IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 2 Ver. I (Mar.- Apr.2016), PP 52-58
www.iosrjournals.org

Restoration Of Pigmentation In Trichogaster Fasciata

Susmita Dey[1] and Umesh C. Goswami[2]
[1]Department of Zoology, Fish Biology and Fishery Science,
Centre for Aquaculture and aquatic Resources Monitoring Centre, Biodiversity and Bioinformatics Centre.,
[2]DST-FIST (Govt. of India) & UGC-SAP Sponsored Department
Gauhati University, Guwahati-781014, Assam, India.

Abstract: *Fishes reared in aquarium or in other containers show gradual loss of pigmentation. This is due to the lack of specific pigments molecules. T.fasciata loss their pigmements from 7 days onwards and become dull after 3rd weeks.Synthetic carotenoids such as beta-carotene and lutein has been added along with fish feed, fish meal (30): rice bran (35): mustard oil cake (35) . The carotenoids added feeds are administered and the fish regain colouration from 2nd week.As the use of synthetic carotenoids are very costly for entrepreneurs and farmers, hence use of natural products / materials of both plant and animal origin such as marigold flower petals (Tagetes minuta), gulmohor (Delonix regia) and algal powder collected from algal blooms. The pigments are isolated from the dried samples and added along with the feed and fed to the fish. The fish starts the regeneration of pigment from 2nd weeks. However the efficiency of gulmohar and deep coloured marigold petals are more effective.The feed additives prepared from animal matters such as dried cruastaceans carapace, exoskeleton of freshwater prawns, and live chironomids larvae showed most effective restoration of pigments.*

Key words: *pigmentation , carotenoids,feed additive,restoration.*

I. Introduction

Trichogaster fasciata inhabit in wetlands, river lets, and ponds (as weed fish) and have both ornamental and commercial values as food fish. The fish live in the stagnant water of ponds, beels, channels, roadside ditches etc. The fish may attain a length of 10 cm. The colour of the fish is reddish brown with a number of bluish-green cross stripes present on the sides.

Trichogaster fasciata is an omnivorous fish and so a variety of foods have been found in its gut. The common food items are algae, higher aquatic plants, insects' larvae and crustaceans. Diatoms, desmids and unicellular algae are recorded highest amount in January. Multicellular algae like *Spirogyra*, *Ulothrix* and *Chara* are also recovered from the gut contents. Higher aquatic plants like *Hydrilla*, *Vallisnaria*, *Salvinia* and Potamogetons formed important food items of *T. fasciata*. Aquatic insects' larvae are dominant food item in the dietary of *T. fasciata*. Most important are lavae of dipterous insects (Culex) and dragonflies. Crustaceans like Daphnia, Bosmia etc. and statoblasts of freshwater bryozoans are also found in the stomachof *T. fasciatus*. It is very well known for its larvicidal nature. It has been found that ornamental fishes after being reared in the aquarium for a long time, there is always fading of colouration. Although regular aquarium feed is being fed to the fishes, yet the fish ultimately lose their brilliant colouration from their skin and fins.Considering the loss of pigments, the proposed investigation has been designed to develop a feed or diet with the inclusion of carotenoids such as beta-carotene, which could act or restore the colouration of the fish.

II. Materials and Methods

Gut content analysis for food preference

After taking the morphometric measurement such as total length and total weight of the fish, the stomachs of the fish were dissected out for analysis. Some live samples were dissected out for food analysis just after collection and the gut content was preserved in 70% ethyl alchol or in diethyl ether and later examine under microscope for gross analysis and identification of different food items(Needham and Needham,1990). Moreover, the fishes were collected during the early hours of the morning to minimize the digestion of food in the gut. The food item was subjected to high degree of mutilation due to the action of strong digestive juices . Therefore the gut contents could only be identified up to higher taxonomic groups**.** Feeding intensity was also assessed by classifying the stomach as full, ¾ full, ½ full, ¼ full empty categories.The occurrence of food item were expressed in percentage value as described earlier (Das and Moitra, 1963; Natarajan *et al*., 1975 Nautiyal and Lal , 1985).

Identification of food items of the fish:

The food preference or food items of the respective fishes are identified from the stomach content of the fish(Needham and Needham,1990).

Feed additive for restoration of colouration:

Different feed additive as shown below were procured and added with the fish feed as mentioned above. The additives used in the present experiments are:

I. Use of synthetic carotenoids in restoration of colour- pigments:

In the present studies 2 carotenoids, such as beta carotene and lutein have been added along with the fish feed. The aqueous suspension of crystalline beta-carotene and lutein has been prepared (Goswami,1984) and added along with the fish feed (30- fish meal: 35 rice bran : 35 mustard oil cake) 10mg / kg. The carotenoids added diets were fed to the fish @10% of the body weight.

II. Addition of high carotenoids content materials as feed additive:

It has been found that although synthetic carotenoids are very effective in restoration of pigment, yet the use of synthetic carotenoids as feed additive is a costly affair and either entrepreneurs or farmers cannot afford it for long term practice. Considering this few plant and amimal matters or products are tried to used as feed additive for restoration of pigments. The following materials are tried along with prepared fish feed.

Plant matters

a) dried marigold petals (both deep and light coloured flowers)*Tagetes minuta*
b) dried anther of Gul Mohor *Delonix regia*
c) algal powder. Freshwater algal bloom are collected and dried in room temperature for7 days and the dried mass of the same is preserved
d)

Animal matters

a. Live feed chironomids larvae
b. Carapace powder of freshwater crustaceans, uses dry powder of shell of freshwater prawn and carapace powder of freshwater crabs.

The feed additives selected as mentioned are mixed with the prepared fish feed and fed to the fish (10% of the body weight of the fish). The re-ocurrence of colourations are measured after 15 and 25 days. The fishes are reared in aquarium (60×40×30cm) and feed.

The experimental fish *Trichogaster fasciata* is collected from the fishermen catch from Chandrapur and Marigoan.

Fish:

The experimental fish are reared rearing in aquarium (90×45×30cm, n=3) 20 fishes are kept in each experiment is set in similar condition in 3 aquarium. The fish were reared in the diet fish meal: rice bran: mustard oil cake (30:35:35). (Goswami 1984, 2007). The restoration of the pigments that are conducted are as shown below.

Table1. Seasonal variation of food of *Trichogaster fasciata*

Food Items	Pre-monsoon	Monsoon	Retreating monsoon	Winter
Phytoplanktons	25%	27%	25%	25%
Zooplanktons	45%	40%	25%	27%
Aquatic plants	25%	25%	40%	30%
Molluscs	NIL	2%	NIL	NIL
Sand & Mud	3%	3%	NIL	8%
UnidentifiedMatter	2%	3%	10%	10%

Isolation of initial pigments / carotenoids:

The lipid extract of the whole fish (n=5) was conducted and their amount of total carotenoids as well as individual carotenoids were identified (Table 2). Further some experiments were repeated after 7 , 15 and 21 days in order to examine any loss of pigment from their body during rearing in the aquarium (Table 2).

Table 2.Estimation of total carotenoids from the initials rearing conditions and later in 7,15,25 days.

Expt. And No. of fish	Initial carotenoids	Isolation of total carotenoids in different days			
	7	15	25		
1.	5	155±2.5	145(±1.5)	110±1.5	90±45
2.	5	162±0.5	140(±2.0)	115±2.0	85±38
3.	5	157(±1.5)	138(±2.5)	187±3.5	88±50

It has been found that the fish loss the carotenoids concentration from its initial conditions in successive periods. The diet require some additive which could be used as restoration.

The individual carotenoids found in the initial rearing fishes and identified and their amount is estimated. The different carotenoids present are shown Table 3.

Table 3. Amount of carotenoid found in the initial rearing fishes

Astaxanthin	20±3.5
β-carotene	25±1.5
Lutein	30±3.0
Cryptoxanthin	22±2.0
Others	23

Considering the amount of carotenoids present and their composition the selected additives are examined and the same are added in their feeds. In Table 3, the total carotenoids and their composition are measured.

Addition of beta-carotene in the feed:

Aqueous suspension of beta-carotene as prepared are mixed with fish feed (10 mg / 1 kg feed) and the same are fed (10 % body weight fish) in the aquarium . The feeding was continued for 25 days and every 7 days interval the amount of carotenoids deposited are estimated (Table 4).

Table 4. Accumulation of pigments after feeding β-carotene added diet

No of days of β –carotene added different administered	Amount of total carotenoids (µg/1000g)	Amount of Retinoids (µg/1000g)	
		Retinol	Dehydro retinol
0	32±2.5	20.0±0.5	30.2±1.2
7	65.0±1.2	35.5±1.5	42±1.5
15	155±2.0	55.2±1.2	68±1.5
25	210±3.5	98.5±1.5	111±3.5

Addition of lutein in the feed:

Crystaline lutein 10mg/1kg diet was added. The aquous suspension of lutein was mixed with the prepared fish fed and the same are fed (10% of the body weight of the fish) to the fish as shown in the Table (5) and the amount of pigments isolated are estimated as shown in the following Table (5).

Table 5. Accumulation of pigments after feeding lutein added diet

No of days of lutein added different administered	Amount of total Carotenoids (µg/1000g)	Amount of retinoid (µg/1000g)	
		Retinol	Dehydroretinol
0	30±1.5	15±1.5	42±3.5
7	55±2.5	28±2.5	59±2.5
15	130±3.5	45±3.0	80±3.0
25	185±15	85±5.0	120±4.0

From the above two experiments as shown in Table 4,5,it has been found that the fish accumulated the carotenoids considerably from 15 days onwards. Further there is deposition of both retinol and dehydroretinol. β-carotene feed fish showed more retinol than the lutein administered fish.

The fish lost pigmentation with 30-32 µg/1000g and almost twice the amount **(**65µg/1000g) and the same fish group showed 210µg/1000g after 25 days of feeding β-carotene added diets. The same way lutein added diets generated the pigmentation in fish 30-35 μg/100g upto 185 μg/100g within 25 days of feeding the same.

Table 6. Amount of total carotenoids and their composition different feeds.

Feed type Total carotenoid mg / 100g. carotenoids		% composition of individual
Marigold(100g)		β-carotene 30, Lutein 40
(Deep yellow)	355	Cryptoxanthin 20, others 10
Marigold(150g)	535	β-carotene 27, Lutein 43
(Deep yellow)		cryptoxanthin 18, others 12
Marigold(100g)	235	β-carotene 25, Lutein 20
(light yellow) 15,others15		Cryptoxanthin20, zeaxanthin
Marigold(150g)	360	β-carotene 22, Lutein 42
(light yellow) 15,others18		Cryptoxanthin18, zeaxanthin
Anther of		
Gul Mohor (100g)	475	β-carotene 30, Lutein 20
Cryptoxanthin30, zeaxanthin 10, others 12		
Anther of		
Gul Mohor (200g)	970	β-carotene 32, Lutein 18
Cryptoxanthin25,zeaxanthin15,others10		
Exoskeletal powder of 10	650	Astaxanthin 65, β-carotene 10, Lutein
Prawn(100g)		Cryptoxanthin 5, others 10
Exoskeletal powder 12	790	Astaxanthin 65, β-carotene 12, Lutein
of prawn (120g)		Cryptoxanthin 5, others 6
Carapace powder 15	575	Astaxanthin 55, β-carotene 20, Lutein
of crab (100g)		Cryptoxanthin 5, others 5
Carapace powder of	295	Astaxanthin 55, β-carotene 22,
crab (50g)		Lutein10Cryptoxanthin 7, others 6
Chironomids	850	Astaxanthin 80, β-carotene 5, Lutein 2
(50g)		others 13
Chironomids 100g	200/100	Astaxanthin 78, β-carotene 5, Lutein 5
Cryptoxanthin 2, others 10		

From the above analysis of various proposed feed additive, an idea has been developed about its application. Considering the same the feed additives are added along with the fish meal: rice bran: muscles cake and the same are administrated in various combination to the fish. The details of the feeding strategies are shown below Table 6.

Table 7.Utilization of different plant and animal matters in restoration of pigments in *T. fasciata*

Feed Additive	No of fish	Amount carotenoids Added in the mg/1kg feed	Initial total carotenoids -FA	Carotenoids accumulation during different days 7d + FA	15d + FA	25 d + FA
Marigold	10	10	38±1.5	45±2.3	68±3.5	90±1.5
Deep colour	10	15	48±2.0	52±3.5	73±2.4	100±3.8
	10	20	35±1.2	62±2.5	80±3.0	110±4.1
Marigold	10	10	35±2.0	42±3.0	50±1.5	65±1.5
Light colour	10	15	38±3.5	50±0.5	58±0.5	70±1.2
	10	20	41±3.0	55±3.5	63±1.0	75±2.5
Gulmohor	10	10	35±1.0	48±1.5	70±2.5	85±3.2
	10	15	38±2.0	58±2.5	75±3.2	90±1.5
	10	20	40±1.5	69±4.0	80±0.5	98±1.5
Algal Powder	10	10	35±0.5	40±2.5	48±1.2	64±2.3

*The initial total carotenoids are expressed from the fish which lost their pigmentation after keeping in general diets which lacks the presence of carotenoids The values are sratistically significance ($P < 0.05$)

The different diet additive showed the deposition of various amount of carotenoids in the reared fish. However there is variation and it has been for the light colour dried marigold flower petal added diet showed less effection in high dose also, whereas the deep coloured marigold petal showed the deposition to some extent, which is higher than the light one. It is most effective from the chironomid and crustaceans carapace powder added feed. This animals products are most effective than the plant products, where 10% per 100 g body additive are enough to restore normal condition of the pigmentation.

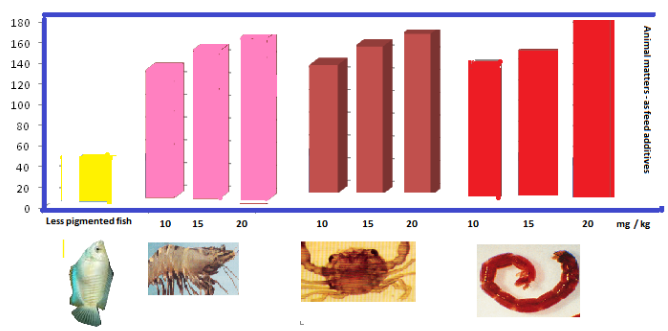

Fig.19:Carotenoid variation in T.fasciata on adding animal matters in feed

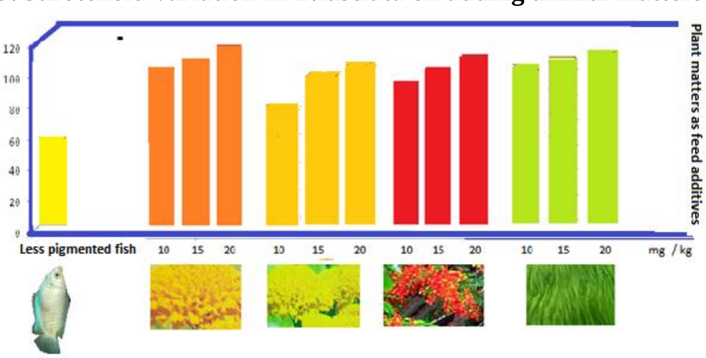

Fig.20:Carotenoid variation in T.fasciata on adding plant matters in feed

III. Discussion

With the proper knowledge about the diet and pigment molecules responsible for bright colours like carotenoids, melanin etc, fishes can be cultivated. Food varies greatly within the species of same family,so food and feeding habits plays an important role in morphological, as well as physiological study of a certain species.It not only differ among species but also within same species with respect to seasonal changes. The food of the fish and the feeding habit has a direct relationship on the development of the colouration.Goodwin(1984) has reported the carotenoids composition of the different aquatic and terrestrial plants and animals and their pigmentation with reference to the presence of carotenoid molecule.Carotenoids which act as the precursors of both forms of vitamin A provide the bright coloration of fishes, which has attracted the attention of biologists and aqua culturists. This lipid class molecule furnishes the red, orange, yellow, pink etc. colouration of the fish (Goodwin, 1984; Pfander, 1992; Gross and Budowski, 1965; Goswami, 2006; 2007).The different diet additive showed the deposition of various amount of carotenoids in the reared fish. However there is variation and it has been for the light colour dried marigold flower petal added diet showed less effection in high dose also, where as the deep coloured marigold petal showed the deposition to some extent, which is higher than the light one. It is most effective from the chironomid and crustaceans carapace powder added feed. This animals products are most effective than the plant products, where 10% per 100 g body additive are enough to restore normal condition of the pigmentation.The fixation of carotenoids in muscle, integument or gonads also depends on many factors. Sometimes a relationship between fish size and pigment level have been established, however there exist large individual variation. In the same way, the age of fish also influence on the pigmentation of fish. On attaining the sexual maturity conspicuous colouration of the fishes are developed, especially during the breeding season. According to Guthrie(1986), a visual recognition of the opposite sex and its breeding condition is important in courtship behavior. The bright red colour of male has dual purpose,as a signal to the female that the male is ready to spawn and as a signal of aggresion and territorial defence to other males(Frischknecht,1993). The sexual dimorphism also occurs according to the colour pattern of the fishes. In *Trichogaster fasciata*, the male fish become more attractive with alternating red and blue bands whereas females are pale and more silvery in colour. Moreover by restoration of these pigments one can cultivate fish which will make them more attractive and increases their value in market. Synthetic carotenoids are very effective in restoration of pigment,but the use of synthetic carotenoids as feed additive is a costly affair and either entrepreneurs or farmers cannot afford it for long term practice. Considering this few plant and animal matters or products are tried to used as feed additive for restoration of pigment.

In the present experiment it has been observed that the restoration of the pigments can be made after feeding carotenoids regularly.However the lack of carotenoids added diet further resulted in loss of the pigmentation.The pigmentation is a complex phenomenon governed by several factors and one of the most important is the influence of sexual maturity.Several fishes attain brilliant colouration during the spawning season . The carotenoids provide the ornamental characteristics of the fish. The fish *T. fasciata* is a high valued ornamental fish. Male fish attain more carotenoids. The female ovaries contain considerable amount of carotenoids.Sexual maturation in fish involves significant changes in the metabolism of carotenoids and there is always mobilization of carotenoids from skin,fins,liver etc to the gonads(especially female)However some fish retain the pigmentation for purposes of attraction to the opposite sex.It is generally found that fish collected from the wetlands that feed on natural avaiable food contain more deposition of carotenoids than the fish reared in the aquariums which are feed on artificial diet. A part of the experiment is an effort made to restore pigmentation using carotenoid added diet.

The different diet additive showed the deposition of various amount of carotenoids in the reared fish. However there is variation and it has been for the light colour dried marigold flower petal added diet showed less effection in high dose also, where as the deep coloured marigold petal showed the deposition to some extent, which is higher than the light one. It is most effective from the chironomid and crustaceans carapace powder added feed. This animals products are most effective than the plant products, where 10% per 100 g body additive are enough to restore normal condition of the pigmentation.The preponderance of dehydroretinol over retinol in freshwater fish has been shown earlier(Goswami,1984).Considering the loss of carotenoid after feeding carotenoid free diets,it can be concluded that there should be a constant addition of such feed ingredient in the diets of ornamental fish.

IV. Conclusion

The efficiency of gulmohar and deep coloured marigold petals are effective but the feed additives prepared from animal matters such as dried crustaceans carapace, exoskeleton of freshwater prawns, and live chironomids larvae showed most effective restoration of pigments.

Acknowledgement

Authors are grateful to the department of zoology Gauhati University for allowing to perform the experiment in their laboratory.

Reference

[1]. Das, S.M. and Moitra, S.K.,(1963). Studies on the food and feeding habits of some freshwater fishes of India, Part IV. A review on the food and feeding habits with general conclusion. Ichthyologica, 2 (1 and 2): 107-115.
[2]. Frischknecht,M.,(1993). The breeding colouration of male three-spined stricklebacks (Gasterosteus aculeatus) as an indicator of energy investment in vigour.Evolutionary Ecology,7:439-450.
[3]. Goodwin , T.W., (1984). The Biochemistry of Carotenoids: Animals, Vo. II Chapman and Hall, London.
[4]. Goswami, U.C.; (1984). Metabolism of cryptoxanthin in freshwater fish. *Brit J Nutr* 52:575-582.
[5]. Goswami, U.C.; (2006). Occurrence of diversedretinoid molecules in freshwaterpiscian diversity. *Fish Research* . 4:157-158.
[6]. Goswami, U.C.; (2007). Vitamin A in freshwater fish. Presidential address of the section Animal, Veterinary and Fisheries, 94 Session of Indian Science Congress. pp 1-26
[7]. Gross,J.; Budowaski, P.; (1966). Conversion of carotenoids into vitamin A and A2 in two species of freshwater fishes. *Biochem.J*. 101:747
[8]. Guthrie, D.M., (1986). Role of vision in fish behavior. In: Pitcher, T.J. (Editor), The behavior of teleost fishes. Croom Gelm, London, pp. 75-113.
[9]. Needham, A.E., (1990). The significance of zoochromes. Springer, Berlin. 429 pp.
[10]. Natarajan, A.V., Ramakrishnaih, M. and Khan , M.A., (1975). The food spectrum of trash fishes in relation to major carp in Konar and Tilaiya Reservoir (Bihar). J. Inland Fish. Soc. India, 6:65-75.
[11]. Nautiyal,P. and Lal, M.S., (1985). Food and feeding habits of Garhwal Himalaya mahseer in relation to certain aboitic factors. Matsya. 11:31-35.
[12]. Pfander,H. (1992) Carotenoids: an overview. Math.. Enzymol., 213:3-13.