

IMPACT OF *CELOSIA ARGENTEA* VAR. *CRISTATA*(COCKSCOMB) EXTRACT ON COLOR ENHANCEMENT IN FISH; *PUNTIUS CONCHONIUS*.

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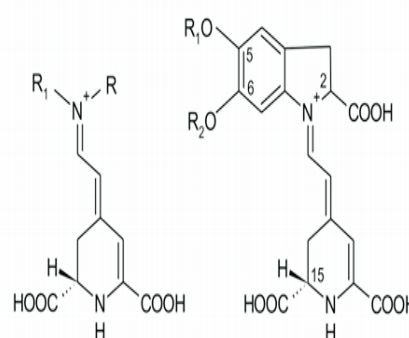
ABSTRACT

The 60-day trial was performed to evaluate the impact of *Celosia argentea* var. *cristata* flower extract in enhancing and maintaining natural pigmentation in Rosy barb, *Puntius conchoni*. The extract was incorporated at the rate of 0% (Control), 0.5% (T₁), 1.0 % (T₂) and 1.5% (T₃) with basal feed (protein 32%) and fed to the fish weighing 0.45 g to 0.49 g initially. It was observed that the muscle pigmentation increased with increasing cockscomb extract with 1.5% extract level fed group showing the highest carotenoid content followed by 1.0% and 0.5 % extract levels which were significantly higher (p<0.05) than that of the control group (0%). The carotenoid retention rate in the muscle was also found to be significantly higher (9.5% ± 0.125) at 1.5% as compared to 1.0% (7.2% ± 0.09) and 0.5% (4.95% ± 0.09) extract levels. No significant change was observed in the physico-chemical parameters of water. The study concluded that locally available *Celosia argentea* flowers can be used to formulate the natural color enhancing diets for less commercially valuable fish, Rosy barb, *Puntius conchoni* at 1.5% level

INTRODUCTION

Fish coloration is one of the important quality attributes of ornamental fishes for consumer acceptability. Fishes contain various pigments that affect the coloration among them. These fishes cannot synthesize such pigments inside their body (Mills and Patterson 2009) and hence depend on the external food source for such pigments. There is a considerable lag in nutritional research on ornamental fish sector due to higher focus on food fish species (Vargas-Abundez *et al.*, 2019). Carotenoid and melanin pigments are commonly studied in the field of aquatics, especially in relation to coloration. It includes work done by Joseph *et al.*, 2011 using *Rosa indica*, *Ixora coccinea* and *crossandra infundibuliformis*; Yanar *et al.*, 2007 using red pepper and marigold flower; Guroy *et al.*, 2012 using Spirulina; Martinez *et al.*, 2013 using marigold meal; Talebi *et al.*, 2013 using red pepper (*Capsicum annum*); Jagadeesh *et al.*, 2015 using Marigold (*Tagetes erecta*) and Patil and Thakare, 2017 using *Penicillium purpurogenum* and *Talaromyces purpurogenum*. Although these are not the only pigments that can be accumulated through the diet. There are various other dietary pigments as well like anthocyanins and betalins. It includes work done by Ahilan and Jeyaseelan, 2001 using Amaranth and Mint; Neverian *et al.*, 2014; Jasmin & Somanath, 2016; Singh and Kumar, 2016 and Adhami *et al.*, 2017 using beetroot. Betalins are the water-soluble nitrogen containing pigments comprises the red-violet betacyanins and

yellow betaxanthins (Strack *et al.*, 2003).



R and R₁: H and amines or their derivatives Betaxanthins
R₁ and R₂: H and glucosyl derivatives Betacyanins

The composition of diet is very crucial factor that directly or indirectly affect the development of pigmentation in fish (Ebenezar *et al.*, 2019). Some producers use hormones and artificial (synthetic) colorations to attract consumers, increase their profit margin and to make the fish more vivid and shiny. However, the color acquired through such methods is not stable and the fish tend to lose their color after a very short span of time. Also the excessive use of such synthetic pigments leads to deteriorating effect on the environment as well. The use of animal based natural carotenoids is also not desirable due to limited supply and declining trend in catches of

crustaceans from marine landing. Also, these are very expensive sources of carotenoids and thus aquaculture feed production becomes costlier. Hence, there is a great demand and need for inclusion of natural pigments in aqua feed to ensure bright coloration in fish.

The rosy barb, *Puntius conchonius* is an indigenous fish, which is missing from the aquariums due to its somewhat dull colors, which can be improved by using natural plant pigment incorporated feeds, such as, cockscomb (*Celosia argentea*), locally known as "Mawal" in Kashmir valley that grows as a weed (Sujathamma, 2015). In Kashmir valley two commonly found flowering varieties of cockscomb are *Celosia argentea* var. *crinata* and *Celosia argentea* var. *plumosa* (Aslam *et al.*, 2010). The main coloring pigment found in these plants are betalins. Appropriate use of a wide variety of carotenoids in the diet can bring large economic benefits to ornamental entrepreneurs as fishes with an assortment of coloration are a priority for fish enthusiasts.

Therefore, the present study is an attempt to see the effect of locally available flower extract of *Celosia argentea* (Cockscomb) on the pigmentation of Rosy barb (*Puntius conchonius*).

MATERIALS AND METHODS

The experiment was carried out in Division of Aquaculture at Faculty of Fisheries, Shuhama (34.1886°N, 74.8286°E), Ganderbal, J&K for a period of 60 days.

Procurement and Acclitimization of fish:-

The experimental fish Rosy barb (*Puntius conchonius*), 240 in number were procured locally from natural water bodies with an average weight of 0.9 ± 0.08 g. After procuring, the fishes were given prophylactic treatment for about 10-20 seconds with potassium permanganate. The dead and weak fishes were discarded and the bath treated fishes were transferred to fish tank for acclimatization to culture conditions for about a week before the start of feeding trial. The fishes were fed with a basal diet during acclimatization period.

Experimental Design

The experiment was carried out in 16 plastic tubs of 25 liters capacity (completely randomized design, CRD with four treatments and three replicates). The fishes were weighed and randomly distributed to these treatment tubs at the stocking density of 15 fishes per tub. The experimental group fishes were fed with their respective diets @ 5% of their body weight twice a day.

Feed Formulation

Initially a basal diet was formulated by mixing the ingredients *viz.* rice bran, soybean meal, fish meal, wheat flour and mustard oil cake with the required amount of distilled water along with vegetable oil using Pearson square method (Table 1). The mixture in the form of dough was steam cooked for 10 minutes in a pressure cooker and subsequently vitamins and mineral premixes were added. In case of treatment diets the cockscomb extract is added as well at this stage of preparation at 0.5 %, 1 % and 1.5% per 100 g of basal feed by replacing the same quantity of rice bran and wheat flour from the basal feed designated as T₁, T₂ and T₃ respectively. The mixture was made into pellets using hand pelletizer of 2.0 mm diameter

and air dried.

The cockscomb extract is prepared by mixing 1gm dried cockscomb inflorescence powder with 5ml of distilled water. The volume of the mixture is then brought down to 1/4th of its original volume by heat treatment at 75°C. The concentrated extract is then cooled and filtered at room temperature using whatman filter paper no.1 and further concentrated in water bath at 100 °C. The concentrated extract was then stored in screw capped test tubes for further use in refrigerator at 4°C.

Carotenoid analysis

Analysis of total carotenoids in fish tissue was administered before the beginning of the experiment and after the termination of the experiment following the procedure by Olson (1979). The total carotenoid content was calculated as microgram per wet weight of tissue as per following equation:

$$\text{Total carotenoid content}(\mu\text{g/g}) = \frac{\text{Absorption at maximum wave length}}{0.25 \times \text{sample weight}} \times 10$$

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

Carotenoid Retention Rate

Assuming a complete ingestion of the diet, the retention rate was calculated by the following equation according to Foss *et al.*, 1984:

$$\text{Retention rate}(\%) = \frac{\text{mg of carotenoid in muscle}}{\text{mg of carotenoid in diet}} \times 100$$

Water Quality Parameters

The various physico-chemical parameters of water *i.e.*, temperature, pH, free carbon-dioxide dissolved oxygen, total alkalinity and total hardness were analyzed every week throughout the trial as per the standard methods described by APHA (2012).

Table 1: Diet ingredients (%) and concentration of cockscomb extract in experimental diets.

| Ingredients | Dietary groups (%) | | | |
|---------------------------|--------------------|----------------|----------------|----------------|
| | T ₀ | T ₁ | T ₂ | T ₃ |
| Fish meal | 15 | 15 | 15 | 15 |
| Mustard oil cake | 16 | 16 | 16 | 16 |
| Wheat flour | 25.5 | 25.25 | 25 | 24.75 |
| Rice bran | 25.5 | 25.25 | 25 | 24.75 |
| Soy bean | 16 | 16 | 16 | 16 |
| Vitamin & Mineral mixture | 1 | 1 | 1 | 1 |
| Vegetable oil | 1 | 1 | 1 | 1 |
| Cocks comb extract | 0 | 0.5 | 1 | 1.5 |

Table 2: Total carotenoid content and Retention percentage in the muscle of *P.conchonius* fed with different experimental diets

| Treatments | Total carotenoid content ($\mu\text{g/g}$) [Mean \pm S.E] | Retention percentage |
|-------------|---|----------------------|
| Control | 2.3 \pm 0.13 | - |
| Treatment-1 | 4.95 \pm 0.09 | 7.6 \pm 0.08 |
| Treatment-2 | 7.25 \pm 0.09 | 10.0 \pm 0.13 |
| Treatment-3 | 9.57 \pm 0.125 | 12.5 \pm 0.20 |
| p-Value | p < 0.05 | p < 0.05 |

Table 3 : Water quality profile of different parameters in different treatment groups

| Parameters | Treatments | | | |
|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | T ₀ (Mean ± SE) | T ₁ (Mean ± SE) | T ₂ (Mean ± SE) | T ₃ (Mean ± SE) |
| Temperature (°C) | 16.31 ± 1.31 | 15.9 ± 1.18 | 16.4 ± 1.34 | 16.0 ± 1.22 |
| Dissolved oxygen (mg/l) | 8.06 ± 0.13 | 8.19 ± 0.115 | 8.21 ± 0.09 | 8.13 ± 0.14 |
| Free CO ₂ (mg/l) | 0.48 ± 0.05 | 0.45 ± 0.04 | 0.58 ± 0.05 | 0.5 ± 0.04 |
| pH | 7.79 ± 0.04 | 7.85 ± 0.03 | 7.8 ± 0.04 | 7.81 ± 0.03 |
| Total Alkalinity (mg/l) | 204.18 ± 1.39 | 203.61 ± 1.50 | 201.65 ± 1.22 | 202.33 ± 1.51 |
| Total Hardness (mg/l) | 129.24 ± 3.87 | 130.4 ± 3.54 | 131.4 ± 4.03 | 131.1 ± 3.73 |

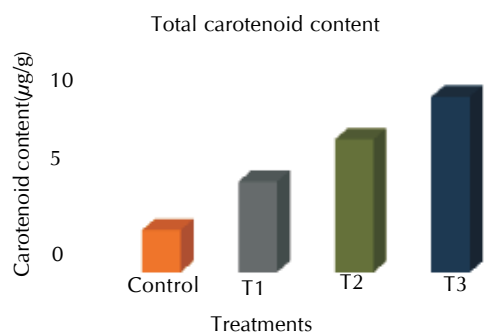


Figure 1: Carotenoid content in the muscle of *P. conchonius* fed with different dietary carotenoid levels

Statistical Analysis

All the experimental data collected were statistically analyzed using mean values of all the parameters through one-way ANOVA by using SPSS version 20.0 and Microsoft Excel 2010 software packages.

RESULTS AND DISCUSSION

Muscle pigmentation of Rosy barb (*Puntius conchonius*)

The total carotenoid content (µg/g of tissue) in the muscle of *P. conchonius* fed with different experimental diets is given in Table 2. The result clearly showed that maximum carotenoid content was found in fish muscle fed with T₃ group followed by T₂ and T₁ which was significantly different (p < 0.05) from fishes fed with control diet (T₀) and increased linearly with the increase in concentration of cockscomb extract (Fig. 1).

Carotenoid Retention Rate:

The carotenoid retention rate in the muscle of the Rosy barb fed with diets containing different concentrations of cockscomb extracts fluctuated from 7.6 to 12.5. The bio-deposition of carotenoid in the muscles of Rosy barb was found to be effective. The retention was found to increase during the experiment for all the diets and the higher values were observed for T₃ group (Fig. 2).

Water quality parameters

The important physico-chemical parameters like water temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and total hardness were monitored on weekly basis in all experimental tubs during the experimental period of 60 days. The overall mean of various water quality parameters monitored during the experiment are shown in Table no.3.

These values of water quality parameters were statistically non-significant to each other at 5% level of significance among the treatment groups. The data clearly revealed that the flower extract incorporation has no negative impact on the water quality during the trial.

The survival percentage was highest (97.7%) in T₃ group and lowest (88.8%) in T₀ group respectively revealing that there was no negative influence of cockscomb extract on the survivability of *Puntius conchonius* (Fig. 3).

Muscle pigmentation

After the fish is subjected to different levels of cockscomb (*Celosia argentea* var. *cristata*) extract for a period of 60 days, the result showed the total carotenoid content in the muscles of *Puntius conchonius* (9.57 ± 0.12 µg/g, 7.25 ± 0.09 µg/g and 4.95 ± 0.09 µg/g wet weight of tissue) in T₃, T₂ and T₁ respectively (Fig. 4). The results have been earlier recorded by Yanar *et al.* (2007) and Buyukcapar *et al.* (2007) with marigold flower meal and red pepper as carotenoid sources to improve pigmentation in rainbow trout. Pham *et al.* (2014) reported that dietary inclusion of paprika (*Capsicum annum* L.) and *Haematococcus pluvialis* extract can increase the entire carotenoid pigmentation of olive flounder (*Paralichthys olivaceus*). The carotenoid concentration in the skin of red porgy (*Pagrus pagrus*) is augmented with increasing amount of shrimp shell meal diet (Kalinowski *et al.*, 2005).

In the present study cockscomb extract fed to the fish at different levels viz., 0.5, 1.0 and 1.5% showed gradual improvement in coloration as compared to the control group fed with without any carotenoid source. This might be due the transforming ability of alimentary carotenoids and subsequently their storage in the muscle of *P. conchonius*. Similar results have been reported by Allaf *et al.* (2014); Nath *et al.* (2013) in the *Barilius*

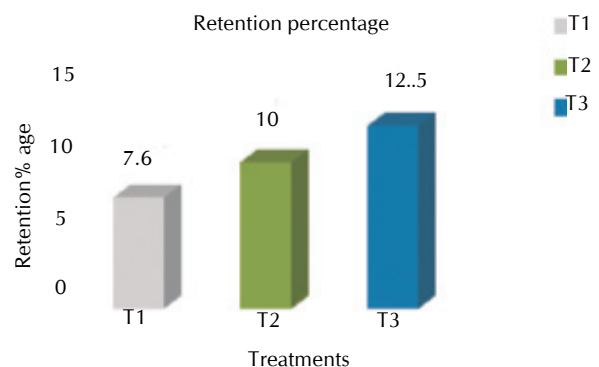


Figure 2: Retention percentage of carotenoid from Cockscomb in the muscle of *P. conchonius*

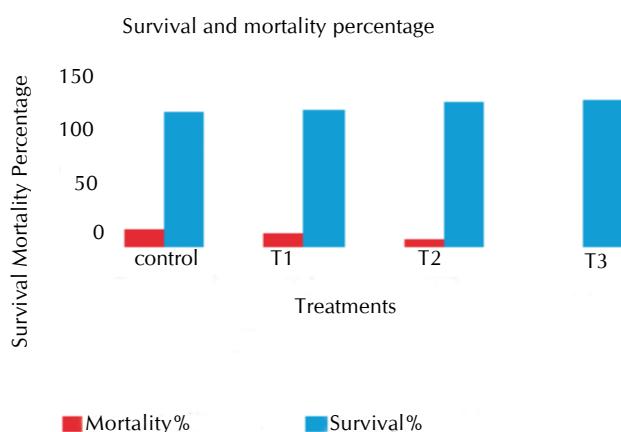


Figure 3: Variation in Mortality/ Survival

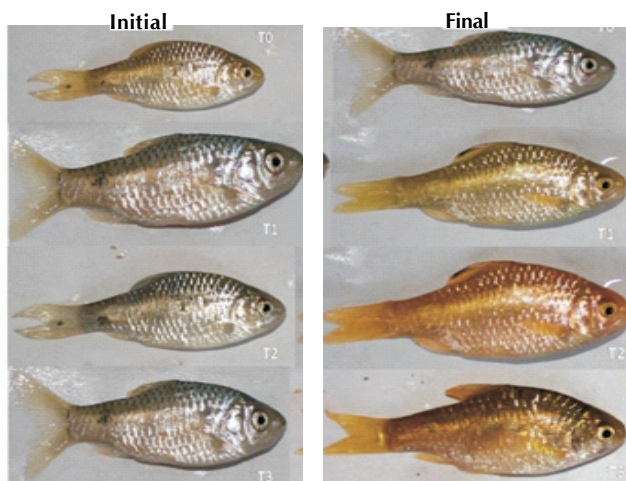


Figure 4: Initial and Final phenotypic appearance

bendelisis; Goswami and Zade (2015) in *Anabas testudineus* and Asadi *et al.* (2014) in Oscar fish using beetroot as a natural source of carotenoid. Ahilan *et al.* (2008) by using amaranth and mint as a natural carotenoid source in gold fish also reported similar results. The findings in the present study are in agreement with the above mentioned works and suggests that rosy barb were able to utilize betalain from cockscomb extract efficiently.

Carotenoid Retention Rate

Carotenoid retention rate in fish muscle increased steadily within the experimental groups with carotenoid retention rate for T₁, T₂ and T₃ as 7.6, 10.0 and 12.5 % respectively. In the diets containing cockscomb extract, the average retention rate increased with increase in the concentration of the extract in the diet. These findings suggest that the carotenoid (betalin) was more efficiently absorbed and bio accumulated in the muscles of Rosy barb, *Puntius conchonius*. Differences in body pigment deposition rate observed in current study may be due to: the dietary carotenoid concentration and the duration of the feeding trial (Storebakken *et al.*, 1986).

Water Quality Analysis

Water quality management has significant importance in aquaculture. It is usually strongly influenced by culture species

combinations, quality and quantity of nutrient inputs and culture system used (Diana *et al.*, 1991). All the water quality parameters were found to vary non-significantly at 5% level of significance and were within the acceptable ranges throughout the experimental period. Water temperature was found to be in the range of 10.8 ± 0.05 to $22.5 \pm 0.02^{\circ}\text{C}$. Pailan *et al.*, 2012 recorded water temperature in the range of $18-22^{\circ}\text{C}$ in Rosy barb culture tanks fed on rose petal meal that were slightly higher than the values found in the present experiment. The lower temperature is due to the cold weather conditions during the season of the year. The pH values in the range of 7.62 ± 0.07 to 8.03 ± 0.03 were suitable as the optimum pH range for fish culture is between 6 to 9 (Sallenave, 2012). The dissolved oxygen was in the range of 7.3 ± 0.17 to 8.6 ± 0.07 mg/l was also under optimum dissolved oxygen range of 6.0 to 9.0 mg/l (Boyd, 1982). Similarly free carbon dioxide, total alkalinity and total hardness found to be in the range of 0.2 ± 0.07 to 0.8 ± 0.10 , 195.8 ± 1.13 to 209.9 ± 2.01 and 115.0 ± 3.81 to 154.3 ± 2.04 mg/l respectively were under the optimum range of < 5 mg/l (Bhatnagar and Devi, 2013) for free carbon dioxide, 50-300 mg/l (Santhosh and Singh, 2007) for Total Alkalinity and for Total Hardness from 5-20 mg/l to over 300 mg/l as most of the ornamental fishes are well adapted for hard water environment.

Thus the incorporation of cockscomb extract in the diet of Rosy barb showed no leaching effect in water due to which all the water quality parameters were found to be under optimum range.

Survival Percentage

The higher survival percentage is the essential aspect in fish culture. The Survival percentage was found to be in the range of 97.7 % to 88.8% .There are several factors that affects the survival like feed availability, water quality parameters, stocking density and nitrogenous compounds (Randazzo *et al.*, 2017). Since all of these parameters were under optimum range the possible reason for better survival of fish can be attributed to the anti-oxidant activity exhibited by betalins. The result concludes that different level of supplementation of cockscomb extract in treatment diets has resulted in better survival percentage of fish.

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