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Effect of Supplementation of Beetroot Waste Extract on the Pigmentation of *Puntius conchonius* (Hamilton, 1822)

Asma Ahad Naik, Tasaduq H. Shah*, Oyas Ahmad Asimi, Farooz A. Bhat, M. H. Balkhi, Adnan Abubakr and Sobiya Gul
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Rangil, Ganderbal, Jammu and Kashmir - 190 006, India

Abstract

Attractive colouration is one of the most important quality attributes of ornamental fish for consumer preference. The present study was conducted to investigate the effect of natural source of carotenoid pigments from beetroot (*Beta vulgaris*) waste on total carotenoid of rosy barb (*Puntius conchonius*). A total of sixty fishes of average body weight (5.5±0.10 g) were selected randomly and distributed into four treatments in triplicate (5 fishes per tank). Four isonitrogenous (32.37±0.48% crude protein) diets, T1, T2 and T3 were prepared with 0.5%, 1.0% and 1.5% of beetroot waste extract respectively along with a control diet without supplementation of beetroot waste extract. Body carotenoid was significantly enhanced ($p<0.05$) by the dietary supplements, increasing linearly with increase in beetroot waste extract (BRWE) concentration. Carotenoid concentration in the muscle was recorded at 10.88+0.08 $\mu g\ g^{-1}$ wet wt tissue for T3 treatment, 9.81+0.2208 $\mu g\ g^{-1}$ wet wt tissue for T2 and 7.38+0.15108 $\mu g\ g^{-1}$ wet wt tissue for T1 as against 4.62+0.0808 $\mu g\ g^{-1}$ wet wt tissue for the control. Changes in chromatophore number in the scales were observed for different concentration of BRWE. The pigmentation was found to be concentration dependent and darkening of scales appeared more with increased number of pigment granule. No effect of BRWE was observed on the quality of water ($p>0.05$) and all water quality parameters were found to be within acceptable ranges in all the treatments Present results indicate that economical and naturally available carotenoid source such as beetroot waste extract can be incorporated into the diet of rosy barb (*P. conchonius*) to enhance pigmentation in order to improve the ornamental value of the fish which otherwise, have little commercial value.

Keywords: Beetroot waste extract, *Beta vulgaris, Puntius conchonius,* pigmentation, carotenoids, chromatophores

Introduction

The success in the ornamental fish trade is very much dependent on the attractive colour of fish and it determines price of aquarium fish in the world market. The intensive culture of ornamental fishes under captivity for long periods results in faded or degraded colouration (Saxena, 1994). There is huge demand of ornamental fishes in foreign countries, hence ornamental fish growers are constantly exploring methods of enhancing skin colouration. Some producers use artificial colorants and hormones to increase their profit margin by attracting customers. However, the colour acquired through such methods is not stable and the fish loses its colour after some time. Pigments responsible for providing colour to fishes include carotenoids, melanins, petridines and purines. Carotenoids being the most dominant pigments are present in the muscles of fishes (Simpson et al., 1981). However, fishes like other animals are not able to perform denovo synthesis of carotenoids (Goodwin, 1984). Thus, they have to obtain them from dietary sources (Storebakken & No, 1992). Different plant and animal sources of carotenoids as well as pure carotenoid pigments have been included in the fish diet in order to achieve consumer acceptance.

Beet root (*Beta vulgaris*) belonging to family Chenopodiaceae is a small sized plant locally

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**E-mail: tasaduqs@gmail.com*

known as *chogander monji* in Kashmir. It contains high concentration of betalains responsible for its intense red colour. The peel carries the main portion of betalains with up to 54%, their content being lower in crown (32%) and flesh (14%) (Kujala et al., 2001).

Puntius conchonius (Hamilton, 1822), commonly called as rosy barb is a subtropical freshwater fish belonging to family Cyprinidae. It is of less economic importance and is mostly used in aquarium. Its potential as an ornamental fish has been reported earlier and just recently it has also made its entry in both domestic as well as international ornamental fish markets of India (Gupta & Banerjee, 2014). Thus, the main utility of this study was to increase the market value of *P. conchonius* by enhancing its pigmentation using beetroot waste extract (BRWE) as a natural source of carotenoids.

Materials and Methods

The experiment was conducted over a period of 60 days at Fisheries Instructional Farm I of Faculty of Fisheries SKUAST-Kashmir at Shuhama, Ganderbal, Kashmir (J&K).

P. conchonius (rosy barb) of uniform size group were collected from Dal lake and were acclimatized to laboratory conditions for one week before the start of experiment. The experiment was carried out in 12 plastic tubs of 25 l capacity each. Five *P. conchonius* fishes were stocked in each plastic tub with three replicates for three experimental diets and a control diet. The experimental groups were fed with their respective diet @ 3% of their body weight daily twice a day. Faecal matter and left-over feed was removed by siphoning and 10% water was exchanged weekly in each tub with fresh well water.

Raw beetroot purchased from the local market, was peeled off and the peel was dried in oven and powdered with the help of grinder. One kg of powdered plant was extracted by hot extraction with 3 l of 99% methanol in Soxhlet apparatus for 72 h. The crude extract thus obtained was filtered and concentrated using rotary evaporator at 40-50°C (Tripathy & Pradhan, 2013).

The various ingredients used for the preparation of basal diet (control diet) are given in Table 1. The ingredients were thoroughly mixed and required amount of distilled water was added. After mixing thoroughly with water, these ingredients were steamed for 5 min and were put into hand pelletizer to make pelleted feed. This feed was then sun dried and stored in air tight containers for further use.

Table 1. Percentage of ingredients in basal diet

S. No.	Ingredients	Percentage (%)
1.	Fish meal	25.81
2.	Mustard oil cake	25.81
3.	Wheat bran	20.19
4.	Rice bran	20.19
5.	Vitamin + Mineral mixture	2.00
6.	Vegetable oil	6.00

The experimental diets were prepared in three treatments T1, T2 and T3 by adding 0.5 g, 1.0 g and 1.5 g of BRWE per 100 g of basal feed as given in Table 2.

Table 2. Percentage of ingredients in three experimental diets

Ingredients (%)	T1	T2	T3
Fish meal	25.81	25.81	25.81
Mustard oil cake	25.81	25.81	25.81
Wheat bran	20.19	20.19	20.19
Rice bran	19.69	19.19	18.69
Vitamin + Mineral mixture	2.00	2.00	2.00
Vegetable oil	6.00	6.00	6.00
Beetroot waste extract	0.5	1.0	1.5

At the end of experiment, scales of the fishes fed on control diet (T_0) and fishes fed experimental diets (T1, T2 and T3) were taken and observed under microscope (Olympus CX21), using Mag Vision software.

The carotenoid content of fish skin was measured according to the method of Torrissen & Naevdal (1984). Three fish were randomly sampled from each diet treatment and used for carotenoid analyses, which were carried out in triplicate. Sample of 100-200 mg skin were collected from dorsal regions and caudal fin of the fish and then transferred to 10 mL pre-weighed glass tubes. After the samples were ground in acetone containing anhydrous sodium sulphate, the extractions were made up to 10 mL with acetone. The samples were stored for 3 days at 4°C in a refrigerator and then extracted three or four times until no more colour could be obtained. The solutions were centrifuged at 5000 rpm for 5 min and then absorptions were measured in a spectrophotometer (Shimadzu, UV 16UA) at 500 nm. The total carotenoid content was calculated as microgram per wet weight of tissue as follows:

Total carotenoid content (µg g^{-1}) =

$$\frac{\text{Absorption at maximum wave length} \times 10}{0.25 \times \text{sample weight}}$$

Where, 10 = dilution factor and 0.25 = extinction coefficient.

Water quality parameters namely water temperature, pH, dissolved oxygen, Free carbon dioxide, total alkalinity and Ammoniacal nitrogen were analysed weekly by standard methods as described by Adoni (1985) and APHA (2012). Water sampling was done at 100 h and the samples were carried to the laboratory for chemical analysis. However, parameters like water temperature and pH were recorded on the site using digital thermometer and pH meter respectively, while the Winkler's modified method was employed for determination of oxygen.

The data collected were analysed using appropriate statistical tools with the help of statistical software SPSS version 20.0.

Results and Discussion

The scales of rosy barb (*P. conchonius*) fed with control diet showed no darkening other than normal, either on the centre or scale ridges (Plate 1) whereas the scales taken from fishes fed with 0.5 g 100 g^{-1} of beetroot waste extract (T1) showed a marked darkening of the scales (Plate 2).

On feeding higher concentration of BRWE (T2) @ 1 mg 100 g^{-1}, the darkening of scales appeared more with increased number of pigment granules (Plate 3). While fish fed on T3 diet @ 1.5 mg g^{-1} in the diet showed further darkening of scales and increased number of pigments/chromatophores (Plate 4). At higher magnification the aggregation of chromatophores is very well seen.

The total carotenoid content (µg g^{-1} of tissue) in the muscle of *P. conchonius* fed with different experimental diets is given in Table 3. The maximum carotenoid content was found in those fishes which were fed with T3 diet containing 1.5 g of BRWE per 100 g basal feed. The muscle pigmentation was found to increase linearly with increase in the concentration of BRWE.

In the present experiment, the values of water quality parameters for the different treatments were found to be not significantly different to each other at 5% level of significance (Table 4). All the physico-chemical parameters of water during the study period were found to be congenial to the fish. The values for water temperature, pH, dissolved oxygen, free carbon-dioxide, total alkalinity and ammonical nitrogen were found to be in the range of 10.2-10.6°C, 7.1-8.01, 8.2-12.6 mg l^{-1}, 2-3 mg l^{-1}, 230-284 mg l^{-1}, 256-298 µg l^{-1} respectively.

One of the essential prerequisites for ornamental fishes in order to fetch higher price in the commercial market is their wide spectrum of colours. Carotenoids are responsible for skin colour in ornamental fishes but fishes cannot synthesis the carotenoids *denovo*, but they can modify alimentary carotenoids and store them in the integument and other tissues (Jha et al., 2012). Since synthetic carotenoids are known to have deteriorating effects on the environment, there is great demand for inclusion of natural carotenoids in feed to achieve bright colouration in fish. In the present study, the deposition of pigments or aggregation of chromatophores in the scales and total carotenoid content in the muscle of *P. conchonius* showed dietary dependent variation. The maximum aggregation of chromatophores was found in the scales of those fishes which were fed with T3 diet containing 1.5 g 100 g^{-1} BRWE, followed by T2 and T1 diet containing 1.0 and 0.5 g 100 g^{-1} BRWE respectively. Fish absorb dietary carotenoids through the intestinal mucosa, transport them through the blood via serum lipoproteins (Bowen et al., 2002) and deposit them into specialized skin cells called chromatophores (Chatzifotis et al., 2005). Similar results were reported by Asimi (2002) in blue gourami, *Trichogaster trichopterus* on feeding diet supplemented with higher dose of methoxsalen (photosensitizer). The dispersing action of melanin pigment within the chromatophores of the fish could be clearly observed to increase with increasing dose of methoxsalen. Odiorne (1957) and Fujii (2000) documented that the multiple colour patterns in ornamental fishes is the result of the combination between the different types of chromatophores which in turn depends upon multiple interactions between the pigments in these chromatophores. Hama & Hasegawa (1967) also reported that pigments in the feed have profound effect on the chromatophores of *Oryzias latipes* (medaka). Presumably, hormonal regulation is exclusively responsible for the motility of the chromatophores (Fujii, 1993). However, in the present study only physiological responses of *P. conchonius* were observed, moreover hormonal regulation needs to be evaluated in detail.

Carotenoid deposition in the muscle of *P.conchonius* was also found to be maximum in fishes fed with 1.5 g 100 g^{-1} BRWE. The higher carotenoid deposition in the groups fed with beet root extract might be due to the transforming ability of alimentary carotenoids which are subsequently stored in the muscle. Similar results documenting intensification of colour in sword tails, rainbow fish and cichlids when fed diet containing 1.5-2.0% carotenoid rich strains of *Spirulina platensis* and 1.0% *Haematococcus pulvialis* has been reported by Ako et al., 2000.

As fish cannot synthesize carotenoids, they rely on a dietary supply of these pigments to achieve their natural skin pigmentation. Attractive colour is the most in demand quality criteria for the market value of ornamental high-value species such as goldfish (Sinha & Asimi, 2007). The effectiveness of a carotenoid source for pigment deposition is species specific (Ha et al., 1993). In rainbow trout (*Oncorhynchus mykiss*) the dietary astaxanthin is partially metabolized to zeaxanthin (Schiedt et al., 1985). Nakazoe et al. (1984) reported that the content of skin carotenoid in fish fed β-carotene-supplemented diets was low and the dietary β-carotene failed to increase the reddish color red porgy. Differing from rainbow trout and red porgy (Nakazoe et al., 1984; Schiedt et al., 1985), the current results indicate that rosy barb were able to utilize betalains from beet root efficiently. Similar results were obtained for goldfish by feeding natural carotenoid source such as alfalfa (*Medicago sativa*)

Table 3. Total carotenoid content in the muscle of *Puntius conchonius* fed with different experimental diets. Each value is the mean of three individual estimates

TREATMENTS	TOTAL CAROTENOID CONTENT($\mu g\ g^{-1}$) Mean ± S.D
CONTROL	4.62 ± 0.08
TREATMENT-1	7.38 ± 0.15
TREATMENT-2	9.81 ± 0.22
TREATMENT-3	10.88 ± 0.08
H value	10.38
p value	$p<0.05$

Table 4. Water quality profile in different experimental treatments during the study period

Parameters	Control Min-Max (Mean ± S.D)	Treatment-1 Min-Max (Mean ± S.D)	Treatment-2 Min-Max (Mean ± S.D)	Treatment-3 Min-Max (Mean ± S.D)	Overall Min-Max (Average)	H-value	p-value
Temperature (°C)	10.20-10.60 (10.43±0.12)	10.20-10.60 (10.43±0.12)	10.20-10.60 (10.43±0.12)	10.20-10.60 (10.43±0.12)	10.20-10.60 (10.43)	NA	> 0.05
pH	7.10-7.90 (7.65±0.30)	7.20-7.90 (7.66±0.23)	7.20-8.00 (7.67±0.32)	7.20-8.00 (7.68±0.25)	7.1-8.00 (7.76)	2.80	> 0.05
DO (mg l^{-1})	8.30-12.40 (9.04±1.27)	8.40-12.20 (9.05±1.18)	8.20-12.40 (9.00±1.29)	8.40-12.60 (9.0±1.35)	8.20-12.60 (9.38)	1.34	> 0.05
Free CO_2 (mg l^{-1})	2.00-3.00 (2.22±042)	2.00-3.00 (2.61±0.48)	2.00-3.00 (2.27±0.44)	2.00-3.00 (2.77±0.44)	2.00-3.00 (2.66)	1.39	> 0.05
Total alkalinity (mg l^{-1})	240.00-284.00 (263.77±14.7)	246.00-274.00 (261.44±7.9)	230.00-280.00 (260.77±16.7)	240.00-280.00 (264.66±11.8)	230.00-284.00 (262.66)	0.52	> 0.05
Ammoniacal nitrogen ($\mu g\ l^{-1}$)	264.00-292.00 (277.77±10.6)	273.00-298.00 (282.88±7.8)	276.00-298.00 (283.55±8.8)	256.00-292.00 (277.44±14.9)	256.00-298.00 (280.41)	1.54	> 0.05

NA-no analysis

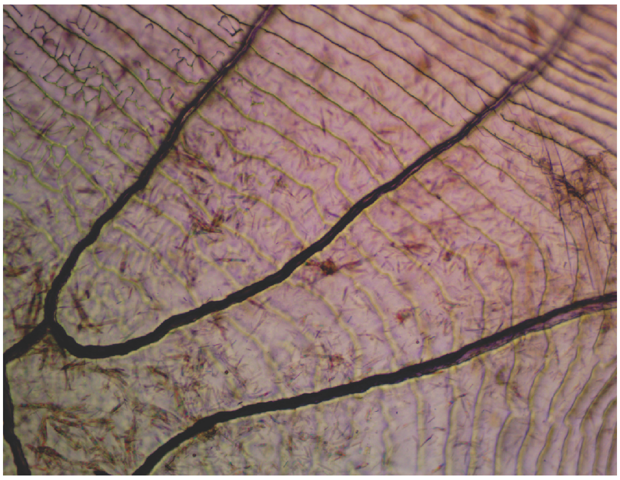

Plate 1. Microphotograph (10X) of scale of *Puntius conchonius* fed with control diet

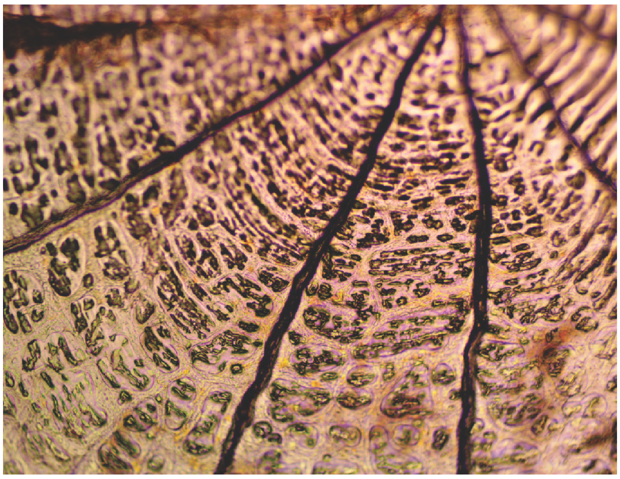

Plate 2. Microphotograph (10X) of scale of *Puntius conchonius* fed with T1 experimental diet

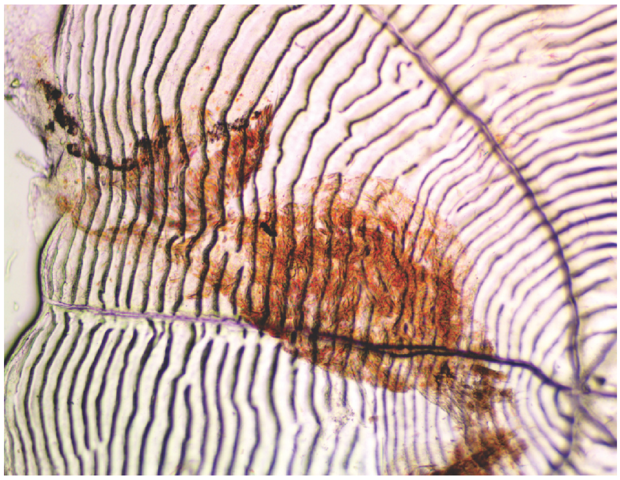

Plate 3. Microphotograph (10X) of scale of *Puntius conchonius* fed with T2 experimental diet

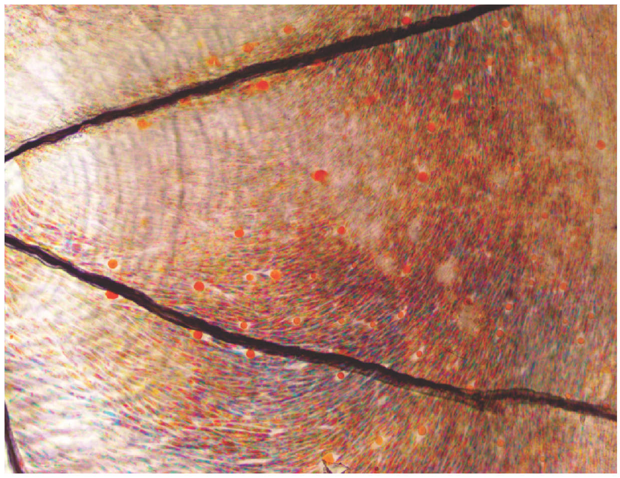

Plate 4. Microphotograph (10X) of scale of *Puntius conchonius* fed with T3 experimental feed depicting increased aggregation of carotenoid pigments

(Yanar et al., 2008). The authors mentioned that this may indicate that carotenoid uptake or transportation to the tissue was saturated due to carotenoid inclusion level in goldfish, only this work reported a saturation level for this specie. The present study showed that beetroot extract, as a natural carotenoid source was effective in increasing skin pigmentation of rosy barb. This study also demonstrated that since beetroot extract led to nearly maximum carotenoid accumulation in the skin of rosy barb, it should be considered as a valuable source of carotenoids, such as zeaxanthin, lutein or astaxanthin (Paripatanont et al., 1999).

In aquaculture, water quality management is of imperative importance. It is usually strongly influenced by culture species combinations, quality and quantity of nutrient inputs and culture system used (Diana et al., 1991). Ornamental fish in captivity need to utilize their dietary protein with the utmost efficiency, as the breakdown products of protein metabolism (mainly ammonia) will directly pollute their living environment (Ng et al., 1993; Earle, 1995; Pannevis & Earle, 1995). In the present experiment, the values of water quality parameters between the different treatments were found to be not significantly different to each other at 5% level of significance. Thus, the incorporation of beetroot waste extract in the diet had no effect on quality of water. Singh & Kumar (2016) also reported non-significant differences in various physico-chemical parameters between different treatments using beetroot as a carotenoid source in red swordtail (*Xiphophorus helleri*). Similar results have been reported by a number of authors (Maske & Satyanarayan, 2012; Shamsuddin et al., 2013). Kaatz & Morris (2007), however reported a significant difference in water quality between treatments which was attributed to the incoming nutrients associated with the fish feed, since feeding fish in an aquaculture setting tends to increase nutrients in the water and can lead to water quality problems (Huner & Dupree, 1984; Barrows & Hardy, 2001; Kaatz, 2003).

The results of this study confirmed increased carotenoid deposition in the muscle tissue of *P. conchonius* with the incorporation of beetroot waste extract (BRWE) at an optimal level of 1.5 g 100 g^{-1} in diet. Synthetic carotenoids, in addition to being expensive, can also cause negative impact on fish metabolism. Thus, it is suggested that the natural colour enhancers such as BRWE could be effectively utilised to increase colouration of *P. conchonius* to increase its acceptability as an ornamental fish.

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