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Improving the Color Quality of the Swordtail Fish through the Supplementation of Butterfly Pea Leaf Meal

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Abstract | This study aims to examine the carotenoid content in Butterfly pea (*Clitoria ternatea* L.) leaves meal (BPLM) as a source of natural pigment and its effect on the tail color quality of Swordtail fish (*Xiphophorus helleri,* Heckel. 1848). Completely Randomized Designed (CRD) was applied consisting of four treatments with three replications. The treatments were added 0 % (A), 1 %, (B), 6 % (C) and 12 % (D) of BPLM. The parameters observed included biochemical compound and carotenoid content of dried BPLM, changes in tail color quality, survival rate and water quality. Kruskal-Wallis analysis was applied to analyzed the data of tail color. Data on the survival rate were analyzed using analysis of variance with the F test with 95 % level of confidence. The result showed that there was 9 076 mg kg^{-1} of carotenoid contained in BPLM. The inclusion of 12 % BPLM to commercial feed was able to enhance the color quality on the tail of the Swordtail fish, i.e average color 5 and a value of color 87.3. Supplementation of BPLM in feed does not significantly affect survival rate and water quality. These result indicated that usage of BPLM is safe as a sources of natural pigment for ornamental fishes, such as Swordtail fish.

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Introduction

Color is one main indicator quality and determining of economic value of ornamental fish. Swordtail fish (*Xiphophorus helleri,* Heckel. 1848) is an ornamental fish, habitats in freshwater, that has beautiful color and varied shape. Fish has chromatophores; cromatophores are located in the skin (Jorjani *et al*., 2019), a type of cell that contains color pigments, these pigments utilize carotenoids to bring forth shades of yellow (xanthophylls), red and orange (carotenoids), and brown and black (melanin) (Kaur and Shah, 2017). Increasing the quality of color in ornamental fish can be done by adding natural carotenoid sources can be obtained from animals and plants, like Carrot [*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens] skins (Andriani *et al*., 2020b), Marigold (*Tagetes* L.) flowers (Jorjani *et al*., 2019), Duckweeds (*Lemna* L.) (Iskandar *et al*, 2019), chitin (Abidin *et al*., 2020) and Blue-green Alga (Spirulina Turpin ex Gomont, 1892) (Asrami *et al*., 2019).

Butterfly pea (*Clitoria ternatea* L.) leaves meal (BPLM) is one of the potential alternative source to improve the color of ornamental fishes, includ-

ing Swordtail fish (*Xiphophorus helleri*) (Hariadi *et al*., 2018). Butterfly pea, commonly known as Asian pigeon wings, bluebell vine, blue pea, cordofan pea and Darwin pea, is a plant species belonging to the Fabaceae family (Karel *et al*., 2018). Based on these facts, a research is needed to determine the effect of giving BPLM on the color quality of Swordtail fish. Thus, it is potent to be cultivated. Besides providing nutrient, feed has also play a role in formation of body color. The pigment sources like caroteonoid in supplementary feed will enhance the color quality of ornamental fish.

Materials and Methods

The main equipment used was 40 cm × 25 cm × 25 cm aquarium, label paper, transparent plastic pipe, tube filter, water pump. The equipment for analysis were Dissolved Oxygen meter (DO) Merk Lutron with an accuracy of 0.01, beaker glass, pH meter merk Lutron with an accuracy of 0.01, millimeter block sheet, thermometer, digital scales MH-200 with an accuracy of 0.01 g, and Toca Color Finder (TCF) Cemani Tuka series 1999. The main materials were 120 kohaku type Swordtail fish length ± 3 cm from Muara Tegalega in Bandung Fish Market, binder Carboxy Methyl Cellulose (CMC), BPLM, and commercial feed merk "Matahari Sakti" (Indonesian product) with type PF 800. The size of feed are 0.7 mm to 1 mm , protein content at least 39 % to 41 % fat at least 5 %, maximum fiber 6 %, maximum ash content 16 %, and maximum moisture content 10 %.

Completely Randomized Designed (CRD) was applied consisting of four treatments with three replications. The number of fish in each replication are 10 fishes. The treatments were added 0 % (A), 1 %, (B), 6 % (C) and 12 % (D) of BPLM. The research lasted for 40 d with feed treatment in the form of pellets. Feed given as much as 3 % of the fish biomass with a frequency of three times a day at 08.00 am, 01.00 pm and 04.00 pm respectively.

The main data observed were the carotenoid content of the BPLM and the color quality of the fish tail which is visually observed using TCF as standard values (Table 1). While the additional data including observation of survival rate and water quality. The measurement of the color of the tested fish was observed by ten panelists of which panelists are fisheries students, the requirements to become a panelist in this test, namely who were not color blind to avoid bias. Before testing, all panelists are trained first to equalize perceptions so that the assessment is objective. Color observations are carried out every 10 d during research. The assessment starts with a gradation of colors from orange to red.

Table 1: *TCF color code used during the experiment of 40 d.*

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

Data on increasing fish color were analyzed using the one-way Kruskal-Wallis test. Analysis of variance with the F test (95 % level of confidence) used to analyze data of survival rate, while the water quality data analyzed descriptively.

Results and Discussion

Biochemical Compound and Carotenoid of Butterfly Pea

Butterfly pea has fine twining stems, 0.5 m to 3 m long. The leaves are pinnate, with five to seven elliptic to lanceolate leaflets (Alok *et al*., 2015). Leaves imparipinnate with two to four pairs of leaflets and a terminal leaflet. Leaflets ovate to elliptic-oblong, up to 6.5 cm × 4 cm, mostly hairless above, pubescent below. Flowers axillary, solitary or two together, resupinate, large and showy, bright blue (Alsanafi, 2016). Butterfly pea contains tannins, phlobatannin, carbohydrates, saponins, triterpenoids, phenols, flavanoids, flavonol glycosides, proteins, alkaloids, antharaquinone, anthocyanins, cardiac glycosides, stigmast-4-ene-3,6-dione, volatile oils and steroids (Alsanafi, 2016; Karel *et al*., 2018). Besides that, parts of the plant such as its leaves, flowers, and roots are believed to possess sought-after medicinal values

such as analgesic, antipyretic and anti-inflammatory properties. The plant also possesses a number of advantages such as antioxidant, antidiabetic, antihelminthic, hepaprotective and antiasthmatic, antibacterial and antifungal, insecticidal, forage for livestock, soil fertility (Afrianto *et al.*, 2020; Ezzudin and Rabeta, 2018; Gollen *et al.*, 2018). Butterfly pea leaves contain flavonoids, triterpenoids and saponins as antibacterial can inhibit the growth of *A. hydrophila* bacteria in Koi fish (Andriani *et al.*, 2020b).

Butterfly pea leaves contain different pigment, which give them respective color, carotenoids and xanthophylls (Arya *et al.*, 2018). Based on preliminary test results Butterfly pea leaves contain high carotenoids was 9 076 mg kg^{-1}. Other sources that contain carotenoids are *Tetraselmis suecica*, the extract of *Tetraselmis suecica* had pigment content 5 380 µg g^{-1} of carotenoids (Sedjati *et al.*, 2019). Fish that live in nature get the supply of carotenoids from natural source, while fish that are cultivated must get carotenoid intake through feed, one of which by using the BPLM in fish feed. Butterfly pea is known to store ternatin in it's blueish petals.

The rate of change of the tail color

The tail is one of the most interesting factors of Swordtail fish, because it has a brighter color compared to the body and head. The attractiveness will increase especially when the fish actively move in the water. The addition of different BPLM in the feed given to the Swordtail fish affected the increase in tail color (Figure 1).

At the end of this study, treatment D gave the highest color enhancement with an average score of 5, while the lowest value in treatment A with an average score of 1.44. Increasing color on treatment control (A) just occurred on the 30 d caused by internal factors, faster than control in treatment B on the 20 d. The higher carotenoid content in feed the faster color enhancement on the fish tail. It is proven by increasing color on treatment C and D that occur from the 10 d with an average score higher than treatment A and B 1.33 and 1.44.

The color of a fish body is mostly influenced by chromatophores cells that distinct the fish group. Melanophore, xanthophore and erythropore store pigments that absorb light (Meilisza *et al.*, 2018). Increasing in the average color score on the tail of a Swordtail fish is presented in Figure 2.

In treatment A, the tail of the Swordtail fish color has initial orange color on day 0 with an average color score of 1 on the TCF code 0615. The color increase was not significant to 1.11 on the 30 d. On the 40 d the average color score increased to 1.44 with a bright orange in the TCF code 0715. Color enhancement without carotenoid sources can occur, because color enhancement is not only formed through external factors but also internal factors, one of this heredity.

Treatment B increased on the 20 d with an average color score of 1.11, increasing from an orange color (TCF Code 0615) to a bright orange color (TCF Code 0715). The score increased to 2.56 on the 30 d. On the 40 d there was a decrease in color in this treatment with a score of 1.89. Fish in treatment C increase color on the 10 d with an average color score of 1.33 with bright orange in TCF code 0715. The increase occurred until the last day reached an average color score of 4.33 to red with the TCF code 1006.

Supplementary of 12 % BPLM (D) has an initial color that is orange with TCF code 0615. The color improvement occurred on the 20 d with an average color score of 1.44, exceeding the color score of treatment C. The color enhancement ends on the 30 d in red on the TCF code 1006. On the 40 d the color of tail of the Swordtail fish began to stabilize, there was no increase or decrease. Utilization and bioconversion of carotenoids into main pigments in fish varies, so their effectiveness varies depending on species (Meilisza, *et al.*, 2018).

Colouration in fishes is influenced by several external and internal factors including dilation of blood capillaries, reflective irridocytes, chromatophores, genetic and environmental factors, stress and array of natural and artificial colour enhancing compounds and fish diets has also been tried by several researchers (Wadges *et al.*, 2018).

Commercial feed containing fat is quite good mixed with Butterfly pea leaf meal containing carotenoids (Oguis *et al.*, 2019). It acts as precursors of vitamin A, increase absorption of carotenoids, and lead to color enhancement of Swordtail fish. Carotenoids have different types, one is carotene. Carotene blending with protein with heating process, namely carotenoprotein, then it will be divided into proteins and carotene that produce a red color. Pigmentation in fish is likewise affected by hormones and the central nervous system.

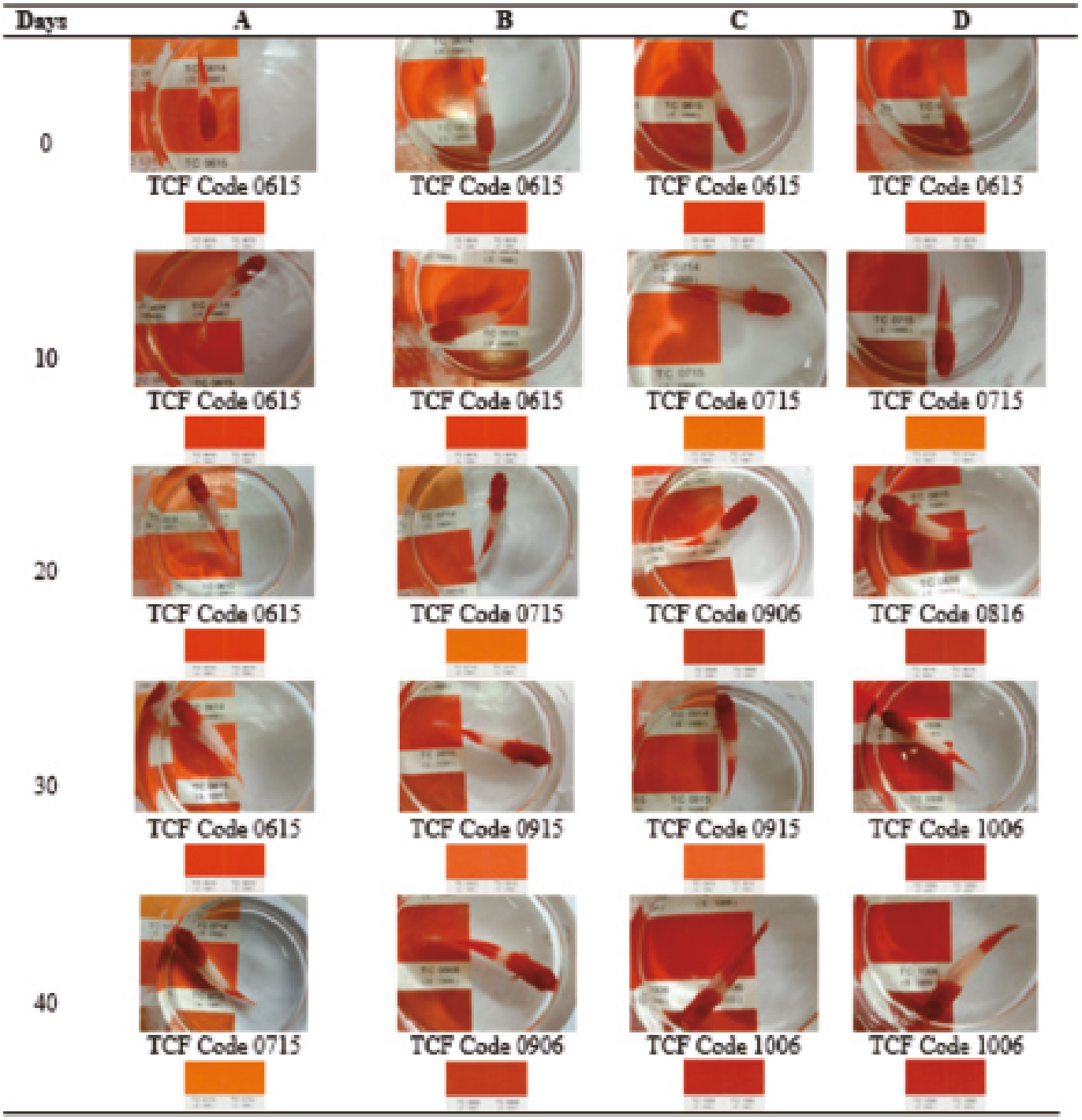

Figure 1: *Increased tail color for 40 d trial.*

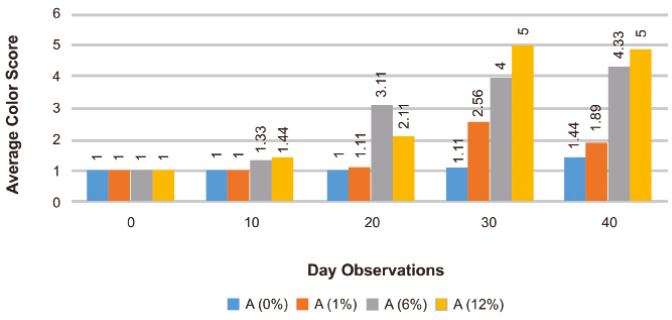

Figure 2: *Increased average color score of the tail of Swordtail fish.*

The pituitary gland produces melanin dispersing hormone (MDH) which influences the color shading and melanin aggregation hormone (MAH) which give an influences to its color (Yustiati *et al*., 2018).

Based on the Kruskal-Wallis Test, it showed significant difference among treatments then continued by the Z test (Table 2). The A treatment (control) was not significant differ from B treatment, meanwhile the C was not significantly different from D treatment, but there were significantly different between treatment A, B and treatment C and D.

Table 2: *Color value of Swordtail fish tails during the experiment of 40 d.*

Treatment	Color Value
A (control/0 % BPLM)	24.667[a] ± 0.192
B (1 % BPLM)	32.167[a] ± 0.692
C (6 % BPLM)	73.833[b] ± 1.522
D (12 % of BPLM)	87.333[b] ± 1.918

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

Supplementation of 12 % BPLM in the fish feed (treatment D) for 30 d indicated the highest color value of the tail (score = 87.3) compared to the control group (score = 24.7). Nevertheless, in treatment C which were given the mixed-feed for 40 d, no increase in color was observed. It is predicted that carotenoids given to Swordtail fish have reached the maximum limit for the tail. Whereas the addition of optimal Butterfly pea to the feed which affects the increase in the color of the head of the swordfish is 6 % (Julia *et al.*, 2019).

The use of carotenoids as a source of pigmentation to improve color quality, especially ornamental fish, has been done in several studies. The use of natural carotenoid sources such as Carrot peel powder as much as 1 %, effective in increasing the coloring and growth of *Cyprinus carpio* Linn. 1758 (Maiti *et al.*, 2017). Carotenoid pigment from *Penicillium purpurogenum* and *Talaromyces purpurogenum* in Golden Koi (*Cyprinus carpio*) fish feed result show that pigments are non-toxic compound to fish, increase the fish weight and body protein content and enhance the color of fish skin (Patil and Thakare, 2017). The addition of another natural carotenoid source, *Phaffia rhodozyma*, improves red skin color fancy goldfish (Ninwichian, 2020). Addition of natural β-carotene sources derived from spinach increases yellow color and natural sources of β-carotene derived from carrots increase red color in Swordtail fish (Wadge *et al.*, 2018).

Survival rate

The F test showed the treatments did not have a significant effect to survival rates of fish. The results of observations of survival rates are presented in Table 3. The percentage survival rate for each treatment was not significantly different. If compared to the four treatment, treatment A or control has a higher mortality rate than the others.

Table 3: *Survival rate of the Swordtail fish during the experiment of 40 d.*

Treatment	Survival Rate (%)
A (control / 0 % BPLM)	73.33[a] ± 11.5
B (1 % BPLM)	86.67[a] ± 5.8
C (6 % BPLM)	83.33[a] ± 11.5
D (12 % of BPLM)	80.00[a] ± 10.0

Note: *Values followed by the same letter are not significantly different based on Duncan's test at a 95 % confidence level.*

This means that the carotenoids in BPLM did not affect on the survival of Swordtail fish. Besides feed, supporting factors that produce good survival values are stocking density. The supplementation up to 30 % of pumpkin meal as natural carotenoid in feed does not affect the survival of the Koi kohaku strain (Yustiati, *et al.*, 2018).

Water quality

Water quality is a variable that affects the productivity of Swordtail fish farming. The parameters observed in water quality were Dissolve Oxygen (DO), pH and temperature, which were observed once every ten days. Based on observations of water quality during the study that is obtained DO value in the average range 4.3 mg L^{-1} to 7.1 mg L^{-1}, average pH of 7.3 to 8.2 to all treatments, while temperature values of 25.8 °C to 26.3 °C (Fullazaky *et al.*, 2010).

Conclusions and Recommendations

Carotenoid has been proven contained in BPLM (9 076 mg kg^{-1}), thus by mixing 12 % of the leaves to commercial fish feed could increase the tail color of the Swordtail fish (average color score = 5; color value = 87.3). However, the supplementation of BPLM in fish feed did not show significant effect on the survival and water quality.

Novelty Statement

Color enhancement in ornamental fish is not only influenced by internal factors, but also by external factors. One of them is feed containing carotenoids. Investigations on this manuscript found that the leaves of Butterfly pea leaves contain carotenoids, and are used as a source of vegetable food which is made into meal and then mixed with commercial feed and the result are indeed influential, adding 12 % of Butterfly pea leaves to commercial feed can improve the color quality of the tail of Swordtail fish.

Author's Contribution

YA and II conceptualized and designed the study, elaborated the intellectual content, performed literature search, data acquisition, data analysis, statistical analysis, manucript preparation and manuscript revision. ROJ and LPSY carried out experimental studies, and manuscript review. YR elaborated the intellectual content, performed literature search, manuscript review and guarantor.

Conflict of interest

The authors have declared no conflict of interest.

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