Short Communication

Effect of Anthocyanin's Extract from Flour of Roselle Calyx (*Hibiscus sabdariffa*) on Growth and Pigmentation of Goldfish (*Carassius auratus*)

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Abstract

Goldfish (*Carassius auratus*) is an ornamental fish. The aim of this work was to evaluate the effect of diet supplemented with anthocyanin from roselle (*Hibiscus sabdariffa*) on survival, growth, and chromatophores identification in skin of goldfish (*C. auratus*). Three experimental diets were prepared from commercial feed for tilapia added with 40, 80 or 160 mg of pigment from roselle/kg. Survival and biometric parameters such a growth, weight gain, feed intake, and feed conversion ratio were evaluated. Histological analyses from some tissues were made. Survival values and biometric parameters were higher in goldfish fed added with roselle in comparison to control diets (96 and 90%, respectively). Melanophores were identified in tissues of all organisms and it was related to anthocyanin concentration. These results showed that roselle flour could be used as alternative source of natural pigments that could provide color to goldfish.

Keywords: anthocyanin, goldfish, *Hibiscus sabdariffa*, roselle

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บทคัดย่อ

ผลของสารสกัด Anthocyanin จากกลีบเลี้ยงกระเจี๊ยบ (*Hibiscus sabdariffa*) ต่อการเจริญเติบโตและสีของปลาทอง (*Carassius auratus*)

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ปลาทอง (*Carassius auratus*) เป็นปลาสวยงาม จุดมุ่งหมายของงานวิจัย คือ ประเมินผลของการเสริม anthocyanin จากกระเจี๊ยบ (*Hibiscus sabdariffa*) ในอาหารต่ออัตราการตาย การเจริญเติบโต และชนิดของ chromatophores ในผิวหนังปลาทอง (*C. auratus*) อาหารทดลองเตรียมจากอาหารสำเร็จรูปสำหรับปลา tilapia จำนวน 3 สูตร ที่มีเม็ดสีจากกระเจี๊ยบปริมาณ 40 80 หรือ 160 มก./กก. ทำการประเมินอัตราการตาย และดัชนีชีวภาพ เช่น การเจริญเติบโต น้ำหนัก ปริมาณอาหารที่กินและอัตราแลกเนื้อ และวิเคราะห์ทางจุลพยาธิวิทยาในเนื้อเยื่อบางส่วน ผลการทดลองพบว่าอัตราการตาย และดัชนีชีวภาพ ในปลาทองที่เลี้ยงด้วยกระเจี๊ยบสูงกว่าอาหารควบคุม (96 และ 90% ตามลำดับ) melanophores ในเนื้อเยื่อสัมพันธ์กับความเข้มข้นของ anthocyanins ผลการทดลองแสดงให้เห็นว่าสารสกัดกระเจี๊ยบสามารถใช้เป็นแหล่งทางเลือกของสีธรรมชาติที่สามารถให้สีปลาทอง

คำสำคัญ: anthocyanin ปลาทอง *Hibiscus sabdariffa* กระเจี๊ยบ

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Introduction

The ornamental fish culture production is an important activity (Livengood and Chapman, 2007). *Carassius auratus* is a traditional ornamental goldfish which the body and fin shape, size, and color are features that affect its market price (Paripatanamont et al., 1999; Gouveia and Rema, 2005). Several products from algae *Arthrospira maxima* (Gouveia et al., 2003), *Chlorella vulgaris* (Gouveia and Rema, 2005), *Haemotococcus pluvialis*, yeast *Xanthophyllomyces dendrorhous* (Xu et al., 2006) and plants *Medicago sativa* (Yanar et al., 2008) are sources of pigments as carotenoids which generates a positive effect on skin pigmentation.

Skin color determination in goldfish involves cellular factors like existence of cells called chromatophores which store specific pigment black or brown (melanophores), yellow or orange (xanthophores), red (erytrophores), iridescent, blue, silver or gold (iridophores) and white (leucophores) (Caliheuque, 2010). However, pigmentation efficiency is associated with different factors such as pigment source, fish size, feeding time, diet composition, sexual maturity and genetics of organisms (Paripatanamont et al., 1999; Xu et al., 2006).

There are pigments such as anthocyanins which may be used as an alternative for goldfish pigmentation. Anthocyanins are molecules responsible of red, purple and blue color of specific plant tissues (Wrolstad et al., 2005). The roselle calyx (*H. sabdariffa*) accumulates anthocyanin that provide their characteristic red color (Prior and Wu, 2006). Those pigments have been used in juices, sauces, jellies, jams, beverages and as additive in food (Hirunpanich et al., 2005).

The aim of this study was to evaluate the effect of diet supplemented with anthocyanin from flour of roselle on survival, some biometric parameters and chromatophores distribution on *C. auratus*, as alternative to improve its market value.

Materials and Methods

Source and analyses of roselle calyx: Dried roselle calyx (*H. sabdariffa*, line R5-4N) were supplied by Colegio Superior Agropecuario del Estado de Guerrero. Calyxes were ground in a commercial food processor (Kintchen Aid, Canada).

Extraction and quantification of anthocyanin from roselle: Anthocyanin extracts were obtained according to Zhao et al. (2008), 8 g of flour was mixed in 100 ml of acidified ethanol solution [0.1 M HCl (v/v)]. This blended was stirred for 24 hours at 200 rpm in dark

condition at room temperature, and was finally filtered through Whatman 42 filter paper. Anthocyanin concentration was quantified by spectrophotometry at 510-550 nm (Yung and Howard, 2003).

Diet preparation: Commercial tilapia diet (crude protein 45%, ether-extract 10%, crude fiber 5%) (Agribrands, Purina México Inc) was used as basal diet and was supplemented with 40, 80 or 160 mg of anthocyanin from calyx/kg diet. Extruded pellets (1.4 mm diameter) were obtained and dried at 50^0 C for 24 hours and stored in containers at 4^0 C until used (Craig and Helfrich, 2002).

Experimental design: Fish were obtained from a commercial farm, in Zacatepec, Morelos, México and were cultured for 8 weeks under laboratory conditions at 25 ± 2^0 C of water temperature, 6-7 ppm of dissolved oxygen, 7.3-7.5 of pH and maintained under controlled photoperiod (12 hour light/12 hour dark). During two weeks fish were fed *ad libitum* with basal diet. Fish (6.3–7.4 g of body weight) were randomly distributed in 10-l tanks; experiment was performed with four treatments and three repetitions (10 org per repetition). Fish were fed on the experimental diets with 6% body weight ratio to satiation three times a day for 8-weeks, food quantity was adjusted every week (Kalinowski et al., 2005, Yanar et al., 2008).

Growth performance parameters evaluated along bioassay included: initial and final body weight, growth rate [(final weight (g)-initial weight (g)/ initial weight (g))*100], specific growth rate [(Ln final weight-Ln initial weight/no days)*100], feed intake [feed intake (g) per fish for the 8-wk period], weight gain, feed conversion rate [feed intake (g)/weight gain (g)] and survival rate [(final live fish/initial live fish)*100] (Gouveia et al., 2003; Kalinowski et al., 2005).

Histological analysis: Once the feeding period ended, three goldfish from each tank were sacrificed and segmented in three portions (head, dorsal and caudal fin, Fig 1). Fixed samples were processed for routine histology, embedded in paraffin according to standard procedures, sectioned at 10 µm, stained with hematoxylin and eosin (H&E) (Luna, 1968, Paripatanamont et al., 1999) and observed through light microscope (NIKON Eclipse, 80i, Japan).

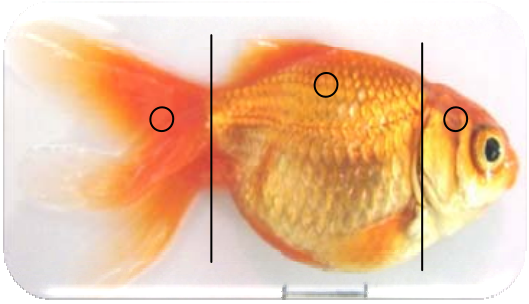

Figure 1 Show cross-sectioned sample of fish (head, dorsal and caudal fin). Analysis were performed on samples taken from the area indicated by the circles.

Chromatophore counting: Dermal tissues were identified and 30 micrographs were taken from each section, three were randomly selected. Quantification was made to three different fields by treatment and repetition.

Data analysis: Data were tested by analysis of variance (ANOVA) with one-way comparison of the means using Tukey with significant level at $p<0.05$ using Sigma plot version 11 software.

Results and Discussion

Anthocyanin concentration of roselle calyx was 3.55±0.35 mg/g, which was higher compared to cornus chilensis (*Aristotelia chilensis*, 2.12±0.6 mg/g) and Chinese purple corn (*Zea mays*, 3.05±0.16 mg/g) (Zhao et al., 2008). An interesting characteristic of roselle calyx is the presence of polyphenols, ascorbic acid (Escribano-Bailón et al., 2006) and red pigments, which show antioxidant capacity (Sáyago-Ayerdi et al., 2007). These components could participate on fish metabolism helping to improve health and growth.

In this work all organisms grew normally, showed high activity and consumed their feed in 5 to 8 min. Specific signs of disease were not detected. Specific growth rate of the *C. auratus* fed on experimental diets were significantly higher ($p<0.05$) than those fed on control diet (Table 1). The highest anthocyanins dose on diet, 160 mg anthocyanin from roselle calyx/kg diet, increased the growth rate, specific growth rate, weight gain, and feed conversion rate of *C. auratus* (Table 1). *Medicago sativa* pigments added to diet for *C. auratus* had an adverse effect on growth rate and weight gain of these organisms. This was attributed to presence of anti-nutrient compounds (Yanar et al., 2008).

Table 1 Effect of diets with anthocyanin's extract from flour of roselle calyx (*Hibiscus sabdariffa*) in nutritional parameters (mean ± SD) in *Carassius auratus*.

	Concentration of anthocyanin (mg/kg food)			
	0	**40**	**80**	**160**
Initial weight (g)	7.43±0.17	7.36±0.23	6.33±0.42	7.43±0.23
Final weight (g)	16.73±0.53	19.66±0.61	17.41±2.03	19.58±2.07
Survival (%)	90.00 ± 0.0^b	96.6 ± 5.10^a	96.6 ± 5.10^a	96.6 ± 5.10^a
Feed conversion ratio	3.70 ± 0.11^b	3.34 ± 0.03^a	3.04 ± 0.20^a	3.35 ± 0.035^a
Feed intake (g)	42.08 ± 2.14^{ab}	47.11 ± 1.64^a	40.70 ± 3.12^b	44.84 ± 2.55^a
Growth (%)	124.63 ± 5.01^b	167.15 ± 12.49^a	163.16 ± 15.91^a	176.26 ± 21.92^a
Specific growth rate	1.26 ± 0.25^b	1.53 ± 0.72^a	1.50 ± 0.12^a	1.56 ± 0.13^a
Weight gain (g)	11.70 ± 0.26^b	14.08 ± 1.25^a	11.69 ± 1.25^b	13.38 ± 0.65^a

Different letter indicates the groups with significant differences ($p<0.05$).

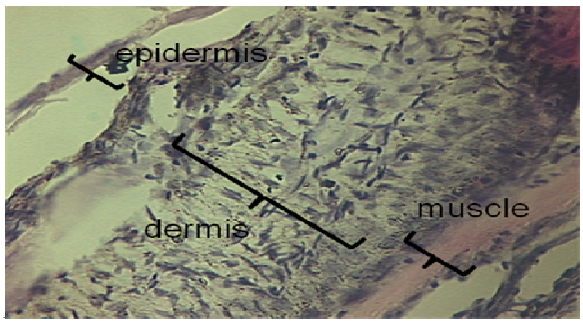

Figure 2 Skin section of goldfish show general structure of the skin, magnification 20X. The chromatophore cells are between the epidermis layer and the muscle layer.

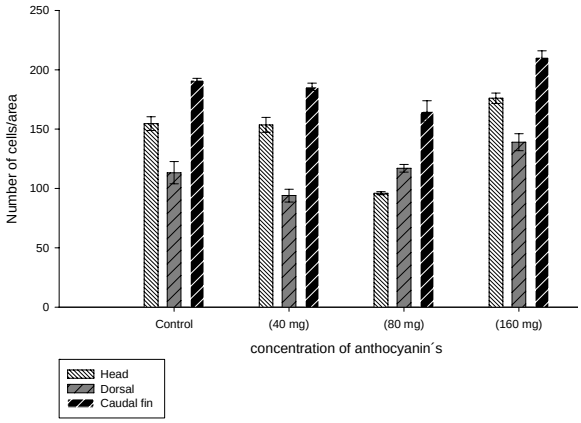

Figure 3 Number of chromatophores of tissue samples (head, dorsal and caudal fin) feeding with diet with anthocyanin's extract from flour of roselle calyx (*H. sabdariffa*) in *C. curatus*. The micrographs are three fields (mean±SD).

Chromatophores were localized in dermal tissues (Fig 2). They were quantified at the end experiment. Chromatophores quantity was higher (p<0.05) with 160 mg of flour roselle/kg compared to all other experimental diets (Fig 3). Fish were stimulated to produce more chormatophores (p<0.05) when fed on diet containing 160 mg. Number of chromatophore cells is highly related to fish color. Paripatanont et al. (1999) reported that diets containing carotenoids produced 6 cells/field. In this study the production of cells was higher probably because of high anthocyanin content from roselle.

Figure 4 shows three sections of fish from control and experimental feeding using 160 mg anthocyanin from roselle/kg. Histological analysis revealed abundant chromatophores which were identified like melanophores (Fig 4). The histological analysis revealed that the distribution and quantity of chromatophores on fish tissues were not found to be homogeneous and depended on body site. The highest concentration was observed in caudal fin.

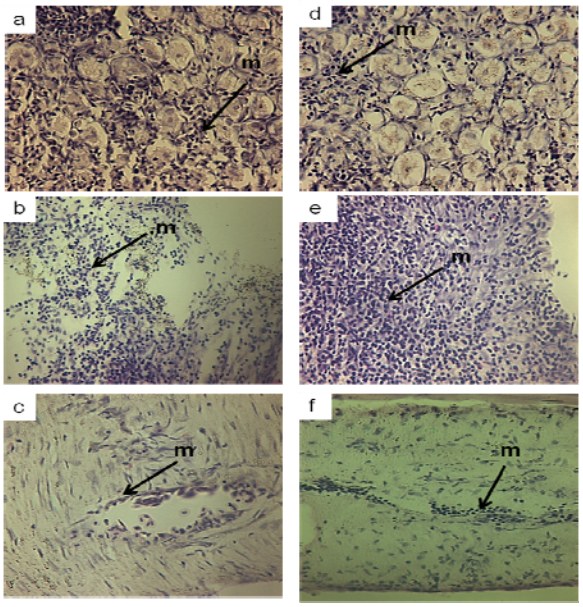

Figure 4 Histological section (H&E) of *C. auratus* feeding with control and experimental (160 mg/kg) diets with anthocyanin's extract of roselle calyx (*H. sabdariffa*). Control and experimental goldfish with melanophore (arrows) in dorsal (a, d), caudal fin (b, e), and head (c, f) section, respectively. Magnification 20x.

None of scientific reports about the use of pigments from roselle on *C. auratus* have been described before. However, *C. lalia* has been used as betalains and anthocyanin source (Baron et al., 2008). In this study, the histological analysis displayed an aggregation of chromatophores cells with high level in caudal fin on *C. auratus*. These pigment cells could be induced by pigment source supplied to diet (Paripatanamont et al., 1999; Gouveia et al., 2003).

According to cell morphology on histological observations, the highest quantity of chromatophores detected were melanophores (Fig 4) which are chromatophores that accumulate black or brown color (Hirata et al., 2003; Kaleta, 2009). Melanophores were observed as aggregated in the cytoplasm which is elliptical in shape and electron dense. They were located in caudal fin, head and dorsal area (Fig 4). A higher number of chromatophores cell were detected in caudal fin in comparison to other analyzed tissues.

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