. The temperature of the water affects the speed of chemical reactions, both in the external media and in the fluids in the body of the fish, the temperature increases, the chemical reaction will increase, while the concentration of gas in the water will decrease, including oxygen. As a result, fish will make a tolerant or intolerant reaction. The effect of low temperatures on fish is the low ability to take oxygen. This low ability is caused by a decrease in heart rate, another effect is the disrupted osmoregulation process. DO (Dissolved Oxygen) is an important element in the metabolic

process. The amount of dissolved oxygen is influenced by temperature. The value of dissolved oxygen during the study obtained an average ranging from 4.3-7.1 mg / L. These results can be said to be still within the feasibility limit.

The chemical process in water is determined by the pH of the water because the pH that is too acidic or basic causes the fish to become stressed (Indarti 2012). The results of pH observations during the study found an average of all treatments from 7.3 to 8.2. The pH level at the beginning of the study shows a value of 8.1-8.2 or outside the optimal limit of SNI, but the value can still be said in the worthiness limit, so that the fish can grow well. Whereas if compared with Rachmawati's study (2016) the pH value ranged from 6.025-7.7.

4. CONCLUSION

The addition of 6% butterfly pea leaf meal on commercial feed was able to improve the best color on the part of swordtail fish head with an average color score of 9.56 and a color value of 82.2. The addition of butterfly pea leaf meal in feed did not significantly influence the growth of absolute weight and length and water quality.

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