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The Effect of Synthetic and Natural Pigments on the Color of the Guppy Fish (*Poecilia reticulata*)

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Abstract: In this study, we have investigated the effects of mix of tomato (*Solanum lycopersicum*) &carrot(*Daucus carota*) and red bell pepper (*Capsicum annuum*) as a natural pigment sources and astaxanthin as synthetic pigment source on the skin color of guppy fish (*Poecilia reticulata*), which are generally silver with red patches in the dorsal skin. The fish were fed diets containing 50 mg kg^{-1}astaxanthin and natural pigment. The amount of both natural and synthetic pigment sources is given as feed was50 mg kg^{-1} and the experiment was continued for 60 days. Total carotenoid content of the fish was determined spectrophotometrically at the end of the experiment. As a result, a visible change of color in the skin of the fish fed on the feed containing astaxanthin was observed with 0.38±0.2mg g^{-1} of pigment accumulation while a relatively change of color was observed in the skin of other fish that were fed on the feed containing powder of tomato &carrot and red bell pepper with 0.30±0.2 mg $g^{-1}$of pigment accumulations, respectively. Therefore, it was determined that these pigment sources have an effect on the color of guppy fish.

Key words: *Solanum lycopersicum* · *Daucus carota* · *Capsicum annuum* · Astaxanthin · Carotenoid

INTRODUCTION

One of the most attractive features of aquatic creatures is arguably their brilliant display of colors. The source of their colors comes from the foods in their natural environment. However, some producers use hormones and artificial colorants to attract consumers, increase their profit margin and to make the fish they produce more vivid and shiny. Nevertheless, the colors acquired through such methods are not stable and the fish lose their color after a while [1]. The studied guppy fish (*Poecilia reticulata*) is one of the most chosen aquarium species. The guppy skin comes in many very different color Combinations. Fish skin color is primarily dependent on the presence of chromatophores (melanophores, xanthophores, erythrophores, iridophores, leucophores and cyanophores), containing pigments such as melanins, carotenoids, pteridines and purines [2]. There are four main pigment groups that give colorto the skin andtissues of animals and plants, namelymelanines, purines, pteridiums and carotenoids. Carotenoids, which dissolve in fat, give the skin the yellow and red colors. They also give the orange and green colors to the egg, skin and flesh of many fish [3]. Carotenoids are synthesized from geranyldiphosphate by all photosynthetic organisms [4]. 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 regarding its potential as a coloring agent [5, 6]. However the use of synthetic pigment sources is more common because they are easy to obtain [7]. There is no study on the effect of natural and synthetic pigments on the color of guppy. Therefore, this study was undertaken to determine the effect of pigment source on the skin coloring in guppy.

MATERIALS AND METHODS

In present study, 450 guppy fish (*Poecilia reticulata*) were used. Their average living weight was 0.063±0.06 g and average total length was 1.3±0.3 cm. Their colors are silver with red patches in the skin. Their sex was not considered. At the beginning of the test, the

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fish were randomly divided into three groups of 50 fish (3 treatments in triplicate). Nine aquaria, which had been dimensioning as 40×25×25 cm and working volume of 15L, were used. Fish were fed with 3 diets containing different pigments. These diets contain natural pigment, synthetic pigment and control group that was without pigment. The experimental diets were formulated to meet the nutritional requirements of guppy fish and prepared with use laboratory type pellet machine. The feed used for feeding of guppy fish included 50% crude protein (CP), 4% crude fat (CF), 2% crude cellulose (CC), 9% ash. So, only the pigment sources vary in the feed, which was prepared in three groups. Astaxanthin (Sigma A9335) was added in the 1st group, tomato &carrot and red bell pepper were added in the 2nd group and the 3th 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 kg^{-1} of each respective pigment source. The fish were fed daily 4% of their total biomass, distributed in two rations at 8:30 and 17:00 h for 60 days. Total carotenoid content of microalgae, synthetic color materials and change in the color of fish were determined at the end of the experiment spectrophotometrically [8].Statistical analysis consisted of one-way ANOVA, using the probability level of 0.05. After ANOVA, significant differences between means were determined by Duncan's multiple range test. All statistical analyses were performed using SPSS 16.0 for Windows.

RESULTS AND DISCUSSION

The coloration 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. Spectrophotometeric analysis was made for the color 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 coloration was 0.06±0.01 mg g^{-1}. In the study, color 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 astaxanthin had significantly brighter red color with 0.38±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 natural pigments, acquired a color among pink and red (0.30±0.2 mg g^{-1}). It was also observed that coloration was less in the control group (0.08±0.01mg g^{-1}). Growth parameters of the fish were shown in the Table 2.

Table 1: Total carotenoid content in the skin of the guppy fish

Parameter	1st (astaxanthin)	2nd (natural pigments)	3th (control)
Total carotenoid content (mg g^{-1})	0.38±0.2[a]	0.30±0.2[b]	0.08±0.01[c]

All values are represented as mean ±SD. (n=3). Different superscript letters indicate significant differences between treatments ($P<0.05$).

Table 2: The growth performance of the guppy fish

Parameters	1st (astaxanthin)	2nd (natural pigments)	3th (control)
First body weight (g)	0.063±0.06	0.066±0.08	0.061±0.02
Final body weight (g)	1.2±0.7	1.4±0.9	1.2±0.3
First total length (cm)	1.3±0.3	1.4±0.5	1.3±0.1
Final total length (cm)	2.1±0.8	2.1±1.1	2.2±0.6

All values are represented as mean ±SD. (n=3). All values were not significantly different ($P>0.05$).

The final maximum body weight was found in fish of the second group that were fed with natural pigments (1.4±0.9 g). However, the maximum total length was detected in the third group (control) of fish (2.2±0.6 cm). In conclusion, there was no statistical differences among all groups in terms of both final body weight and final total length ($P>0.05$).

Carotenoids are known to have a positive role in the intermediary metabolism of fish [9, 10]. Coloration is controlled by the endocrine and nervous system, but dietary sources of pigment also play a role in determining the color 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 [2]. Synthetic pigment materials brought about more accumulation in the tissue according to the results that were obtained regarding color 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 [11]. According to the obtained results of this study, Fish fed an astaxanthin supplemented diet were more colorful then those of natural pigments. It seems in order to achieve more favorable results whether herbal materials of contents or duration of nourishment must be increased. Astaxanthin was efficiently used for deposition and coloration of the skin in red sea bream, Australian snapper, cichlid and Oscar [1, 12]. Also, in gilthead sea bream synthetic astaxanthin and cantaxanthin or pigments from algae were efficiently absorbed [13]. Although these two synthetic

pigment sources have no carcinogenic effect and are permitted to be used in many countries, there is a search for alternative coloring materials, because they are expensive and add about an extra 10-15% to the cost of feed [1]. Plant material containing the pigment is one of the most favorite of these alternative materials both effects nutritive quality and it's being a good source of Carotenoid [14]. Mix of tomato, carrot and red bell pepper that was used in this work, contains natural carotene and It was observed that these plants, has an important effect on the color of the skin. However, the ratio of tomato, carrot and red bell pepper in the feed for the optimum coloration is the subject of another work. It is necessary to search about the other variables that may be effective on the accumulation of pigments, such as the species of fish, the size of fish, color types and the duration of feeding on pigment sources.

Also carotenoids could enhance nutrient utilization and may ultimately result in improved growth [15]. In the present study, fish feed a carotenoid source did not differ with control fish in growth. These results are in accordance with another study carried out with cichlid and red porgy [1, 16]. Furthermore, our results are in agreement with Gomes *et al*. [13]. Gomes *et al.* reported that there is no growth or feed efficiency enhancement when gilthead sea bream were fed on different sources of carotenoids for 9 weeks. Also, a study with rainbow trout found no significant differences between fish feed an astaxanthin source did not differ with control fish in growth [17]. Nevertheless, this study with rainbow trout, found that fish fed an astaxanthin supplemented diet from6.5 and 25.5 until 400 g exhibited higher wet weight than fish fed astaxanthin from 120.5 up to400 g; these results suggest supplementing for longer periods are needed to evaluate a possible role on growth.

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