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Effect of Natural Pigment Sources on Colouration of Cichlid (*Cichlasoma severum* sp. Heckel, 1840)

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Abstract: This study, examined influence of carrot (*Daucus carota*) and red pepper (*Capsicum annuum*) as natural pigment material on colouration of cichlid (*Cichlasoma severum* sp. Heckel, 1840). Mean weight of 120 fish were 1.16±0.22 g and they were divided into 3 groups with duplication each. Three types of experimental feed were prepared; the first group was added carrot, second one red pepper to provide 50 mg of total pigments kg^{-1} in diets and control feed do not have any pigment material. Fish were fed by 1% of their live weight and trial lasted for 50 days. Early in the trial, total carotenoid quantities were measured in carrot and red pepper, fish and their feeds and the end of it only in fish. Carotenoid amount in the fish samples by fed with red pepper and carrot diets were 5.25±0.90 and 5.60±0.29 mg g^{-1}, respectively. Consequently a significant difference was found between individuals fed by natural pigment material and those by unpigmented feeds ($p \leq 0.05$). It was demonstrated that natural pigment substances have an impact on coloration of cichlid and the groups did not exhibit any distinctions in feed conversion and growth rates.

Key words: Aquarium fishes, *Cichlisoma* sp., natural pigment, total carotenoid, skin pigmentation, fish feed

INTRODUCTION

The red colouration of salmonids, crustaceans and some aquarium fish have become of interest in the cultivation. Dietary carotenoids play significant part in the regulation of skin and muscle color in fish (Selong, 2005; Chatzifotis *et al*., 2005). Astaxanthin is the main carotenoid pigment of red-pink coloured aquatic animals, being widely used in aquacultural processes because it is a standardized and chemically stable product with a high carotenoid concentration (De La Mora *et al*., 2006). Astaxanthin is an expensive product with an average cost of $200 USD per kg for and active agent of 10% is in the products available in the market (Hardy, 2005).

Dietary natural pigments are obtained from fruits, vegetables and flowers (Mangels *et al*., 1993). Therefore, a great many research has been conducted involving employment of natural carotenoid material to pigment aquatic organisms (Kamata *et al*., 1990; Carter *et al*., 1994; Yanar *et al*. 1997).

The studied cichlid fish (*Cichlasoma severum* sp., Heckel, 1840) is one of the most chosen aquarium species. These fish were bright orange in colour, when first imported tend to preserve their original pigments as long as they ingest pigment added feeds. Later generations can hardly display as well pigmentation as their ancestors even when they have been supplied with the same feed. Carrot is a natural beta carotene source and red pepper is of dark red colour due to it's capsantine in it content, being used for flesh pigmentation of salmonids given capsorobin in it (Torrisen *et al*., 1989; Gurocak, 1983; De La Mora *et al*., 2006), both of which are cheaply available considering their high level of carotene.

This study designed for this purpose used carrot and red pepper as natural carotenoid agents. Experimental diets tested whether due to their very features, they could have any positive effects as pigment sources in pigmentation of parrot cichlid fish or not.

MATERIALS AND METHODS

In this research, 120 cichlid fishes (*Cichlasoma severum* sp., Heckel, 1840) were used and their average living weight was 1.16±0.22 g. Their sex was not taken into consideration. In this study, a random design with three treatments and two replicates were utilized. There were 20 fishes in each aquarium, which had dimensions as 40×25×25 cm and working volume 20 L. Two pieces of air pump and one sponge filter were used in the aquariums for filtration and air flow. Artificial illumination system and heater was used. While water temperature was measured

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everyday, the pH values were measured in every 2 days for observing water parameters. Average water temperature and pH value were measured 24.50±0.28°C and 7.8±0.1 as the result of the measurements, respectively.

The experimental diets were formulated to meet the nutritional requirements of cichlid fish and prepared with use laboratory type pellet machine in the fish nutrition and fish feed technology laboratory in Ege University (Turkey). The feed that used for the feeding of cichlid fish includes 41% Crude Protein (CP), 6%, Crude Fat (CF), 2% Crude Cellulose (CC), 9.5% ash. So, only the pigment sources show differences in the feed, which were prepared as 3 groups; carrot and red pepper was added in 1st group and 2nd group, respectively. The feed of 3rd group was separated as the control group and no pigment material was added into it. All diets except the control diet were formulated to include 50 mg g^{-1} of each respective pigment source. The fish were fed daily at 1% of their total biomass, distributed in three rations at 0900 and 1700 h for 50 days. Feed Conversion Ratio (FCR) was calculated according to Rice *et al*. (1994).

At the initial and the end of the experiment, fish were measured and weighed. During the study the specific growth rate whose formulation is given below was used to determine growth rate of the fish (Jensen, 1985):

$$\text{SGR} = \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{Day}} \times 100$$

Total carotenoid content of fish (skin and flesh) was determined at the initial and the end of the experiment spectrophotometrically (Choubert and Storebakken, 1989; Yanar and Tekelioglu, 1999). After 10 mg dry sample passed through the homogenization procedure with the addition of 5 mL acetone (98%, Merck Germany), centrifuge procedure was applied for 10 min at 3500 rpm. After that these samples were read at 475 nm wavelength on the spectrophotometer (JENWAY 6305). In order to determine the quantity of β-carotene, calibration curve was used which was based on the absorbance values of 5 mL acetone solution which had 0.16, 1.63, 2.04, 3.27 and 4.09 mg g^{-1} of β-carotene values alternately.

Assuming a complete of diet, the carotenoid retention rate were calculated by the following equation (De La Mora *et al*., 2006):

$$\text{Retention rate (\%)} = \frac{\text{mg of carotenoid of skin+flesh}}{\text{mg of carotenoid in diet}} \times 100$$

The crude protein, crude fat and ash in diets were determined with methot of AOAC (1990).

In addition colour changes on the fish skins were photographted by a Sony DSC-W80 model camera.

Statistical analysis consisted of one-way ANOVA, using the probability level of 0.05 for rejection of the null hypothesis. After ANOVA, significant differences among means were determined by Tukey's multiple range test. All statistical analysis was performed using SPSS 11.0 for Windows.

RESULTS AND DISCUSSION

All experimental diets were equally accepted by fish. The carotenoid-supplemented diets did not appear to have any effect on chiclid's growth rate. All the three groups are similar in weight increase ($p \geq 0.05$). Variation in growth for the feeding trial is shown Table 1. In this study, no mortality was observed. Some research purpose that red pepper has been used as carotein source in pigmentation of fish (Peterson *et al*., 1966; Gurocak, 1983; Carter *et al*., 1994; Yanar *et al*., 1997; De La Mora *et al*., 2006). And also Yanar and Tekelioğlu (1999) suggest that carrot has been used as a carotein source of diets in aquarium fish. Kop and Durmaz (2008) were studied same fish species as used different pigment sources in diets. Pigment sources were used 50 mg kg^{-1} as astaxanthin β-carotene and *Porphyridium cruentum* (Algae). They determined that synthetic carotenoid agents are expensive and adviced that tries to assay different pigment sources for diet composition. Not only can natural carotenoids sources have a positive impact on growth and development thanks to their very nutrient contents but some other natural agents with various carotenoids not commercially used for pigmentation can be added to livelihood as well as.

Feed conversion ratio among groups ranges from 1:2.30-1:2.27 (Table 1), with negligible differences among them ($p \geq 0.05$). But some authors (Gurocak, 1983; Carter *et al*., 1994; Yanar *et al*., 1997) have suggested that capsanthin can increase the segregation of gastric juice, causing an improvement in nutrient assimilation and increased growth. All the three groups are similar in spesific growth rate ($p \geq 0.05$).

In this study, spectrophotometric analyses in experimental diets were made to determine any changes

Table 1: The growth performance of fish fed experimental diets (mean±SEM, n = 20)

Groups[1]	Initial fish weigth (g)	Final fish weigth (g)	FCR[2]	SGR[3]
			-----------%--------	
Control	1.14±0.212	3.29±0.385	2.27	2±0.1
1st group	1.21±0.234	3.51±0.412	2.15	2±0.2
2nd group	1.19±0.219	3.48±0.398	2.13	2±0.2

[1]Control = no pigment material; 1st group = carrot; 2nd group = red pepper; [2]Feed Conversion Ratio (FCR); [3]Spesific Growth Rate (SGR)

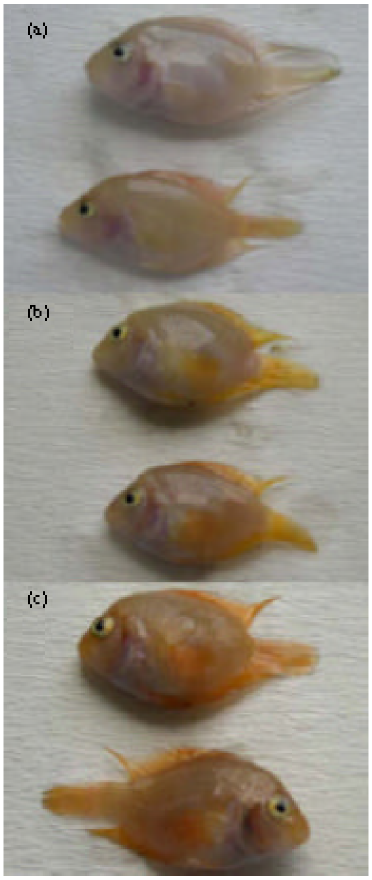

Fig. 1: The photos of the cichlid fish at the end of study. (a) Control = no pigment material, (b) 1st group = carrot and (c) 2nd group = red pepper

likely to emerge in the quantities of carotenoid agents in the feed depending on process variables during pellet feed manufacturing.

The group of fish which were fed by carrot-contained feeds began to have pigmentation of bright orange two weeks after the onset of the study, whereas that fed by red pepper content started to be pigmented from the 3th week on, including some reddening on the abdomen as well. The areas that all the pigment materials provided coloration are nearly same. Firstly, it is observed that it started from dorsal, anal and deep of tail fins and then appeared in the area of abdomen (Fig. 1). Fish fed with the carrot and red pepper diets presented significantly higher ($p \leq 0.05$) color average values compared to the control group at the end of the experiment. The best pigmentation percentage is in red pepper followed by that of carrot. The total carotenoid concentration and carotenoid retention rate of the cichlid fish (skin+flesh) are shown in Table 2.

Carotenoid retention rates for 1st group 2nd group are 10.5 and 11.2%, respectively. Rate of retention of

Table 2: Total carotenoid content and retention rate in the skins + flesh of the cichlid fish at the initial and the end of experimental trial, (mean±SEM, n = 20)

Groups[1]	Initial carotenoid content (mg g^{-1})	Total carotenoid content (mg g^{-1})	Carotenoid retention rate (%)
control	0.270±0.12	0.272±0.87[b]	-
1st group	0.271±0.11	0.416±0.20[a]	0.554
2nd group	0.270±0.09	0.454±0.94[a]	0.605

[1]Control = no pigment material; 1st group = carrot; 2nd group = red pepper; [a,b]Average values in the same row with different superscripts are significantly different at $p \leq 0.05$

dietary carotenoids in fish depends on the efficiency of absorption from the digestive tract, transport capacity, deposition mechanisms in the various tissues, metabolism and rate of excretion. Yanar and Tekelioğlu (1999) and Hata and Hata (1972), reported that after absorbing and oxidizing different forms of carotenoids, goldfish accumulate them in the form of astaxanthin in tissues, especially skins. Similarly parrot cichlid fish was found to be able to accumulate various carotenoids in the form of astaxanthin in that the study established total carotenoid content in their flesh and skin.

CONCLUSION

Consequently, the study concerned found that uses of vegetable carotenoid sources (carrot and red pepper) tend to have many positive effects on the pigmentation of the species, cichlid. For they are cheap and readily available however, it seems necessary to process them into raw material usable (powdering after drying and grinding and extraction for concentration) before adding into feeds so that more positive consequences could be obtained.

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