Bangladesh J. Fish. (2021) **33**(2) : 235-241

Effects of natural carotenoids on the body coloration and growth performance of Blue Gourami fish (*Trichogaster trichopterus)*

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Abstract. The effect of natural carotenoid on body coloration and growth of Blue Gourami (*Trichogaster trichopterus)* was investigated using china rose petal (T_1), marigold petal (T_2), and carrot (T_3). Sources were incorporated with fish feed with four treatments, including T_0 control treatment (without natural carotenoid sources) at a 15% ratio. Fishes were purchased and conditioned for two weeks and then randomly distributed among the 12 plastic tanks, and stocking density was maintained at eight fishes/tank. Fishes were fed twice/daily with four different treatments for eight weeks. Sampling was done every week and carotenoid contents, weight, and length measurements were recorded. The average final weights in four treatments were found 5.455±0.821 gm, 4.504±0.2196 gm, 4.764±0.727 gm, and 3.776±0.418 gm in treatments T_0, T_1, T_2, and T_3, respectively. In the case of weight gain, a higher result was found in T_0 (5.455±0.821 gm). Mean final lengths obtained were 4.094±0.250 cm, 4.005±0.233 cm, 4.083-0.297 cm, and 4.083±0.311 cm, respectively. The average final carotenoid absorptions of the fishes in four treatments were 0.1226±0.0115 mg/100 kg, 0.1296±0.0169mg/100 kg, 0.1669±0.0298 mg/100 kg, and 0.1487±0.0228 mg/100 kg in treatments T_0, T_1, T_2 and T_3, respectively. T_2 treatments (marigold) had the highest carotenoids gain. The highest survival rate (100%) was observed at T_2. Study suggested that marigold petal could be used to make natural color enhancer feeds at a cheaper cost.

Keywords: Carotenoid, Body Coloration, Blue Gourami.

Introduction

Ornamental fish come in a wide range of colors and patterns, and the fish's vibrant color is vital to its success in the ornamental fish market. Color is a significant factor in determining aquarium fish price globally (Saxena 1994, Torrissen 1989). Color pattern and intensity influence market demand and customer preference. Colors have psychological and physiological effects on consumers' minds (Kaushik 2011). Fish that are naturally colored frequently exhibit faded coloration under intensive farming conditions (Pulcini *et al.* 2020). Like other animals, fish cannot synthesize carotenoids on their own and must obtain them through diet. As a result, they exhibit a direct relationship between carotenoids and coloration (Hatlen 1997). The principal source of color in the skin of ornamental fish is carotenoids (Sinha and Asimi 2007). Three factors affect fish coloration; genetics, diet, and the nervous system. Carotenoid enhancers could be both natural and synthetic, and naturally occurring carotenoids are preferable to synthesized carotenoids because synthetic carotenoids can pollute the environment (Kaur and Kumar 2017). Different natural carotenoid sources such as plant's petals, bacteria, microalgae, and flower extract are often used as color enhancers and influence fish growth (Maiti 2017). However, a few studies were conducted on the effects of natural color enhancers on body coloration and development, e.g., *Spirulina* was used for rainbow trout (Choubert 1979) and carrot for goldfish (Tiewsoh 2019). If natural carotenoid pigment-enriched feed like carrot, china rose, and marigold flower can improve coloration, it will enhance the quality of fish and value in the aquarium fish market. Since, synthetic additives are expensive, recent research has

https://doi.org/10.52168/bjf.2021.33.26

focused on natural substances. Blue gourami (*Trichogaster trichopterus*) is an attractive colored fish in the ornamental fish industry and has potential market demand worldwide (Degani 2013). The current study evaluates the effects of marigold and china rose petal and carrot mixed diet on the growth and pigmentation of Blue Gourami.

Materials and Methods

Experiment setup: The study was carried out in the twelve circular plastic tanks (height 65 cm & diameter 52 cm) for four months. An adequate level of oxygen in each tank was maintained through artificial aerators. The underground water for the experimental tank was stored in two plastic barrels. Table I represents the experimental layout with four different treatment groups.

Table I. The layout of the experiment

Dietary treatment groups	Treatment×Replication (Tn × Rn)	No. of fishes per tank	Total no. of fish per treatments
T_0 (Control)	T_0R_1	08	24
	T_0R_2	08	
	T_0R_3	08	
T_1 (China rose flower mixed feed)	T_1R_1	08	24
	T_1R_2	08	
	T_1R_3	08	
T_2 (Marigold flower mixed feed)	T_2R_1	08	24
	T_2R_2	08	
	T_2R_3	08	
T_3 (Carrot mixed feed)	T_3R_1	08	24
	T_3R_2	08	
	T_3R_3	08	

Collection of natural carotenoid sources and feed preparation: Carrot (*Daucus carota*), China rose (*Hibiscus rosa sinensis*) and marigold (*Tagetes erecta*) were collected and dried for the preparation of experimental diet. After drying, the sources were powdered and sieved (Laboratory test sieve, ASTM E-11) and then stored at -20°C to preserve carotenoid from oxidation. Then the sources were thoroughly mixed with commercial feed (Tiger Brand EON Nursery Powder Feed-1) at a rate of 15g/100g before pelletization. The control feed consisted of 100g commercial feed (Tiger Brand EON Nursery Powder Feed-1). Proximate analysis and carotenoid content were determined of the natural carotenoids mixed feed and control feed before the experimentation. Table IIdescribes the proximate analysis and carotenoid content of the dietary experimental feed.

Table II. Proximate analysis and carotenoid content of the experimental feed

Content	T_0	T_1	T_2	T_3
Protein %	30	27.82	27.34	28.8
Lipid %	06	5.47	5.11	5.58
Ash %	16	14.53	14.681	14.44
Moisture %	12	11.38	11.47	12.24
Carotenoid(mg/100g)	0.70	1.30	2.80	16.50

Collecting experimental fishes and conditioning: The experimental fish were purchased from local ornamental fish traders and conditioned to laboratory conditions for two weeks. Optimal water quality parameters and sufficient oxygen concentration were maintained during the conditioning period.

Stocking and feeding: The experimental fish were stocked, then experimental feed was applied, according to the experiment layout (Table 1). Feeding was done twice/daily and at a rate of 5% body weight. Feeding management was controlled according to fish body weight.

Sampling: Sampling was done every week throughout the experiment period. The fish length, weight, and carotenoid content were measured in every sampling. Water quality parameters (Dissolved oxygen, pH, and temperature) were measured every week to ensure a suitable environment for the experimental fishes.

Growth performance: In each sampling, fish length and weight were recorded. Mean weight and length were determined and recorded in order to assess growth performance. Furthermore, survival rates were determined in order to assess the impact of natural carotenoids on survivability of fish.

Carotenoid Analysis: The carotenoid content of experimental fish skin was determined using Torrissen and Naevdal's method (1984). One gram of skin was taken from both sides of the fish, between the abdominal and dorsal areas, and transferred into 10-ml pre-weighed glass tubes. The samples were homogenized in acetone with 1.5g anhydrous sodium sulfate and extracted up to 10 ml with acetone. The samples were kept at 4°C for three days before being extracted for three times. The solution was centrifuged (HERMLE Labortechnik GMBTA) for 5 minutes at 5000 rpm, and then absorption was measured by a spectrophotometer (OptizenPoP). The following formula was used to measure the carotenoid content of the fish body:

- Carotenoid content (mg/100kg): $\frac{Abs \times 1000 \times V \times 100\ kg}{1900}$, where V= 10, Abs= Pigment Absorption rate which measured by spectrophotometer

Statistical analysis: Statistical analysis was done using Microsoft Excel (Version-2016) and IBM SPSS (Version-25). Standard deviation was used to express values (SD). The data were analyzed using one-way ANOVA with a significance level of 0.05.

Results

Growth performance: The average final weights in four treatments were found 5.455±0.821 gm, 4.504±0.2196 gm, 4.764±0.727 gm, and 3.776±0.418 gm in treatments T_0, T_1, T_2, and T_3, respectively (Table III). In the case of mean weight, a higher result was found in T_0 (5.455±0.821 gm) followed by T_2, T_1, and T_3. Mean final lengths were found 4.094±0.250 cm, 4.005±0.233 cm, 4.083-0.297 cm, and 4.083±0.311 cm, respectively (Table III). According to the values, it was clear that control feed (T_0) provides better growth performance in terms of length and weight gain ($p<0.05$).

Table III. Growth performance of Blue Gourami

Treatment	Mean weight (gm)	Mean length (cm)
T_0	5.455 ± 0.821^c (5.046-5.864)	4.094 ± 0.250^b (3.969-4.219)
T_1	4.504 ± 0.219^a (4.394- 4.613)	4.005 ± 0.233^b (3.889- 4.121)
T_2	4.764 ± 0.727^a (4.402- 5.126)	$4.083\text{-}0.297^b$ (3.935-4.231)
T_3	3.776 ± 0.418^b (3.568- 3.984)	4.083 ± 0.311^b (3.928- 4.238)
Level of significance	0.001	0.0232

***Total carotenoid gains in fish tissue**:* The amount of natural carotenoids gained by fish bodies during the experimental period is shown in Fig.1. The initial carotenoid contents in T_0, T_1, T_2 and T_3 were 0.1109 mg/100kg, 0.1035 mg/100kg, 0.123 mg/100kg and 0.1158 mg/100kg, respectively. At the end of experimental trials, the mean carotenoid contents of the fishes of four treatments were 0.1226 ± 0.0115 mg/100kg, 0.1296 ± 0.0169 mg/100kg, 0.1669 ± 0.0298 mg/100kg, and 0.1487 ± 0.0228 mg/100kg in treatments T_0, T_1, T_2 and T_3, respectively. T_2 treatments (marigold) showed the highest carotenoids gain (0.16690.0298 mg/100kg) ($p < 0.05$) (Fig. 1).

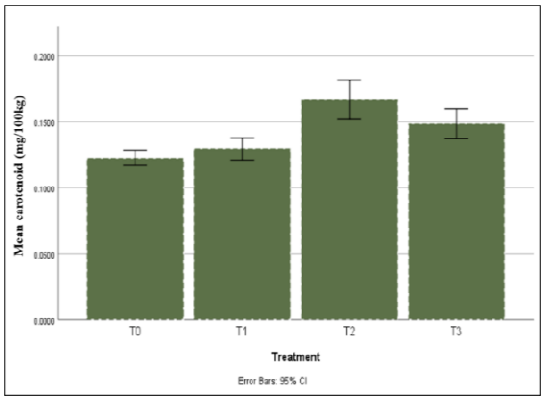

Fig 1. Final carotenoid contents of experimental treatments.

Survival rate: The survival rate of experimental fish of different treatments is given in Table IV. The highest survival rate was observed in the T_2 (100%). Experimental fish showed good performance in T_0 & T_3 treatment in which survival rate is about 87.50%, and the lowest performance was shown in T_1 treatment (75%).

Water quality parameters: Water quality parameters were monitored weekly throughout the experimental period. The range of mean value of water quality parameters during the research work are shown in Table V.

Table IV. Water quality parameters

Treatments	pH	Dissolved oxygen (mg/L)	Temperature (□)
T_0	7.5-8.5	5-7	24-27.8
T_1	7.2-8.0	6-7.2	24-27
T_2	7.5-8.2	5.5-7.5	25-28
T_3	7.0-8.0	5-7.5	23-28

Discussion

Growth performance: The present study found that control feed (T_0) showed higher growth in terms of length and weight gain, but in the case of carotenoid sources mixed feed, marigold petal showed better growth performance compared to other natural carotenoids mixed feed treatment groups (Table III). Fish fed with china rose enriched diets grew the slowest. The present study also showed that high protein content in the control feed (30%) shows high growth at the end of the study. According to Sommer *et al.* (1992), inclusion of carotenoids-rich microalgae and krill meal in the diet could aid trout growth. Ezhil *et al.* (2008) found that feeding marigold petal 15 g/100 g to Red Swordtails fish, enhanced their growth rate, which matched with the findings of the present study. The control diet in this study had a higher protein percentage than the others, which could be cause for the fishes' faster growth under this treatment.

Total carotenoid gains in fish tissue: In this study, marigold, china rose, and carrot were chosen as natural carotenoid sources to improve pigmentation in blue gourami fish. T_2 showed higher carotenoid gain, while T_0 showed the lowest mean carotenoid gain (Figs. 1 and 2). Swian *et al.* (2014) found that feeding marigold petal as a carotenoid stimulate with feed has effective influences on the koi carp growth and pigmentation. Liang *et al.* (2012) discovered that when fish were fed a diet supplemented with carotenoids with a dose of 180 mg/kg Marigold oleoresin, there was a significantly higher deposition of carotenoids in the fish body compared to the other sources of dietary carotenoid. According to Matuno *et al.* (1981), the marigold meal is a valuable source of carotenoids. It has significant influences on the body of ornamental fishes, which also agrees with the present study findings. The study suggested that using natural plant pigments like marigold petals might improve the body coloration of ornamental fishes.

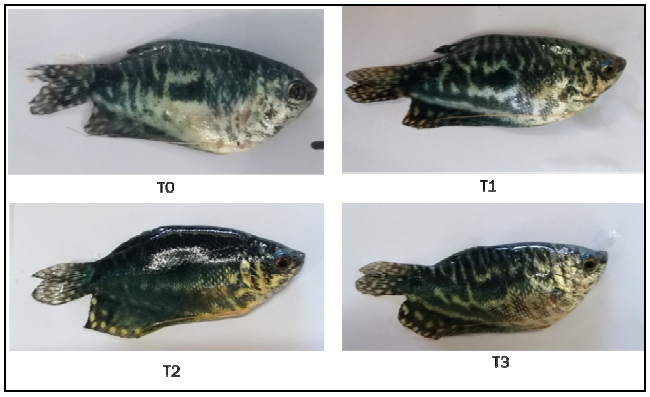

Fig. 2. Effects of natural carotenoid on the body coloration of Blue Gourami.

Survival rate and water quality parameters: This study found the highest survival rate in T_2 treatment (100%), and the lowest survival rate was found in T_1 treatment (75%). Natural carotenoids did not affect water quality in this study. All the water quality parameters were within the suitable ranges. Natural carotenoid sources such as paprika in the diet do not influence water quality or survival in dwarf cichlids (Padowicz and Harpaz 2007) which agreed with the present study. Still, Arulvasu *et al.* (2013) reported natural carotenoid source have a negative influence on fish survival.

Based on the study's findings, it can be concluded that color enhancer feeds may be manufactured at a lower cost utilizing the readily accessible source 'marigold petal' to enhance the body coloration of Blue Gourami fish.

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(Manuscript Received: 22 November 2021)