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Effect of dietary natural carotenoid sources on colour enhancement of Koi carp, *Cyprinus carpio* L.

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Abstract

A feeding trial of 45 days was conducted to observe the effects of dietary natural source of carotenoids on growth and colour enhancement of koi carp, *Cyprinus carpio* fingerlings. Five diets were prepared with different natural carotenoids supplementation such as beet root powder (BP), carrot peel powder (CP), tomato peel powder (TP) and their mixture (MP) and designated as C (0% supplementation), T1 (1% BP), T2 (1% CP), T3 (1% TP) and T4 (1% MP). The growth parameters was analysed and significantly ($P < 0.05$) higher weight gain percentage was reported in T2 however, it was comparable with T3 group and lowest weight gain percentage observed in T1. Specific growth rate (SGR) was manifested significantly ($P < 0.05$) highest in T3 followed by T2 was found lowest in T1 group. Food conversion ratio (FCR) reported lower in T2. The survival percentage was manifested highest in T3 followed by T4 and T2 groups. The colour analysis of the fishes was done by colorimetry and the three colours such as lightness, redness and yellowness were manifested highest in T4 group fed with mixture of carotenoids powder followed by T2. The carotenoid deposition in the fish body was also analysed and was observed significantly ($P < 0.05$) highest in T1 followed by T4 group while lowest was found in T2. The overall study revealed that natural carotenoid sources such as beet root, carrot peel, tomato peel and their powder mixture gave good growth and coloration at 1% however in present study, the fish group fed with 1% CP was more effective in enhancing the growth and colouration of koi carp, *Cyprinus carpio*.

Keywords: Growth, carotenoids, specific growth rate, lightness, redness etc.

1. Introduction

Ornamental fish farming is an emerging sector of aquaculture which provides high income and employment opportunity to the people across the globe. It has potential to contribute to the economic development in underdeveloped countries, especially tropics (Yanar *et al.*, 2008) [1]. The price of the ornamental fish is largely determined by the colour and fishes with good colour pattern fetches higher price in the market. The fishes in their natural environment get coloration from the wild source however, culture of ornamental fish under high density in captive condition without supplementation of dietary carotenoids leads to faded coloration which decrease the commercial value of the fish (Harpaz and Podowicz, 2007) [2]. Fish use carotenoids, as one of the major groups of natural pigments for their pigmentation and besides this carotenoid has a diverse function in fishes such as antioxidant activity, as nutraceuticals and also plays an important role in reproduction. Koi carp (*Cyprinus carpio*) is characterised by wide diversity of colour and colour patterns and more than hundreds of colour has been developed (Tamadachi, 1990) [3] for this fish and its value increase with the intensity of skin colour which is because of absorption and deposition of carotenoids.

There are different source of carotenoids such as natural and synthetic origin which have been used to enhance the coloration of the fishes. The different synthetic carotenoids include (β -Carotenoids, canthaxanthin, zeaxanthin and astaxanthin) and natural carotenoids such as plant materials, bacteria, algae, crustaceans, microalgae etc.) are used as colour enhancer. However, the high price of synthetic carotenoid forces the researcher to explore the natural sources and their application as colour enhancer. In natural sources spirulina have been used as cheapest sources of carotenoid pigment for colour enhancement in rainbow trout, fancy carp and yellow tail cichlid, *Pseudomonas acei* (Choubert, 1979) [4]. Marigold petal meal was used for the tiger barb, and red sword tail (Ezhil *et al.*, 2008) [5]. Swain *et al.* (2014) [6] reported 180 ppm dietary marigold oleoresin enhanced growth and colour of koi carp, *Cyprinus carpio*.

Ramamoorthy *et al.* (2010)^[7] have used natural carotenoid sources such as carrot (*Dacus carota*) and China rose petal (*Hibiscus rosasinensis*) for the colour enhancement of marine ornamental fish *Amphiprion ocellaris*. Similarly, dietary beet root powder at 15% had highest carotenoid deposition in the flesh and enhance the colouration in Red sword tail, *Xiphophorus helleri* (Singh and Kumar, 2016)^[8]. The mixture of dietary tomato (*Solanum lycopersicum*) and carrot (*Daccus carota*) at 50 mg/kg had enhanced the colour and carotenoid content of flesh in case of Guppy fish (*Poecilia reticulata*) (Mirzaee *et al.*, 2012)^[9]. The natural carotenoid sources can be effectively used for enhancement of the colour in ornamental fishes which will be highly economical and user friendly. Owing to the above facts the present experiment has been conducted to evaluate the effects of dietary natural carotenoids sources such as beet root (*Veta vulgaris*), carrot (*Daccus carota*) and tomato (*Solanum lycopersicum*) for the colour enhancement and growth of the koi carp, *Cyprinus carpio*.

Materials and Methods

1. Experimental site and experimental animals

The experimental set up was maintained in the wet laboratory of the Central Institute of Fisheries Education (CIFE), Mumbai, India, and the laboratory analyses were carried out at CIFE, Mumbai. Koi carp, *Cyprinus carpio* fingerlings (average weight 6.32 ± 0.02 g) were procured from the Kurla fish market, Mumbai, Maharashtra, India, during the month of October, 2016. Fish were transported, stocked in FRP tank (1000 L capacity) and left undisturbed during the whole night. The next day, fish were given a salt treatment (5%) to ameliorate the handling stress. The stock was acclimatized under aeration, provided through compressed air for 15 days. During acclimation, fish were fed with controlled diet having (35% CP and 7% Lipid) at 3% of the body weight. Round the clock aeration was provided. The physico-chemical parameters of the water were within the normal range of carp rearing (dissolved oxygen: 5.56–7.1 mg l⁻¹; pH: 7.25–7.8; temperature: 26.6–28.2°C; alkalinity 46–58 mg l⁻¹ and hardness 48–64 mg l⁻¹) throughout the experimental period.

2. Experimental design, feed and feeding

One hundred and fifty fingerlings of koi carp were randomly distributed in five treatment groups in triplicates following a completely randomized design (CRD). The experimental rearing system consisted of 15 uniform size rectangular fibre tubs (150 L capacity) containing 10 fish per tank. The total volume of the water in each tank was maintained at 110–120 L throughout the experimental period.

Five isonitrogenous and isocaloric experimental diets were prepared. The protein percentage was maintained 35% in all the treatments while lipid percentage in all the treatments was kept around 7%. The Fingerlings of koi carp required 34–35 % protein and around 7–9 % lipid which come under the normal requirement of the carp including Koi carp, *Cyprinus carpio* fingerlings. The different natural carotenoid sources such as Beet root, carrot peel, tomato peel and their mixture were used for the enhancement of colour of the experimental fish. The fish were divided into five different treatment groups as control (0% supplement), T1 (1% BP), T2 (1% CP), T3 (1%TP) and T4 (1% MP) (Table 1). The protein and lipid percentage of 35% and 7% respectively were maintained in all the diets. Continuous aeration was provided to all the tanks throughout the experimental duration. Casein and gelatin were

used as protein sources, sunflower oil was used as lipid source, starch and dextrin powder were used as carbohydrate sources. All the ingredients were thoroughly mixed with water to make dough form. The dough was steam cooked for 10 min in a pressure cooker. Vitamin-mineral premix and different natural carotenoids source in the powder form were mixed in the different treatments after cooling of dough. Finally, the dough was pressed through the pelletizer to get uniform size pellets and dried under fan for 4 h. The pellets were then kept in a hot air oven (50–60 °C) for 3–4 hrs for complete drying, packed in polythene bags, and stored at 4 °C. Feed was given to approximate satiation level for 60 days twice daily at 3% of the body weight and adjusted accordingly based on the biomass gain during the trial. Daily ration was divided into two parts: about 2/3rd of total ration was given at 09:00 h and the rest 1/3rd at 18:00 h. The uneaten feed and faecal matters were removed by siphoning out about 50% of the tank water on alternate days.

3. Proximate Analysis

Proximate analysis of feed, and tissue and performed as per the prescribed method of AOAC (1995)^[10] in the Nutrition Laboratory of CIFE.

3.1. Moisture: The moisture content of the diets and animal tissue were determined by taking a known weight of the sample in a moisture cup and drying it in a hot air oven at 100–105° C till a constant weight was achieved. The difference in weight of the sample indicated the moisture content, which was calculated by using the following formula. Moisture % = (Wet weight of sample – Dried weight of sample)/Wet weight of sample x 100

3.2. Crude protein (CP): The Nitrogen content of the sample was estimated quantitatively by using semi-automated distillation system using titration as the means for determining nitrogen percentage. The crude protein percentage was obtained by multiplying the nitrogen percentage by a factor of 6.25.

Crude Protein (%) = N₂ (%) X 6.25

3.3. Ether Extract (EE): Ether extract was estimated by Soxhlet apparatus using petroleum ether (Boiling point 40–60°C) as the solvent. The calculation was made as follows. Ether extract% = (Weight of the ether extracts /Weight of the sample) × 100

3.4. Ash: Total ash content was estimated by taking a known weight of sample in silica crucible and placing it in a muffle furnace at 600°C for 6 hrs. The calculation was done as follows: Ash (%) = (Weight of ash /Weight of sample) x 100.

4. Carotenoid Analysis

Spectrophotometer analysis: The carotenoid content of fish skin was extracted according to the method of Torrissen and Naevdal (Torrissen *et al.*, 1984)^[11]. Five fish were randomly sampled from each diet treatment per sampling period and used for carotenoid analysis which was carried out in triplicate. The sample of 200–300 mg skin was collected from both sides between abdominal and dorsal regions of the fish. The samples were transferred into 10 ml pre-weighed glass tubes. After the samples were ground in acetone containing 1.5 g of anhydrous sodium sulphate with a homogenizer, the extractions were made up to 10ml with acetone. The samples

were stored for three days at 4°C refrigerator and then extracted three or four times until no more colours could be obtained. The solution was centrifuged at 5000 rpm for 5 minutes and then absorption was measured in a spectrophotometer.

5. Growth study

Fish were weighed at the start and termination of the experiment on the 60th day. The growth performances of *Cyprinus carpio* fingerlings were evaluated in terms of weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR) by using following equation:

Weight gain (%) = (final weight – initial weight) / initial weight x 100;

SGR = 100 x (log_e average final weight – log_e average initial weight)/number of culture days;

FCR = Total feed given (dry weight) (g) / weight gain (wet weight) (g)

6. Colour Analysis

Three fish from each treatment were selected for the colour analysis. In this study, skin colour was assessed with

reflectance spectroscopy with transformation into colour parameters based on the tristimulus values, L*, a*, b* and dE, representing lightness, redness, yellowness and chromatic aberration, respectively (Skrede, 1987)^[12]. The measurements were performed on the largest zone of black, red and white from each fish. L* and dE were measured in the black colour zones; L* and b* were measured in white colour zones; L*, a* and dE were measured in red colour zones.

7. Water quality analysis

The different water quality parameters such as temperature, pH, dissolve oxygen, carbon dioxide and ammonia in all the experimental tubs were analysed by standard methods (APHA, 1995)^[13].

8. Statistical Analysis

The data were statistically analysed by statistical package SPSS version 16 and data were subjected to one way analysis of variance and Duncan's multiple range tests were used to determine the significant differences if any, between the means. Comparisons were made at the 5% probability level.

Table 1: Ingredient composition of the different experimental feeds

Ingredients	Experimental diet (%) inclusion				
	Control	T1	T2	T3	T4
Casein ^a	29.7	29.7	29.7	29.7	29.7
Gelatin ^a	10	10	10	10	10
Dextrin ^a	18.3	18.3	18.3	18.3	18.3
Cellulose ^a	10	10	10	10	10
Starch ^a	21.5	21.5	21.5	21.5	21.5
Choline ^a	0.5	0.5	0.5	0.5	0.5
Veg oil	6	6	6	6	6
Vitamin-mineral Premix ¹	1.0	1.0	1.0	1.0	1.0
CMC ^a	1.5	1.5	1.5	1.5	1.5
BHT ^a	0.5	0.5	0.5	0.5	0.5
BP ^b	-	1.0	-	-	-
CP ^b	-	-	1.0	-	-
TP ^b	-	-	-	-	-
MP ^b	-	-	-	1.0	-
<i>Biochemical composition</i>					
Protein (%)	35.11	35.06	35.12	35.21	35.14
Lipid (%)	7.12	7.13	7.06	7.21	7.01
Crude fibre (%)	5.12	5.42	5.54	5.68	6.65
Total ash (%)	7.24	8.68	7.65	6.86	7.87
Dry matter (%)	93.61	92.01	93.12	93.12	93.24

^aSupplied by Himedia, Mumbai (India)

^bBP (Beet root peel powder); CP (Carrot peel powder); TP (Tomato peel powder); MP (Mixed powder); all these are procured from local market Andheri, Versova Mumbai, India

¹Composition of vitamin mineral mix (PREEMIX PLUS) (quantity/2.5kg) Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L- lysine, 10 g; DL- Methionine, 10 g; Selenium, 50 ppm; Satwari, 2500 mg;

Results and Discussion

1. Growth and survival performance

The growth performance of the koi carp at the end of experiment is shown in table 2. The weight gain % was recorded significantly (P<0.05) higher in T2 group fed with 1% dietary carrot peel powder followed by T3 group. Significantly (P<0.05) lowest FCR was recorded in T2 group followed by T3 group fed with dietary 1% tomato peel powder. The SGR recorded significantly (P<0.05) higher in T3 group followed by T2 and T4 group (Table 2). The survival % reported higher in all treatment groups compared to control. The highest survival % was found in T3 group

followed by T4 group. (Fig. 6)

Table 2: Growth parameters of Koi carp, *Cyprinus carpio* fed with different carotenoid sources for 45 days

Treatments	Weight gain (%)	Feed Intake (g)	FCR	SGR
C	13.62 ^a ±0.66	3.11 ^a ±0.03	3.87c±0.03	0.22 ^a ±0.02
T1	17.69 ^b ±0.94	3.07 ^a ±0.02	3.48 ^c ±0.03	0.37 ^b ±0.02
T2	37.75 ^c ±0.58	3.90 ^b ±0.05	2.25 ^a ±0.20	0.58 ^c ±0.01
T3	35.98 ^c ±0.61	3.89 ^b ±0.05	2.33 ^a ±0.28	0.71 ^d ±0.02
T4	19.95 ^b ±0.27	3.89 ^b ±0.06	2.90 ^b ±0.01	0.41 ^b ±0.02

2. Body coloration

The spectrophotometric analysis was made for color change in the skin of fishes fed with different natural carotenoid sources. The intensity of the fish colour viz., lightness, redness and yellowness (Fig. 1, 2, 3 and 4) was measured. The lightness of the black zone of fish body was higher in all the treatment groups compared to control and it was recorded significantly ($P < 0.05$) highest in T4 group fed with mixture of carotenoids followed by T2 and T3 groups. The T4 group showed significantly ($P < 0.05$) higher redness of koi carp followed by T2. Similarly, the yellowness of the skin colour reported higher in all the treatment groups compared to control. Significantly ($P < 0.05$) higher skin yellowness recorded in T4 group followed by T2 and T3.



Fig 1: Koi carp, *Cyprinus carpio* fish fed with different natural carotenoid sources

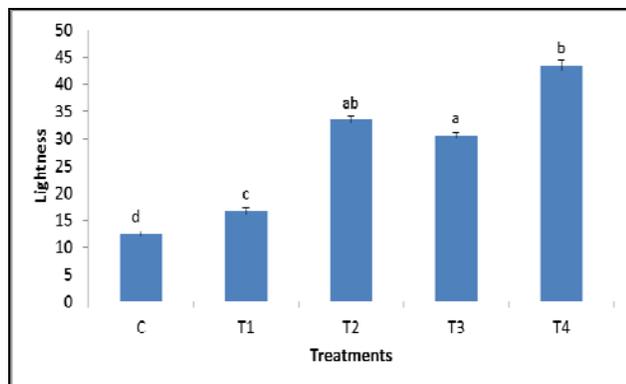


Fig 2: Lightness of koi carp, *Cyprinus carpio* fed with different carotenoid sources

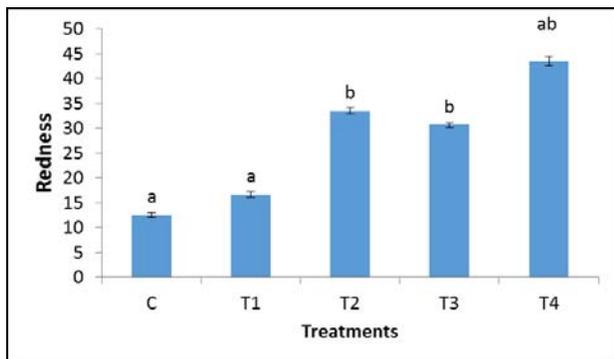


Fig 3: Redness of koi carp, *Cyprinus carpio* fed with different sources of carotenoids

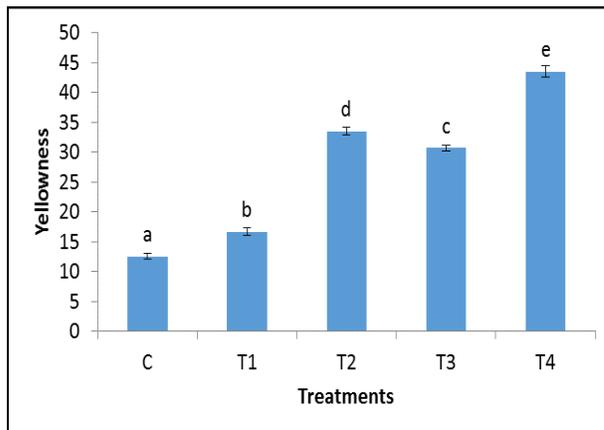


Fig 4: Yellowness of koi carp, *Cyprinus carpio* fed with different source of natural Carotenoids

3. Carotenoid Deposition

The concentration of carotenoid deposition in the body of the koi carp in different treatment groups were higher than control group. The carotenoid deposition in T1 group was reported significantly ($P < 0.05$) higher followed by T4. Significantly lowest ($P < 0.05$) carotenoid deposition was found in T2 group (Fig.5)

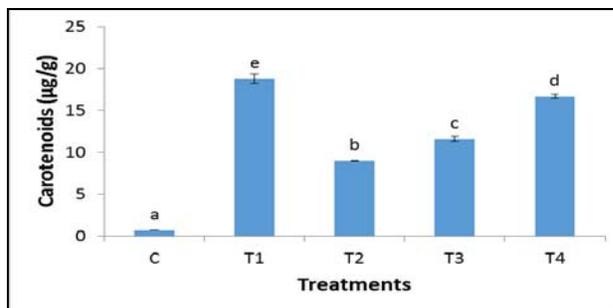


Fig 5: Carotenoids contents in skin of Koi carp fed with different carotenoid source diets

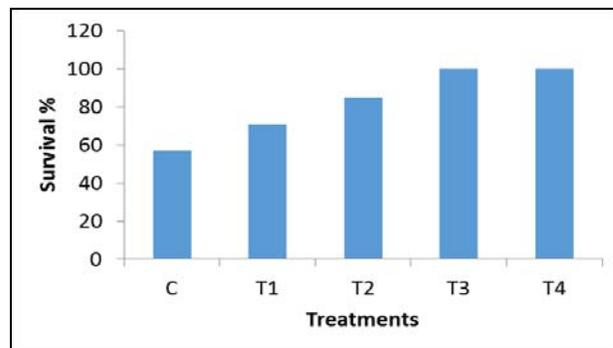


Fig 6: Survival % of koi carp fed with different source of carotenoids

4. Water quality parameter

The water quality parameters of the different experimental group is shown in table 3. The water temperature in different group varies from 23 °C to 28 °C. The pH was found in the range of 7.5 to 8.5. The dissolved oxygen level fluctuated in the range of 5.4 to 7.6 mg/L. The total ammonia content of different experimental groups was recorded in the range of 0.15 to 0.27 mg L⁻¹. Hardness in the different treatment group was found in the range from 249-264 mgL⁻¹ (Table 3)

Table 3: Water quality parameters of different experimental groups during the experiment

Parameters	C	T1	T2	T3	T4
Temperature (°C)	24.5-25.6	26.0-27.5	26.4-28.0	24.0-26.5	23.5-25.6
pH	7.6-8.1	7.5-8.2	7.4-8.2	7.5-8.2	7.6-8.2
Dissolved oxygen (mg L ⁻¹)	5.4-6.6	5.8-7.6	5.6-7.4	5.8-7.9	5.4-7.2
Ammonia (mgL ⁻¹)	0.15-0.22	0.22-0.27	0.24-0.26	0.16-0.27	0.23-0.26
Hardness (mgL ⁻¹)	252-259	249-258	249-264	250-261	252-259

Carotenoid is the important source of colour pigmentation in fishes rearing in the captive condition unlike wild condition where they receive carotenoids from the environment and hence dietary supplementation in feed is essentially required in captive culture. Carotenoids are known to have a positive role in the intermediary metabolism of fish (Segner *et al.*, 1989)^[14] that could enhance nutrient utilisation and may ultimately result in improved growth (Amar *et al.*, 2001)^[15]. The Metabolic path way of carotenoids in different fishes vary and hence there is no universal transformation of carotenoids in the fish tissue (Chatzifotis *et al.*, 2005)^[16]. The use of artificial carotenoids is not cost effective and sustainable hence many natural products such as beet root powder (Singh & Kumar, 2016)^[8], tomato (*Solanum lycopersicum*) & carrot (*Daucus carota*) (Mirzaee *et al.*, 2012)^[9] have been used as the colour enhancement in different fishes. In the present study the natural carotenoid sources such as beet root powder, carrot peel powder, tomato peel powder and their mixture powder were used which had significant effects on growth, feed conversion ratio and survival percentage of koi carp, *Cyprinus carpio*. The highest growth percentage reported in group fed with carrot peel powder in present study might be due to the natural carotenoids presence and its growth promoting role. Carotenoid act as a precursor for the vitamin A synthesis and bioconversion of carotenoids into other nutrients in fishes signify its role as growth promoter. Similar kind of result was obtained by Singh and Kumar (2016)^[8] in Red sword tail (*Xiphophorus helleri*) fed with 15% beet root incorporated diet and Mirzaee *et al.* (2012)^[9] in guppy fish (*Poecilia reticulata*) fed with mixture of tomato & carrot in the diet. The results are also supported by link carotenoids to growth enhancement in Atlantic salmon fry (*Salmo salar*) (Christiansen *et al.*, 1995)^[17], rainbow trout (*Oncorhynchus mykiss*) (De la Mora *et al.*, 2006)^[18] and goldfish (*Carassius auratus*) (Sinha and Asimi, 2007)^[19]. Data on feeding trial showed that addition of beet root, carrot peel, tomato peel powder and their powder mixture in the different diet had increased the lightness, redness and yellowness of koi carp which might be because of the natural carotenoid deposition in the integument. In colorimetry and colour theory, hue is one of the main properties of a colour, defined technically (in the CIECAM02 model), as “the degree to which a stimulus can be described as similar to or different from stimuli that are described as red, green, blue, and yellow” (Sun *et al.*, 2010)^[20]. The fish group fed with carrot peel powder and mixed source affected skin colouration and received higher lightness, redness & yellowness whereas fishes fed beet root powder received lowest colouration of lightness, redness & yellowness. Red sea bream, fed β -carotene or canthaxanthin, showed a decrease in the carotenoid level in the integuments (Lorenz, 1998)^[21]. Decrease in deposition can be explained by a limitation in the rate of absorption (torrissen *et al.*, 1990)^[22]. Uneven colour distribution was also noticed in the salmonids muscle, where a longitudinal variation in carotenoid content and red colour was observed, with more

astaxanthin deposited in the caudal than in the anterior part (Bierkeng, 2000)^[23].

The Koi carp received higher carotenoids deposition in the skin in all the treatment groups compared to control which clearly showed that dietary carotenoid leads to the carotenoid deposition in the fishes and consequently increase colouration. Similar kind of results were obtained for goldfish by feeding different natural carotenoid sources, such as Spirulina (Kiriratnikom *et al.*, 2005)^[24], microalgal biomass (Gouveia and Rema, 2005)^[25] and red yeast (*Xanthophyllomyces dendrorhous*) (Xu *et al.*, 2006)^[26] and alfalfa (*Medicago sativa*) (Yanar *et al.*, 2008)^[1]. The effectiveness of carotenoid sources in terms of deposition and pigmentation is species specific (Ha *et al.*, 1993)^[27]. The higher carotenoid deposition in the group fed with beet root powder and mixed sources might be due to the transforming ability of alimentary carotenoids and subsequently stored them in the skin of fish.

Conclusion

The present results suggest that koi carp can efficiently utilize the natural source of carotenoids along with deposition of carotenoids in the integument and increasing coloration. In present study, the fish group fed with 1% CP was more effective in enhancing the growth and colouration of koi carp, *Cyprinus carpio*.

Future perspective

The effects of natural source of carotenoids such as beet root, tomato peel, carrot peel and their mixture at higher doses beyond 1% need to be analysed and ascertain about further growth promoting ability as well as colour enhancement in koi carp, *Cyprinus carpio*.

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