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The effect of synthetic and natural pigments on the colour of the cichlids (*Cichlasoma severum* sp., Heckel 1840)

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Abstract In this study, we have investigated the effects of *Porphyridium cruentum* (Rodophyta) as a natural pigment source and astaxanthin and β-carotene as synthetic pigment sources on the skin colour of cichlid fish (*Cichlasoma severum* sp., Heckel 1840), which are generally light orange with white patches and becomes shiny orange in the reproductive phase. The fish were fed diets containing 50 mg kg^{-1} astaxanthin and β-carotene, and *P. cruentum* powder. The amount of both natural and synthetic pigment sources given as feed was 50 mg kg^{-1}, and the experiment was continued for 50 days. Total carotenoid content of the fish was determined spectrophotometrically at the end of the experiment. As a result, while a visible change of colour in the skin of the fish fed on the feed containing astaxanthin was observed with 0.34 ± 0.2 mg g^{-1} of pigment accumulation, a relatively small change of colour was observed in the skin of other fish that were fed on the feed containing *P. cruentum* and β-carotene with 0.22 ± 0.2 mg g^{-1} and 0.26 ± 0.1 mg g^{-1} of pigment accumulations, respectively. Therefore, it was determined that these pigment sources have an effect on the colour of cichlid fish.

Keywords Cichlid · Pigments · *Cichlisoma* sp. · *Porphyridium cruentum* · Astaxanthin · β-carotene

Introduction

One of the most attractive features of aquatic creatures is arguably their brilliant display of colours. The source of their colours comes from the foods in their natural environment. The most important problem for the producers of these commercial species and aquaculturists is that most of these species lose their colours in the production process; therefore, consumer demand for them is low. The feed given to these species must provide the necessary ingredients for the species to acquire the desired colours.

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However, some producers use hormones and artificial colorants in order to attract consumers, increase their profit margin, and to make the fish they produce more vivid and shiny. Nevertheless, the colours acquired through such methods are not stable and the fish lose their colour after a while.

The cichlid fish (*Cichlasoma severum* sp., Heckel 1840) that was studied is one of the most preferred species. Although it has only recently come to be known in Turkey, it is widely popular in other countries. The cichlid's skin comes in many very different colour combinations. For example, while male emperor cichlids (*Aulonocara* sp.) have shiny and beautiful colours, females have less vivid colours. Since consumers prefer males, females are transformed into males using hormones so that they become more colourful. It is both possible and more useful to use microalgae or synthetic colorants. Because, in addition to affecting the skin colour of fish, these materials strengthen the immune system of fish and help them grow faster (Tanaka et al. 1976; Tacon 1981). But there is not sufficient information on which material and which dose are optimum for the sole reason that these subjects are not attractive for researchers.

Fish colour is primarily dependent on the presence of chromatophores that contain coloured pigments. There are four main pigment groups that give colour to the skin and tissues of animals and plants, namely melanines, purines, pteridiums and carotenoids. Carotenoids, which dissolve in fat, give the skin the yellow and red colours. They also give the orange and green colours to the egg, skin and flesh of many fish (Fuji 1969). Carotenoids, which are produced primarily by phytoplankton and plants, are divided into two groups as carotens and xantofilles. Although more than 600 carotenoids in nature have been defined, only a few of them are used in animal feeds, pharmaceuticals, cosmetics and food colouring (Bricaud et al. 1998; Ong and Tee 1992).

Microalgae, which are important in the production of larval fish because of their nutritive ingredient, can be used as a natural pigment source in fish feeds. The use of microalgal biomass has been recently investigated with regard to its potential as a colouring agent (Gouveia et al. 1997; Raymundo et al. 2005). But the use of synthetic pigment sources is more common because they are easy to obtain (Sales and Janssens 2003). So, new research on the use of microalgae as fish feed must be done. The unicellular red alga *P. cruentum* is a member of the Rodophyta, order of Porphyridiales. The spherical *P. cruentum* cells lack a cell wall and their diameter ranges between 4 μm and 9 μm. The cells of *P. cruentum* can be either solitary or massed into irregular colonies held in the mucilage liquid (Vonshak 1988). Rodophyta tends to have a simple pigment composition with β-carotene, zeaxanthin and chlorophyll as the predominant thylakoid pigments (Grabowski et al. 2000).

There is no study on the effect of natural and synthetic pigments on the colour of cichlids. Therefore, this study was undertaken to determine the effect of pigment source on the skin colouring in cichlids (*Cichlasoma severum* sp., Heckel 1840) using feeds containing *P. cruentum* as natural pigment source as well as astaxanthin and β-carotene as synthetic colorants.

Materials and methods

In this research, 200 cichlid fishes (*Cichlasoma severum* sp., Heckel 1840), which were produced in aquarium at the Ege University, Faculty of Fisheries, Department of Aquaculture, were used. Their average living body weight was 0.62 ± 0.01 g, and average total length was 2.86 ± 1.12 cm (Table 1). It was found that body of the fish has approximately no colour. Their sex was not taken into consideration.

Table 1 Total carotenoid content in the skins of the cichlid fish

Groups	Total carotenoid content (mg g^{-1})
1st (astaxanthin)*	0.34 ± 0.2
2nd (β-carotene)	0.26 ± 0.1
3rd (*P. cruentum*)	0.22 ± 0.2
4th (control)*	0.06 ± 0.01

*$P < 0.05$

This fish, which is of American (Amazon) origin, has no specific season for reproduction and females ovulate in every 30–45 days. Males always tend to copulation. Their colour changes between white-cream and orange. They have brighter colours in their copulation phase. Their length can be 8–10 cm, their width can be 4–5 cm, and their body is close to the shape of a disc.

The feed which was used in the research has been prepared in the Fish Nutrition and Fish Feed Technology Laboratory at Ege University, Faculty of Fisheries, and Department of Aquaculture. The feed was prepared with an attention to the nutritive needs of cichlid fish. The feed used for the feeding of cichlid fish included 43% crude protein (CP), 6% crude fat (CF), 2% crude cellulose (CC), 9.5% ash. So, only the pigment sources vary in the feed, which was prepared in four groups. While astaxanthin (Sigma A9335) was added in the 1st group, β-caroten (Fluka 22040) was added in the 2nd group, and dried biomass of *P. cruentum* powder in the 3rd group; the 4th group was separated as the control group and no pigment material was added ito it. *P. cruentum* was grown in continuous mode in the plankton unit at Ege University, Faculty of Fisheries and Department of Aquaculture. The total carotenoid amount was determined as 50 mg kg^{-1} in all groups of feed.

In the study, eight aquariums, which had dimensions as 40 × 25 × 25 cm and working volume of 20 l, were used. There were 25 fishes in each aquarium. Two air pumps and one sponge filter were used in the aquariums for filtration and airflow. The aquariums were placed side by side in two lines. No artificial illumination system was used, but aquariums were kept from direct sunlight. Since the experiment was done in summer, no heater was used.

A laboratory-type pellet machine was used in preparing feed. The fish were fed twice in the morning and afternoon ad libitum.

While water temperature was measured everyday, pH values were measured in every 2 days for observing water parameters. The experiment was done twice for each group and lasted for 50 days.

Total carotenoid content of microalgae, synthetic colour materials and change in the colour of fish were determined at the end of the experiment spectrophotometrically (Choubert and Storebakken 1989). After 10 mg of dry sample was passed through homogenisation process with the addition of 5 ml acetone (98%, Merck Germany), centrifuge procedure was applied for 10 min at 3,500 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, we calculated the retention rate by the following equation (Ingle de la Mora et al. 2006); retention rate (%) = (mg of carotenoid of muscle) × (100)/(mg of carotenoid in diet).

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

Water temperature in all aquariums was measured daily, but pH values were measured once every 2 days throughout the experiment. The average water temperature was determined as 26.01 ± 0.6°C, and pH as 7.9 ± 0.09.

The colouration areas in all the pigment materials were nearly the same. First, it was observed that it started from the ends of dorsal, anal and tail fins and then spread to abdomen. The spectrophotometer analysis was made for the colour change in the skin of the fish, which were fed on the feed that included different colorants, and the results are shown in the Table 1. At the beginning of this study, it was found that all fish colouration was 0.05 ± 0.01 mg g^{-1}. In the study, colour changes appeared exactly in the same body parts (abdomen, fins, tail area and ventral lateral) in all the groups. However, it was determined that the fish fed on the feed that included astaxanthin had significantly brighter red colour with 0.34 ± 0.2 mg g^{-1} ($P < 0.05$). It was observed that the abdominal area, tail, dorsal and anal fins of the fish fed on the feed including β-carotene, acquired a colour between pink and red (0.26 ± 0.1 mg g^{-1}). It was also observed that coloration was less in the fish which were fed on the feed including *P. cruentum* (0.22 ± 0.2 mg g^{-1}). On the other hand, the least colouration was found in the control group (0.06 ± 0.01 mg g^{-1}).

At the end of the study, retention rates of the pigments in the muscles of the cichlid which were fed on these diets were recorded at approximately the same levels in the 1st, 2nd and 3rd groups of experiments; 0.67%, 0.52% and 0.44%, respectively. Therefore, it was found that there was a relationship between the amount of carotenoid in the diet and the retention rate of muscle of fish.

Growth parameters of the fish were shown in the Table 2. The maximum final body weight was found in fish of the second that were fed with β-carotene (2.28 ± 0.9 g). However, the maximum total length was detected in the first group (Astaxanthin) of fish (3.49 ± 1.2 cm). In conclusion, there was no statistical difference among all groups in terms of both final body weight and final total length ($P > 0.05$). The main goal of the research was to determine the scale and duration of colour change in the fish with relation to pigment sources. So, nutritional change in the feed that included *P. cruentum* was ignored.

Discussion and conclusion

Carotenoids are known to have a positive role in the intermediary metabolism of fish (Segner et al. 1989). Colouration is controlled by the endocrine and nervous system, but

Table 2 The growth performance of the cichlid fish

Groups	First body weight (g)	Final body weight (g)	First total length (cm)	Final total length (cm)
1st (astaxanthin)	0.63 ± 0.1	2.26 ± 1.1	2.86 ± 0.1	3.49 ± 1.2
2nd (β-carotene)	0.62 ± 0.9	2.28 ± 0.9	2.85 ± 1.1	3.45 ± 1.7
3rd *(P. cruentum)*	0.62 ± 0.3	2.27 ± 0.7	2.85 ± 1.8	3.47 ± 1.1
4th (control)	0.63 ± 0.5	2.25 ± 0.6	2.86 ± 1.2	3.48 ± 3.1

dietary sources of pigment also play a role in determining the colour of fish. The effectiveness of carotenoid source in terms of deposition and pigmentation is species-specific. In addition, all fish species do not possess the same pathways for the metabolism of carotenoids, and therefore, there is no universal transformation of carotenoids in fish tissues (Chatzifotis et al. 2004).

Synthetic pigment materials brought about more accumulation in the tissue according to the results that were obtained regarding colour especially during the working period of astaxanthin and this influence is easily observed visually. The absorption and accumulation of astaxanthin in the fish is higher than the other carotenoids (Torrisen 1989). Besides, β-carotene caused less accumulation as compared to astaxanthin in the same period. It was also observed that fish fed on feed, which included astaxanthin became more colourful than the other groups and their colour was red but β-carotene gave pinkish-red colour. As a result, the 1st group of fish fed on feed including astaxanthin was significantly more colourful than the other groups. In the group that included *P. cruentum* less pigment accumulation occurred than in the other groups. It seems necessary either to increase the amount of *P. cruentum* in the feed or to prolong the feeding time in order to have better results.

According to the results obtained from the experiment, it was observed that the cichlid fish responded to colouration effected by the use either of synthetic or of natural pigment sources. This difference between two synthetic sources of pigment can be ascribed to the difference in quality, ingredients and accumulation period. Astaxanthin was efficiently utilized for deposition and coloration of the skin in red sea bream and Australian snapper (Lorenz 1998; Booth et al. 2004). Also, in gilthead sea bream synthetic astaxanthin and cantaxanthin or pigments from algae were efficiently absorbed (Gomes et al. 2002).

Although these two synthetic pigment sources have no cancerogenic effect and are permitted to be used in many countries, there is a search for alternative colouring materials, because they are expensive and add about an extra 10–15% to the cost of feed. Microalga is one of the most favourite of these alternative materials both because ofits nutritive quality (its protein content ranges from 28% to 39%, the available carbohydrates vary between 40% and 57% and total lipids may reach 9–14 %) and its being a good source of carotenoid (Becker 1994).

P. cruentum that was used in this work is an alga, which contains β-carotene, and the experiment tried to make use of this ingredient. It was observed that this alga, which was given together with the feed, has an important effect on the colour of the skin. However, the ratio of *P. cruentum* in the feed for the optimum coloration is the subject of another work. It is necessary to research the other variables that may be effective on the accumulation of pigments, such as the species of fish, the size of fish, colour types, and the duration of feeding on pigment sources.

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