



Effect of Dietary Supplementation of Carrot Meal on Survival, Growth and Pigmentation of Freshwater Ornamental Fish, Koi Carp, *Cyprinus Carpio* (L.)

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ABSTRACT

The study was conducted to assess the efficacy of experimental diets supplemented with carrot meal (CM) @ 1, 3, 5 and 7 % with respect to pigmentation, survival and growth in freshwater ornamental fish koi carp, *Cyprinus carpio* (Linnaeus) for 120 days. Five diets including one control (CM0) without any supplemented carotenoid source and four supplemented experimental pelleted diets with 1% (CM1), 3% (CM3), 5% (CM5) and 7% (CM7) of powdered carrot meal were prepared. At the termination of the experiment, colouration studies indicated significantly higher ($P < 0.05$) skin and muscle carotenoid ($\mu\text{g g}^{-1}$ wet weight basis) content in fish fed with CM5 (4.87) followed by diets CM3 (3.28), CM7 (2.78), CM1 (1.95) and CM0 (1.24). RGB colour intensity based on digital photographs analysis was also found to be highest in CM5 followed by CM3, CM7, CM1 and CM0. Fish growth in terms of % NWG, PER and SGR was highest in CM5 (94.09%, 1.48, 0.55), with lowest values of NWG and SGR in CM7 (65.71% and 0.41), while PER in CM1 (0.75). Further, supplementation of CM @ 5% in Koi carp diet also improved the FCR (2.16) as compared to control (4.17). 100% survival of fish was recorded with diets supplemented with CM @ 3, 5 and 7 %, while it was 80 % in CM0 and CM1. During the experimental period, the water quality parameters did not vary significantly among control and treatments and remained in optimum range for fish growth. Hence, carrot meal can be supplemented in koi carp diet up to 5 % level both as colour enhancer as well as growth promoter under indoor rearing conditions.

Key words: Carotenoids, Carrot meal, Colour enhancement, Koi carp, Pigmentation

INTRODUCTION

Ornamental fish are nature's wonderful creation and ornamental fish keeping is the second most preferred hobby in the world and this sector plays a vital role in the international fish trade, having high export value (Jain *et al.*, 2016). The value of ornamental fish is determined by their unique body characteristics, good health and coloration. The beautiful and flamboyant coloration exhibited by ornamental fishes is due to the presence of carotenoid pigments along with overall nutrition. Therefore, there is a direct relationship between dietary carotenoids and pigmentation in fish (Halten *et al.*, 1997). Carotenoids are the main pigments, representing a class of over 600 natural lipid soluble pigments found in plants, algae, photosynthetic, some non-photosynthetic bacteria and animals, which are required for healthy growth, metabolism, photosynthesis and reproduction, besides colour development. Chemical composition of

carotenoids include approximately 20% β -carotene, 30% γ -carotene and 30 % lycopene (oxygen free hydrocarbons) and 15% of oxygen containing carotenoids *i.e.* xanthophylls (Zeb, 2004). Fish contain various kinds of carotenoids, the proportion of which depends upon their physiological status and dietary conditions. Synthetic or natural carotenoids are required to be incorporated @ 50-400 mg kg^{-1} in diet of indoor reared fish to develop colour similar to fish feeding on live food like algae, tubifex, zooplankton, *etc.* (Boonyaratapalin and Lovell, 1977) under outdoor conditions.

For supply of carotenoids, various synthetic as well as natural sources (animal and plant) are available and are being developed for use in aquaculture for food as well as ornamental fishes. Synthetic processes results in production of only specific carotenoids like β -carotene or astaxanthin, whereas, natural sources are always a combination of several carotenoids

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like α -carotene, β -carotene, zeaxanthin, lutein, cryptoxanthin *etc.* (Gupta *et al.*, 2007). Moreover, synthetic carotenoids are expensive and their use in aqua feed is limited due to species specificity. Natural carotenoids of animal origin are mostly extracted from crustacean such as crayfish meal, shrimp meal, crab meal, *etc.* (Torrissen and Naevdal, 1984) and are rich source of astaxanthin. The major concerns with the production of animal-based carotenoid are limited supply due to declining catches of crustaceans along with high expenses, which leads to increase in cost of carotenoid incorporated aquaculture diets. Plant based natural carotenoids are mainly derived from flowers, fruits, vegetables, algae, yeast, *etc.* (Tsushima and Matsuno, 1998; Chapman, 2000), which are not only inexpensive, but also a potential source of mixed carotenoids.

Few studies have demonstrated the effect of natural carotenoids on the growth, survival, colour development and antioxidation responses in ornamental fishes. Among natural vegetable pigment source, carrot (family Apiaceae) is one of the important root vegetables rich in bioactive compounds *i.e.* carotenoids along with several other functional components having significant health promoting properties (Sharma *et al.*, 2012). The total carotenoid content in the edible portion of the carrot root range from 6-55 mg/100g (Simon and Wolff, 1987) with β -carotene (44-79 % of total carotenoids, 5.3 – 10 mg/100 g) as major component (Holland *et al.*,

1991) and is the single major source of β -carotene providing 17% of total vitamin A. Due to these properties, carrot alone or in combination with other natural antioxidants, is found to induce better growth, enhance colour intensity and improve health of ornamental fish in a natural way. In view of unique properties of carrot, the present study was designed to assess the efficacy of carrot (*Daucus carota*) meal on survival, growth and pigmentation of ornamental Koi, *C. carpio* (Linn.).

MATERIALS AND METHODS

The experimental fish, Koi carp, *C. carpio* L. were procured from ornamental fish culture unit of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The fishes were acclimatized under indoor conditions for one month with control diet. Ten uniformly sized fishes (av. length 2-4 cm, av. weight 1-2 g) were stocked in glass aquaria, having 50 liters of water with continuous oxygen supply. The experiment (in triplicate) was carried out for 120 days.

Four experimental supplemented pelleted diets were prepared by adding carotenoid source *i.e.*, carrot meal (CM) @ 1 % (CM1), 3 % (CM3), 5 % (CM5) and 7 % (CM7) in the basal diet (CM0) by adjusting the level of other feed ingredients (Table 1). Carrot was procured from market, washed, grated and dried under shade and grounded to prepare the powdered meal. All the feed ingredients were powdered, mixed thoroughly

Table 1. Composition (%) of experimental diets

Ingredients	Experimental diets				
	CM0(Control)	CM1	CM3	CM5	CM7
Soybean meal*	25	25	24.5	24	24
Groundnut meal*	25	25	24.5	24	23
Mustard meal*	25	25	24	24	23
Rice bran*	20	19	19	18	18
Vegetable oil	02	02	02	02	02
Vitamin-mineral mixture	02	02	02	02	02
Salt	01	01	01	01	01
Carrot meal	-	1	3	5	7

*Solvent extracted

and palletized. The proximate composition of feed ingredients and experimental diets (Table 2) was analyzed by following the methods of AOAC (2000). Carotenoid analysis of pigment source (carrot meal) was done by following method of Cyanotech (2002). Pelleted feed was provided to the fish @ 5 % fish body weight twice a day. Amount of feed was adjusted at every sampling (fortnightly interval) according to increase in fish weight.

Water quality parameters in terms of water temperature, pH, dissolved oxygen, total alkalinity, total hardness and ammonical nitrogen were measured at fortnightly intervals by following the methods of APHA (1991). Survival of the fish was calculated by subtracting the number of fishes harvested at the end of the experiment from the fish stocked at the initiation of the experiment.

Fish growth in terms of total body length and body weight was recorded at fortnightly intervals. At the end of the experiment, total length gain (TLG), per cent total length gain (%TLG), net weight gain (NWG), percent net weight gain (% NWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and condition factor were calculated as follows

TLG = Final total body length (cm)-initial total body length (cm)

%TLG = Final total body length (cm)-initial total body length (cm)/Initial total body length (cm) × 100

NWG = Average final body wt. (g) - Average initial body weight (g)

%NWG = Final body weight (g) - initial body weight (g) /initial body weight (g) × 100

SGR (% increase in weight /day) =

$$\frac{\ln \text{ final body weight} - \ln \text{ initial body weight}}{\text{Culture days}} \times 100$$

Where, ln = Natural logarithm

FCR = Feed given (g) / Weight gain (g)

PER = Weight gain (g) / Protein intake (g)

Condition factor (K) =

$$\text{Body weight} / (\text{Body length})^3 \times 100$$

Carotenoid analysis of fish muscle and skin was done by following method of Olson (1979) at initiation, middle and termination of the experiment. The digital photographs of experimental fish were taken at monthly intervals with a Sony full HD camera (DSC-HX 300). Colour of the fish skin was measured from opercular region, base of dorsal fin and caudal peduncle area of at least three fishes from each replicate. While taking the photographs all camera conditions were kept constant.

Table 2. Proximate composition (% DM basis), gross energy (kcal/g) and total carotenoid content (mg/100g) of carrot meal and carrot meal incorporated diets

Ingredients/ feed	Crude protein	Ether extract	Crude fibre	Ash	NFE	Gross energy (kcal/g)	Carotenoid content (mg/ 100g)
Rice bran*	21.94	1.46	10.86	7.00	58.72	3.77	-
Ground nut meal*	35.62	0.76	20.26	8.40	34.94	3.50	-
Mustard meal*	34.67	0.76	14.40	8.00	41.56	3.78	-
Soybean meal*	39.37	1.46	9.20	6.13	43.82	4.14	-
Carrot meal	17.33	1.33	10.33	8.50	59.03	3.50	16.45
CM0	31.93	1.04	13.20	7.60	46.46	3.79	-
CM1	31.20	1.06	13.06	6.95	47.73	3.81	0.152
CM3	31.38	1.08	13.04	7.12	47.38	3.81	0.431
CM5	31.20	1.06	12.93	7.16	47.65	3.81	0.670
CM7	30.85	1.00	12.81	7.11	48.23	3.80	0.987

*Solvent extracted

Photographs were analyzed in adobe photoshop (Hancz *et al.*, 2003; Tlusty, 2005) to compare these on the basis of RGB (Red, Green and Blue) values. To quantify the colour of the fish skin from digital photographs, CIE (Commission International de l'Eclairage) Lab is applied. Adobe Photoshop has a colour space called "Lab colour mode" which is based on CIE Lab. It consists of three parameters: L*, a* and b*. Where L* stands for luminosity (lightness ranging from 0 for black to 100 for white), channel a* is the balance between red / green and channel b* describes the balance between yellow/ blue.

RESULTS AND DISCUSSION

The study revealed that during the experimental period, water quality parameters (Table 3) such as temperature, pH, dissolved oxygen, total alkalinity, total hardness and ammonical-nitrogen did not vary significantly among the treatments and were within the recommended range for ornamental fish culture (Wurts and Durborow, 1992; OATA, 2008; Rinna *et al.*, 2013).

At the termination of the experiment, 100% survival of fish was recorded with diets supplemented with CM @ 3, 5 and 7 %, while it was 80 % in control (CM0) and CM1 (Table 4), indicating positive effect of carrot on survival of fish. According to Krinsky (1993),

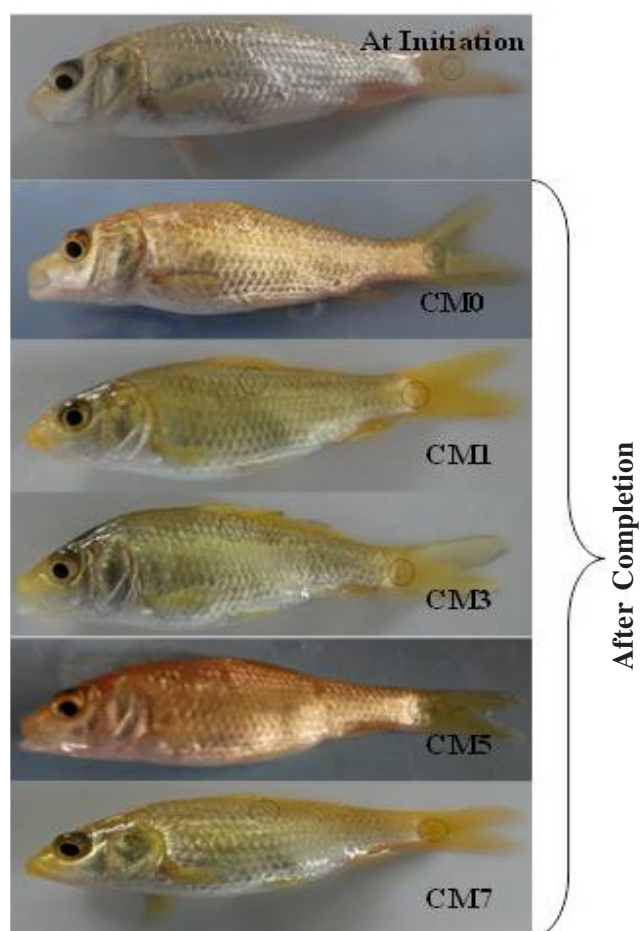


Fig. 1. Changes in skin colour of Koi carp, *C. carpio* L. before and after feeding

Table 3. Physico-chemical parameters of water in different treatments

Parameters	Diets				
	CM0(Control)	CM1	CM3	CM5	CM7
Water temperature (°C)	29.28 ^a ±0.50 (27.50-31.76)	29.15 ^a ±0.62 (26.66-31.70)	29.36 ^a ±0.57 (26.73-31.93)	29.00 ^a ±0.65 (26.20-31.86)	29.24 ^a ±0.64 (26.33-32)
pH	8.59 ^a ±0.08 (8.33-8.90)	8.50 ^a ±0.07 (8.24-8.76)	8.51 ^a ±0.07 (8.24-8.75)	8.57 ^a ±0.09 (8.20-8.97)	8.61 ^a ±0.09 (8.18-9.02)
Dissolved oxygen (mg l ⁻¹)	6.78 ^a ±0.29 (5.86-8.40)	6.86 ^a ±0.28 (5.86-8.40)	6.91 ^a ±0.28 (5.80-8.20)	7.04 ^a ±0.28 (6.20-8.20)	7.07±0.22 (6.33-8.20)
Total alkalinity(mg CaCO ₃ l ⁻¹)	211.14 ^a ±12.2 (140-254)	203.33 ^a ±9.98 (154-254)	214.67 ^a ±11.79 (160-256)	214.22 ^a ±10.92 (150-257)	219.44 ^a ±14.1 (148-277)
Total hardness(mg CaCO ₃ l ⁻¹)	266.56 ^a ±5.5 (235-282)	269.33 ^a ±6.1 (246-298)	275.89 ^a ±5.7 (255-296)	274.11 ^a ±4.0 (252-292)	266.22 ^a ±6.68 (229-296)
NH ₃ -N (mg l ⁻¹)	0.185 ^a ±0.020 (0.116-0.308)	0.179 ^a ±0.022 (0.063-0.251)	0.159 ^a ±0.027 (0.045-0.260)	0.188 ^a ±0.024 (0.049-0.268)	0.171 ^a ±0.024 (0.044-0.249)

Values are Mean ± S.E.; Values with different superscript (abcd) in a row differ significantly (P<0.05); values in parentheses indicate range

absorption of carotenoids enhances fish survival. In agreement to present study, Mirzaee *et al.* (2013) recorded colour enhancement in jewel cichlid (*Hemichromis bimaculatus*) with carrot as compared to red pepper. Likewise, Kop *et al.* (2010) reported carrot as an efficient carotenoid source in comparison to red pepper (*Capsicum annum*) for colour enhancement in cichlid (*Cichlasoma severum*). Comparison of carrot (*D. carota*) with three flower pigment sources *i.e.* marigold petal (*Tagetes erecta*), China rose petal (*Hibiscus rosasinensis*), rose petal (*Rosa cinensis*) also revealed that carrot resulted in significantly higher carotenoid content in fish skin/muscle (Ramamoorthy *et al.*, 2010). Yanar and Tekelioglu (1999) also suggested utilization of carrot as potential carotenoid source for colour development in gold fish, *Carassius auratus*.

At the end of culture period, maximum average final total body length (cm) was observed in CM1 followed by CM7, CM5 and CM0 and CM3, respectively and the differences among treatments were significant ($P < 0.05$). TLG and % TLG were also

maximum in CM1 and minimum in CM3 and the differences among different treatments were significant ($P < 0.05$). The final body weight was highest in CM5 followed by CM1, CM0, CM3 and CM7 with significant differences. Likewise, NWG, %NWG and SGR was highest in CM5 and minimum in CM7. PER and CF were highest in CM5, while PER was lowest in CM0, and CF was lowest in CM1. Feed efficiency in terms of FCR also improved maximally in CM5 as compared to all other treatments and control.

Improved fish growth has been found in pigment supplemented diets as carotenoids are known to play a positive role in the metabolism of fish and might have improved nutrient utilization leading to improved growth (Amar *et al.*, 2001). In the present study also, it is clear that carotenoid do play a role in fish growth, as increasing level of CM (1-5%) resulted in positive effects on growth, in terms of increased weight gain and reduced FCR. Mirzaee *et al.* (2013) conducted comparative study to see the effect of carrot and red pepper (both providing 60 mg/kg of total pigment) on growth of jewel cichlid. Feeding of carrot resulted in

Table 4. Growth performance and of koi carp, *C. carpio* L. in different treatments

Parameters	Diets				
	CM0(Control)	CM1	CM3	CM5	CM7
% Survival	80	80	100	100	100
Final total body length (cm)	6.24 ^{bc} ±0.20	6.48 ^a ±0.19	6.13 ^c ±0.21	6.24 ^{bc} ±0.11	6.26 ^{bc} ±0.06
TLG	0.70	0.96	0.62	0.82	0.67
	---	(+26.32%)	(-11.43%)	(+17.14%)	(-4.28%)
%TLG	12.63	17.39	11.25	14.49	11.98
Final body weight (g)	3.83 ^{cd} ±0.44	4.18 ^b ±0.26	3.62 ^{cd} ±0.21	4.27 ^a ±0.24	3.48 ^{cde} ±0.16
NWG	1.60	1.96	1.44	2.07	1.38
		(+22.50 %)	(-10.00 %)	(+29.37 %)	(-13.75 %)
% NWG	71.74	88.28	66.05	94.09	65.71
FCR	4.17	3.40	3.32	2.16	3.46
PER	0.75	0.94	0.95	1.48	0.93
SGR	0.45	0.53	0.43	0.51	0.42
CF	1.57	1.53	1.57	1.75	1.41

Values are Mean ± S.E.; Values with different superscript (ab...d) in a row differ significantly ($P < 0.05$); Values in parentheses indicate % change over control; TLG = Total length gain; NWG = Net weight gain, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR= Specific growth rate, CF = Condition factor

significantly higher growth as compared to red pepper. Ramamoorthy *et al.* (2010) too showed enhanced growth of marine ornamental fish, *Amphiprion ocellaris* fed on four pigment sources (carrot, *D. carota*; marigold meal, *T. erecta*; China rose petal, *H. rosasinensis* and rose petal, *R. chinensis*) as compared to non-pigmented diet, where *D. carota* resulted in maximum growth rate as compared to other diets as well as control. Positive effects in terms of growth through carotenoid were also observed by Ahilan *et al.* (2008) in Gold fish, *C. auratus*, when amaranth and coriander were given @ 1 and 3 %, respectively. *Spirulina*, a rich source of carotenoids, has also proved to be protein rich nutrient and resulted in significantly higher growth (@ 30 mg kg⁻¹ of diet) in blue gourami, *Trichogaster trichopterus* (Rinna *et al.*, 2013) Similar to natural carotenoid sources, synthetic carotenoid source such as astaxanthin was also reported to influence fish and shellfish growth in a positive manner

(Thongrod *et al.*, 1995). Liang *et al.* (2012) showed significant higher weight gain and specific growth rate along with significantly reduced FCR in red white koi carp with 150, 200 and 250 mg of astacin kg⁻¹ of diet. Similarly, Nguyen *et al.* (2014) found that supplementation of astaxanthin @ 80 mg kg⁻¹ in koi carp resulted in higher growth and feed utilization. However, few of the earlier reports suggested the role of carotenoids only on colour enhancement without having much effect on the fish growth (Kop *et al.*, 2010; Mirzaee *et al.*, 2013; Seyedi *et al.* 2013).

The result of the present study revealed maximum colour enhancement in terms of carotenoid content at 5% incorporation level of carrot meal as compared to both low (1% and 3%) and high incorporation level (7%). The digital photographs also exhibited improved values for RGB along with increased a* and b* values in CM5. In the present study, carrot meal resulted in significantly increased carotenoid

Table 5. Colouration parameters in skin of Koi carp (*C. carpio* L.) in different treatments

Days	Particulars	Diets*				
		CM0	CM1	CM3	CM5	CM7
Carotenoid content (µg/g)						
120		1.24 ^c ±0.011	1.95 ^d ±0.008 (57.25)	3.28 ^b ±0.035 (164.52)	4.87 ^a ±0.384 (292.74)	2.78 ^c ±0.018 (124.19)
RGB Values						
0	R	59.00 ^a ±1.17	59.00 ^a ±1.10	60.00 ^a ±1.11	59.00 ^a ±1.12	58.00 ^a ±1.15
	G	51.00 ^a ±1.21	50.00 ^a ±1.12	52.00 ^a ±1.15	52.00 ^a ±1.14	51.00 ^a ±1.16
	B	28.00 ^a ±1.20	29.00 ^a ±1.13	29.00 ^a ±1.14	30.00 ^a ±1.16	29.00 ^a ±1.19
120	R	75.66 ^d ±2.60	179.00 ^{ab} ±5.77	187.66 ^a ±3.17	189.00 ^a ±9.81	150.66 ^{bc} ±5.48
	G	66.66 ^{de} ±0.33	166.00 ^{bc} ±2.30	155.66 ^{cd} ±3.17	185.00 ^a ±0.57	150.00 ^c ±4.61
	B	51.66 ^d ±3.17	143.66 ^{bc} ±1.45	174.66 ^a ±7.79	179.00 ^a ±1.73	167.00 ^{ab} ±5.19
Lab values						
0	L*	22.00 ^a ±0.25	21.00 ^a ±0.30	22.00 ^a ±0.25	21.00 ^a ±0.24	21.00 ^a ±0.26
	a*	1.00 ^a ±0.26	1.00 ^a ±0.21	1.00 ^a ±0.24	1.00 ^a ±0.23	1.00 ^a ±0.28
	b*	16.00 ^a ±0.15	15.00 ^a ±0.19	17.00 ^a ±0.21	18.00 ^a ±0.22	18.00 ^a ±0.20
120	L*	31.00 ^{cd} ±0.57	69.00 ^{ab} ±1.15	64.66 ^b ±0.88	62.00 ^{bc} ±1.15	75.66 ^a ±0.33
	a*	1.89 ^{de} ±0.57	4.00 ^{bc} ±0.00	3.66 ^{cd} ±0.88	7.66 ^a ±1.45	3.00 ^{cd} ±0.57
	b*	15.00 ^e ±2.00	34.00 ^{cd} ±0.57	35.66 ^{bc} ±0.33	39.00 ^a ±2.00	34.66 ^{bc} ±2.02

Values are Mean ± S.E.; Values with different superscript (ab...d) in row differ significantly (Pd" 0.05); Values in parentheses indicate % change over control

concentration at 5 % incorporation level, showing direct relationship with concentration of carotenoids on deposition of carotenoids. A positive relationship between feed dose and deposition of carotenoids in muscle of Atlantic salmon was also observed by Baker (2002). In agreement to present studies, Mirzaee *et al.* (2013) also recorded more colour enhancement in jewel cichlid (*H. bimaculatus*) with carrot as compared to red pepper. Likewise, Kop *et al.* (2010) also reported carrot (*D. carota*) as a more efficient carotenoid source in comparison to red pepper (*Capsicum annum*) for colour enhancement in cichlid (*C. severum*). Comparison of carrot with three flower pigment sources *i.e.* marigold petal (*T. erecta*), China rose petal (*H. rosasinensis*), rose petal (*R. cinensis*) also revealed that carrot resulted in significantly higher carotenoid content in fish skin/muscle (Ramamoorthy *et al.*, 2010). Yanar and Tekelioglu (1999) also suggested utilization of carrot as potential carotenoid source for colour development in gold fish, *C. auratus*.

Thlusty (2005) while feeding of American Lobster (*Homarus americanus*) also found the colouration values on RGB scale were proportional to amount of astaxanthin carotenoid in the diet. Liang *et al.* (2012) analyzed the red white ornamental koi carp by visual evaluation, photography as well as total carotenoid concentration after feeding with different levels of astacin. Red areas of skin, scale, head and fins showed marked increase in carotenoid content, when fed with 200 and 250 mg kg⁻¹ of astacin. In addition, highest deposition of carotenoid was observed in red skin followed by scales, red head and fins. Sun *et al.* (2012) compared the effectiveness of synthetic and natural pigment sources in colouration of Japanese ornamental koi carp (*C. carpio*). Synthetic carotenoid *i.e.* carophyll red @ 1.5 g kg⁻¹ and natural pigment source *i.e.* *Spirulina platensis* @ 75 g kg⁻¹ of diet resulted in similar colour improvement. These studies clearly indicate the effectiveness of natural carotenoids similar to synthetic sources in colour enhancement of koi.

The results in terms of CIE colouration were in agreement with the findings of Yeider *et al.* (2014) in

Zebra cichlid (*Maylandia estnerae*) with *spirulina* feeding, in which decreased L* values (lightness) along with significantly increased a* and b* values (red and yellow) were observed. Similarly, in the present study also, increased a* and b* values resulted in enhanced orange appearance of fish skin. These results are in accordance with the study of Nguyen *et al.* (2014) on koi carp, in which significantly higher values of a* and b* were reported, when fish were provided with synthetic astaxanthin @ 80 mg kg⁻¹ of the diet. Skin pigmentation in terms of total carotenoids (0.9 µg/g) and L*a*b* values were significantly affected by paprika @ 16 % in the diet of pale chub (*Zacco platypus*). The skin lightness (L* values) significantly decreased with increased values of a* and b* in fish fed diets containing paprika (Lee *et al.*, 2010).

CONCLUSION

It can be concluded that the carrot meal has a positive effect on growth as well as colour enhancement of koi carp, *Cyprinus carpio* L. in indoor rearing conditions. Hence, carrot meal can be supplemented in koi carp diet at 5 % level both as colour enhancer and growth promoter.

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