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Effect of dietary supplementation of marigold oleoresin on growth, survival and total muscle carotenoid of Koi carp, *Cyprinus carpio* L.

Himanshu S. Swian*, S. Ratnamanjari Senapati[1], S. J. Meshram[2], R. Mishra[3] and H. Shivananda Murthy[2]

Central Inland Fisheries Research Institute, Barrackpore, Kolkata- 700120, INDIA
[1]Central Institute of Fisheries Education, Mumbai- 400061, INDIA
[2]College of Fisheries, Karnataka Veterinary Animal and Fisheries Sciences University, Mangalore-575002, INDIA
[3]College of Fisheries, Odisha University of Agriculture and Technology, BBSR-760007, INDIA
*Corresponding author. E-mail: himanshufishco@gmail.com

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Abstract: The experiment was conducted to evaluate the effect of dietary supplementation of carotenoid (marigold oleoresin) on growth, survival and total body carotenoid of Koi carp (*Cyprinus carpio*). The experiment was carried out in 12 fiber aquarium tanks of size 12"×6"×6"(20l capacity). Each tank was stocked with15 fishes of uniform size. The marigold oleoresin were as dietary supplement at levels 60, 120, 180, 240, 300 ppm/kg of the feed and designated as treatment T_1, T_2, T_3, T_4 and T_5 respectively and diet without marigold oleoresin supplementation served as a control (T_0). The result of the experiment showed that there was significant difference found in absolute growth rate and specific growth rate of the fish (P >0.05). However, 180ppm marigold oleoresin fed fishes showed higher mean weight gain of 3.98±0.22g and lower feed conversion ratio (FCR) of 2.81±0.04 than the other treatment groups. The body coloration and total carotenoid concentration of muscle tissue (30.16±0.60µg/g) was significantly higher in fish fed with 180 ppm marigold oleoresin diet. The study showed that incorporation of 180ppm of marigold oleoresin in diet was found better to enhance the growth and coloration in *C. carpio*.

Keywords: Carotenoid, *Cyprinus carpio*, Koi carp, Marigold oleoresin

INTRODUCTION

Ornamental fish sector has potential to contribute to the economic development in underdeveloped countries, especially tropics (Yanar *et al.*, 2008). These are gaining importance because of their attractive coloration and aesthetic value. The pigments in ornamental fish are one of the most important quality criteria dictating the market value. Like other animals, fishes are unable to produce de novo (inside the body) synthesis of pigments. So the pigmentation of fish is due to ingested carotenoids (Goodwin, 1984). Among the important ornamental fishes, Koi carps are characterized by a wide diversity of colors and color patterns (Gomelsky *et al.*, 2003). Koi value increases with intensity of skin color and this is because of absorption and deposition of carotenoids in body.

In nature, carotenoids have been implicated in diverse functions such as pigmentation, antioxidant activity, immunostimulation and reproduction, and they play a positive role in intermediary metabolism (McGraw and Ardia, 2003; Watanabe and Vasallo-Agius, 2003; Chatzifotis *et al.*, 2005). In the natural environment, fish depends on aquatic vegetation to meet their carotenoid requirement. But culture of ornamental fish under high density in captive condition without supplementation of dietary carotenoids leads to faded coloration, disease and decrease the commercial value of the fish (Harpaz and Padowicz, 2007). Different sources of carotenoids pigments like pure carotenoids pigments, animal sources and plant sources are included in fish diet. Various synthetic pigments like β carotene, castaxanthine, zeaxanthine and astaxanthine and also natural sources such as yeast, bacteria, algae, higher plants and in animal source, crustacean meal have been used as dietary supplements to enhance the pigmentation of fish and crustaceans (Shahidi *et al.*, 1998; Kalinowski *et al.*, 2005)

Among these natural compounds, many of the botanical additives have been used as cheapest sources of pigmentation in fish e.g. *spirulina* have been used as a source of carotenoid pigment for rainbow trout, fancy carp and yellow tail cichlid *Pseudotropheus acei* (Choubert, 1979; Boonyarapatin and Phrom kunthony, 1986). Marigold petal meal was used for the tiger barb and red swordtail (Boonyarapatin and Lovell, 1977; Ezhil *et al.*, 2008). Ramamoorthy *et al.* (2010) have used natural carotenoid sources such as carrot (*Daucus carota*), marigold petal (*Tagetes erecta*), China rose petal (*Hibiscus rosasinensis*) and rose petal (*Rosa*

chinensis) and found that they can enhance colour of marine ornamental fish *Amphiprion ocellaris.* Similarly *H. rosasinensis, R. indica, Ixora coccinea and Crossandra infundibuliformiss* have been utilized to enhance the growth and body colouration of an ornamental fish red sword tail, *Xiphophorus hellerei* (Baby Joseph *et al.*, 2011). Marigold oleoresin, which is the hexane extract of the dehydrated marigold flowers (*T. erecta*) contain free fatty acids, waxes, sterols and the esterified lutein. The marigold contains various carotenoids, of which leutin is the principal component (Navarrete-Bolanos *et al.*, 2005). Keeping this in view, the present study was conducted to know the effect of marigold oleoresin on growth, survival and total muscle carotenoid of Koi carp, *C. carpio*.

MATERIALS AND METHODS

Experimental design and experimental fish: The study was carried out in an indoor system of fiber glass aquaria tanks. Koi carps (*C. carpio*) were obtained from local commercial aquarium hatchery, kept under quarantine condition for three weeks and then acclimatized to the experimental condition for two weeks. During this period fishes were fed with basal diet (Control diet). Fishes of same color hue having initial weight of 2.60-2.68g were selected for the experiment.

Experimental diet: A basal diet without adding carotenoid was used as control diet and five test diets namely T_1, T_2, T_3, T_4 and T_5 with inclusion level of marigold oleoresin 60, 120, 180, 240 and 300 mg/kg were formulated using the square method of Hardy (1980). The experimental diets had 30% protein and the ingredients used in the formulation of different experimental diets were fishmeal, soybean meal, groundnut oil cake, wheat flour, rice bran, tapioca flour, vitamin and mineral premix. All the ingredients were analyzed for proximate composition prior to formulation of the test diets employing standard methods (AOAC, 2005). Moisture content was estimated by heating samples at 105 ^{0}C for 30 min and then cooling and weighing to a constant weight. Crude protein was analyzed using Kjeltec system (Tecater 1002 Distilling Unit), fat content by Soxtech system (Tecater 1043 Extraction Unit), fibre content by using Fibretech system (Tecater 1017 Hot Extractor). The ash content was determined by first drying the sample and then heating it in a Muffle furnace at 550 ± 10 ^{0}C for 6h. Marigold oleoresin was procured from M/S Avesthagen Ltd, Bangalore. A suspension of marigold oleoresin was made in soybean oil by heating it upto 55°C and was mixed with experimental diets on a slow mixer. Proximate composition of the ingredients and experimental diets are given in Table 1.

Fish feeding and sampling: Fish were fed at the rate of 5% of their body weight till the end of the experiment. The feed was broadcasted over the surface of water twice daily at 10.00 h and 17.00 h. After each sampling, the quantity of feed given was re-adjusted based on the increased weight of fish. Water in the experimental tanks was replaced with fresh and clean water every day. The fishes were sampled once in a fortnight and measured individually on electronic balance (Essae, India) to assess the growth. The stocked fishes were collected during each sampling and measured individually for growth parameters. After the experimental period of 60 days all the survived fishes were collected and their weight and survival data were recorded. The other growth parameters such as Specific growth rate (SGR) and Feed conversion ratio (FCR) were calculated by using the following formulae.

Specific growth rate (%) = [(ln final weight – ln initial weight) / Rearing period (day)] ×100

Feed conversion ratio = Total dry feed offered (g) / Total wet weight gain (g)

Total carotenoid concentration (TCC) in the fish muscle tissue was analysed immediately after the completion of experiment following the pigment extraction method as described in Olson (1979). One gram body tissue of *C. carpio* was taken from headless and degutted fish and stored in a 10 ml screw capped clear glass vials then added with 2.5 g of anhydrous sodium sulphate. The sample was gently meshed with a glass rod against the side of the vial and then 5 ml of chloroform was added and left for overnight at 0^{0}C. when the chloroform formed a clear layer of 1-2 cm above the caked residue, the optical density was read at 380, 450, 470 and 500 nm, in a spectrophotometer (Systronics, India) taking 0.3 ml aliquots of chloroform diluted to 3 ml with absolute ethanol. A blank prepared in a similar manner was used for comparison. The wave length at maximum absorption was recorded and used for the calculation.

Total carotenoid concentration (µg/g wet wt.) = [Absorption at maximum wave length / (0.25 sample weight (g)] ×10

(Where, 10 = dilution factor; 0.25 = Extinction coefficient)

Water quality analysis: The physico-chemical |parameters of water were analyzed for temperature (thermometer), pH (Hanna) and dissolved oxygen, carbon dioxide and ammonia in all the experimental tanks were estimated by standard methods (APHA, 1995).

Statistical analysis: The data is presented in the form of mean and standard error (± S.E.).The experimental results were tabulated and analyzed statistically by using One-way analysis of variance (ANOVA) and Duncan's multiple range tests was used for mean separation.

RESULTS

The changes in the growth during the 60 days of experiment are shown in the table 2. The mean weight of Koi carp on the first day of stocking in the control (T_0) was 2.62g and mean weight of carotenoid fed fishes group at 5 different concentrations such as 60, 120, 180, 240 and 300 mg/kg were 2.68g, 2.60g,

Table 1. Ingredients and chemical composition of experimental diets.

S. N	Diet ingredients	% of inclusion					
		T_0	T_1	T_2	T_3	T_4	T_5
1	Fish meal	22	22	22	22	22	22
2	Soybean meal	20	20	20	20	20	20
3	Groundnut cake	18	18	18	18	18	18
4	Rice bran	15	15	15	15	15	15
5	Wheat flour	12	12	12	12	12	12
6	Tapioca flour	10	10	10	10	10	10
7	Soyabean oil	2	2	2	2	2	2
8	Vitamins and mineral mix[1] (Agrimin forte)	1	1	1	1	1	1
9	Marigold oleoresin	-	60mg	120mg	180mg	240mg	300mg
	Proximate composition (%)						
	Dry matter	91.86±0.21	92.02±0.16	91.62±0.21	92.12±0.04	91.60±0.21	91.37±0.31
	Moisture	8.14±0.21	7.98±0.13	8.38±0.31	7.88±0.11	8.40±0.10	8.63±0.13
	Protein	30.36±0.29	30.80±0.11	30.80±0.02	30.45±0.12	31.02±0.16	29.80±0.25
	Crude lipid	7.34±0.14	8.12±0.12	8.12±0.07	7.76±0.05	7.92±0.22	8.19±0.18
	Ash	10.55±0.08	10.80±0.17	11.15±0.20	10.78±0.29	11.26±0.14	10.62±0.20

[1]Vitamins and mineral mix (mg kg^{-1} feed) (Vitamin A 700000 IU; Vitamin D_3 70000 IU; Vitamin E 250mg; Nicotinamide 1000mg; Cobalt 150mg; Copper 1200mg; Iodine 325mg; Iron 1500mg; Magnesium 6000mg; Potassium 100mg; Sodium 5.9mg; Manganese 1500mg; Sulphur 0.72%; Zinc 9600mg; Calcium 25.5%; Phosphorus 12.75%)

Table 2. Growth, survival, specific growth rate (SGR) and food conversion ratio (FCR) of Koi carp (*C. carpio*) during the experiment.

Treatment	Mean weight (g)					Survival (%)	SGR	FCR
	Initial	15 days	30 days	45 days	60 days			
T_0	2.62±0.01	3.38±0.08	4.84±0.12	5.45±0.16	6.08±0.18^{a}	83.34±4.06^{b}	1.40±0.04^{a}	2.95±0.01^{bc}
T_1	2.68±0.03	4.12±0.01	4.66±0.15	5.03±0.19	6.10±0.13^{d}	86.67±7.57^{c}	1.37±0.01^{a}	3.04±0.02^{cd}
T_2	2.60±0.02	2.96±0.06	4.13±0.20	5.38±0.11	6.06±0.14^{e}	90.33±4.46^{d}	1.41±0.02^{a}	2.98±0.01^{a}
T_3	2.60±0.00	3.68±0.08	4.90±0.14	5.84±0.17	6.58±0.22^{f}	86.76±2.56^{c}	1.64±0.02^{b}	2.81±0.04^{ab}
T_4	2.63±0.04	3.23±0.10	4.81±0.12	5.74±0.20	6.10±0.15^{c}	80.0±6.66^{a}	1.40±0.03^{a}	3.10±0.03^{cd}
T_5	2.62±0.03	3.78±0.11	4.48±0.12	5.13±0.14	5.98±0.11^{b}	84.0±3.16^{b}	1.38±0.01^{a}	3.18±0.04^{d}

Different superscript indicates significant difference (P<0.05)

2.60g, 2.63g and 2.62g respectively. At the end of the experiment, the mean weight of the control fishes were found to be 6.08g and the fishes fed with carotenoids, the mean weight observed to be 6.10g, 6.06g, 6.58g, 6.10g and 5.98g in T_1,T_2,T_3,T_4 and T_5 respectively. The food conversion ratio in different treatment varies from 3.18 in T_5 to 2.81 in T_3. The survival rate of fishes fed in different treatment with different level of marigold oleoresin and control diet ranged from 80.0 (T_4) to 90.33 (T_2) is shown in table 2. Specific growth rate and Food conversion ratio was significantly varied among the treatments as shown in Table 2.

Total carotenoids concentration in body of *C. carpio* fed with experimental diets are presented in Table 3. T_3 exibits highest total carotenoid (30.16 µg/g) content in body muscle followed by T2 (25.33 µg/g), T1 (21.98 µg/g), T4 (20.82 µg/g), T5 (18.96 µg/g) and T_0 (4.10 µg/g) at the end of the experiment. Significant ($p>0.05$) increased trend of body carotenoids were observed in T3 group after fifteen days and which was continued throughout the experimental period. The physio-chemical parameters of water of the experimental tanks during the experimental period are presented in Table 4. The water temperature of the experiment varied between 27^0c to 29.5^0c. The pH fluctuated from 7.2 to 7.9. The dissolved oxygen (DO) level showed variation from 5.5mg to 6.80 mg/l. Total alkalinity varied from 70 ppm to 90 ppm. The presence of free carbon dioxide and ammonia was in trace level throughout the experimental period.

DISCUSSION

Fishes use carotenoid as one of the most important group of natural pigment for pigmentation of the skin and flesh. As fish cannot synthesize these pigments de novo, they depend on a dietary supply of carotenoids to achieve their natural skin pigmentation, one of the quality criteria most in demand for the market value of ornamental high-value species such as goldfish (Lovell, 2000; Gouveia *et al.*, 2003; Sinha and Asimi, 2007). Many commercial products extracted from marigold flower (*Tagetes erecta*) are being used throughout the world by poultry and nutraceutical industries. These products are rich in lutein and zeaxanthin, which play an important role as precursors of astaxanthin and enhance pigmentation of animals (Del Villar-Martínez *et al.*, 2007).

In the present study survival rate of fish is not markedly different within the treatment but growth performance with respect to final mean weight and specific growth was significantly improved in fish fed with 180 mg/kg marigold oleoresin than the other lower and higher inclusion levels. Also the fish fed with 180 mg/kg marigold oleoresin supplemented diets showed lowest food conversion rate. These results are also in agreement with reports that link carotenoids to growth enhancement in Atlantic salmon fry (*Salmo salar*) (Christiansen *et al.*, 1995), rainbow trout (*Oncorhyncus mykiss*) (De la

Table 3. Total carotenoids concentration in body of Koi carp (*C. carpio*) fed with experimental diet (µg of carotenoids / g of tissue).

Treatments	Carotenoids (mg/kg)	Days				
		Initial	15days	30days	45days	60days
T_0	0	4.3±0.0[a]	4.45±0.04[a]	4.21±0.22[a]	4.13±0.24[a]	4.10±0.28[a]
T_1	60	4.3±0.0[a]	5.26±0.11[c]	7.26±0.21[b]	12.54±0.37[b]	21.98±0.26[d]
T_2	120	4.3±0.0[a]	6.06±0.13[d]	9.10±0.30[c]	16.18±0.40[d]	25.33±0.8[e]
T_3	180	4.3±0.0[a]	6.52±0.20[e]	10.95±0.41[d]	18.0±0.21[e]	30.16±0.60[f]
T_4	240	4.3±0.0[a]	5.10±0.09[b]	8.86±0.18[c]	13.20±0.24[bc]	20.82±0.54[c]
T_5	300	4.3±0.0[a]	5.12±0.16[b]	9.08±0.20[c]	14.45±0.34[c]	18.96±0.39[b]

Different superscript indicates significant difference (P<0.05)

Table 4. Physico-chemical parameters of water during the experimental period.

Parameters	Values
Temperature (°C)	27.0°C – 29.5°C
DO (mgl^{-1})	5.5 – 6.80
pH	7.2 - 7.9
Free carbon dioxide (mgl^{-1})	1.40 – 2.80
Total alkalinity (mgl^{-1})	70 - 99
Ammonia- nitrogen (μgl^{-1})	0.02 - 0.32

Mora *et al.*, 2006) and goldfish (*Carassius auratus*) (Sinha and Asimi, 2007) Further, observation was made by Ahilan *et al.* (2008) in gold fish fed with coriander incorporation feed at 3 percent level showed better biological performance like weight gain and specific growth rate when compared to other coriander in corporate diet and control. According to Tveranger (1986) and Sommer *et al.* (1992), the addition of carotenoids rich microalgae and krill meal enhanced the growth of trout.

The carotenoids deposition was significantly higher in fish body fed with carotenoids supplemented diet (with 180mg/kg of Marigold oleoresin) diet than unsupplemented control and other inclusion level of carotenoid in diet. It is observed that 250 mg of astain supplementation per kg of diet showed highest carotenoids deposition in Koi carp (Liang *et al.*, 2012) and at a low concentration (60mg/kg) a saturation point in the accumulation of carotenoids was reached in adult Dwarf cichilid (Harpaz, 2007). Similar results were obtained for goldfish by feeding different natural carotenoid sources, such as Spirulina (Kiriratnikom *et al.*, 2005), microalgal biomass (Gouveia and Rema, 2005), red yeast (Xanthophyllomyces dendrorthous) (Xu *et al.*, 2006) and alfalfa (Medicago sativa) (Yanar *et al.*, 2008). The results obtained in the current study concludes that the pigmentation in skin of Koi carp increased with a level up to 180ppm of carotenoids /kg of feed from marigold oleoresin, over that level there was no additional carotenoid accumulation in fish body. Current findings are supported by Yanar *et al.* (2008) mentioned that carotenoid uptake or transportation to the tissue was saturated due to carotenoid inclusion level (200mg) in goldfish. The effectiveness of a carotenoid source for pigment deposition is species specific (Ha *et al.*, 1993).

Conclusion

The present study concluded that marigold oleoresin (180 mg/kg of feed) as a carotenoid source was effective on growth and skin pigmentation of Koi carp as it led to nearly maximum carotenoids accumulation in the boy of goldfish.

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