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Changes in color of betta fish (*Betta splendens*) by feeding of *Artemia salina* enriched with *Tagetes erecta* flower flour

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Abstract. *Kiswara CA, Budiharjo A, Sari SLA. 2020. Changes in color of betta fish (*Betta splendens*) by feeding of* Artemia salina *enriched with* Tagetes erecta *flower flour. Cell Biol Dev 4: 46-50.* Betta fish are attractive due to their varied and vibrant colors and aggressive temperament. Carotenoids are the pigments found in betta fish. They are kept in chromatophores. While fish cannot synthesize carotenoids in their bodies, they can absorb them from their diet and use them as pigments to enhance the color intensity of their bodies. The purpose of this study was to determine the degree of color change and survival of betta fish fed with *Artemia salina* (Linnaeus, 1758), which has *Tagetes erecta* L. flower flour as their food. This study used a completely randomized design (CRD) and three treatments with varying compositions of rice flour: *T. erecta* flower flour, namely, 5:1, 2:1, and 1:1, respectively. *Artemia salina* fed simply with rice flour was a control. Betta fish require 40 days of care. Color intensity was determined once every ten days using a color scale with a score of 1 (white), 2 (weak red), 3 (medium red), and 4 (dark red) (red). The results indicated that *A. salina* fed in a 1:1 ratio had the best effect on improving the color brightness of betta fish. All parts of the betta fish's body received a score of 4 in this treatment, including the head, fins, and tail.

Keywords: *Artemia salina,* Betta, *Betta splendens,* degree of discoloration, *Tagetes erecta*

INTRODUCTION

Betta fish (*Betta splendens* Regan, 1910) are freshwater fish native to various tropical Asian nations, including Indonesia, Thailand, Malaysia, and Vietnam (Witte and Schmidt 1992). Male betta fish have long tails, gorgeous colors, and a tendency to defend their territory aggressively, frequently used as ornamental fish or fighting fish. This fish's primary attraction is its long dangling fins with vibrant colors. Round (wild type) fins, half-moon (shovel-shaped), spade-tailed (lanced form), long fin (flowing and extended), delta (triangular with vertical end), and ribbon (elongated flowing) fins are found in betta fish (Goldstein 2004). Betta fish are classified according to their color into three categories: solid color, combination, and mascot.

Moreover, it is called solid color if all body parts and fins are the same color, such as red, blue, gray, black, yellow, or white. However, if the body or fins are a combination of two or more different hues, such as blue-red, black-red, red-steel, or green-red, it is classified as a color combination. At the same time, it is referred to as a mascot if the betta fish's body is predominantly red and white or other hues such as gray, blue, or green (Goldstein 2011).

Carotenoids are the pigments found in betta fish. They are kept in chromatophores. Carotenoids are organic pigments found naturally in the chromoplasts of plants. Although animals, including fish, lack chromoplasts, they can retain carotenoids in their bodies (Britton et al. 2001). By and large, fish will absorb the carotenoids in their meal and use them as a pigment-forming agent, thereby intensifying the color of their bodies (Torrlssen et al. 1989). Carotenoids are absorbed into tissue cells and affect the chromatophores in the epidermal layer of fish. Fish can change color due to chromophores found in their skin. Chromatophore cells are pigment cells with a spherical form that are distributed throughout the fish skin's epidermal cell layer. Chromatophores produce distinct hues; each chromatophore has only one color (Sally 1997; Anderson 2000). Fish modify the pigment their food receives physiologically, resulting in color differences. Color changes generated by the movement of pigment grains or chromatophores are physiological color alterations (Evan 1993). Chromatophores are pigment-containing cells that store, manufacture, absorb, and reflect light in a specific color form. There are six types of chromatophores, which are generally classified according to their color range when observed under light: melanophores (black-brown), erythrophores (red-orange), xanthophores (orange-yellow), iridophores (metallic), leucophores (beige-white), and cyanophores (bright green-blue) (Sköld et al. 2013).

Carotenoid-rich feeds can accelerate pigment production in fish and modify the brightness of color. For example, Çapar et al. (2007) studied rainbow trout feeding (Oncorhynchus mykiss) with *Tagetes erecta* L. flower flour, red chili, and pure astaxanthin. The results indicated that using *T. erecta* flower flour at a concentration of up to 1.6% (carotene about 65 mg/kg) in the feed was the most effective way to raise the carotene content in the fish body while reducing the fish performance (fish weight) just little when compared to control feed. According to Kusuma's (2012) research, adding 1.5% marigold flower flour to total artificial feed increased the color intensity by up to 127.53%, with a final chroma content of 54.54.

Carotenoids are abundant in *T. erecta* flowers; moreover, the carotenoid concentration of *T. erecta* flower petals is around 7,000 mg/kg dry weight. This number is more than the carotenoids found in algae (2,000-4,000 mg/kg) or yeast (30-800 mg/kg), which are frequently added to the fish diet as supplements (Hertrampf and Puscual 2008). Therefore, the purpose of this study was to examine the effect of adding *T. erecta* flowers to Betta's fish native diet, *Artemia salina* (Linnaeus, 1758), on the color brightness of Betta fish.

MATERIALS AND METHODS

Research materials

The main ingredients used were 56 betta fish seeds with an age of ± 60 days and size of ± 1 cm, 2 kg of *T. erecta* flowers, 2 kg of rice flour, and *A. salina* eggs.

Research design

This study used a completely randomized design (CRD) and three enrichment treatments with *T. erecta* flower flour on the *A. salina* diet. The composition of *A. salina* feed is shown in Table 1.

The making of *Artemia salina* feed

Tagetes erecta flowers are carefully washed under running water and separated into petals and other parts. After washing, the petals were weighed wet with a scale. The petals were then dried in an oven set to 70-80°C for 15 minutes. Next, they were blended until smooth and in the form of flour after drying. Then the feed was made by diluting a mixture of rice flour and *T. erecta* flower flour into 10 mL of salt suspension water with a concentration of 55% according to the variation of treatment, namely 3 mg of rice flour and a mixture of rice flour and *T. erecta* flower flour with the composition according to the research design.

Maintenance of *Artemia salina*

Artemia salina eggs are incubated in bottles until they hatch. Bottles for egg incubators are constructed with two sections of insulated space, one for darkness (on top) and the other for illumination (bottom). An aerator is used to aerate saltwater that has a concentration of 55%. The water is placed in a container and aerated. *Artemia salina* eggs are laid in a single chamber, after which the chamber is sealed. As a result of the phototactic properties of the hatched *A. salina*, the other side of the bulkhead was left exposed, and a lamp was used to pull it through the opening. The eggs will hatch into larvae after approximately 24 hours. Larvae were fed as much as 1 mL of feed suspension twice a day when they were 48 hours old, in the morning at 08.30 and in the afternoon at 15.00. After being kept for two days, *A. salina* was fed betta fish as food.

Betta fish care

Eight pieces were used to construct the aquarium, which measured 20 cm in length, 15 cm in height, and 10 cm in width. First, the aquarium is cleaned with alcohol and thoroughly rinsed, filled with water to a height of up to 12 cm.

Betta fish seeds are acclimated in advance for approximately one night, after which they are placed in the aquarium that has been previously prepared. Betta fish seeds were maintained over 40 days by feeding them one teaspoon of sugar daily (5% of fish biomass). Therefore, it is necessary to feed the brine shrimp three times a day, in the morning at 09.00, in the afternoon at 13.00, and in the evening at 17.00. Daily, 25% of the water was siphoned and replaced to keep the system running correctly during maintenance.

Color change observation

Every ten days, the body color parameters of betta fish were observed. The head (from the mouth to the front of the operculum), the middle body (from the tip of the operculum to the base of the anal fin), and the fin were studied. Color parameters were observed using the scoring method, explicitly calculating the color scale on the body of betta fish, as Satyani and Sugito (1997) and Brake et al. (2013) did. The color scheme is depicted in Figure 1.

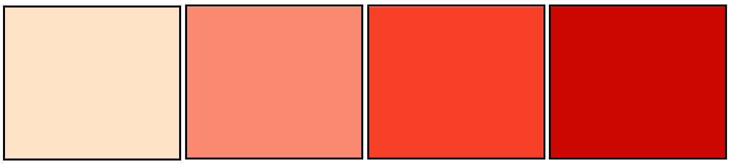
Identification of carotenoid compounds in *Tagetes erecta*

Tagetes erecta flower flour was soaked in a solvent mixture of acetone and methanol (7:3) with a sample-solvent ratio of 1:10 (w/v). Next, $CaCO_3$ was added as a neutralizing agent and ascorbic acid as an antioxidant to prevent oxidation during extraction. Next, the extracted pigments were filtered, and the residue was re-extracted with the same solvent using a stirrer at a speed of 750 rpm for 10 minutes or until all pigments were lifted (became colorless). Next, the extraction findings were partitioned using diethyl ether at a sample ratio of 1:1 (v/v), which is compatible with distilled water and concentrated sodium chloride (if the pigment solution is difficult to separate). Furthermore, to bind H_2O, the ether layer containing the pigment (colored) was removed, and anhydrous Na_2SO_4 was added. Following that, a filter was applied to this layer. Finally, the filtrate was concentrated using a rotary evaporator (Britton et al. 1995).

The carotenoid pigment extract was briefly affixed to the silica gel TLC plate 60 F254 and then eluted with a 3:2:1 (v/v/v) mixture of hexane, diethyl ether, and acetone. After observing the TLC pattern of each pigment, the Rf was calculated and compared to the standard marker's TLC pattern.

Table 1. Combination of treatments

Feed concentration *Artemia salina*	**Weight (mg)**			
	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**
T. erecta flour	0	0,5	1	1,5
Rice flour	3	2,5	2	1,5



A B C D

Figure 1. Color Intensity Scale on Betta fish body: A. scale 1 (white), B. scale 2 (weak red), C. scale 3 (medium red), and D. scale 4 (red)

RESULTS AND DISCUSSION

Discoloration

All treatments were classified according to their color appearances in categories: body, fins, tail, and head. The results indicated that adding *T. erecta* flower flour imparted a reddish hue to betta fish. This condition was demonstrated by the rise in color intensity observed in each treatment throughout the investigation (Table 2).

At the commencement of the study, the fish's tail and fins were a medium red, while the head and body were weak red. On day 10th, after treatment with *A. salina* fed with *T. erecta* flower meal, betta fish experienced a higher color intensity than the control treatment. Colors occurred exclusively on the control treatment's body, tail, and fins. In contrast, other treatments that included *T. erecta* flower flour underwent an increase in color intensity on the tail, fins, body, and head. The initial treatment resulted in a color alteration of weak red into a medium red on the head and body, while the fins and tail changed from a medium red to a red rise.

On the tenth day of treatment, the color intensity of betta fish increased. The highest increase in color was noted in the head of betta fish fed with *A. salina,* which was given by rice flour and *T. erecta* flower flour at a 1:1 ratio. Meanwhile, the greatest average increase in color intensity ratings was recorded in the body and tail at 5:1 and 2:1 feed compositions.

On the twentieth day, the greatest rise in color intensity occurred. Color changes, particularly on the head and body, were found in betta fish fed with *A. salina,* which had *T. erecta* flower flour as the meal. In addition, the tail and fins developed pigment more subtly than on the tenth day. The greatest rise was recorded in betta fish fed with *A. salina*, which had a diet of rice flour and *T. erecta* flower flour at a ratio of 2:1. This color change happened on the head, tail, fins, and body which started on day 10th and culminated on day 20th (Figure 2). In contrast, the control treatment had a lower average score in the head, body, and tail than the other treatments.

Observations from the 30th to 40th days revealed a reasonably consistent color intensity, practically identical to that on the 20th day. There was a small drop in color intensity in certain treatments. After 20 days of treatment, betta fish development was at its peak. On the 30th day, the Kruskal Wallis test using SPSS revealed that the findings were substantially different only for the body part for which the calculated F (0.005) was less than the F table (0.05).

Table 2. Changes in color intensity of betta fish fed *A. salina* feed enriched with *T. erecta* flower flour

Fish body parts	Feed composition rice flour: *T. erecta* flower flour	The average color intensity on the day-				
		0th	10th	20th	30th	40th
Head	1:0	2.0	2.0	3.0	3.4	3.3
	5:1	2.0	2.4	3.9	3.9	3.1
	2:1	2.0	2.6	4.0	4.0	3.7
	1:1	2.0	2.9	3.7	3.7	4.0
Body	1:0	2.0	2.1	3.0	3.0	3.1
	5:1	2.0	2.7	3.9	3.7	3.6
	2:1	2.0	2.7	3.9	4.0	3.7
	1:1	2.0	2.6	3.9	3.9	4.0
Tail	1:0	3.0	3.4	3.6	3.9	3.7
	5:1	3.0	3.6	3.9	4.0	3.9
	2:1	3.0	3.6	4.0	4.0	4.0
	1:1	3.0	3.3	4.0	4.0	4.0
Fins	1:0	3.0	3.4	3.9	3.7	3.7
	5:1	3.0	2.9	3.6	4.0	3.7
	2:1	3.0	2.9	4.0	4.0	4.0
	1:1	3.0	3.3	4.0	4.0	4.0

A B C D

Figure 2. Color of betta fish before treatment (A), after treatment with *A. salina* feed fed with rice flour and *T. erecta* flower flour with a composition of 2:1 on day 10th (B), 20th (C), and 30th (D)

According to Evan (2008), as cited in Indarti et al. (2012), carotene in the form of lutein found in *T. erecta* flower flour is altered by fish into astaxanthin and is used as a source of red pigment. Fish can brighten their color physiologically by the food they ingest, and the pigments are dispersed throughout the body's chromatophore cells via the blood. On the tenth day, it is conceivable that the red pigment has not been spread evenly throughout the body, resulting in the coloration of the fins of certain Betta fish remaining unchanged.

Hormones and the central nervous system also affect fish pigmentation. Sally (1997), cited by Puspita (2012), states that the pituitary gland generates three types of hormones: Melanocyte Stimulating Hormone (MSH), Melanin Concentrating Hormone (MCH), and Melatonin (MT). MSH allows the pigment to permeate throughout the cells, giving the scales a brilliant and clear appearance. MCH causes the pigment to collect in cells, giving fish scales a lighter appearance. MT, produced in the epiphyseal gland, is the third hormone that affects fish color. These hormones act on chromatophores of pigment cells, causing pigment granules to aggregate within cells, resulting in a decrease in color. The food consumed by fish highly controls these hormones' activity. According to Fujaya (2004), dietary sources contribute significantly to the secretion of hormones that directly produce and store a variety of colors in the fish body.

Betta fish convert astaxanthin to zeaxanthin in their bodies, and zeaxanthin also exists in three optical isomers in the fish body (Maoka 2009). Tunaxanthin is most abundant in fish of the order of Perciformes. The fish's tail and skin are brilliant red due to the presence of tunaxanthin, whereas tunaxanthin is the product of astaxanthin metabolism via zeaxanthin, according to research.

Color changing pattern

The direction of the color change pattern in betta fish is determined by comparing the average color change score on each body part of the fish after 40 days of observation, beginning with the fins, tail, body, and head. According to the observations, the most noticeable color changes occurred on the fins and tail, while the head and body remained relatively unchanged (Figure 3).

Betta fish that consumed *A. salina* fed on rice flour (control), and a mixture of rice flour and *T. erecta* flower flour in a ratio of 5:1 and 2:1 would almost result in the same color change pattern; that is, the head is better than the body. However, Betta fish fed *A. salina* fed rice flour, and *T. erecta* with a ratio of 1:1 showed a better pattern of color change throughout the body.

Identification of carotenoid compounds in *Tagetes erecta* flowers

Thin Layer Chromatography (TLC) technology was used to identify carotenoid pigments. Figure 4 illustrates the color separation pattern. TLC analysis revealed three yellow and yellow-orange spots with Rf values of 0.8, 0.68, and 0.25 in the carotenoid extract *T.erecta*.

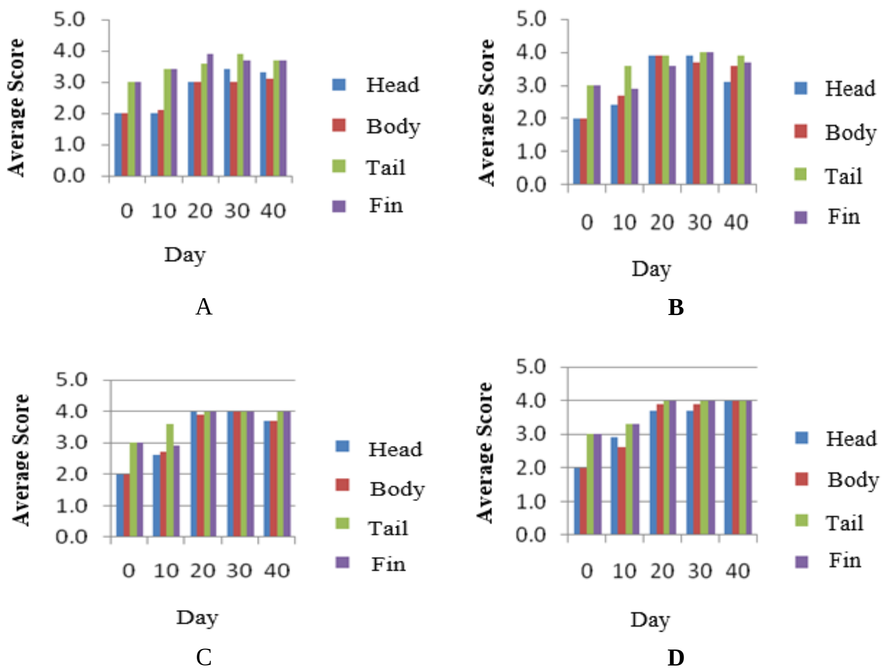


Figure 3. The average distribution of the total color of betta fish on each limb after 40 days of treatment by feeding *A. salina* fed rice flour (control) (A), rice flour, and *T. erecta* in a ratio of 5:1 (B), 2: 1 (C) and 1:1 (D)

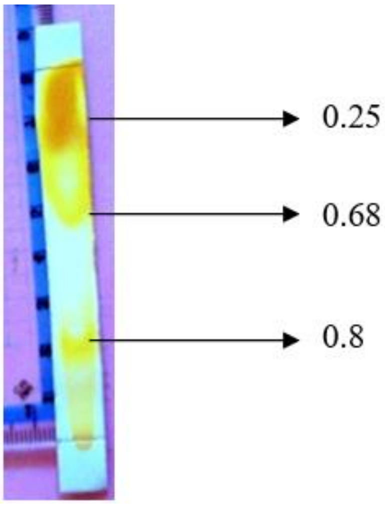


Figure 4. TLC Results of *T. erecta* carotenoid pigment extract

The diversity of pigment Rf values is closely related to the pigment content of the *T. erecta* flower. Spot 1 produces an orange color on the TLC plate, which is thought to be beta-carotene, as the mobile phase used is predominantly nonpolar, which is also a property of beta-carotene, as beta-carotene has a higher Rf value and a faster rate of movement than other pigments. Spot 2 with yellow-orange color is astaxanthin, while spot 3 with yellow-orange color represents lutein. The research results of Monika (2009) show that lutein, astaxanthin, and beta-carotene have an RF values of 0.3, 0.6, and 0.8. This RF value is almost the same compared to this study's results.

In conclusion, feeding *T. erecta* flower flour to betta fish natural food can cause the color change of the betta fish to become more noticeable. The *A. salina* feed, composed of rice flour and *T. erecta* flower flour in a 1:1 ratio, was shown to be the most effective in boosting the color of betta fish. The initial direction of color change in betta fish is on the limbs, but the fastest color changes are on the fins and tail. The body and head tend to be slower to change color.

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