EFFECT OF CAROTENOID SUPPLEMENTED DIET ON BODY COLOURATION AND GROWTH OF THE ORNAMENTAL FISH *PUNTIUS TETRAZONA*

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ABSTRACT

The impact of diet supplemented with five plant materials of varying carotenoid content, namely leaves of *Amaranthus tricolor* and petals of *Chrysanthemum indicum*, *Hibiscus rosasinensis*, *Ixora coccinea* and *Tagetes erecta*, on food consumption, growth rate and body colouration of the ornamental fish *Puntius tetrazona has been* was studied. Feed supplemented with *Hibiscus rosasinensis* petals increased the growth rate with simultaneous increase in biochemical constituents of *P. tetrazona* but better body colouration was obtained with *Tagetes erecta* petal supplemented feed. Improved assimilation efficiency was observed for *Amaranthus tricolor* leaf supplemented feed compared to other supplemented feeds. The results obtained in the current study will be of immense help to those interested in home aquaria and can be considered for future research on ornamental fish feed formulation focussed towards improving the market value that would ultimately increase the commercial trade of ornamental fishes.

Key words

Carotenoids, Colouration, Growth, Petals, *Puntius tetrazona*

INTRODUCTION

Ornamental fish culture in glass tanks is a very common custom around the globe and has become an integral part of modern interior decoration (Oliver, 2001). Ornamental fishes are referred to as "living jewels" due to their beautiful coloration, shape and behavior. Their attractive bright colouration not only helps to relax the mind and body of this hobby-lovers but also serves as a natural stress reliever for heart patients as it helps to decrease the blood pressure and reduce the risks of cardiac ailments. Ornamental fish culture also has immense potential to create substantial job opportunities (Daniel *et al*., 2017). Ornamental fish farming is one among the most valuable industries in recent times. The small size, attractive color, gentle and quite movement and adaptability to live within a confined space in captivity have made ornamental fish culture a flourishing industry.

Pigments are responsible for the wide spectrum of colours in the fishes and colour is one of the major factors, which determine the quality and the market value of aquarium fish in the world market (Gouveia *et al*., 2003). As fishes cannot synthesize their own colouring pigments *de novo*, the colouring agents which are synthesized by some plants, algae and micro organisms, need to be incorporated in their diet (Jhonson and An, 1991). Many of the naturally bright coloured fishes may show fading in colouration due to various factors like constant exposure to sunlight, starvation, chlorine, pH, hardness and turbidity of water, stress, toxicants and pollutants and also low quality of water, poor quality feed, nutrient-deficient live feed etc (Kirwan, 2008; Geens *et al*., 2009).

Carotenoids are a class of 800 natural fat-soluble pigments found principally in plants, algae, photosynthetic bacteria and some non-photosynthetic bacteria and they play a critical role in the photosynthetic process, resistant and protective function, anti-oxidative characteristics and also in reproduction (Bell *et al*., 2000; Anderson, 2000). Various synthetic pigments (β-carotene, canthaxanthin, zeaxanthin and astaxanthin) and natural sources (yeast, bacteria, algae, higher plants and crustacean meal) have been used as dietary supplements to enhance the pigmentation of fish and crustaceans (Kalinnowski *et al*., 2005). Previous studies have shown that *Chlorella vulgaris* is as efficient as synthetic pigments in the pigmentation of *Oncorhynchus mykiss* (Gouveia *et al*., 1996b), *Sparus aurata* (Gouveia *et al*., 2002), *Cyprinus carpio* and *Carassius auratus* (Gouveia *et al*., 2003). Pigments obtained from *Phaffia rhodozyma* (Bon *et al*., 1997), *Agrobacterium aurantiacum* (Yokoyama and Miki, 1995), *Chlorococcum* sp. (Zhang *et al*., 1997), *Haematococcus pluvialis* (Yuan and Chen, 2000), *Chlorella zofingiensis* (Bar *et al*., 1995) and *Chlorella vulgaris* (Gouveia *et al* 1996a) have been used as sources of dietary carotenoids.

MATERIAL AND METHODS

Collection and maintenance of the experimental animal

Active and healthy *Puntius tetrazona* were purchased from a commercial aquarium in Nagercoil and they were transported safely to the laboratory in oxygenated bags. Extreme care was taken during transportation, to avoid injury or damage to the fish. The fish were then acclimatized to the laboratory conditions in plastic troughs with tap water for 10 days.

Preparation of the experimental feed

The required quantities of various ingredients were dried, powered and mixed well. To the experimental (carotenoid supplemented) feeds, 500 mg of each dried powder of the various carotenoid sources (*Amaranthus tricolor* leaves / *Chrysanthemum indicum* petals / *Hibiscus rosasinensis* petals / *Ixora coccinea* petals / *Tagetes erecta* petals) were added separately and mixed well. The mixed content of each feed type was steamed and pressed through a hand pelletizer having a perfected disc. The noodles were dried and broken into pieces of about 1cm. Care was taken, so that the pelleted feeds were free from moisture. Different feed pellets were stored in separate containers and labeled as A_{Cont} (control feed with no plant material), B_{Amar} (feed with *A.tricolor* leaf powder), C_{Chry} (feed with *C. indicum* petal powder),

D_{Hibi} (feed with *H. rosasinensis* petal powder), E_{Ixor} (feed with *I. coccinea* petal powder) and F_{Tage} (feed with *T. erecta* petal powder).

Experimental setup

Active and healthy fishes were selected and divided into six groups, each group with three fish. Each group of fish was introduced into a plastic trough of three litre capacity containing two litre of tap water. Before the animals were released into the experimental troughs, they were weighed in a balance. The animals were fed daily in the morning with weighed quantity of control / supplemented feed. The unconsumed food remaining in the aquarium after the feeding time was collected in separate petridishes and dried. Everyday prior to the changing of water, faecal matter was collected separately from each experimental setup and dried. Extreme care was taken that except for the feeding and water change disturbance, the experimental animals were least disturbed. The experiment was carried out for a period of 28 days. To avoid the interaction of faecal matter in the gut with the feeding parameters the experimental animals were starved for 24 hours, before starting of the experiment and after finishing the experiment (Rajamani and Joeb, 1976).

To estimate the growth of the fish "Sacrifice method" (Maynard and Loosli, 1962) was followed. The scheme of energy balance followed in the current study was the IPB formula of Petrusewicz and Macfadyen (1970). The animals were weighed before and after the experiment separately. Mean body weight (g) was calculated as wet weight of the test animals divided by total number of animals in the aquarium at a time. Food consumption was estimated gravimetrically in terms of the dry weight, subtracting the weight of unconsumed food from that of food offered. Rates of feeding and conversion were calculated by dividing the respective quantities by the product of initial wet weight of the fish and duration (day) of the experiment. Production (or) conversion rate was estimated as the difference between the dry weight of the fish at the commencement and at the termination of the experiment. Gain in weight was calculated as the difference between the wet weight at the beginning of the experiment and that on the day of calculation. Gross conversion efficiency was calculated as the quantum of production to consumption.

Analysis of biochemical parameters

Biochemical analysis was carried out in the tissues of dried fish in both the control and the experimental groups. Standard methods for estimation of protein (Lowry *et al*., 1951), carbohydrate (Seiffer *et al*., 1950) and lipids (Folch *et al*., 1957) were adopted to quantify the biochemical constituents.

Estimation of carotenoids

Spectrophotometric analysis method as applied by Akhtar *et al*. (1999) was used to determine the total carotenoid of the feed and fish. 10mg of the sample was homogenized in 2 ml of acetone containing 100mg of anhydrous sodium sulphate. The samples were left overnight at 4^{o}C and then centrifuged at

5000 rpm for 5 minutes. An extraction coefficient of acetone was taken as 1922 (Erdem *et al*., 2009). Total carotenoid in the sample was calculated by the following expression,

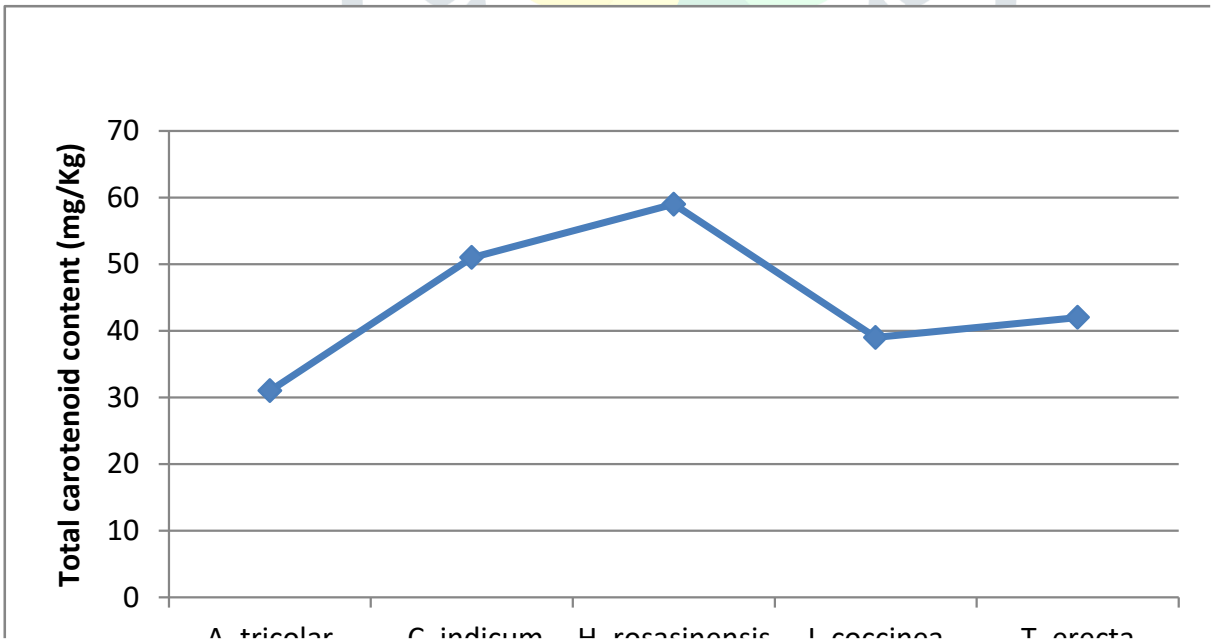
$$\text{Total carotenoid (mg)} = \frac{\text{OD of the sample} \times \text{Vol. of the sample}}{\text{Extinction coefficient of acetone} \times \text{Sample wt.}}$$

RESULTS AND DISCUSSION

Carotenoid content of the plant sources used

Carotenoid content of the five plant materials used as carotenoid sources (Figure 1) were 31.87±07.01 mg/kg (*A. tricolor*), 51.14±03.44 mg/kg (*C. indicum*), 59.69±10.05 mg/kg (*H. rosasinensis*), 39.59±01.09 mg/kg (*I. coccinea*) and 42.68±09.54 mg/kg (*T. erecta*). Carotenoids are the most widespread and structurally diverse pigmenting agents. In combination with proteins, they are responsible for many of the brilliant yellow to red colour in plants and the wide range of blue, green, purple, brown and reddish colour of fish and crustaceans (Lorenz, 2010) that may be obtained through organisms rich in the aquatic food chain. The conspicuousness of the carotenoid-based colouration is considered as a reliable indicator of the foraging ability of the individuals for carotenoid-rich foods (Endler, 1980). Commercial feed ingredients that are are rich sources are carotenoids are yellow corn, corn gluten meal and alfalfa (zeasanthin and lutein) (Lovell, 1992), marigold meal (lutein), red pepper extracts (capsanthin) and krill or crustacean meals (astaxanthin) (Boonyaratpalin and Unprasert, 1989). A direct relationship exists between dietary carotenoid and pigments in fishes (Halten *et al*., 1997).

Figure 1 Total carotenoid content in the carotenoid sources used as feed supplements



Biochemical constituents in the feed

Biochemical constituent namely total carotenoid, protein, carbohydrate and the lipid were analyzed in the feed (Table 1). The maximum quantity of carotenoid content (22.54±2.62 mg/kg), protein content (30.47±0.06 mg%), carbohydrate content (38.53±6.11 mg%) and lipid content (21.33±1.28 mg%)

were found in feed D_{Hibi} . Generally, feed materials rich in nutrients promote the growth of edible as well as ornamental fishes. Fish for proper growth and maintenance, requires protein and the inclusion of plant materials improve the quality of fish diets (Mohanty and Swamy, 1986). Composition and sequence of amino acids determines a protein quality. The optimum protein requirements for ornamental fishes range from 30-45%. The protein content of the prepared feed after incorporating *T. erecta* (38.33-38.41%), *H. rosasinensis* (38.34-38.36%) and *Rosa centifolia* (38.35-38.36%) (Bagre *et al*., 2011).

The effect of dietary carbohydrate on fish growth seems to depend on the source, dietary concentration and digestibility, the level of dietary intake, rearing conditions and fish species (Krogdahl *et al*., 2005). Carbohydrates are economical inexpensive resources of energy with protein sparing and lipid sparing effects. Lipids are primarily included in formulated diet to maximize their protein sparing effects (Hasan, 2001) by being a source of energy. In general 10-20% of lipid in most freshwater fish diets gives optimal growth rates without producing an excessively fatty carcass (Cowey and Sargent, 1979).

Table 1 Biochemical constituents in the carotenoid supplemented feed

Feed	Total carotenoid content (mg/kg)	Protein (mg%)	Carbohyrate (mg%)	Lipid (mg%)
A_{Cont}	0.99±0.58	19.03±0.02	30.40±.58	18.28±0.75
B_{Amar}	16.37±3.16	23.34±0.12	31.06±2.00	16.95±0.65
C_{Chry}	09.62±2.57	17.91±1.09	30.10±1.62	17.71±0.76
D_{Hibi}	**22.54±2.62**	**30.47±0.06**	**38.53±6.11**	**21.33±1.28**
E_{Ixor}	09.17±1.81	18.05±1.01	36.00±2.27	16.95±0.43
F_{Tage}	20.12±1.59	27.65±0.01	35.60±2.00	17.91±0.59

Effect of carotenoid supplementation on consumption, assimilation, metabolism and conversion

The feeding behavior of the fish depends on various factors such as appetite, visual and chemosensoryability and restriction in food searching area. A higher value was obtained for consumption (1.40±0.16 g) and consumption rate (75.66±4.90 mg/g.wt./day) with feed group B_{Amar} (Table 2 and Table 3). Food consumption in fish is regulated to maintain a constant energy intake and indeed, the energy requirement remains constant. When fish are fed on diets differing in “digestible energy” and “digestible protein”; they exhibit different growing rates. The consumed energy at allocated to catabolic processes (maintanance and activity metabolism), then to waste losses (faeces, urine and specific dynamic action) and that left over is allocated to somatic storage (body growth). Some fishes have shown high growth efficiencies (approaching 50%) and are known to exhibit strikingly negative energy budgets, as in the case of migrating salmon (Brett, 1995). The food energy requirements of fish are only about 10% of what is necessary for mammals and birds (Smith, 1989).

Table 2 Influence of dietary carotenoid supplementation on consumption, adsorption, conversion and metabolism of the fish, *Puntius tetrazona*

Feed	Consumption (g)	Assimilation (g)	Conversion (g)	Food metabolized (g)
A_{Cont}	1.05±0.11	1.01±0.07	0.02±0.01	0.98±0.12
B_{Amar}	**1.40±0.16**	**1.37±0.15**	0.03±0.01	**1.34±0.32**
C_{Chry}	0.95±0.05	0.91±0.05	0.05±0.01	0.86±0.14
D_{Hibi}	1.30±0.08	1.24±0.10	**0.12±0.05**	1.12±0.07
E_{Ixor}	1.10±0.02	1.08±0.74	0.06±0.01	1.02±0.08
F_{Tage}	1.02±0.01	0.99±0.04	0.08±0.01	0.91±0.04

The higher consumption rate may be considered to be due to the presence of *A. tricolor* in the feed at the optimum amount, for promoting enhanced feeding or consumption of the fish *P. tetrazona*. Our results indicate, the other feed groups show comparatively less consumption rate. Thus it is evident that the feed containing *A. tricolor* leaf is preferred better than the other feeds provided and hence it can be concluded that *A. tricolor* leaf meal enhances the feeding behavior of *P. tetrazona*. The active principles in food may result in inducing secretion of digestive enzymes that will result in stimulates the appetite an increasing food consumption (Citarasu, 2010).

Table 3 Influence of dietary carotenoid supplementation on the rate of consumption, assimilation, conversion and metabolism of the fish, *Puntius tetrazona*

Feed	Consumption rate (mg/g.wt./day)	Assimilation rate (mg/g.wt./day)	Conversion rate (mg/g.wt./day)	Metabolic rate (mg/g.wt./day)
A_{Cont}	47.66±3.21	47.33±2.55	1.25±0.32	46.08±02.63
B_{Amar}	**75.66±4.90**	**66.25±7.30**	1.59±0.25	**63.99±15.35**
C_{Chry}	46.00±2.12	43.95±1.67	2.59±1.87	41.37±01.47
D_{Hibi}	52.66±2.59	50.82±2.84	**5.15±0.70**	45.77±05.40
E_{Ixor}	68.50±8.13	64.43±8.27	3.64±0.19	63.78±16.75
F_{Tage}	54.50±2.47	53.10±2.60	4.59±0.96	48.50±04.26

The higher value was obtained for assimilation rate (66.25±7.30 mg/g.wt./day) and assimilation efficiency (97.33±0.27 %) with feed group B_{Amar} (Table 2, 3 & 4). Assimilation is the transformation of external substances and materials that are eaten, into substances and materials internal to the body that can later be used in the metabolic process (Wikipedia, 2006).

Table 4 Influence of dietary carotenoid supplementation on the efficiency of assimilation and conversion of *Puntius tetrazona*

Feed	Assimilation efficiency (%)	Gross conversion efficiency (%)	Net conversion efficiency (%)
A_{Cont}	96.33±0.27	2.60±0.37	2.69±0.82
B_{Amar}	**97.33±0.27**	2.41±0.20	2.46±0.20
C_{Chry}	95.00±0.47	5.43±1.78	5.73±1.91
D_{Hibi}	95.33±0.72	**9.49±0.30**	**9.92±0.29**
E_{Ixor}	97.10±0.35	5.47±1.59	5.61±1.67
F_{Tage}	96.50±0.35	8.31±0.97	8.59±0.95

The feed with *A. tricolor* leaf meal that was consumed more was also assimilated better compared to the control and the other feeds with *C. indicum*, *H. rosasinensis*, *I. coccinea* and *T. erecta*. The decrease in the food consumption and assimilation in other feeds may be due to slow rate of digestion (Christopher and Mathavan, 1980). The dietary ginseng herb greatly enhanced the diet utilization efficiency in the Nile tilapia, *Oreochromis niloticus* fingerlings (Ashruf and Goda, 2008).

Food quality has been reported to have a significant influence on the growth of the fish *Heteropneutes fossilis* (Arunachalam *et al*., 1985). Production of an animal depends on the growth range and biomass (Brocksen *et al*., 1968). In our present study, a higher value was obtained for metabolic rate (63.99±15.35 mg/g.wt./day) with feed group B_{Amar} (Table 2 and 3). A higher value was obtained for conversion rate (5.15±0.70 mg/g.wt./day), gross conversion efficiency (9.49±0.30 %) and net conversion efficiency (9.92±0.29 %) with feed group D_{Hibi} (Table 3, 4 and 5). The *H. rosasinensis* petal supplemented meal has been found to improve the growth of *P. tetrazona* as revealed by the experimental results. Similar observation was made in rainbow trout offsprings using 10 % krill meal as a source of carotenoids (Tveranger, 1986). Carotenoids influenced the growth of fishes and crustaceans (Thongrod *et al*., 1995). In trouts, the addition of carotenoid rich microalgae, *Haemetococcus pluvialis* enhanced the growth (Sommer *et al*., 1992). *Spirulina* induced the growth and body colour of the crucian carp (Peimin *et al*., 1999). Conversion efficiency is found to have been influenced by the quality of the food (Pandian and Raghuraman, 1972).

Total carotenoid and biochemical constituents in the fish, *P. tetrazona*

The body colour of fish predominantly depends on the presence of special cells in the tissue, called chromatophores (melanophores, xanthophores, erythrophores, iridophores, leucophores and cyanophores), containing pigments such as melanins, carotenoids (eg. astaxanthin, canthaxanthin, lutein and zeaxanthin), pteridines and purines. Fish can exhibit different colour patterns as a result of the dispersion or aggregation of chromatophores in the skin (Withers, 1992). A combination of environmental, neural endocrine and husbandry-related factors influences the chromatophores mobility and pigment deposition in cultured fish (Fujii, 2000). In the present study the total carotenoid content (20.76±1.48 mg/kg) of *T. erecta* petal supplemented diet fed group was comparatively higher than the other group (Table 6). The maximum quantity of protein (21.06±2.41 mg%), carbohydrate (32.13±6.11 mg%) and the lipid (25.05±2.31 mg%) were found in fishes fed with feed D_{Hibi} (Table 5). Diets not only affect the feeding energetics but have a profound influence on the body composition of the animals (Lee and Putman, 1973). *Haematococcus pluvialis* as a safe natural source of astaxanthin derived from microalgae resulted in extensive pigmentation in koi and tropical fishes (Ronneberg *et al*., 1979). The addition of *spirulina* was effective in producing deeper colouration in the fancy carp *Trichogaster tricopterus* Pallas (Alagappan *et al*., 2004). The effectiveness of carotenoid sources in terms of deposition and pigmentation is species specific (Ha *et al*., 1993). The rate of colour development seems to depend on

the amount and nature of the carotenoid present in the pigment source/ingredient. Continuous feeding of fish with marigold increased pigmentation in *Xiphophorus helleri* (Ezhil *et al*., 2008). Amaranth and mint at 1% level enhanced the colouration of adult gold fish. The natural carotenoid source of carrot was found to be an effective colour enhancer at a cheaper prize (Ramamoorthy *et al,* 2010). Our present study suggests that *T. erecta* supplemented feed which contained comparatively higher carotenoid content like *H. rosasinensis* produced improved colouration as revealed by the increased carotenoid content on the body of *Puntius tetrazona*.

Table 5 Total carotenoid, protein, carbohydrate and fat content of *Puntius tetrazona* fed with carotenoid supplemented feed

Feed	Total carotenoid (mg/kg)	Protein (mg%)	Carbohydrate (mg%)	Lipid (mg%)
A_{Cont}	0.73±0.38	16.80±2.37	12.13±6.11	16.75±0.71
B_{Amar}	12.89±3.37	17.50±3.04	17.60±4.00	17.04±0.87
C_{Chry}	10.67±3.26	16.20±1.61	28.50±6.11	16.66±1.86
D_{Hibi}	18.97±4.36	**21.06±2.41**	**32.13±6.11**	**25.05±2.31**
E_{Ixor}	13.77±1.06	19.30±5.33	29.73±2.31	11.91±0.43
F_{Tage}	**20.76±1.48**	18.40±1.55	30.40±4.00	23.62±1.02

CONCLUSION

In our present study we focussed on incorporating natural carotenoid sources in the fish feed, since synthetic carotenoids are too expensive. The study was undertaken to know the effect of five feed supplements namely *A. tricolor, C. indicum, H. rosasinensis, I. coccinea* and *T. erecta* on the nutrition, body constituents and colouration of the ornamental fish, the tiger barb, *P. tetrazona*. Our findings suggest that *H. rosasinensis* petal supplemented meal enhances the growth and increase the carbohydrate and protein content while *T. erecta* improved the colour of the fish, *P. tetrazona*. Considering both nutritional efficiency and colour enhancing potential of the supplemented feed, the above observations reveal that *T. erecta* and *H. rosasinensis* can be used as better feed supplements for the ornamental fish *P. tetrazona* compared to others. Appropriate combination of these supplements in the feed would result in improved growth and enhanced colouration of the ornamental fish *Puntius tetrazona.*

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