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COLOUR RETENTION IN ORNAMENTAL FISH, INDIAN ROSY BARB (*Pethia conchonius*), USING PETAL MEAL OF ASIAN PIGEONWINGS *(Clitoria ternatea)*

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ABSTRACT

The use of synthetic carotenoids in fish feed to potentiate the colouration, along with some health benefits has become exorbitant to mitigate the demand for lucrative ornamental fish within budget. Switching the source of pigmentation and colouration from synthetic to natural sources is all time preferable choice for biological system as well as for fish farmers. Petal dust of Asian pigeonwings (*Clitoria ternatea*) was incorporated in basal diet to retain the colour of rosy barb (*Pethia conchonius*) within captive conditions. Varied concentrations of petal dust (0.5, 1.0, 1.5 and 2 g 100 g^{-1} basal feed) were added to the basal feed, fed twice a day at 4% body weight. Water quality parameters and proximate analysis of feed and growth parameters were measured and carotenoid concentration in fish tissue were measured spectrophotometrically. At the completion of 60 day's experiment a spectacular increase in growth, survival, and carotenoid concentrations (1.88 to 2.42 μg g^{-1}) in body tissue was observed. The best survival and growth of fish were obtained from D4 (0.5%) at 5% level of significance. In proper captive conditions, it is recommended to include 0.5% Asian Pigeonwings dust in prepared feed for better growth and colour retention of rosy barb.

Keywords: Asian pigeonwings, colour retention, formulated feed, natural carotenoids, rosy barb

INTRODUCTION

Diverse skin colour and patterning is one of the significant attracting factors for ornamental fishes influencing their commercial value. The development of alluring colour is due to the accumulation of different carotenoids in fish tissue, which also play vital roles in healthy growth, metabolism, and reproduction (Miki, 1991). Chromatophores present in the skin of fish are the main pigment bearing cells that perform several cellular reactions to reflect variety of colours through different pigments. Chromatophores like melanophores, leucophores, cyanophores, iridophores, xanthophores, erythrophores, etc. assemble pigments such as carotenoids, melanin, pteridines, and purines. Among these, carotenoids are the most appreciable contributors to coloration, growth, immunity, and reproduction.

Fishes, like other vertebrates, are unable to perform *de novo* synthesis of carotenoids (Goodwin, 1984). Carotenoids possibly perform a biological function similar to that of α-tocopherol, protecting tissues and reactive compounds from oxidative stress. Environmental factors, genetic background, and dietary routes are the major concerns for colour development and retention in fish. Genetic and environmental factors are beyond cost-effecting manipulation for retaining vibrant colours, thereby dietary inclusions are the most convenient way to ensure a continuous supply of carotenoids in fish tissue. Harpaz and Padowicz (2007) indicated that 60 mg oleoresin paprika kg^{-1} diet was sufficient for good coloration in *M. ramirezi.* The 1.5% marigold meal gave best colour intensity value of 5.97 in tail region and 6.5 in head portion of Koi fish Kohaku strain (Lili *et al.*, 2020). Higher carotenoid deposition was observed in *Xiphophorus helleri* and *Cyprinus carpio* due to natural pigment enriched feed (Ezhil *et al*., 2008; Swian *et al*., 2014). *P. conchonius* fed with 4% rose petal meal supplemented diet showed weight gain and increase in length and skin coloration (Pailan *et al*., 2012). The improvement in fillet colouration of rainbow trout has been reported when the fish were supplemented with beetroot juice feed (Neverian *et al.*, 2014).

Indian rosy barb (*Pethia conchonius*), a very familiar name in the ornamental fish industry, have aquarium-soothing salient features *viz*., bright coloured skin and fins with approximate length of 5 to 6 cm. The colour of the species deepens in the course of their reproductive season. Males possess significantly brighter colour while females are a little plump. Being the hardest of the barbs, this species is well suited to the community aquarium for its beauty, activeness, and peaceful in nature.

Asian pigeonwings (*Clitoria ternatea)* is known for various medicinal values like antimicrobial, anti-depressant and anti-inflammatory activities (Mukherjee *et al.*, 2008). The purplish blue colour of petals is rich in anthocyanins along with several groups of carotenoids. The plant parts *viz*., root, shoot, flowers, and leaves, provides worthy compounds, especially secondary metabolites like saponin, cardiac glycosides, tannin, alkaloids and phytosterol (Mukherjee *et al*., 2002; Malic *et al*., 2008). Secondary metabolites are benevolent when administered in appropriate amount, otherwise it may lead to certain complications. Besides phytochemicals, the presence of ascorbic acid may create a new domain of application in several other animal feed. In present study, Asian pigeonwings petal dust was incorporated in varied concentrations in the feed of rosy barb as a source of carotenoids to assess the improvement in body colour and retention of skin colour in captive condition along with growth.

MATERIALS AND METHODS

Collection of flowers

Fresh Asian pigeon wings (Aparajita) flowers (*Clitoria ternatea*) of family Fabaceae were collected locally and got identified by the taxonomists in the Department of Botany and Forestry, Vidyasagar University. Immediately, the sepals were removed, washed thoroughly, and allowed to dry, followed by fine grinding. Powdered petals were preserved in an airtight container for future studies.

Experimental design

The fish used in present study was supplied by a local fish farmer and transferred to the laboratory of Department of Fisheries Sciences, Vidyasagar University, West Bengal (India) where they were allowed to adapt the captive conditions for 10 days. The fish were fed on basal feed two times a day during acclimatization. Then fish were grouped in five experimental tanks for feeding treatment and the experiment was carried out in a completely randomized block design in triplicate. The monitoring of water quality parameters, proximate analysis of feed and carotenoid measurements were taken for convenience. On completion of feeding trial (60 days), the growth performances and carotenoid estimation from fish tissue were observed.

Preparation of feed

The 40% protein feed was prepared by calculating the protein percentage in feed ingredients using Pearson's square method. The different feed ingredients for basal diets were rice bran (27 g), fish meal (25 g), soybean meal (18 g), wheat flour (22 g), tapioca flour (4 g), sunflower oil (2 g), vitamins and minerals mix (2 g). The varied concentrations of Asian pigeonwings dust included in feed were 2.0, 1.5, 1.0, and 0.5 g, respectively, in four diets marked as D1 to D4 along with a control feed (without any petal dust). The ingredients were thoroughly sieved, ground and mixed, followed by dough formation by using the required amount of water, and autoclaved at 121°C for 10 min. The dough was put into a hand pelletizer for pelleting, and the pelleted feed was dried in hot air oven and kept in air-tight containers to minimize microbial infections.

Experimental aquaria set up

Rosy barb fish (*Pethia conchonius)*, an indigenous variety of ornamental fish species belonging to the family Cyprinidae, was chosen; and 85 live specimens were brought to the laboratory and kept in 5 aquaria (size: 60 cm x 45 cm x 30 cm) for their acclimatization for 10 days. Five experimental tanks were set up with 10 species each, marked as D1, D2, D3, and D4 plus a control, in addition to air stones for maintaining soluble oxygen throughout the experimental period and kept away from sunlight. Uneaten food particles and faeces were siphoned out after every 7 days.

Assessment of water quality parameters

Water quality parameters like temperature, dissolved oxygen, pH, total hardness, and alkalinity from all the aquaria were analyzed at 15 days-interval as per the standard methods (APHA, 1998).

Feeding and growth performance

The fish were fed twice a day @ 4% body weight during the experimental period of 60 days. Body weight and length were measured at the start of experiment and after every week, and carotenoid content estimated after every 20 days.

Proximate analysis of feed

The proximate composition of the prepared feed was assayed as per the AOAC guidelines. Each analysis was performed at least 3 times. Protein estimation by Kjeldahl method and crude lipid estimation was done by Soxhlet extraction method.

UV-vis spectrophotometric analysis of total carotenoids

Total carotenoids in fish tissue were calculated as per Olson (1979). The absorbance was read at 400, 450, 475, and 500 nm in a UV-vis spectrophotometer (Shimadzu-1800). The total carotenoid content was calculated in terms of $\mu g\, g^{-1}$ tissue. Carotenoids predominantly show peaks between 450 and 475 nm.

$$\text{Total carotenoids} = \frac{\text{Absorption} * 10}{0.25 * \text{sample weight}}$$ wherein 10 = dilution factor, 0.25 = Extinction factor

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Mean values of treatment groups were subjected to one-way ANOVA to determine the significant differences between two means. Comparisons were made at 5% probability level. Critical difference (CD) was calculated for crude protein and crude lipid to find out the significant differences among treatments. All the data were analysed using statistical package Graphpad Prism version 8.4.2.

RESULTS AND DISCUSSION

Water quality assessment

Ornamental fish adopted well in culture condition and were capable of living in varied environments. The results of water quality parameters revealed that during the period of experiment the temperature, pH, and dissolved oxygen did not fluctuate significantly (Table 1).

Proximate composition of experimental diets

The proximate composition of test diets remained the same throughout the experimental tenure. (Table 2). Similarities of the constituents was maintained, except for carotenoid sources in various diets. The proximate analysis of each test diets demonstrated the adequate compositions of protein and lipids. The target protein in all diets was 40%, resulting in 39.76% for all feeds that were adequate for rearing and maintaining the good health of ornamental fish. Both crude lipid and

Table 1: Water quality of experimental aquaria

Parameters	Minimum	Maximum
Temperature (^{0}C)	20	31
pH	7.2	8.3
Alkalinity (ppm)	286	313
Dissolve oxygen (ppm)	5.4	7.5
Total hardness (ppm)	320	375

Table 2: The proximate analysis (%) of the formulated test feeds

Experiments	Control	D1	D2	D3	D4
Moisture content (%)	10.47 ± 0.015	9.65 ± 0.015	10.02 ± 0.012	10.31 ± 0.040	8.66 ± 0.009
Ash content (%)	8.83	8.50	8.75	8.50	8.70
Crude protein (%)	39.70	39.71	39.76	39.76	39.73
Crude fat (%)	8.11	8.10	8.12	8.10	8.08

D1, D2, D3, D4 and control treatments were supplemented with 2.0, 1.5, 1.0, 0.5 and 0 g Asian pigeonwings dust 100 g^{-1} feed, respectively.

crude protein showed insignificant differences (CD values: 0.017 and 0.21, respectively).

Feed utilization and growth performance

All the experimental diets, along with control, were accepted by fish throughout the experiment. At the start, fishes varied in weight from 0.79 to 0.86 g and their initial length was between 2.97 to 3.0 cm. Table 3 reveals the data of growth performance at the end of the experiment. Feed conversion ratio (FCR) is considered as an index for feed utilization. In present study, the control diet showed FCR of 1.98, which indicates quite good utilization of feed. The increase in length and weight in each formulated diet may be attributed to the enough feed intake.

Table 3: Growth performances of rosy barb fish fed on diets supplemented with Asian pigeonwings dust

Parameters	Control	D1	D2	D3	D4
Initial weight (g)	0.79 ± 0.19	0.80 ± 0.16	0.86 ± 0.19	0.82 ± 0.19	0.85 ± 0.17
Final weight (g)	2.60 ± 0.49	2.92 ± 0.54	2.90 ± 0.38	3.00 ± 0.35	3.26 ± 0.46
Feed conversion rate	1.98 ± 0.50	1.82 ± 0.59	1.96 ±.043	1.80 ± 0.49	1.77 ± 0.52
Survival (%)	86.66	46.66	53.30	67.30	87.20
Initial length (cm)	2.97 ± 0.31	2.99 ± 0.34	2.98 ± 0.39	2.97 ± 0.38	2.98 ± 0.39
Final length (cm)	3.59 ± 0.37	3.61 ± 0.52	3.74 ± 0.28	3.84 ± 0.49	4.14 ± 0.50

D1, D2, D3, D4 and control treatments were supplemented with 2.0, 1.5, 1.0, 0.5 and 0.0 g Asian pigeonwings dust 100 g^{-1} feed, respectively. Initial and final weight and length were estimated at before and at the end of 60 days experimental period.

The survival rate of fish was 86.6% in control and 87.2% in D4, however least survival was observed in D1treatment in spite of maintaining the water quality (Fig. 1). Dead were collected and checked for any microbial infections. Both D1 and D2 showed lesser survival but no health disorder. The FCR was highest in control group was 1.98 followed by D2 (1.96) and lowest FCR was in D4 (1.77). The total carotenoid content in every group varied significantly.

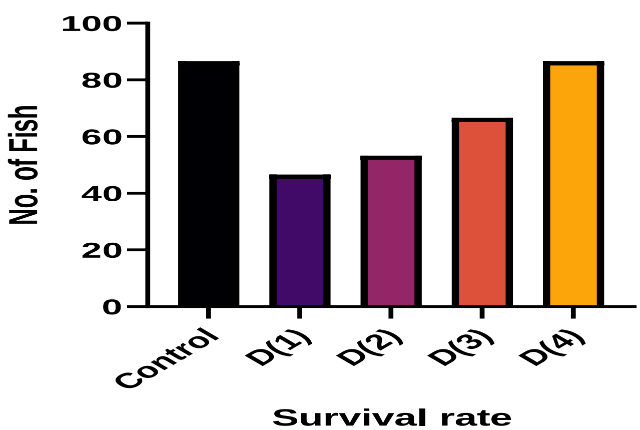
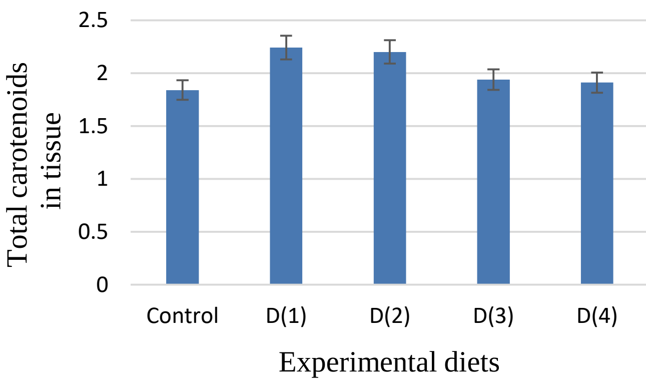


Fig. 1: Survival rates of fish in different experiments
D1, D2, D3, D4 and control treatments were supplemented with 2.0, 1.5, 1.0, 0.5 and 0.0 g Asian pigeonwings dust 100 g^{-1} feed, respectively.

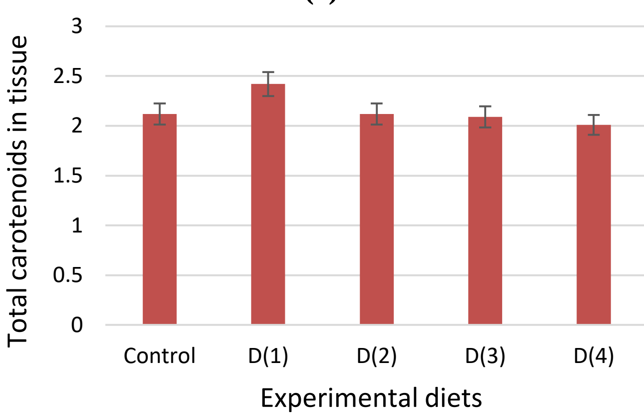
Total carotenoid contents in fish tissue

Carotenoids play positive key role in the metabolism of fish (Tacon, 1981). The concentration of dietary inclusion of pigments and duration of continuation with that particular diet affected the colouration and pigmentation (Torrissen, 1985). Several experimental studies on the effect of addition of carotenoid in fish diets like Atlantic salmon fry, rainbow trout (*Oncorhyncus mykiss*), goldfish (*Carassius auratus*) and characins (*Hyphessobrycon callistus*) have shown significant impact on fish survival and growth (Sinha and Asimi, 2007; Pan *et al.*, 2010).

The carotenoid concentrations of each test diets are given in Fig. 2. Based on the experiments, the control group of fish had 1.83 µg g^{-1} at 450 nm and 2.12 µg g^{-1} at 475 nm. D1 fish had 2.23 and 2.42 µg g^{-1} carotenoids at 450 and 475 nm, respectively. Contrarily, the D1 which had maximum concentration of Asian pigeonwings showed least survival, whereas D4 with least inclusion exhibited maximum survival. Statistical analysis of control, D3 and D4 showed significant differences among means ($p<0.05$) with F value of 8.612. The total carotenoid in fish tissue ranged from 1.88 to 2.42 µg g^{-1}. Jagadeesh *et al*. (2015) studied the effect of marigold oleoresin on pigmentation of rosy barb where fish were fed with test diets supplemented with marigold oleoresin @ 0, 60, 120 and 180 ppm. The results showed the total carotenoid concentration in fish muscle to be significantly higher ($p<0.05$) in both male and female fish fed diet supplemented with 120 ppm marigold oleoresin. The present study revealed that D4 diet comparatively supplied less carotenoids to body tissue but gave highest survival rate with proper FCR. D4 and D3 diets were found best suited for enhancing the fish length and weight. The proximate composition showed that FCR values, length and weight amongst the test diets was highest in D1 but showed least fish survival. Prioritizing the survival over growth and colouration revealed D4 diet to be suitable which may be attributed to the presence of significant amount of phytochemicals in the flowers of Asian pigeonwings, especially saponins which is a major anti-nutritive factor. Increasing the content of flower dust increases accumulation of saponin in fish body. The use of saponin and other phytochemicals in a certain range is desirable for fish health.



(a)



(b)

Fig. 2: Total carotenoids content in fish tissue at (a) 450 nm (b) 475 nm, D1, D2, D3, D4 and control treatments were supplemented with 2.0, 1.5, 1.0, 0.5 and 0.0 g Asian pigeonwings dust 100 g^{-1} feed, respectively.

Conclusions: Carotenoid source can be incorporated through natural flowers of medicinal plants. The preparation of artificial fish feed with different concentrations is both farmer friendly and eco-friendly as the carotenoid source is purely natural. Besides supplementing with carotenoid pigments, Asian pigeonwings serve as growth enhancers and water soluble vitamin C sources. D4 0.5 g 100 g^{-1} Asian pigeonwings furnishes the highest lifetime without any biological disturbances with maximum potential length and weight.

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Conflict of interest: The authors declare that they have no conflicts of interest in the research work. All the authors have thoroughly reviewed the manuscript, and there is no financial interest to report.

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