

Effect of Natural Carotenoids on the Pigmentation of Ornamental Fishes

Phadke S. V.

Department of Zoology

Shivaji Mahavidyalaya, Renapur, Latur, Maharashtra, India

Abstract: *The present study revealed the impact of four natural β -carotene sources viz., beetroot (*Beta vulgaris*), carrot (*Daucuscarota*), spinach (*Spinaciaoleracea*) and turmeric(*Curcuma longa*). The experimental fishes(Koi carp and Molly fish) were cultured for 35 days in an aquarium. The experimental diets were prepared with four natural carotenoid sources and fed to fish twice a day at 3% of their body weight. Five experimental diets were used and four of them were with β catroenoid sources and one was common control diet (i.e., devoid of β carotene). The prominence of orange colour increased in the fish which was fed with diets enriched with natural beta carotenoid sources. The orange colour was more in fish fed with turmeric. From this study, it could be concluded that the easily available natural β -carotene sources incorporated feed can be used as a colour enhancer of fish at lower cost.*

Keywords: β -carotene sources

I. INTRODUCTION

Ornamental aquaculture, a fast developing 'Aquatic Rainbow Sector' in India demands true innovations and implementation of advanced technologies to promote itself to the next level to compete in the international market. Ornamental fishes are characterized by a wide diversity of colours and colour patterns and success in the ornamental fish trade is very much dependent on the vibrant colour of fish. Colour is one of the major factors, which determines the price of aquarium fish in the world market.

Fishes are coloured in nature often show faded colouration under intensive culture conditions. Fish like other animals do not synthesize carotenoid and depend on dietary carotenoid content for colouration. Hence, a direct relationship between carotenoids and pigmentation exists in them. If enhancement of colouration can be done by administrating pigment enriched feed, it will definitely improve the quality and cost of the fish because it contains the colourenhancing pigments like carotenoids, xanthophylls etc. (Ramamoorthy,2010). Ornamental fishes are nowadays rapidly gaining importance because of their aesthetic value and also due to their immense commercial value in the export trade world over. Feed additives facilitate feed ingestion and consumer acceptance of the product. Carotenoids are the primary source of the pigmentation on the skin of fishes. In natural environment, the fishes meet their carotenoid requirements by ingesting aquatic plants or through their food chains. But, fishes cannot synthesize carotenoid de novo. Carotenoids are responsible for many of the red, orange and yellow hues of plant legumes,fruits and flowers. The colour enhancing diets should contain additional natural pigments to enhance the colour of ornamental fishes (Ahilan,2008).

II. PIGMENTS AND PATTERNS OF COLOURATION

Color patches in fish, reptiles, and amphibians are multilayer, multicomponent signals (Grether *et al.*,2004). The basic unit of color in these taxa is the dermal chromatophore, which is generally composed of three cell layers: the xanthophore (contains carotenoid and pteridine pigments), the iridophore (reflects color structurally), and the melanophore (contains melanin). Pigments are compounds that absorb particular wavelengths of light and can contribute to the color of biological patches. Two classes of pigments that are commonly studied in fish are carotenoids (usually yellows, orange, and red) and melanin (browns, blacks, and grays); they are deposited in the integument. Short wavelength (blue and violet) and silvery coloration in vertebrates are almost always structurally based, the result of selective light scatter owing to variable refraction within the integument, and only one blue pigment has been described

in fish (Bagnara *et al.*, 2007). This type of structural coloration, which occurs in the iridophores, is undoubtedly an important component of animal color patterns.

Pigments (or their precursors) cannot be synthesized and must be obtained from the diet. In many cases, the amount of pigment expressed is dependent on an individual's foraging success and physiological efficiency. The level of coloration an individual expresses can therefore indicate their quality as a potential mate or competitor, or of their escape potential in the eyes of predators. In addition to this variation in expression based on diet, pigments can be expressed differentially depending on ontogeny (juveniles can possess color patterns that are very different from adult conspecifics), seasonally, and even ephemerally (Anna *et al.*, 2008).

Pigment-based color patterns can change through direct regulation of pigment containing cells, or indirectly through adjustment of the light interacting with the pigment through regulation of iridophores. By changing the structure of the iridophore, the paradise whiptail (*Pentapodusparadiseus*) can change which wavelengths of light are reflected back through the xanthophore, turning from blue to red in a fraction of a second. A more common mechanism of color change in fish involves changes in the intensity and or area of black, brown, or gray melanic colors through melanocyte-stimulating hormone (MSH)-induced stimulation of melanin granule dispersion in the melanocytes (Metz *et al.*, 2006); the size and shape of melanin-based pattern elements can be changed in seconds, with the diameter of completely dispersed and completely aggregated cells often differing by several fold. The behavior of pigment-containing cells is controlled by both the nervous and endocrine systems, with more rapid changes typically reflecting neural control (Fujii, 2000). This flexibility in the expression of color patterns means that coloration can go beyond signaling static properties such as species identity, sex, or developmental stage, but can also indicate an individual's current quality and motivational state.

Carotenoids are synthesized by photosynthetic algae and plants, fungi and bacteria while other organisms must obtain the necessary carotenoids either directly from the diet, or modify the dietary carotenoid precursors through metabolic reactions. In autotrophs, carotenoids act mainly as photoprotectors related to the photosynthetic process, while other organisms in the food web benefit from their protection against ultraviolet (UV) radiation, their antioxidant properties against reactive oxygen species (ROS) and free radicals (Caramujo *et al.*, 2012), their role in vision, as precursors of transcription regulators, and in the immune system (Anbazhane *et al.*, 2014). In vertebrates, body coloration plays important roles in camouflage, communication, ecological interactions and speciation.

Colouration in fishes provides them power of adjustment with surroundings and also enables them to survive. The colour of fishes is used for concealment, communication, camouflage, sexual recognition and advertisement, warning or threat. Colouration also has taxonomic value. Different colour pattern in fish is often considered as character for distinction among species and subspecies. The specific pattern due to exact distribution of chromatophores is under genetic control.

In the present study, natural carotenoid sources from beetroot, carrot, spinach and turmeric were used instead of synthetic astaxanthin. The use of these carotenoids not only enhance the colour of fish, but also reduce the cost of feed preparation.

III. MATERIAL AND METHODS

EXPERIMENTAL FISH

Experimental ornamental fishes, Koi carp (*Cyprinus carpio*) and Molly fish (*Poecilia sphenops*) were procured from local ornamental fish trader and acclimatized to laboratory condition in aquaria. The experiment was run with 18 fishes of each type (Molly fish, Carp fry, Carp fingerlings). The initial body length and weight of Molly fish ranged between 3.6 cm to 4.8 cm and 0.42 gm to 0.51 gm respectively. The initial body length and weight of Carp fry ranged between 5.5 cm to 6.7 cm and 1.5 gm to 2.6 gm respectively. The fishes were conditioned and fed with control diet (devoid of beta carotene source) for one week to equalize their body carotenoid content in indoor conditions. The water exchange and aeration were given sufficiently. Before administering the experimental diet, the fishes were grouped to five aquaria, in which each aquaria has three fishes of each species.



Koi carp

Molly fish

EXPERIMENTAL FEED PREPARATION

The experimental feed were prepared with basic ingredients such as wheat flour, rice flour, wheat bran flour, fish flour and groundnut oilcake. All ingredients were split into five equal parts. The experimental diets were prepared using carrot (*Daucus carota*), spinach (*Spinacia oleracea*), beetroot (*Beta vulgaris*) and turmeric (*Curcuma longa*) mixed in basal diet. For this purpose, the beta carotenoid sources are added in diet, by replacing rice flour and wheat bran. All the locally available ingredients were collected and mixed well according to the proportions (Table 1). It was then pelletized and dried under direct sunlight (Minalet *al.*, 2013).

TABLE 1: FORMULATION OF EXPERIMENTAL FEED (Ingredients in gm/100gm)

INGREDIENTS	COMPOSITION (%)				
	T0	T1	T2	T3	T4
Wheat bran	25	15	15	15	15
Fish meal	25	25	25	25	25
Oil cake	25	25	25	25	25
Rice flour	15	10	10	10	10
Wheat flour	10	10	10	10	10
Beetroot	-	15	-	-	-
Carrot	-	-	15	-	-
Spinach	-	-	-	15	-
Turmeric	-	-	-	-	15
Total	100	100	100	100	100



Beta carotenoid sources Grinding Mixing with basal ingredients



Pelletization and drying of feed Dried feed

FEEDING EXPERIMENTAL DIET

This experiment was carried out for one control and five treatments in five experimental aquaria in indoor system. All the fish were fed at the rate of 3% of their body weight once a day with the experimental diet. The experiment was carried out for 35 days. The water quality in aquaria was maintained by aeration, removal of fecal matter and replacement of at least 20% of water every day.

CAROTENOID ANALYSIS

PIGMENT EXTRACTION FROM FISH TISSUE

Pigments were extracted from fillet and waste of fish. The fillets included the flesh of the fish and the waste included the tail, scales, and fins. 5gm of entire fish body tissue (fillet) and waste (scales, fins, and tail) were collected and homogenized with 20 ml ethanol in a blender for 5 minutes. The commercial ethanol was used since it enables a better extraction with less cost and it is a less toxic solvent (Santos *et al.*, 2012).

CENTRIFUGE

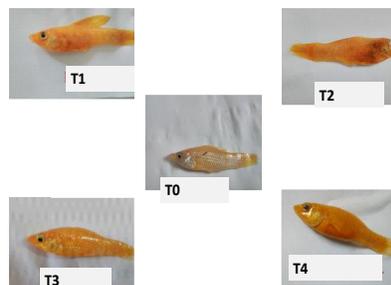
Each crude extract of fillets and wastes were then centrifuged for 3800 rpm for 10 minutes separately and the supernatant (liquid phase) were collected in test tubes.

COLORIMETER

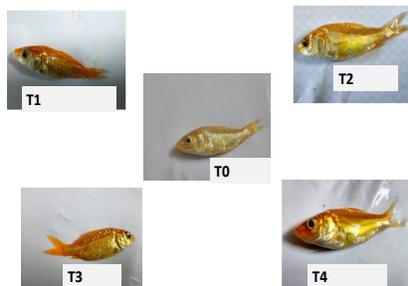
The carotenoid content was determined by the absorbance of each ethanolic extract which were measured at 470 nm using calorimeter. The 'blank' reference corresponding to 1 ml ethanol (Schiedt and Liaen-Jensen, 1995).

OBSERVATION AND RESULT

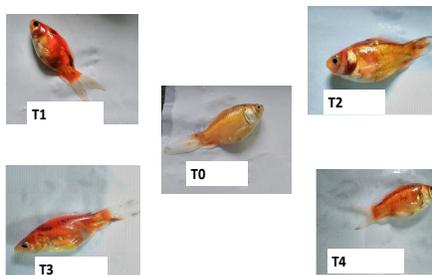
After 35 days of feeding, enhancement of both colour and size of fishes were observed. By comparing the OD value, the amount of carotenoid was higher in turmeric fed fishes (0.44 in Molly, 0.45 in Carp). By visual analysis of colour, beetroot fed carp fingerlings had a more reddish-orange colour. Spinach and carrot fed fishes developed more yellow colour.



External morphology of Molly fish (*Poeciliasphenops*) after treated with experimental feeds (T0 - Control, T1- Beetroot, T2- Carrot, T3- Spinach and T4- Turmeric).



External morphology of Carp fry (*Cyprinus carpio*) after treated with experimental feeds (T0-Control, T1- Beetroot, T2- Carrot, T3- Spinach and T4-Turmeric).



External morphology of carp fingerlings (*Cyprinus carpio*) after treated with experimental feeds (T0-Control, T1- Beetroot, T2-Carrot, T3-Spinach and T4-Turmeric).

Table 2: OD values obtained after colorimetry

SR. No.	Fish	Carotenoid source	O.D.VALUE	
			WASTE	FILLET
1	Molly	Beetroot	0.24	0.03
2	Carp fry	Beetroot	0.33	0.17
3	Carp fingerlings	Beetroot	0.38	0.28
4	Molly	Spinach	0.10	0.05
5	Carp fry	Spinach	0.42	0.31
6	Carp fingerlings	Spinach	0.31	0.21
7	Molly	Turmeric	0.44	0.06
8	Carp fry	Turmeric	0.20	0.27
9	Carp fingerlings	Turmeric	0.45	0.16
10	Molly	Carrot	0.20	0.17
11	Carp fry	Carrot	0.38	0.20
12	Carp fingerlings	Carrot	0.40	0.22
13	Molly	Control	0.03	0.04
14	Carp fry	Control	0.13	0.01
15	Carp fingerlings	Control	0.16	0.04

The present work was carried out to determine whether carotenoids of beetroot, carrot, spinach and turmeric could induce pigmentation to make Molly fish and Koi carp more colourful. By the end of feeding period (35 days), a marked increase in colour was observed in experimental fishes, comparing to the control fed fishes. All the four plant additives were found to be an effective colour enhancer at a cheaper price. An ornamental fish does not have the capacity of synthesizing the carotenoids itself, hence it must be supplied through the diet.

The calorimetric analysis was made for the measurement of colour change in skin of fishes fed with different plant carotenoids. The data regarding the colour(ODvalue) of both fish species fed with four different botanical additives are presented in Table 2. The greatest colour enhancement was observed in turmeric fed fishes. By the visual analysis, beetroot fed fishes developed more reddish orange colour. Turmeric and carrot fed fishes developed more yellow colour. By comparing with other plant additives spinach had the least colour enhancement among them. This is also proved by calorimeter results. According to the OD value, a higher colour enhancement was observed in scales than fillet.

According to the colorimeter results, turmeric(T4) proved to be the best plant additive in fish feed. The turmeric enhanced the golden yellow colour of molly fish(OD value=0.44), carp fry(OD value=0.42) and carp fingerlings(0.45). Carrot(T2) enhanced the orange yellow colouration in fishes. The OD values obtained were 0.38 for molly fish, 0.38 for carp and 0.40 for carp fingerlings.

By visual analysis, the beetroot(T1) fed fishes had the highest reddish orange colour. The OD value obtained was 0.24 for molly, 0.33 for carp fry and 0.38 for carp fingerlings. Spinach enhanced the yellow colouration of fishes. The OD value obtained was 0.28 for molly, 0.42 for carp fry and 0.31 for carp fingerlings. The OD value for control fed fishes indicates that all the plant carotenoids enhanced the colour of both fishes significantly. The OD value for control fed fishes were 0.16 for molly fish, 0.20 for carp fry and 0.16 for carp fingerlings. The fishes received higher carotenoids deposition in the skin in all the treatment groups compared to control which clearly indicates that dietary carotenoid supplementation leads to the carotenoid deposition in the fishes and consequently increase colouration.

IV. CONCLUSION

From the present experiment it could be concluded that dietary supplementation of beetroot, carrot, spinach and turmeric at 15% of diet can be used for enhancing the orange colouration in Koi carp (*Cyprinus carpio*) and Molly fish (*Poecilia sphenops*). The use of turmeric as a colour enhancer laid out the best colour. The antiseptic, antifungal and antibacterial activity of turmeric should be also mentioned. Since synthetic carotenoids are pricey, cheap and readily accessible natural carotenoid sources such as beetroot, carrot, spinach and turmeric can be incorporated into the diet to enhance better colouration in fishes. This will help ornamental fish sellers to get superior cost in trading of this fish. Synthetic carotenoids have several limitations, firstly, synthetic processes have only specific carotenoids such as beta carotene; moreover they involve petrochemical solvents as well as complex organic solvents causing residual problems. The use of natural carotenoids instead of synthetic carotenoids do not results in such side effects and promotes the healthy growth of fishes.

REFERENCES

- [1]. Ahilan B., Jegan K., Felix N. and Ravaneshwaran K. (2008). Influence of botanical additives on the growth and colouration of adult goldfish, *Carassius auratus* (Linnaeus), Tamil Nadu. J. Vet. & Ani. Sci, 4(4):129-134.
- [2]. Anna C. Price, Cameron J. Weadick, Janet Shim, and Helen Rodd (2008). Pigments, patterns and fish behavior. Zebrafish, Volume 5: Number 4.
- [3]. Anbazhahan S. M., Mari L.S., Yogeshwari G., Jagruthi C., Thirumurugan R. and Arockiaraj, J. (2014). Immune response and disease resistance of carotenoids supplementation diet in *Cyprinus carpio* against *Aeromonas hydrophila*. Fish Shellfish Immunol, 40:9-13.
- [4]. Bagnara J.T., Fernandez P.J. and Fujii R. (2007). On the blue coloration of vertebrates. Pig Cell Res, 20:14-26.
- [5]. Caramujo, M.J., de Carvalho C. C., Silva S. J. and Carman K. R. (2012). Dietary carotenoids regulate astaxanthin content of copepods and modulate their susceptibility to UV light and copper toxicity. Mar. Drugs 10, 998-1018.
- [6]. Fujii R. (2000). The regulation of motile activity in fish chromatophores. Pigment Cell Res 13:300-319.

- [7]. Grether G.F., Kasahara S., Kolluru G.R., Cooper E.L.(2004). Sexspecific effects of carotenoid intake on the immunological response to allografts in guppies (*Poeciliareticulata*). Proc. R. Soc. B.,271:45–10.
- [8]. Metz J.R., Peters J.M.J., Flik G. (2006). Molecular biology and physiology of the melanocortin system in fish: a review. Gen Comp Endocrinol ,148:150–162.
- [9]. Minal S.Wagde, Subodh K. Sharma, Bhanu K. Sharma, Amrita P.Shivani and Naresh R.Keer. (2013). Effect of natural β -carotene from-carrot (*Daucuscarota*) and Spinach (*Spinaciaoleracea*) on colouration of an ornamental fish - swordtail (*Xiphophorushellerii*). Journal of Entomology and Zoology Studies , 6(6): 699-705.
- [10]. Ramamoorthy K., Bhuvaneswari S., Sankar G. and Sakkaravarthi K. (2010).Proximate composition and carotenoid content of natural carotenoid sources and its colour enhancement on marine ornamental fish Amphiprionocellaris (Cuveir 1980). World J of Fish & Mar. Sci, 2(6):545-550.
- [11]. Santos Lisia Maria G.dos, RenataJ.Medeiros, AlineS.Freire, Ricardo E.Santelli, Ana Maria C.B.Braga, Thomas M.Krauss&Silvana do C.Jacob (2012). Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. Food Control, 23: 535-541.
- [12]. Schiedt K. and Liaaen-Jensen S. (1995). Isolation and Analysis. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids. Vol. 1A: Isolation and analysis. Birkh ¨ auserVerlag, Basel, pp 81–108.