

The effects of Marigold as natural carotenoids on scale chromatophores' variations in blue gourami under different stocking densities

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Abstract

Skin coloration is important in ornamental fish. Fish coloration is due to the presence of chromatophores, which contain pigments and are usually located in dermis. In this study, the effects Marigold as natural carotenoids was investigated on fish scale chromatophores' variations under different stocking densities. For this, diet containing 2.5% Marigold along with a control diet were fed to the fish held at two stocking densities of 20 and 30 fish (0.6 and 0.9 fish/Liter). After 70 d, changes in chromatophores were monitored in fish scales upper lateral line. The fish initial weight was 0.80 ± 0.02 g. Each treatment had three replications. According to the results, four different chromatophores, including Punctate, Punctate-stellate, Stellate, Stellate-reticulate were observed in the fish at the higher density. The results suggest that increase in stocking density alters chromatophores type and results in hypermelanosis that affects body surface and neural system; thus chromatophores indicate stress contamination.

Keywords: Blue gourami, Marigold, Chromatophores, Density, Skin color

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Introduction

Skin coloration is important in ornamental fish like blue gourami. Color is an important factor determining ornamental fish price in world market. Color of the naturally-colored fish often fades under intensive rearing conditions. Ornamental fish producers are always seeking methods for skin coloration increase. Also, they are looking for natural pigments to replace synthetic pigments, because the synthetic pigments are expensive (Ramamoorthy *et al.*, 2010). Fish coloration is due to the presence of chromatophores, which contain pigments and are usually located in dermis. Chromatophores also present in epidermis, peritoneum, eyes and other organs (Sattari, 2002). Chromatophores are divided into five groups: Punctate, Punctate-stellate, Stellate, Stellate-reticulate and Reticulate (Muralidharan and Pillai, 2012).

There are four main groups of pigments responsible for tissue and skin coloration in animals and plants namely melanin, purine, pyridium and carotenoid. Carotenoids are the main pigment in fish. Carotenoids belong to a family with more than 600 fat-soluble natural pigments. Fish and other animals are not able to synthesize carotenoids, thus they are depended on dietary source. After dietary intake, carotenoids can be transformed to other compounds. Due to some adverse effects of dietary

additives, use of plant pigments has been studied extensively for example red cabbage (Asadi and Allaf Noveirian, 2015), carrot and beetroot (Adhami *et al.*, 2016). Marigold might be important in fish chromatophores increase and consequently color change because it is cheap, easily available, natural and rich in pigments.

Blue gourami (*Trichogaster trichopterus*) is a species found in ponds, lakes, rice farms, channels and rivers in South-East Asia (Rainboth, 1996). Degani *et al.* (1992) stated that this species can be a candidate for aquaculture, because it is consumed in some countries such as Thailand. Thus, change in coloration can play an important role in marketability of this species.

Aquaculture has been developing as a global industry; it includes production of different types of marine and ornamental fish, and mollusks (Meyers, 1994). To meet increasing demands for freshwater fish, fish producers should keep the fish under maximum allowable density to produce high quantity of fish (Olivier and Kaiser, 1997). Optimum stocking density in aquaculture is an important factor in determination of production costs and investment (Metusalach *et al.*, 1999). In addition to production cost, stocking density affects fish survival, growth, welfare and nutrition (Braun *et al.*, 2010). Over-crowding may result in oxidative

stress, and consequently vitamin and mineral depletion; these compounds have important roles in immune system, thus fish go weak and susceptible to diseases (Braun *et al.*, 2010). Van der Salm *et al.* (2004) reported that stocking density has an important role in skin coloration of several fish species. In the present study, the effects of Marigold dietary supplementation was investigated on chromatophores type and number in blue gourami.

Materials and methods

Fish and rearing conditions

Blue gourami larvae were progenies of broodstocks propagated in a local ornamental fish propagation farm (Shahriyari farm, Golestan, Iran). Larvae were transferred to laboratory (Inland Water Aquatic Stocks Research Center, Gorgan, Iran) and were kept in holding aquaria to reach 0.80 ± 0.02 g in weight. Then the fish were stocked in 12 aquaria (60×30×30 cm; 35 L water) at the densities of 20 and 30 fish per aquarium (0.6 and 0.9 fish/Lit). The fish were fed either control, or control diet supplemented with either 2.5% Marigold for ten weeks. Each treatment consisted of three aquaria. Feeding rate was 2.5-3.5% of biomass divided into two meals at morning and evening (Degani *et al.*, 1990).

Diets

To prepare the diets, a commercial feed, specific for ornamental fish (0.5 mm in diameter; Biomar, France), was weighed and then mixed with 2.5% Marigold. Desired volume of water was added to the mixture to form dough (Asadi and Allaf Noveirian, 2015). The dough was transformed to sticks using a meat grinder (1 mm die). The sticks were dried overnight, because carotenoids are susceptible to high temperature (Niazmand, 2015). The control die was prepared in similar way, but without Marigold supplementation. The diets were kept in refrigerator until use. At each meal, required amounts of the diets were weighed and offered to corresponding aquaria. Wastes were siphoned daily and 30-50% new water was added to each aquarium.

The diets proximate composition was determined according to AOAC (1995). Crude protein, lipid and ash were measured using Kjeldahl, Soxhlet, calorimeter bomb and oven. Proximate compositions are presented in Table 1. Total carotenoid was determined using Torrissen and Naevdal (1984).

Water physic-chemical parameters during the experiment

The aquaria were filled with tape water of city Gorgan. Water was aerated (24 h) for dechlorination. An air stone connected to a central air pump was placed in each aquarium.

Table 1: Proximate analysis of blue gourami diet containing 2.5% Marigold.

Ingredients	Crude protein (%)	Fat (%)	Ash (%)	Energy (Cal/g)	Crude Fiber (%)	Total carotenoid (mg/kg)
Control	41.43	8.63	11.35	4598.6	2.09	20
Marigold-diet	40.62	8.32	11.39	4501.76	2.69	40.47

Water temperature was fixed using a heater (100 W) in each aquarium. Average water temperature, dissolved oxygen and pH were 26.50 ± 0.02 , 7.15 ± 0.02 and 8.35 ± 0.02 , respectively.

To determine change in chromatophores of blue gourami at different stocking densities

To determine alteration in chromatophores' count, 18 scales were sampled from each treatment. The samples were taken from upper lateral line and placed in separate pockets. To count chromatophores, the scales were placed into water to remove fatty materials easily. The scales were then dried and monitored under light microscopy connected to an LCD.

Statistical analysis

Variances equality was checked by Levene's test. Data were analyzed using Independent T-test. Significance was determined at confidence limit of 95%. Data are presented as mean \pm SEM. Software packages SPSS20 and Excel were used for analysis.

Results and discussion

Results showed that amount of punctate significantly decreased at M0.6 and M0.9 in comparison with CG0.6 and CG0.9 ($p < 0.05$). Punctate amount

decreased at 42% and 40%, respectively. No significant difference ($p > 0.05$) in the contents of punctate-stellate has been found between M0.6 compared to CG0.6 (Table 2). However, results indicated that punctate-stellate at M0.9 increased at 89% compared to CG0.9. No significant differences ($p > 0.05$) in the contents of stellate have been found between M0.6 compared to CG0.6 (Table 2). However, results indicated that stellate at M0.9 increased 4 times compared to CG0.9. Comparative analysis for stellate-reticulate also showed same trend. Significant difference ($p < 0.05$) in the contents of stellate-reticulate has been found between M0.6 compared to CG0.6 (Table 2). Results indicated that stellate-reticulate at M0.9 significantly increased compared to CG0.9. Stellate-reticulate amounts revealed increasing trend in M0.6 and M0.9 in comparison with their controls. M0.6 and M0.9 enhanced 3 and 20 times compared to CG0.6 and CG0.9, respectively (Table 2).

In the scale chromatophores analysis, four out of five chromatophores comprising Punctate, Punctate-stellate, Stellate and Stellate-reticulate chromatophores were observed in blue gourami fish as shown in Fig. 1.

Table 2: The number of chromatophores of blue gourami fed with 2.5% Marigold powder at different stocking densities.

Treatments	Punctate	Punctate - Stellate	Stellate	Stellate - Reticulate
CG0.6	36.66 ± 12.18 ^A	51.88 ± 11.29	12.55 ± 7.73	6.88 ± 2.69 ^B
M0.6	21.11 ± 5.80 ^B	53.00 ± 10.59	8.66 ± 3.17	21.88 ± 6.04 ^A
CG0.9	52.21 ± 10.79 ^a	33.88 ± 3.55 ^b	7.77 ± 2.88 ^b	1.22 ± 1.10 ^b
M0.9	31.55 ± 8.04 ^b	64.11 ± 10.06 ^a	31.22 ± 9.39 ^a	24.55 ± 6.47 ^a

*Each value is a mean ± S.E of three replicates

CG0.6 = Control blue gourami reared at 0.6 fish/L stocking density,

M0.6 = Blue gourami fed with 2.5% Marigold at 0.6 fish/L stocking density,

CG0.9 = Control blue gourami reared at 0.9 fish/L stocking density,

M0.9 = Blue gourami fed with 2.5% Marigold at 0.9 fish/L stocking density.

Uppercase letters (A and B) show significant difference between CG0.6 and M0.6 (P<0.05).

Lowercase letters (a and b) show significant difference between CG0.9 and M0.9 (P<0.05).

Mean values in columns with different superscripts are significantly different (P<0.05).

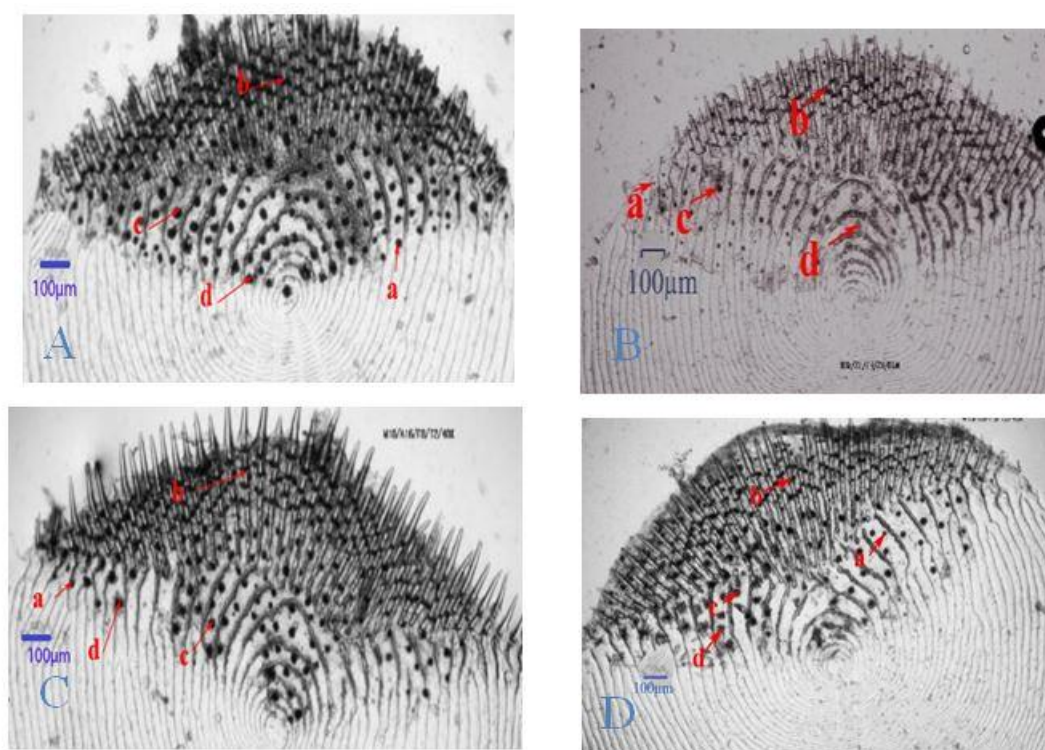


Figure 1: Comparison of chromatophores of in blue gourami fish fed with 2.5% Marigold at different stocking densities (0.6 and 0.9 fish/L) (Scale Bar:100 µm).

A: Scale of control blue gourami reared at 0.6 fish/L stocking density,

B: Scale of blue gourami fed with 2.5% Marigold at 0.6 fish/L stocking density,

C: Scale of control blue gourami reared at 0.9 fish/L stocking density,

D: Scale of blue gourami fed with 2.5% Marigold 0.9 fish/L stocking density.

a: Punctate, **b:** Punctate-stellate, **c:** Stellate, **d:** Stellate- reticulate

Fish exhibit two types of skin color change in nature; the first is physiological color change (e.g. camouflage), which the fish adapt their skin color to ambient patterns,

and the second is permanent skin color change as a result of development and differentiation of chromatophores. The first color change is physiological consisting of

reversible movement of pigments within chromatophores, which skin color is rapidly adapted with ambient media along with time progression (Ellis *et al.*, 1997). But the second is morphological color change which is irreversible and resulted from increase in pigment quantity and chromatophores' development.

There are limited studies on the effects of carotenoids and stocking density on chromatophores' deposition and density in ornamental fish especially blue gourami. In the present study, 4 types of chromatophores (Punctate, Punctate-stellate, Stellate and Stellate-reticulate) were observed in blue gourami in different stocking densities and diet. However, Morioka *et al.* (2012) observed two types of chromatophores (Punctate-stellate and Stellate) under normal conditions in blue gourami. Kang *et al.* (2011) reported that stocking density is an environmental factor which may affect hypermelanosis, pigment and epigenetic mechanisms. The present results are in line with Kang *et al.* (2011) which reported that increased stocking density induced hypermelanosis in olive flounder. Takahashi *et al.* (1994) reported that abnormal pigments increase under increased stocking density. Kim *et al.* (2008) found that ambicolored flounders exhibit higher stress levels than fish with normal coloring.

Results of the present study suggest that high stocking density induce alteration in chromatophores' type capable to affect fish body surface and neural system; thus the chromatophores indicate stress. Also, the results suggest low stocking density along with natural carotenoid supplementation should be used to improve blue gourami color and marketability.

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