



Effect of Different Live Foods Source (Culex Larvae, Chironomus Larvae and Artemia) on Pigmentation of Electric Yellow (*Labidochromis Caeruleus*)

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Abstract

The orange-red spectrum of the skin of electric yellow (*Labidochromis caeruleus*) is one of the idealistic and necessary schemas sought by aquarists and commercial producers. In this study, the effect of live foods meal on the skin coloration of juvenile electric yellow was examined. 270 fish with an average living body weight was 0.42 ± 0.11 g, and average total length was 3.3 ± 0.35 cm. Their sex was not taken into consideration. The fish were fed twice in the morning and afternoon by 3-5 percent of the biomass for 8 weeks. The six different treatments (three replicates/treatment) used in the experiment were used. Skin color was measured in below the dorsal fin of all fish. Measurements were recorded at the end of the feeding trial using a Konica Minolta Chroma Meter CR400. At the end of the trial, the carotenoid supplemented diets significantly increased the values of redness (a^*), yellowness (b^*), and chroma (C^*), and decreased the values of lightness (L^*) and hue (H^{o}_{ab}) on the tail, body, and head areas ($p < 0.05$). luminosity (L^*) and hue (H^{o}_{ab}) were less in fishes fed the diets with live foods and astaxanthin than in fishes fed the control diet with no pigment sources ($p < 0.05$). yellowness (b^*), and Chroma (C^*) were greater in fish fed the culex and were less in fish fed the control diet ($p < 0.05$). Results show that live foods used in this trial can be used as an alternative natural carotenoid source in electric yellow diets.

Keywords: Culex larvae, Chironomus larvae, Live food, Artemia, Pigment, Electric yellow fish

Introduction

Ornamental fish aquaculture is a growing industry segment. While the market value of aquarium fish is closely related to the size and shape of the fish body and fin, Perhaps the most important factor affecting customer choice is skin color (Yılmaz and Ergün, 2011). The cichlid family, with a total of 1330 species, is the second largest family in the perciformes order (Nelson, 2006). In this family species have a various colors (Sugie et al., 2004). Skin coloration in animals is related to carotenoids, melanines, pteridiums and purines (Kop and Durmaz, 2007). Xanthophores (yellow), melanophores (black and brown), leucophores (whitish), erythrophores (red), and iridophores (metallic) are the pigmentation cells of ornamental fish (Fujii, 2000). In plants, carotenoids are respondent for the colors of

orange, yellow, and red. Since most synthetic carotenoids are expensive, inclusive 10-15% of the total feed cost, natural carotenoids (Buttle et al., 2001) or components such as algae (James et al., 2006, 2009) and live foods are added to fish diets as an alternative. A feeding trial on electric yellow (*Labidochromis caeruleus*) was undertaken to assess the effect of different dietary live food as a natural carotenoid source on pigmentation of its body's color. The electric Yellow is a freshwater perciform fish, a cichlid and called the Electric Yellow, the Blue Streak Hap, Lemon Yellow Lab, the Electric Yellow African or Yellow Prince. Electric Yellow is a bright yellow freshwater cichlid and one of the most commercially valuable ornamental fish species (Ergün et al; 2010).

Natural and synthetic carotenoids are used in fish diets to upraise skin and fillet coloration. Oral administration of red pepper meal, shrimp by-product meal, and astaxanthin in *Oncorhynchus mykiss* (Yanar et al., 1997; Diler et al., 2005), astaxanthin, canthaxanthin, and *Gammarus* spp. in *Carassius auratus* (Yeşilayer et al., 2011), beetroot and marigold in *Schizothorax richardsonii* (Jha et al., 2012) and red pepper meal in (Yılmaz and Ergün, 2011) was successfully used for coloration. Live food organisms include all animal (zooplankton) and plants (phytoplankton) lives grazed upon by financially important fishes (Das et al, 2012). Live foods are able to swim in water column and are constantly accessible to fish and shellfish larvae are likely to stimulate larval feeding response (David, 2003). The success in the hatchery production of fish fingerlings for stocking in the grow-out production system is largely dependent on the availability of appropriate live food for feeding fish larvae, fry and fingerlings (Lim *et al.*, 2003). Chironomids, are a group belong to insects of order *Diptera*, phylum *Arthropoda*, are of benthic fauna living in aquatic ecosystems (Bernard, 2011). They are called blood worm because they have hemoglobin in their body and look red in color (Madlen, 2005). Chironomid larvae is a good source of vitamins, nutrients and iron (Mclarney et al., 1974) and is used in frozen form in aquaculture and as a live food for many fish species, including sturgeons (Sahandy, 2011). Chironomid larvae nutritional value is very high and biochemical analyses showed that their dry weight contains the high digestibility (73.6%) as mentioned by De La Noue and Choubert (1985), 56% protein (De La Noue et al., 1985) and the apparent function in small quantities as a growth promoter in fish and crustacean diets (Tidwell *et al.*, 1997) make chironomid larvae a rich food for many organisms. Larvae and pupa mature in the aquatic habitat and contribute as a good natural live food for larvivorous as well as carnivorous fish (Blaustein 1992, Espinoza et al. 1997, Singaravelu et al. 1997) and other aquatic predators (Marian et al. 1983), called Mosqui. In addition, mosquito larvae are nutrient due to their high protein content (50%) and that they could be used to formulate artificial diet for fish (Degani and Yehuda 1996), at least to fill out to some extent the increasing demand of protein (Habib et al. 1992). The *Culex* mosquito, contrast to *Anopheles* and *Aedes*, usually lays its eggs in polluted water containing decomposed organic matter (Pennak 1978, Blaustein 1992) with high organic loads. *Artemia* normally known as brine shrimp are zooplankton, like copepods and *Daphnia*, which are used as live food for marine finfish and crustacean larval culture and in the aquarium trade. It is an organism closely related to shrimp belonging to the order - Anostraca of the class - Crustacea and phylum - Arthropoda. (Das et al, 2012). Technically speaking the benefit of using *Artemia* is that one starts from an apparently motionless product, namely the dry cysts. These cysts which are in fact immobile embryos are commercially accessible, can be stored for years and only have to be incubated for 24 hr in sea water to produce free-swimming larvae. Besides, brine shrimp are very well accepted as a food source (Bookhout and Castlow. 1970). *Artemia* has high conversion efficiency and high nutritive value. All the life Phases of *Artemia*, i.e. cysts (after decapsulation), nauplii, juveniles, sub-adults are used as feed. Today, in majority of the commercial aqua hatcheries, *Artemia* nauplii is virtually used as a sole diet. Frozen adult *Artemia*, are widely used by fish breeders, aquarists and aqua culturists (Das et al, 2012). Astaxanthin (AX) is a pigment belonging to the family of the xanthophylls, the oxygenated derivatives of carotenoids whose synthesis in plants derives from lycopene. AX is one of the main pigments included in salmonids, crustacean, and other farmed fish feeds. Its principal role is to supply the favorable reddish-orange color in these organisms since they do not have access to natural sources of carotenoids. The use of AX in the aquaculture industry is important from the viewpoint of pigmentation and consumer requisition but also as a fundamental nutritional

ingredient for adequate growth and reproduction (Higuera et al; 2006). Enthusiasts prefer an orange-red color in electric yellow and the price of orange-red adult electric yellow is high. Thus, the aim of this study was to determine the effect of live foods meal on the skin color of electric yellow fry. It is obviously agreed that the production of live food organisms continues to be a very important first step in exacerbation of aquaculture, both horizontally as well as vertically (Das et al, 2012)..

Materials and Methods

Fish and rearing conditions

This experiment was conducted over an 8 weeks period at aquaculture research center of Gorgan University of agricultural sciences and natural resources, Gorgan, Iran. In this research, 270 cichlid fishes, which was purchased of ornamental hatchery and education center, were used. Their average living body weight was 0.42 ± 0.11 g, and average total length was 3.3 ± 0.35 cm. Their sex was not taken into consideration. 15 aquariums, which had dimensions as $70 \cdot 40 \cdot 30$ cm, were used. There were 15 fishes in each aquarium. The aquariums were placed side by side in two lines. 1 heater in each aquariums was used. To supply oxygen to the tanks, air pumps and air stones (5 cm in length) were used. The water exchange rate was 50% of the volume per day. Temperature was maintained at $27.40 \pm 2.50^\circ\text{C}$, with an artificial photoperiod of 12L: 12D.

Feed preparation

The fish were fed twice in the morning and afternoon by 3-5 percent of the biomass for 8 weeks. The six different treatments (three replicates/treatment) used in the experiment were as follows:

Treatment 1: commercial normal diet (negative control)

Treatment 2: Diet supplemented with astaxanthin 150 mg/kg (positive control diet which has been proven as a supplementary pigment source)

Treatment 3: 50% commercial normal diet + 50% *Chironomus*

Treatment 4: 50% commercial normal diet + 50% *Culex*

Treatment 5: 33.33% commercial normal diet + 33.33% *Chironomus* + 33.33% *Culex* (mixture)

Treatment 6: 50% commercial normal diet + 50% *Artemia*

For preparation treatment 2, initially 1g of liquid oil with astaxanthin are well mixed and eked powder of basal diet. The amount of astaxanthin added to ration, was 150 mg/kg.

Frozen package of *Chironomus* was provided of aquarium market. Since it is hard to supply *Culex* larvae from the wild and there aren't in bazaar, it is necessary to provide a place for spawning. To this, 6 tanks were placed at different locations of the University, which each tank contained 20 g of wheat straw and 15 cm water. Since *Culex*es spawn in the mediums rich in organic substance, they spawned in the provided tanks. The tanks were checked every morning and *Culex*'s spawn that were attached to the tanks were transferred to laboratory by the spoon. The eggs in each cocoon were counted using a loupe. Average number of eggs per cocoon was 100-250. For culturing *Culex*'s larvae, cocoons were added to each experimental tanks filled with dechlorinated tap water (26°C). *Culex* eggs usually hatch after 24 to 36 hours. 15 cocoons laid in each tank and soybean meal powder 3 grams in each tank for feeding was considered, after 36 hours feeding began. First hatching of larvae was observed in eighth day, then larvae within the pan harvested with a small spring tour and weight by sensitive digital scale with an accuracy of 0.01 and poured into a sealed disposable containers and hold on in freezer with Grade -20. *Artemia* provided of Gomishan farmed shrimps located in the Golestan province and weighed in sealed plastic containers and packaging was frozen.

Color measurement

Skin color was measured in below the dorsal fin of all fish. Measurements were recorded at the end of the feeding trial using a Konica Minolta Chroma Meter CR400. Recordings were made according to the color system mode of the International Commission on Illumination (CIE): L^* = lightness, where dark = 0 and

white = 100, a^* = red, where positive values = red and negative values = green, and b^* = yellow, where plus values = yellow and negative values = blue. Hue (H_{ab}^0), chroma (C^*), and color difference (ΔE_{ab}^*) were calculated using the following formulae: if $a^* > 0$ then $H_{ab}^0 = \tan^{-1}(b^*/a^*)$ but if $a^* < 0$ then $H_{ab}^0 = 180 + \tan^{-1}(b^*/a^*)$; $C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$; and $\Delta E_{ab}^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Sharma, 2003). While water temperature was measured every day, pH values were measured in every 2 days for observing water parameters.

Statistical Analysis

Statistical analysis consisted of one-way ANOVA, using the possibility level of 0.05 for refusal of the null hypothesis. All statistical analysis was performed using SPSS 19.0 and Excel 2013 software for Windows.

Results

Given the importance of water physicochemical parameters, including dissolved oxygen, temperature, pH and its effect on pigmentation all the time these factors were carefully controlled (Table 1). Results of water quality parameters, no significant difference was found during the breeding period ($p > 0.05$). The six diets were equally accepted by the fish and there was no mortality or disease in any treatment. The result of pigmentation show in table 2. In general, the carotenoid supplemented diets significantly increased the values of redness (a^*), yellowness (b^*), and chroma (C^*), and decreased the values of lightness (L^*) and hue (H_{ab}^0) on the tail, body, and head areas ($p < 0.05$).

Az showed in the fig 1 and table 2, the fish fed with pigmentation source had significantly lower lightness values than fish fed the control diet in their body regions ($P < 0.05$). Red/green tonality (a) of skin from the body varied from -9.84 ± 0.46 (Control) to -4.27 ± 0.99 (*Chironomus*) ($P < 0.05$). The red/green tonality (a) of skin from the body of fish fed diet *Artemia* (-9.55 ± 0.34) was not significantly different compared to control group (-9.84 ± 0.46). The yellow/blue tonality (b) in the body was different in groups ($P < 0.05$). The highest yellow/blue tonality (b) was in the fish fed diet *Culex* and following with treatment mixture, Astaxanthin, *Chironomus* and *Artemia*. Results show that live foods used in this trial can be applied as an alternative natural carotenoid source in diets to ensure good pigmentation.

Discussion

Fish skin color is primarily dependent on chromatophores (melanophores, xanthophores, erythrophores, iridophores, leucophores, cyanophores) containing pigments such as carotenoids (e.g., astaxanthin, canthaxanthin, lutein, zeaxanthin), melanins, purines, pteridines and (Chatzifotis *et al.*, 2005). In general like other animals, fish are disable to biosynthesize carotenoids *de novo*, but they can modify alimentary carotenoids and store them in the skin and other tissues (Nath Jha *et al.*; 2012). Live food organisms contain all the nutrients such as lipids, essential proteins, vitamins, carbohydrates, amino acids, minerals and fatty acids (New, 1998) and since are commonly known as “living capsules of nutrition”. This present study is the first investigation to evaluate selected live foods in diets for electric yellow cichlid and was performed to check the feasibility of including dietary with different live foods in practical diets for electric yellow cichlid. The diet containing the live foods were the most successful in inducing the yellow coloration in the skin of the electric yellow cichlid. In the nutrition trial, supplementation of *Culex* had more beneficial effects on the coloration of electric yellow cichlid. Yılmaz and Ergün (2011) checked the effect of red pepper (*Capsicum annuum*) on pigmentation of yellow electric and they essayed that L^* and H_{ab} decreased as the dietary concentration of the carotenoid increased, that this result is similar to our result in this survey. Also Similar results were obtained when astaxanthin, *Capsicum annuum*, or its oleoresins were supplemented in diets for (Yılmaz and Ergün 2011), rainbow trout (Diler *et al.*, 2005; Kouakou and Choubert, 2006; Yeşilayer and Erdem, 2011) and *Zacco platypus* (Lee *et al.*, 2010), showing that the concentration of carotenoids in fish skin lowers lightness and hue. Sornsupharph *et al.* (2013) surveyed the effects of dried fairy shrimp (*Streptocephalus sirindhornaemeal*) on pigmentation

and carotenoid deposition in flowerhorn cichlid, and their results demonstrate an increase in the flowerhorn cichlid skin pigmentation from alternative carotenoid feeding. Fish fed the fairy shrimp (FS) diet displayed higher ($P \leq 0.05$) chroma and redness values than those fed with a SP diet. The hue value (measure for skin pigmentation) was high when fish were fed with treatments of FS at 20% (FS20) for 30 and 60 days ($P \leq 0.01$). However, fish also showed high hue values when fed for 90 days with treatments of FS at 10% ($P \leq 0.01$). The FS20 treatment gave better results than other treatments in terms of total carotenoid, canthaxanthin, astaxanthin and b-carotene concentration in the skin and musculature. The optimum level of FS in flowerhorn cichlid diets for achieving the highest skin pigmentation was 20%. *Spirulina* meal has been used successfully to increase the skin coloration of red sword tail (James et al. 2006), blue gourami, *Trichogaster trichopterus* (Alagappan et al. 2004) and goldfish, *Carassius auratus* (Gouveia et al. 2003; James et al. 2009). availability of large quantities of live foods organisms such as *Artemia* nauplii and marine rotifer (*Brachionus plicatilis* and *Brachionus rotundiformis*) to meet the different stages of fry production has contributed to the successful fry production of at least 60 marine finfish species and 18 species of crustaceans (Dhert, 1996). Providing appropriate live food at proper time play a major role in achieving maximum growth and survival of the young ones of finfish and shellfish (Das, et al 2012). Supplemented artificial feed can not meet all the elements required for the growth of fish. So, fish and shellfish must be fed with live food (Das, et al 2012). Also in others survey had proved the positive effect of live foods on fishes, as in common carp exposed to *Microcystis* by feeding with boom scum (Li & Chung 2004) and in planktivorous fish by natural ingestion of *M. aeruginosa* (Rai, 2000). Sornsupharp et al (2013) showed dried fairy shrimp (*Streptocephalus sirindhornaemeal*) increase growth parameters of flower horn cichlid. Then it is necessary to research about effect of selected live foods in this trial on growth parameters of electric yellow.

These studies show how well a much cheaper pigmentation affects skin color in fish within a short period. The results clearly show that live foods specially *culex* could be used in electric yellow cichlid diets to replace up to 50% of the dietary fish meal with positive effect on coloration. Indeed, all fish fed with live foods and astaxanthin diets exhibited more coloration values. This preliminary study confirms a basis for more extensive investigations on electric yellow cichlid.

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Table 1: Water physicochemical parameters

Factor	Average	Min.	Max.
Oxygen	7.12±0.329	6.79	7.42
Temperature	27.40 ± 2.50	26.04	28.12
pH	7.9 ± 0.06	6.38	8.27

Table 2. Color parameters (L, a, b) in below of dorsal fin of Electric Yellow fed experimental diets for 8 weeks

	Control	astaxanthin	<i>Chironomus</i>	<i>Culex</i>	mixture	<i>Artemia</i>
L	81.88±0.95 ^a	74.73±0.95 ^b	71.43±0.63 ^d	73.64±1.01 ^b _c	72.41±0.62 ^c _d	73.72±0.99 ^b _c
a	-9.84±0.46 ^d	-5.51±0.92 ^b	-4.27±0.99 ^a	-7.30±0.18 ^c	-5.24±0.24 ^{ab}	-9.55±0.34 ^d
b	35.37±0.98 ^d	52.17±0.64 ^c	51.79±0.57 ^c	60.92±0.48 ^a	54.57±0.64 ^b	46.22±0.59 ^c
H ^o _a	105.57±1.11 _a	96.04±1.07 _d	94.72±3.77 ^b	96.84±0.22 ^c	95.49±0.31 ^c _d	101.7±0.55 ^b
H ^o _b	36.71±0.82 ^c	39.79±0.54 ^c	51.96±0.49 ^c	61.35±0.45 ^a	54.82±0.62 ^b	47.19±0.51 ^d

**Figure1:** Electric yellow fed diets containing T1= Commercial diet without pigment, T2= commercial diet containing astaxanthin, T3= *Chironomus*, T4= *Culex*, T5 = Mixture containing *culex*, *chironomus* and commercial diet, T6= *Artemia*