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Enhancement of Pigmentation in Blue Morph, *Pseudotropheus lombardoi* Through Feeding Different Carotenoid Sources

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Abstract: Aquarium fishes are well-liked by everyone because of their magnificent coloration and graceful movement. Fishes that are colored in nature often get faded under intensive culture conditions where the chance of rejuvenating and replenishing the carotenoid source is meagre. The possibility of enriching the fish feed with various energy rich and different concentrations of carotenoid containing ingredients like Spirulina, mango peel, curry leaves and beet root have been studied. The quantitative estimation of carotenoid in the experimental fish tissue by spectrophotometric method revealed a better coloration in fish fed with the carotenoid incorporated diet than the control diet. Among the various carotenoid ingredients used, Spirulina (15%) incorporated feed ranks first in enhancing the colour of *Pseudotropheus lombardoi.* It is evident from the present investigation that an extrinsic carotenoid source had more influence in enhancing the colour of the tropical fishes, in a simulated environment.

Key words: Pigmentation · Carotenoid · Phycocyanin · *Pseudotropheus lombardoi*

INTRODUCTION

Pet fishes are now a craze and have generated a profitable business throughout the world. The popularity of aquarium is also driven by its relevance in Vasthu and Fengshui, the ancient systems of architecture and interior design. In pet fish species, emphasis should be given to achieving high levels of skin pigmentation, which together with body shape, fin shape and body size, are the important quality criteria giving their due in market [1]. Fish, like other animals are unable to perform *de novo* synthesis of carotenoids [2] and, therefore, rely entirely on dietary supply to achieve their natural pigmentation. Under intensive rearing conditions fish are fed exclusively on compound feeds which must, therefore, be supplemented with carotenoids.

Carotenoids are a group of over 600 natural lipid-soluble pigments that are primarily synthesized within phytoplankton, algae and plants. Carotenoids are absorbed in animal diets, sometimes transformed into other carotenoids and incorporated into various tissues [3]. Earlier, carotenoids were incorporated in the diets of edible fishes such as salmon, rainbow trout, red sea bream, prawn and lobsters to increase the flesh pigmentation which gave them the additional market value. Later, the same technique albeit for different reasons was followed in aquarium fishes, which often show faded coloration under captivity. In order to enhance pigmentation in aquarium fishes crustacean wastes, micro algae like *Dunaliella salina*, *Chlorella vulgaris, Haematococcus pluvialis, Spirulina* sp., vegetable extract, probiotic bacteria like *Pseudomonas aeruginosa* and marigold petal meal have been used [4-9]. Present day aquariculture is turned towards production of not only variety of tropical fishes but also fishes with special colors. This is possible only with enriching the fish feed with various carotenoid rich and calorific ingredients. This being the foundation of the present investigation and it dwells on its application in the experimental cichlid, *Pseudotropheus lombardoi*.

MATERIALS AND METHODS

[Brood blue morphs (*Pseudotropheus lombardoi*, Cichlidae) were purchased from a commercial aquarium fish farm and were acclimatized to laboratory conditions in large cement tanks in Aquatic Biodiversity Centre, St. Xavier's College. They were allowed to breed and the

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Table 1: Proximate and biochemical composition of the experimental diets (dry weight basis)

Ingredients	Quantity (g/100 g feed)	Protein (%)	Lipid (%)	Carbohydrate (%)
Sepia	13.04	74	14	5.2
Soyabean	15.95	45.6	9.7	8.7
Fish meal	15.95	60	5.2	3.7
Groundnut oilcake	13.04	44.6	9.1	30.7
Wheat flour	13.04	11	3.5	44
Tapioca flour	13.04	3	0.7	87.9
Rice bran	15.95	13.2	2.37	59.17

fishes required for the present work were taken from the stock. In the present investigation, 90 fishes were taken and subjected to four different variations. Four carotenoid sources such as curry leaves (C), *Spirulina* sp. (S), mango peel (M) and beet root (B) each with three concentrations of 5%, 10% and 15% were experimented along with a control. For the present investigation, non-conventional feeds were prepared in the form of dry pellets. The ingredients used for the feed preparation are given in Table: 1. Using these ingredients, a basal diet was prepared following the square method [10]. In all experiments, the same basic feed ingredients were used except for the incorporation of carotene. All these experiments were done in triplicates and were carried out in plastic troughs of 24.0 L capacity. Duration of the experiment was 21 days. The fishes were fed with 5% of their body weight daily. The remnants of food if present were collected while changing the water the next day and were oven-dried at a constant temperature of 50°C. Water in the experimental troughs was changed every day for ensuring sufficient supply of oxygen to the fish. At the end of the experiment, all fishes were starved for one day to take the final wet weight. At the same time, three fishes were sampled for carotenoid analysis following the method of Olson [11]. One gram of fish tissue was taken in a 10 ml screw capped clear glass vial and 2.5g of anhydrous sodium sulphate was added. The sample was gently meshed with a glass rod against the side of the vial and then 5ml of chloroform was added and left overnight at 0°C. When the chloroform formed a clear 1- 2cm layer above the caked residue, the optical density was read at 380nm, 450nm, 470nm and 500nm, in a spectrophotometer taking 0.3ml aliquots of chloroform diluted to 3ml with absolute ethanol. The wavelength, at which maximum absorption obtained, was used for calculation.

The total carotenoid content was calculated as µg per wet weight of tissue as follows:

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

where,

10 = Dilution factor
0.25 = Extinction coefficient

The data were subjected to one way ANOVA and the difference between the mean treatments were tested with Tukey test [12].

RESULTS

Results of the current investigation showed that the pellet feed exhibit better response on enhancing colour rather than energy enrichment. The maximum carotenoid content to the tune of 2.27±0.019 µg/g wet wt. was obtained in fish fed with 15% of *Spirulina* sp. (S) incorporated diet. This is followed by the fish fed with 10S which showed 2.22±0.027 µg/g wet wt. carotenoid (Table 2). Concentration of carotenoid content increased linearly with the quantity of carotenoid sources in the feed. Contrary to this, in 15% of beet root (B) incorporated feed, the fish showed decreased carotenoid content. For instance, the carotenoid content in 5B was 0.22±0.25 µg/g wet wt. and in 10B, it was increased to 0.80±0.035 µg/g wet wt whereas fishes fed with 15B feed, the carotenoid content got decreased to 0.51±0.072 µg/g wet wt.

Better growth was attained in fish fed with 15% of *Spirulina* sp. (15S). The highest SGR was observed for fish fed with 15S diet i.e. 1.19±0.051% and lowest value (0.41±0.061%; Fig.1) was obtained for fish fed with control diet.

DISCUSSION

Color plays a major role in the overall preference and acceptability of any pet fish. As fish cannot synthesize pigments, they rely on dietary supply of carotenoids to achieve their natural skin pigmentation. Diet supplementation with carotenoids revealed that maximum carotenoid content was obtained in fish fed with *Spirulina* sp. (15S). The results obtained are in conformity

Table 2: The carotenoid content of *Pseudotropheus lombardoi* fed with pellet feed fortified with various concentrations of different carotenoid sources.

	Carotenoid content		
Variations	Initial content	Final content (µg/g wet weight)	Increase in carotenoid content (µg/g wet weight)
Control	0.036±0.03	0.096±0.01	0.06±0.012[a]
5S	0.036±0.03	0.436±0.06	0.40±0.064
10S	0.036±0.03	2.260±0.01	2.22±0.027[b]
15S	0.036±0.03	2.304±0.02	2.27±0.019[b]
5C	0.036±0.03	0.124±0.12	0.08±0.128[ac]
10C	0.036±0.03	0.128±0.01	0.09±0.041[ac]
15C	0.036±0.03	0.176±0.02	0.14±0.062[d]
5B	0.036±0.03	0.252±0.02	0.22±0.025[e]
10B	0.036±0.03	0.836±0.03	0.80±0.035
15B	0.036±0.03	0.516±0.07	0.51±0.072
5M	0.036±0.03	0.212±0.07	0.18±0.131[de]
10M	0.036±0.03	0.724±0.04	0.69±0.043
15M	0.036±0.03	0.860±0.08	0.82±0.078

S-*Spirulina* sp., B-Beet root, C- Curry leaves, M- Mango peel.

Values in the same column sharing a common superscript letters are not significant (P>0.05)

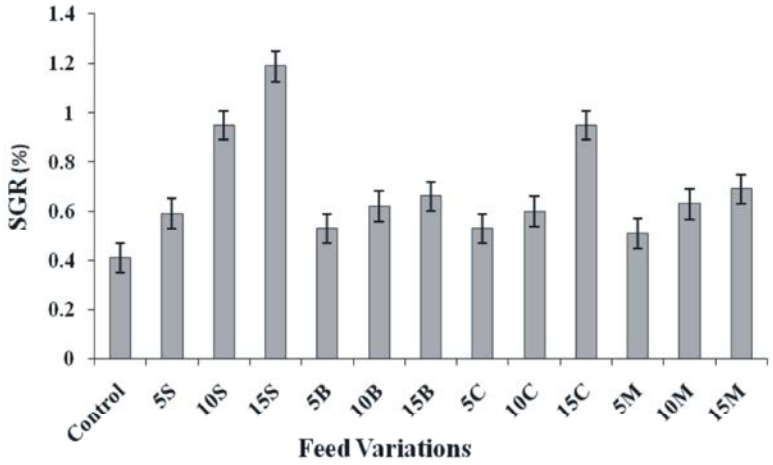

Fig. 1: SGR(%) of *Pseudotropheus lombardoi* fed with pellet feed fortified with various concentrations of different carotenoid sources

with the results obtained by other authors. Increase in carotenoid content was also obtained by Boonyaratpalin and Unprasert [13] in *Oreochromis niloticus* fed with *Spirulina* sp. when compared with other sources such as marigold petal meal, shrimp head meal and turmeric. Gouveia *et al*. [14] acquired carotenoid increment in *Cyprinus carpio* and *Carassius auratus* when fed with *Spirulina* sp. and blue gouramis fed with *Spirulina* sp. diet also showed a distinctive carotenoid pigmentation [6]. Similarly, Ako *et al*. [15] also reported that *Spirulina* sp. was an efficient colour enhancer.

Carrasius auratus fed with carotenoid sources such as shrimp head meal, drumstick leaves, turmeric powder and curry leaves have opined that curry leaves incorporated diet had a significant effect on pigmentation [5]. In the present investigation also feeding *P. lombardoi* with incorporated curry leaves elicited an increase in carotenoid content than the control fish. Carrot was found to be an effective colour enhancer in the marine ornamental fish, *Amphiprion ocellaris* [16]. However, there is no report on incorporation of beet root and mango peel as colour developing agents in fish feed formulation. Nevertheless, feeding the experimental fishes with mango peel and beet root as carotenoid sources showed positive effect on pigmentation. Forsberg [17] opines that fish size and dietary factors affect pigmentation in Atlantic salmon appreciably. Besides, there are also other factors known to affect pigmentation viz., genetic origin [18] and stress level prior to slaughtering [19]. In the present evaluation of beet root as a carotenoid source, the carotenoid content in 10% concentration of beet root (10B) was four times higher than 5% concentration of beet root (5B).

However, a higher concentration of beet root (15B) showed a decreased pigmentation. Similar trend was also reported by Yamada *et al*., [20] on pigmentation of prawn with astaxanthin. They explained that once a plateau level in pigmenting capacity or carotenoid uptake or transportation to tissues was reached, a further increment in carotenoid level was arrested. This might be the reason for the variation in carotenoid content with respect to the concentration of beet root in the present investigation. Mirzaee *et al*., [21] opined that the rate of retention of dietary carotenoids in fish depends on the efficiency of absorption from the digestive tract transport capacity, deposition mechanism in the various tissues, metabolism and rate of excretion.

Carotenoid pigments play a decisive role in intermediary metabolism and that could also enhance nutrient utilization thereby resulting in growth [22, 23]. Though various sources of carotenoid pigments were tried for enhancing pigmentation and growth, the latter did not differ from the control fish in improving the growth of the fish. These results are in accordance with the report carried out in red porgy juveniles fed with a source of astaxanthin through krill meal [24]. From the study of Nickell and Bromage [25] rainbow trout fed on astaxanthin containing diet one could conclude that supplying supplemented food for longer periods are needed to evaluate a possible role on growth. In the present study dietary supplementation of carotenoid pigments failed to show significant effect on the growth related parameters. Similar trend was also reported by various authors [23, 26, 27] and Teimouri *et al*., [28] have also inferred similarly on the carotenoid supplementation study carried out in rainbow trout. But it was clear that *Spirulina* sp. incorporated diet enhanced growth of *P. lombardoi* than other carotenoid sources incorporated diet. Phycocyanin enriched diet might enhance blue coloration [29]. This view corroborates with the enhanced blue coloration achieved in *Pseudotropheus lombardoi* fed with Spirulina enriched moina i.e 53% than the normal moina [8]. Hence, from the present investigation it is concluded that bright coloration obtained in spirulina enriched feed is due to the blue pigment phycocyanin in spirulina. So aquarium fishes should fed with exact carotenoid sources to enhance and retain their beautiful coloration.

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