The Bioscan 13(2): 719-724, 2018
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

EFFECT OF SUPPLEMENTATION OF DIFFERENT CAROTENOID SOURCES ON GROWTH AND COLOUR ENHANCEMENT OF KOI CARP (*Cyprinus carpio*) Fry

D. CHOUDHURY[1]*, B. B. J. SAHOO[2], S. S. DAS[3] AND S. NANDA[1]
[1]College of Fisheries, Orissa University of Agriculture and Technology (OUAT), Rangailunda, Berhampur, Ganjam, Odisha, 760 007, INDIA.
[2]Management Society-Fishing Harbour, Sector-21, Nehru Bunglow, Paradeep, Jagatsinghpur, Odisha -754 143.
[3]Krishi Vigyan Kendra Ganjam-II,
Orissa University of Agriculture and Technology (OUAT), Berhampur, Odisha - 761 001, INDIA.
e-mail: dharitrichoudhury@rediffmail.com

KEYWORDS
Carotenoid
Koi carp fry
Color enhancement
China rose petal

Received on :
09.03.2018

Accepted on :
16.05.2018

***Corresponding author**

ABSTRACT
To know the effect of dietary supplementation of different carotenoides (marigold petal, rose petal, china rose petal and carrot) on growth and color enhancement, one hundred fifty number of koi carp (*Cyprinus carpio*) fry of average weight (0.63-0.75g) were distributed in five experimental groups and fed with five isonitrogenous diets such as T_0 (control), T_1 (15% marigold petal meal), T_2 (15% rose petal meal), T_3 (15% china rose petal meal) and T_4 (15% carrot meal) for a period of 90 days. Significantly higher daily weight gain (0.03 ± 0.001), weight gain % (425.12 ± 32.01), SGR (0.794 ± 0.019) and lower FCR (5.25 ± 0.03) were observed in T_3 group. Among the treatments maximum total carotenoid in the muscle was found in T_3 group ($17.791 \pm 0.395 \mu g$/g wet tissue) and the minimum was found in T_0 group (4.782 ± 0.412 μg/g wet tissue). The scores obtained in the panel test also showed highest value in T_3 group (7.725 ± 0.79). The computer assisted image analysis of the skin showed highest r (red) value in T_3 group (0.454 ± 0.024) and lowest in T0 group (0.320 ± 0.017). Thus it is concluded that growth and color in koi carp fry can be enhanced using china rose petal meal at 15% level of inclusion in the diet.

INTRODUCTION

Ornamental fish keeping and rearing in aquarium is an age old practice which is reflected by *ubiquitous aquaria* that feature an integral part of modern interior decorations in 21st century (Katia, 2001). The sector also plays an important role in international fish trade, fisheries, aquaculture and development (FAO, 2014). The ornamental fish trade and its demand is very much dependent on achieving vibrant color. Thus emphasis should be given to achieve high level of skin pigmentation which along with body shape, fin shape, body size determines their price in the international market (Paripatananont *et al*., 1999). Intensive culture of colorful fishes in captivity resulted in faded or degraded color of their skin which is a major concern and needs to be addressed. Carotenoides is a group of lipid soluble organic molecule and are the primary source of pigmentation (red, orange, yellow) on the skin of fishes and are believed to play positive role in immunostimulation, reproduction, intermediary metabolism *etc*. (Chatzifotis *et al*., 2005). In natural environment, the fishes meet their carotenoid requirement by ingesting aquatic plants or through their food chain, but in culture system they rely entirely on dietary supplementation to sustain and achieve natural coloration as fishes like other animals cannot biosynthesis carotenoids *de novo* (Goodwin, 1984). The bright and beautiful coloration of the fishes is a combination of carotenoids especially astaxanthin, zeaxanthin and leutin when these are supplied through diet in confinement (Shahidi *et al*., 1998). Different sources of carotenoids like synthetic carotenoides (β- carotene, castaxanthine, zeaxanthine, astaxanthine), animal sources and plant sources are used in fish diet for enhancing pigmentation (Kalinowski *et al*., 2005).

A number of works have already been carried out on incorporation of different natural plant origin carotenoids in feed to induce growth and coloration in many ornamental fishes such as dried flowers (Torrissen *et al*., 1989), yeast (Savolainen & Gyllenberg, 1970), algae (Hekimoglu *et al*., 2017), beet root meal (Jha *et al*., 2012; Ezhil and Narayan, 2013), coriander, mint and amaranth leaves (Ahilan *et al*., 2008), red pepper (Maede *et al*., 2013), paprika (Hancz *et al*., 2003), spirulina (Bakshi *et al*., 2017; Behera *et al*., 2018) etc. Marigold petal has been used as a crotenoid source in many ornamental fishes like red sword tail (Ezhil *et al*., 2008), gold fish (Alma *et al*., 2013), koi carp (Swain *et al*., 2014), sword tail (Golandaj *et al*., 2015) and rosy barb (Jagadeesh *et al*., 2015) for enhancing pigmentation. Rose petal meal was used for *Amphiprion ocellaris* (Ramamoorthy *et al*., 2010), orange sword tail (Joseph *et al*., 2011), rosy barb (Pailan *et al*., 2012 a) and dwarf gouramy (Pailan *et al*., 2012 b) to increase coloration. Ramamoorthy *et al*. (2010), Joseph *et al*. (2011), Somanath and Jasmin (2013) used china rose petal meal as a

dietary source of carotenoid in *Amphiprion ocellaris*, orange sword tail and gold fish respectively. Ramamoorthy *et al*. (2010) found carrot meal as a good source of natural color enhancer as compared to marigold petal, china rose petal and rose petal for marine ornamental fish, *Amphiprion ocellari*. Among the important ornamental fishes Koi carp (*Cyprinus carpio*) are characterized by a wide diversity of color and color pattern (Gomelsky *et al*., 2003). Koi value increases with intensity of skin coloration and the natural color of the fish can be sustained and enhanced by dietary supplementation of carotenoids.

Keeping this in view, the present study was conducted to evaluate the efficacy of natural carotenoid sources like marigold petal, rose petal, china rose petal and carrot on growth and coloration of koi carp, *Cyprinus carpio*.

MATERIALS AND METHODS

Experimental design and experimental fish

Koi carp fry were brought from the Ornamental fish breeding and rearing unit of State Fisheries Department, Kausalyagang in oxygen packing polybags to the laboratory and were acclimatized under aerated condition for a period of 30 days. During the period, the fishes were fed with the control diet. One hundred and fifty fry of koi carp (avg weight of 0.63-0.75 g) of same color hue were randomly distributed in 15 aquaria of 30 lit. capacity (10 fish/ aquarium), following a completely randomized design. Constant aeration facilities were provided except the period of feeding. The experimental aquaria were cleaned manually with 30% water exchange daily and siphoning was done to remove excess left over feed and remaining faecal matter. Water quality parameters viz., temperature, pH, dissolved oxygen, free CO_2, total alkalinity, hardness were recorded (APHA, 1995) at forth night interval.

Diet formulation

Marigold (*Tagetes erecta*) petals, rose (*Rosa chinensis*) petals, china rose (*Hibiscus rosasinensis*) petals and carrot (*Daucus carota*) were air dried in a dark room under fan to prevent denaturation of carotenoids. After drying the sources were powdered, sieved and used as an ingredient for preparation of experimental diets. Five isonitrogenous diets were prepared by using the square method (Hardy, 1980) namely diet without carotenoid (T_0), diet containing 15% marigold petal meal (T_1), diet containing 15% rose petal meal (T_2), diet containing 15% china rose petal meal (T_3) and diet containing 15% carrot meal (T_4). The proximate composition of the experimental diets were analysed as per the standard methods of (AOAC, 2005). The ingredient and proximate composition of the experimental diets were given in Table 1.

Feeding and sampling

The fishes were fed @ 5% of the body weight for a period of 90 days. The daily ration was divided into two equal parts and was fed at 6.00 AM in the morning and 6.00 PM in the evening. At the end of the experiment the wet weight of the fishes was recorded. The following growth parameters were calculated by using the standard formulas.

$$\text{Daily weight gain(DWG)} = \frac{\text{Final weight of fish - Initial weight of fish}}{\text{Total no.of experimental days}}$$

$$\text{Weight gain \% (WGP)} = \frac{\text{Final weight of fish - Initial weight of fish}}{\text{Iniitial weight of fish}} \times 100$$

$$\text{Specific growth rate(SGR)} = \frac{\log_e \text{Final body weight} - \log_e \text{Initial body weight}}{\text{Total no.of experimental days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total dry food intake(g)}}{\text{Ttoal live weight gain(g)}}$$

Total carotenoid content in the muscle of the fishes were determined at the end of the experiment following spectrophotometric method (Hongxia *et al*., 2005). About 2-3g of fish muscle (without head and alimentary canal) was taken in a clean mortar, homogenized and extracted with acetone containing BHT as an antioxidant. The acetone extraction was repeated until the tissue became white. The acetone extract was pooled, mixed and centrifuged at 3,500 rpm for 5 minutes at 4ºC. The supernatant liquid was collected in a calibrated centrifuge tube and evaporated to dryness with anhydrous Na_2SO_4 in a dark and cold room. Then the dried residue was re-suspended in ethanol and the absorbance was recorded at 450 nm. The total carotenoid content was calculated as β carotene equivalent using the following equation:

μg β carotene / g wet tissue = (10,000 x V x A) / (W x $E_{\%, 1cm}$)

where,

V is the total volume of extract

W is the weight of the sample

A is the recorded absorbance at 450 nm and $E_{\%,1\ cm}$ is the extinction coefficient of β carotene as 2620 at 450 nm in ethanol.

Color of the experimental fishes were also judged by a test panel consisting of persons randomly recruited from in and around College of Fisheries, Rangailunda. At the end of the experiment random samples were collected from the experimental tanks along with the control and kept in different glass beakers containing clear water. The treatments were not revealed to the individuals and were asked to rank the fishes according to intensity of color. Color ranking was done by a score of 1-10 (Ako *et al*., 2000).

Fish skin color was assessed by capturing its colour image with a high resolution USB CCD colour camera (Model No. DFK51AU02), the major component of the Colour Machine Vision System (CMVS) following the method by Wallat *et al*. (2003).Four fishes were randomly chosen from each experimental unit for capturing the image. For each fish, four images were captured from different regions of the fish body. Digital images were processed using colour.exe software. Grayscale values of 'r' (Red), 'g' (Green) and 'b' (Blue) components of the RGB scale were measured within 1600×1200 pixel area of red coloured dorsal body surface of fish.

Statistical analysis

The data were statistically analyzed by statistical package SPSS version 20.0, in which data were subjected to one way ANOVA and Duncan's Multiple Range Test to determine the significance differences between the mean values at the 5% probability level.

RESULTS AND DISCUSSION

Physico chemical parameter

The physico chemical parameters of water (Table 2) like temperature, pH, after dissolved O_2,free CO_2 hardness and total alkalinity of the different experimental groups ranged from 23.1ºC to 28.3 ºC, 7.6-8.4, 5.5 to 7.9 mg L^{-1}, 224 to 245 mg L^{-1} and 120 to 136 mg L^{-1} respectively during the experimental period of 90 days. The result showed all the parameters were within the ideal range suitable for culture of the warm water fishes (Boyd., 1988) and therefore was not thought to have influenced the result of the study.

Proximate composition of the experimental diets

The crude protein (%) and crude fat (%) of different experimental diets (Table 1) ranged from 34.72-36.11 % and 7.77-9.66 % respectively, which is supported by NRC (2011), where the protein and lipid requirement of koi carp (0.5-10g) ranged from 31-38% and 7-9% respectively.

Growth parameters and FCR

The body weight gain of koi carp fry fed with different experimental diets at 15 days interval is shown in fig 1. The growth parameters of the fishes at the end of the experiment are shown in Table 3. The effect of carotenoids on growth and survival of aquatic animals are controversial (Jha *et al*., 2012). Fishes fed with 15% china rose petal meal showed significantly increased values in weight gain % (425.12 ± 32.01), daily weight gain (0.031 ± 0.001) and SGR (0.794 ± 0.019). Significantly lower FCR was observed in the fishes fed with 15% carrot meal (4.20 ± 0.14), fishes fed with 15% marigold meal (4.24 ± 0.42) and fishes fed with 15% china rose petal meal (5.25 ± 0.03). The results were in agreement with the reports that link the carotenoides to growth enhancement of Atlantic salmon fry (Christiansen *et al*., 1995), rainbow trout (De la Mora *et al*., 2006) and gold fish (Sinha and Asmi, 2007). In the present study, dietary supplementation of china rose petal meal have significant effect on growth compared with other treatments, which can be compared with the study done by Joseph *et al*. (2011), where incorporation of china rose petal meal in diets of sword tail promotes growth. The positive effect of marigold petal meal on growth is reported in red sword tail (Golandaj *et al*., 2015), common carp (Swain *et al*., 2014). Carot meal incorporation in diets had also a positive

Table 1: Ingredients and proximate composition (% dry matter basis) of experimental diets

Ingredients	T_0	T_1	T_2	T_3	T_4
Fish meal	27	27	27	27	27
GNOC	27	27	27	27	27
Rice bran	20	20	20	20	20
Wheat flour	20	20	20	20	20
Oil	4	4	4	4	4
Vit-mineral mixture	2	2	2	2	2
Marigold petal	-	15	-	-	-
Rose petal	-	-	15	-	-
China rose petal	-	-	-	15	-
Carrot	-	-	-	-	15
Proximate composition of experimental diets					
Moisture	7.68 ± 0.19	7.56 ± 0.46	8.22 ± 0.14	8.22 ± 0.47	7.56 ± 0.30
Crude protein	35.34 ± 0.19	34.98 ± 0.03	35.46 ± 0.66	34.72 ± 0.24	36.11 ± 0.14
Crude fat	9.22 ± 0.37	7.77 ± 0.16	8.83 ± 0.38	9.66 ± 0.30	8.44 ± 0.29
Crude fiber	2.49 ± 0.54	2.65 ± 0.46	3.43 ± 0.26	2.66 ± 0.17	2.99 ± 0.17
Total ash	12.18 ± 0.31	12.62 ± 0.08	12.38 ± 0.86	11.71 ± 0.24	12.50 ± 0.08
Acid insoluble ash	0.80 ± 0.07	0.61 ± 0.07	0.70 ± 0.10	0.93 ± 0.07	0.72 ± 0.08

Table 2 : Physico-chemical parameters of water during the experimental period for different experimental groups.

Parameters	T_0	T_1	T_2	T_3	T_4
Temperature (ºC)	23.4-27.9	23.1-28.2	24.1-28.5	23.7-27.3	23.8-28.3
pH	7.9-8.0	7.6-8.1	8.0-8.4	7.7-8.1	7.8-8.2
DO_2 (mgL^{-1})	6.7-7.5	5.5-7.9	5.6-6.8	5.9-7.2	7.6-7.8
Free CO_2 (mgL^{-1})	ND	ND	ND	ND	ND
Total alkalinity (mgL^{-1})	120-125	127-134	134-136	122-135	126-130
Hardness (mgL^{-1})	224-235	238-241	240-244	234-245	232-238

Table 3: Growth parameters of koi carp fry fed with different experimental diets

Treatments	Daily weight gain	Weight gain %	SGR	FCR
T_0	0.019^{c} ± 0.001	249.57^{c} ± 7.44	0.603^{c} ± 0.010	6.03^{b} ± 0.37
T_1	0.028^{b} ± 0.003	351.12^{b} ± 8.17	0.726^{b} ± 0.008	4.24^{a} ± 0.42
T_2	0.017^{c} ± 0.004	209.40^{c} ± 29.39	0.545^{d} ± 0.047	7.66^{c} ± 0.11
T_3	0.031^{a} ± 0.001	425.12^{a} ± 32.01	0.794^{a} ± 0.019	5.25^{ab} ± 0.03
T_4	0.026^{b} ± 0.001	340.66^{b} ± 24.71	0.715^{b} ± 0.028	4.20^{a} ± 0.14

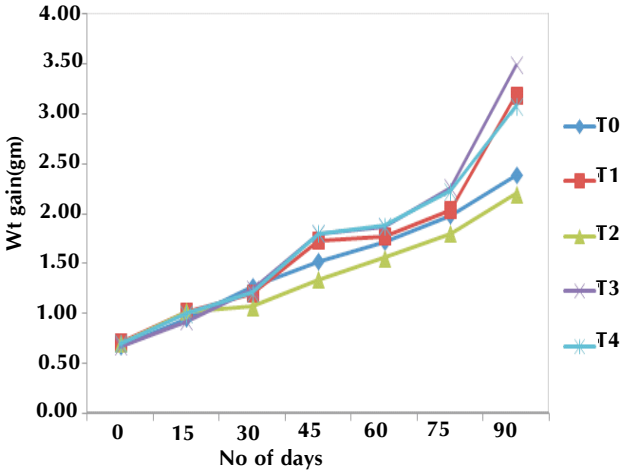

Figure 1: Body weight gain of koi carp fry fed with different experimental diets at 15 days interval.

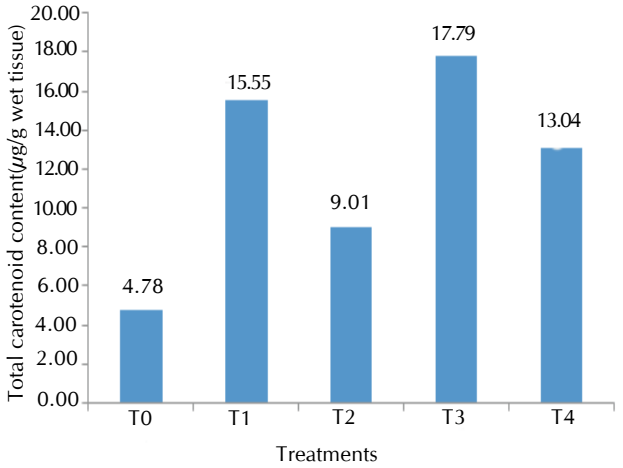

Figure 2 : Total carotenoid content in the muscle (μg/g wet tissue) of koi carp fry fed with different experimental diets at the end of the experiment

Table 4: Visual inspection of colour (Panel test) of koi carp fry fed with different experimental diets

Treatment	No of panelist	Score
T_0	20	$4.375^b \pm 1.68$
T_1	20	$7.300^a \pm 1.19$
T_2	20	$4.750^b \pm 1.16$
T_3	20	$7.725^a \pm 0.79$
T_4	20	$5.000^b \pm 1.25$

The means with different superscript in each column indicate a significant difference ($P<0.05$)

Table 5: Red, Green and Blue (r, g and b) values in skin of koi carp fry fed with different experimental diets.

Treatment	r value	g value	b value
T_0	$0.320^d \pm 0.017$	$0.343^b \pm 0.006$	$0.344^a \pm 0.014$
T1	$0.444^a \pm 0.014$	$0.358^a \pm 0.007$	$0.198^d \pm 0.023$
T2	$0.362^c \pm 0.013$	$0.343^b \pm 0.009$	$0.297^b \pm 0.015$
T3	$0.454^a \pm 0.024$	$0.349^b \pm 0.012$	$0.195^d \pm 0.026$
T4	$0.409^b \pm 0.017$	$0.348^b \pm 0.009$	$0.247^c \pm 0.009$

The means with different superscript in each column indicate a significant difference ($P<0.05$)

effect on growth in *Amphiprion ocellaris* (Ramamoorthy *et al*., 2010). Though rose petal meal incorporated diets have a positive effect on weight gain and SGR in *Xiphophorus helleri* (Arulvasu *et al*., 2013), rosy barb and dwarf gourami (Pailan *et al*., 2012 a and b), in the present study rose petal meal incorporated diets did not affect the growth significantly as compared to the control group. This is in agreement with the work done by Ramamoorthy *et al*. (2010) which reveals no significant effect of rose petal meal on growth of marine ornamental fish *Amphiprion ocellaris*. The positive effect of different carotenoids on growth of koi carp fry is attributed due to the fact that carotenoids have a positive role in intermediary metabolism of fish (Tacon, 1981) which enhanced the nutrient utilization associated with improved growth (Amar *et al*., 2001).

Color analysis

The total muscle carotenoid content (fig.2) showed the significantly highest carotenoid concentration (17.79 ± 0.395) in fishes fed with 15% china rose petal meal (T_3) and the lowest (4.782 ± 0.412) in control group (T_0). From the panel test results (Table 4), the highest score (7.725 ± 0.79) was obtained by the fishes fed with 15% china rose petal meal which did not differ significantly from the group fed with 15% marigold petal meal. The result obtained in Computer assisted image analysis showed a significant effect of carotenoid supplementation on coloration in fish (Table 5). The red values were significantly higher in the fishes fed with 15% china rose petal meal (0.454 ± 0.024) and 15% marigold petal meal (0.444 ± 0.014). In the present study the 'b' value decreased as carotenoid feed additives seems to have no effect in the process. The 'g' value slightly decreased as redness increased due to dietary carotenoids in the experimental groups. Sinha and Asmi (2007) reported an increased level of carotenoid content (4.01μg/gm) in skin of gold fish, *Carassius auratus* fed with diet containing china rose petal meal. Somanath and Jasmin (2013) stated that the dietary addition of china rose petals and spirulina influenced the total carotenoid content in skin and muscle tissue of *Carassius auratus*. Marigold petal meal has already been proven as a good color enhancer for many of the ornamental fishes (Ezhil *et al*., 2008; Swain *et al*., 2014; Golandaj *et al*., 2015). Ramamoorthy *et al*. (2010) reported highest (7.681mg/kg) total carotenoid in the group fed with carrot meal followed by the group fed with marigold petal meal (7.235 mg/kg), hibiscus petal meal (5.236 mg/kg) and rose petal meal (4.254 mg/kg) in marine ornamental fish *Amphiprion ocellaris*. The result of the present study is in contrast to this study where the highest carotenoid content in the muscle of koi carp was found in the group fed with hibiscus petal meal followed by marigold petal meal, carrot meal and rose petal meal. This may be due to the fact that effectiveness of a carotenoid source for pigment deposition is species specific (Ha *et al*., 1993).

Though, there are many methods for quantifying color intensity are available, the test panel method however offers the advantage of cost effective and convenience. A similar attempt has been made by Ako (2000) to evaluate the effect of carotenoid rich strain of *Spirulina platensis* and

Haematococcus pluvialis incorporated diets on colouration of red velvet sword tails (*Xiphophorus helleri*), rain bow fish (*Psuedomugil furcatus*) and topaz cichlids (*Cichlasoma myrnae*). Similarly, computer assisted image analysis for evaluating color intensity was followed by Hancz *et al*., (2003) to evaluate the color intensity in gold fish and koi carp by using paprika as a feed additive and by Mitra *et al*., 2013 for evaluation of color development in rosy barb, *Puntius conchonius* during ontogeny. The 'r', 'g' and 'b' values are proposed to be used for detection of phenotypic difference in the experimental groups fed with diets of different pigment sources. The results of color analysis in the present investigation reveals that the fish has capacity to utilize these pigment sources (china rose petal, marigold petal, rose petal and carot) efficiently. But supplementation of 15% china rose petal meal in the diet gives the best result.

ACKNOWLEDGEMENT

The authors are thankful to Director, College of Fisheries, OUAT and Director, CIFA, Kaushalyaganga, Bhubaneswar for providing facilities and guidance towards successful completion of the research work.

REFERENCES

Ahilan, B., Jegan, K., Felix, N. and Ravaneswaran, K. 2008. Influence of botanical additives on the growth and colouration of adult goldfish, *carassius auratus* (Linnaeus). *Tamil Nadu J. Vet. Ani. Sci.* **4(4):** 129 -134.

Ako, H., Asano, T.L., Yuen, B and Yamamoto, M. 2000. Achieving natural colouration in fish under culture, VINR Tech. Report. **28:**

Alma, A. del Villar-Martinez, Juan, C. Orbe-Rogel, Pablo, E., Vanegas-Espinoza, Adrian G. Quintero- Gutierrez and Maurilio Lara-Flores. 2013. The effect of marigold (Tagetes erecta) as natural carotenoid source for the pigmentation of goldfish (*Carassius auratus* L.). *Res. J. Fish. Hydrobio*. **8(2):** 31-37.

Amar, E. C., Kiron, V., Satoh, S. and Watanabe, T. 2001. Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum), *Aquacult. Res.* **32(1):** 162-163.

AOAC. 2005. Official methods of analysis of AOAC, 18th edition, In: Horwitz, W. (Ed.), 1094, AOAC, Washington DC.

APHA. 1995. Standard methods for the Examination of water and Wastewater. 18th. Edn., American Public Health Association, Washington DC, *Aquacult.* **17:** 63-92.

Arulvasu, C., Ramya, M. S., Chandhirasekar, D and Sivaganam S. 2013. Evaluation of natural sources of carotenoid pigments from *Rosa rubiginosa* on growth, survival and coloration of *Xiphophorus helleri* fish fry. *European J. Biol. Sci.* **5(2):** 44-49.

Baksi, S., Behera, S., Bhakta, D., Kumar, S., Jomang, O., Saha, S. and Modal, A. 2017. Effects of spirulina powder in colouration and growth enhancement of an indigenous ornamental fish *Trichogaster lalius*. *Int. J. Adv. Biol. Res.* **7(2):** 263-267.

Behera, S., Baksi, S., Saha, S., Mondal, A., Mondal, A. and Patra, P. 2018. Spirulina powder as a source of carotenoid on pigmentation in the skin of dwarf gouramy *Trichogaster lalius*. *Int. J. Fauna. Biol. Studies*. **5(1):** 91 - 95.

Boyd, C. E. 1988. Water Quality in Warm water Fish Pond, Vol. **4**, 359, Alabama, USA: Agricultural Experiment Station, Auburn University.

Chatzifotis, S., Pavlidis, M., Jimeno, C. D., Vardanis, G., Sterioti, A. and Divanach, P. 2005. The effect of different carotenoid sources on skin coloration of cultured red porgy (*Pagrus pagrus*). *Aquacult. Res.* **36:** 1517-1525.

Christiansen, R., Lie, O. and Torrissen, O. J. 1995. Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. *Aquacult. Nutr.* **1:** 189-198.

De la Mora, G. I., Arredondo-Figueroa, J. L., Ponce-Palafox, J. T., Barriga-Sosa, I. A. and Vernon-Carter, J. E. 2006. Comparison of red chilli (*Capsium annuum*) oleoresin and astaxanthin on rainbow trout (*Oncorhynchus mykiss*) fillet pigmentation. *Aquacult.* **258:** 487-495.

Ezhil, J. and Narayanan, M. 2013. Enhancement of Pigmentation in Blue Morph, *Pseudotropheus lombardoi* through feeding different carotenoid sources. *World J. Fish Mar. Sci.* **5(6):** 655-659.

Ezhil, J., Jeyanthi, C. and Narayanan, M. 2008. Marigold as a carotenoid source on pigmentation and growth of red swordtail, *Xiphophorus helleri*. *Turkish J. Fish. Aqua. Sci.* **8**: 99-102.

FAO. 2014. www.fao.org/fishery/topic/13611/en.

Golandaj, A., Shyama, P. S., Dinesh, K., Sreenath, V.R. and Swain, S. 2015. Colour enhancement potential of selected local flowers in Sword tail, *Xiphophorus helleri* through dietary incorporation. *Res. J. Rec. Sci.* **4:** 37-43.

Gomelsky, B., Cherfas, N., Hulata, G. and Dasgupta, S. 2003. Inheritance of the white-red (kohaku) color complex in ornamental (koi) carp *(Cyprinus carpio* L.). *The Israeli J. Aquacult. – Bamidgeh*. **55(3):** 147-153.

Goodwin, T.W. 1984. The Biochemistry of carotenoids. Volume II. Animals. Chapman and Hall, New York.

Ha, B. S., Kang, D. S., Kim, J. H., Choi, O. S. and Ryu, H. Y. 1993. Metabolism of dietary carotenoids and effects to improve the body color of cultured flounder and red sea bream. *Bulletin of Korean Fish. Soc.* **26:** 91-101.

Hancz, C., Magyary, I., Molnar, G., Safo, S., Horn, P. and Taniguchi, N. 2003. Evaluation of colour intensity enhanced by paprika as feed additive in gold fish and koi carp using computer assisted image analysis, *Fishery Sci.* **69:** 1158-1161.

Hardy, R. 1980. Fish feed formulation, In: ADCP (Eds.), Fish feed Technology, Agriculture development coordination programme. FAO/ADCP/Rep. **80(1-1):** 233-239.

Hekimoglu, M. A., Firat, K., Saka, S., Suzer, C., Kope, A. and Durmaz, Y. 2017. Effect of supplemented algal carotenoid diets on skin colour of tomato clownfish *Amphiprion frenatus*. *Pakistan J. Zool.* ***49(2):*** 663-668.

Hongxia, L., Seline, T., Daniel, T., Heath, D., Robert and Letcher J. 2005. Determination of carotenoids and all trans-retinol in fish eggs by liquid chromatography-electrospray-ionization-tandem-mass spectrometry, *J. chromatography B.*

816: 49 -56.

Jagadeesh, T. D., Murthy, H. S., Surendranath, S. V., Panikkar, P., Manjappa, N. and Mahesh, V. 2015. Effects of supplementation of marigold (*Tagetes erecta*) oleoresin on growth, survival and pigmentation of rosy barb, *Puntius conchonius* (HAMILTON). *The Bioscan*. **10(3):**1431-1435.

Jha, G. N., Sharma, D., Qureshi, T. A. and Akhtar, M. S. 2012. Effect of beetroot and marigold flower meal on growth performance, carcass composition and total carotenoids of snow trout (*Schizothorax richardsonii*), *The Israeli J. Aquacult. – Bamidgeh*. **64:** 752-759.

Joseph, B., Sujath, S., Jemima, J. S. and Palavesam A. 2011. Influence of four ornamental flowers on the growth and colouration of orange sword tail Chicilidae fish (*Xiphophorus hellerei*, Heckel. *Int. J. of Biol. Med. Res.* **2(3):** 621-626.

Kalinowski, C. T., Robaina, L. E., Fernadez-Palacios, H., Schuchardt, D. and Izquierdo, M. S. 2005. Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. *Aquacult.* **244:** 223-231.

Katia, O. 2001. Ornamental fish trade – overview. *Infofish Int.* **3:** 14-17

Maede Talebi, Hossein Khara, Jalil Zoriehzahra, Shayan Ghobadi, Arezo Khodabandelo and Elham Mirrasooli. 2013. Study on Effect of Red Bell Pepper on Growth, Pigmentation and Blood Factors of Rainbow Trout (*Oncorhynchus mykiss)*. *World J. Zool*. **8(1):** 17-23.

Mitra, G., Jena, J. K., Jayasankar, P., Das, P. C. and Swain, S. K. 2013. Evaluation of colour development of rosy barb, *Puntius conchonius* (Hamilton) during ontogeny. *Communications in agricultural and applied biological sciences*, Larvi, 13-Fish and shell fish larviculture symposium. PP.279 - 282.

NRC. 2011. Nutrient requirements of fish and shrimp, 1-179, National Academy Press, Washington, D.C.

Pailan, G. H., Sinha, A. and Kumar, M. 2012a. Rose petal as natural carotenoid source in pigmentation and growth of rosy barb (*Puntius conchonius*). *Indian J. Ani. Nutr*. **29(3):** 291-296.

Pailan, G.H., Sinha, A. and Borkar, A. 2012b. Rose petal meal as natural carotenoid source for pigmentation and growth of dwarf gourami (*Colisa lalia*). *Ani. Nutr. Feed Tech*. **12(2):** 199 -207.

Paripatananont, T., Tangtrongpairoj, J., Sailasuta, A. and Chansue, N. 1999. Effect of astaxanthin on the pigmentation of goldfish *Carassius auratus*. *J. World Aquacult. Soc.* **30(4):** PP.454-460.

Ramamoorthy, K., Bhuvaneswari, S., Sankar, G. and Sakkaravarthi, K. 2010. Proximate composition and carotenoid content of natural carotenoid sources and its colour enhancement on marine ornamental fish *Amphiprion ocellaris*. *World J. Fish and Mar. Sci*. **2(6):** 545-550.

Savolainen, J. R. T. and Gyllenberg, H. G. 1970. Feeding of rainbow trout with *Rhodotorula sanneii* preparations. II. Amounts and qualities of carotenoids. *Lebensm-Wiss Technology*. **3:** 18-20.

Shahidi, F., Metusalach and Brown, J. A. 1998. Carotenoid pigments in seafoods and aquaculture. *Critic. Rev. Food Sci. Nutr*. **38:** 1- 67.

Sinha, A. and Asimi, O. A. 2007. China rose (*Hibiscus rosasinensis*) petals: a potent natural carotenoid source for goldfish (*Carassius auratus* L.). *Aquaculture Res*. **38:** 1123 - 1128.

Somnath, B. and Jasmin, K. J. 2013. Hibiscus petals and spirulina supplemented diet induced carotenoid changes in freshwater gold fish *carassius auratus*. *Int. J. Pure and Applied Zoology*. **1(4):** 352-362.

Swain, H. S., Senapati, S. R., Meshram, S. J., Mishra, R. and Murthy, H. S. 2014. Effect of dietary supplementation of marigold oleoresin on growth, survival and total muscle carotenoid of Koi carp, *Cyprinus carpio* L. *J. Applied and Natural Science*. **6(2):** 430-435.

Tacon, A. G. J. 1981. Speculative review of possible carotenoid function in fish, *Progressive Fish Culturist*. **43:** 205 - 208.

Torrissen, O. J., Hardy, R. W. and Shearer, K. D. 1989. Pigmentation of salmonids: carotenoid deposition and metabolism. *Rev. in Aqua. Sci*. **1:** 209 - 225.

Wallat, G.K., Lazur, A. M., Balaban, M. O. and Chapman, F. A. 2003. Analysis of skin color development in live Goldfish using a color machine vision system, *North American J. Aquacult*. **64:** 79 - 84.