Indian Journal of Animal Sciences **82** (3): 336–340, March 2012

Effect of spirulina (*Spirulina platensis*) and marigold (*Tagetes erecta*) fortified diets on growth, body composition and total carotenoid content of *Barilius bendelisis*

GHANSHYAM NATH JHA[1], DEBAJIT SARMA[2] and T A QURESHI[3]

Directorate of Coldwater Fisheries Research, ICAR, Bhimtal, Nainital, Uttarakhand 263 136 India

Received: 8 October 2010; Accepted: 28 October 2011

ABSTRACT

The effect of *Spirulina platensis* and *Tagetes erecta* at different incorporation levels of 0, 3, 5, 7, and 10% were tested on growth, body composition and total carotenoid content in *Barilius bendelisis* (an endangered upland fish species) for 60 days in triplicate. An overall reduction from 3.29 (control, T_0) to 1.57 (7% marigold, T_3), 1.49 (5% spirulina, T_6) and 1.34 (10% spirulina, T_8) in FCR was observed with increase in either spirulina or marigold in the diet. Further, RGR (%) increased from 93.37 (T_0) to 191.17 (T_3), 203.09 (T_6) and 224.359 (T_8) while SGR (%) from 1.09 (T_0) to 1.77 (10% marigold-T_4), 1.84 (T_6) and 1.96 (T_8) for both the additives. Crude protein increased from 14.9% (T_0) to 16.93% (for both T_6 and T_8) while, potassium from 1115 mg 100^{-1}g (T_0) to 1276 mg 100^{-1}g (T_6). Proximate and mineral composition of the fish was significantly affected by Spirulina while marigold has less significant impact. On the other hand, total carotenoids were significantly affected by marigold and it improved from 2.09 µg g^{-1} (T_0) to 4.58 µg g^{-1} (T_4) than the Spirulina, which has less impact on it. *B. bendelisis* fed on 5% spirulina fortified diet not only improve the growth but also enhanced its total carotenoid content and body composition. However, marigold was found to improve the total carotenoid content of the fish with least significant impact on growth and body composition.

Key word: *Barilius bendelisis,* Marigold, Proximate composition, Spirulina, Total carotenoid

Barilius bendelisis is widely distributed in hilly regions of India (Sahoo *et al.* 2009). Inadequate pigmentation, retarded growth with degraded muscle composition is always noticed in this type of cultured fishes due to lack of carotenoid and other nutrient content in their artificial diets (Huner and Meyers 1979, Harpaz *et al.* 1998, Jha *et al.* 2010).

Fish skin colour is primarily dependent on the presence of chromatophores containing pigments, pteridines and purines (Chatzifotis *et al.* 2005). Fish can modify alimentary carotenoids and store them in the integument and other tissues (Halten *et al.* 1997). Farmed fish (particularly, in intensive culture system) have no access to carotenoids-rich feed and therefore, the necessary carotenoids must be added to the diet (Gupta *et al.* 2007). The effectiveness of carotenoid source in terms of deposition and pigmentation is species specific (Ha *et al.* 1993). The loss of pigments, due to inadequate carotenoid reached diets, can be overcome by their addition to the artificial diet. Several studies addressed this issue and have evaluated different potential pigment sources like red pepper (*Capsicum annum*), marigold flower-*Tagetes erecta* and Spirulina-*Spirulina platensis* (Boonyaratpalin and Lovell 1977, Alagappan *et al.* 2004, Buyukcapar *et al.* 2007). These additives, besides pigments, influence muscle quality, survival, resistance to diseases and growth (Harpaz *et al.* 1998, Boonyaratpalin *et al.* 2001, Rema and Gouveia 2005, Ezhil *et al.* 2008).

Therefore, the purpose of the present study is to investigate the effect of different levels of *S. platensis* and *T. erecta* incorporated diet on the growth, body composition and total carotenoid content of *B. bendelisis* in order to increase their production with enhanced pigmentation and muscle composition.

MATERIALS AND METHODS

Experimental animal

Fingerlings of *B. bendelisis* (5.63g, average weight) were collected from a local stream of Nainital (Uttarakhand), transported in a circular container (500 L) with sufficient aeration, to the experimental site at hatchery complex of Directorate of Coldwater Fisheries Research, Bhimtal and were acclimatized to the experimental rearing conditions for one week. During acclimation, fish were fed with control diet. After acclimatization, fishes were transferred to 27 uniform size experimental fiberglass tanks of 100 l capacity and reared for 60 days.

Present address: [1]Research Associate, (ghan_shark @yahoo.com),[2]Principal Scientist (dsarma_sh@yahoo.co.in; dsarma@dcfr.res.in). [3]Consultant (qureshita@yahoo.com), Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal, Madhya Pradesh 462 026, India.

Experimental design and feeding

Nine iso-nitrogenous (35.25±0.9% crude protein) diets were prepared with graded levels (0, 3, 5, 7 and 10% of diet) of either marigold (MG) or spirulina (SP) meal. Fishes (275) were randomly distributed in 9 experimental groups in triplicates following a completely randomized design (CRD). There were 9 treatment groups, viz. T_0 (control), T_1 (3%MG), T_2 (5%MG), T_3 (7% MG), T_4 (10% MG), T_5 (3%SP), T_6 (5% SP), T_7 (7% SP) and T_8 (10% SP). The physicochemical parameters of water were analyzed by APHA (1995) methodology, and were within the optimum range (dissolved oxygen: 6.0–8.5 mg/l, pH: 7.3–8.2 and temperature: 18–20 °C) throughout the experimental period. All the groups were fed their respective diets. Feeding was done at 5% of the body weight. Daily ration was divided into 2 split doses: about 2/3rd of total ration was given at 09:00 h and the rest at 18:00 h. The fecal matters were removed by siphoning and a constant water flow (2-3 $lmin^{-1}$) was maintained by providing inlet at one and outlet at the other end to ensure optimum dissolved oxygen throughout the experimental period. A very few accidental mortality (10%) was observed during the 60 day experimental feeding trial.

Proximate composition analysis

The proximate composition of the experimental diets and fish muscle was determined following the standard methods of AOAC (1995). The moisture content was determined by drying at 105 °C to a constant weight. Nitrogen content was estimated by Kjeldahl method and crude protein was estimated by multiplying nitrogen % by 6.25. Crude fat (ether extract) was measured by solvent extraction method using diethyl ether (boiling point, 40–60 °C) as a solvent and ash content was determined by incinerating the samples in a muffle furnace at 600 °C for 6 h.

Total carotenoids and minerals

Total carotenoids were determined following the methodology given by Olson (1979). The samples (skin and muscles) were gently meshed with a glass rod against the side of the vial and then 5 ml of chloroform was added and left overnight at 0°C. When the chloroform formed a clear 1–2 cm layer above the caked residue, the optical density was read at 380, 450, 470 and 500 nm, in a spectrophotometer 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 wavelength, at which maximum absorption observed, was used for the calculation. Calcium, sodium and potassium were analyzed by atomic absorption spectroscopy using AOAC (1995) methods. Ashed samples were dissolved in 2 ml of concentrated acid (HCl: HNO_3, 1:1), diluted with distilled water (Shearer 1984). The diluted mixture was analyzed for calcium, sodium and potassium.

Growth study

Growth rate of fish was measured in terms of weight gain/ absolute growth rate (AGR,%), specific growth rate (SGR) and feed conversion ratio (FCR) using the following equations.

Relative growth rate (RGR)% = [(final weight – initial weight) / initial weight] × 100

SGR (%) = [(Log_e final weight – Log_e initial weight) / number of experimental days] × 100

AGR (g) = final mean weight – initial mean weight

FCR= feed given (dry weight) / body weight gain (wet weight)

Statistical analysis

Mean values of all parameters were subjected to one way ANOVA to study the treatment effect and Tukey's test (HSD) were used to determine the significant differences between two means. Comparisons were made at 5% probability level. All the data were analyzed using statistical package SPSS (Version 12.01).

RESULTS AND DISCUSSION

The quantities of ingredients used for diet preparation and the proximate composition along with total carotenoids of the diets are given in the Table 1. The initial average weight, final weight, SGR, AGR, RGR and FCR for the experimental fishes and tested diets were calculated and recorded in Table 2. Amongst the marigold fortified diets, T_3 showed highest RGR (191.17%) and lowest FCR (1.57), while, lowest RGR (93.37%) and highest FCR (3.29) were recorded for T_0 (Table 2). SGR was observed highest (1.77%) for T_4 and lowest for T_0 (1.09%). In the present study, improved growth rate and decreased FCR were noticed with the diet containing higher quantity of carotenoids in the form of marigold. These findings are in conformity with those of Amar *et al.* (2001) who reported that utilization of carotenoids through marigold flower led to improved growth and decreased FCR. Marigold flower contains carotenoids and carotenoids have a positive role in metabolism in fish as reported by Tacon (1981). In resemblance with the present observation, the growth of fish and prawn were achieved higher when carotenoids from different sources were added in their diets by Harpaz *et al.* (1998) and Ezhil *et al.* (2008).

FCR reduced from 3.29 (T_0) to 2.03 (T_5) and 1.34 (T_8) with the increase in spirulina % in the diet. Christiansen *et al.* (1996) used astaxanthin as a source of carotenoid and observed lower FCR in Atlantic salmon juveniles, similarly, lower FCR was matter-of-fact in *Penaeus monodon* fed on the diet containing spirulina as a source of carotenoid (Boonyaratpalin *et al.* 2001). Spirulina in the diet augmented RGR from 93.37 (T_0) to 203.09% (T_6) and 224.35% (T_8) and SGR from 1.09 to 1.84 and 1.96 respectively, while AGR increased from 5.76 to 10.15 and 11.65 respectively. The consequences of the present study are in conformity with those of Peimin *et al.* (1999), who reported that more quantity of *S. platensis* in diet increased the length and weight of the fish.

Table 1. Diet composition (%) and its proximate analysis (% dry matter basis)

Ingredients	Diets								
	1	2	3	4	5	6	7	8	9
Fish meal	20.27	20.27	20.27	20.27	20.27	17.27	15.27	13.27	10.27
Soybean meal	20.27	20.27	20.27	20.27	20.27	20.27	20.27	20.27	20.27
Rice bran	29.73	26.73	24.73	22.73	19.73	29.73	29.73	29.73	29.73
Wheat bran	21.73	21.73	21.73	21.73	21.73	21.73	21.73	21.73	21.73
Vitamin-Mineral Mix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vegetable oil[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil[3]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sodium alginate[4]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Marigold flower meal	0	3.00	5.00	7.00	10.00	0	0	0	0
Spirulina meal	0	0	0	0	0	3.00	5.00	7.00	10.00
Total	100	100	100	100	100	100	100	100	100
Proximate analyses									
Moisture	13.28 ±0.02	12.89 ±0.03	12.50 ±0.01	12.85 ±0.05	12.69 ±0.02	11.85 ±0.02	12.15 ±0.02	12.54 ±0.03	11.90 ±0.02
Crude protein	35.21 ±0.08	35.40 ±0.05	35.95 ±0.02	36.25 ±0.01	36.70 ±0.02	34.72 ±0.04	34.51 ±0.03	34.35 ±0.04	34.09 ±0.01
Ether extract	6.65±0.02	6.23±0.01	6.45±0.02	6.15±0.03	6.20±0.02	5.96±0.02	6.05±0.01	6.27±0.03	6.33±0.03
Ash	10.85±0.05	12.05±0.02	12.96±0.04	13.89±0.03	14.90±0.05	10.05±0.07	10.70±0.03	10.25±0.05	10.18±0.04
Dry matter	86.72±0.25	87.11±0.41	87.50±0.23	87.15±0.33	87.31±0.54	88.15±0.02	87.85±0.02	87.46±0.03	88.1±0.02
TC[3] (µg/g diet)	29.1±0.94	110.12±2.32	178.74±3.13	292.32±7.84	411.28±6.98	100.23±3.41	170.24±4.63	250.14±3.74	350.25±5.51

[1]Agrimin forte (Virbac Animal Health India Pvt. Ltd., Mumbai-59, India). Each kg contains- Vitamin A-7, 00,000 I.U., Vitamin D_3–70, 000 I.U., Vitamin E-250 mg, Nicotinamide-1000 mg, Cobalt-150 mg, Copper-1200 mg, Iodine-325 mg, Iron-1500 mg, Magnesium-6000 mg, Manganese-1500 mg, Potassium-100 mg, Selenium-10 mg, Sodium-5.9 mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5%, Phosphorus-12.75%.

[2]Pvt. ltd. industry, India. [3]Total carotenoids procured from local market, [4]Commercial purchase, India.
Data expressed as Mean±SE, n=3.

Table 2. Growth performance of *B. bendelisis* fingerlings fed with graded levels of marigold flower and spirulina meal

Treatment	Initial Weight (g)	Final Weight (g)	AGR (g)	SGR (%)	RGR (%)	FCR
T_0	6.26±0.8	12.03±0.76	5.76±0.55^d	1.09±0.15^d	93.37±17.12^c	3.29±0.65^c
T_1	5.66±0.35	13.53±0.85	7.86±0.55^c	1.45±0.04^c	138.87±6.38^c	2.16±0.10^b
T_2	6.13±0.3	14.83±1.0	8.7±0.7^c	1.47±0.03^c	141.7±4.62^c	2.11±0.06^b
T_3	5.8±0.81	16.8±1.41	11±0.6^b	1.78±0.10^b	191.17±16.61^b	1.57±0.13^a
T_4	6±0.1	17.36±0.55	11.36±0.45^a	1.77±0.03^b	189.39±4.38^b	1.58±0.03^a
T_5	5.17±0.1	12.8±0.56	7.63±0.46^c	1.51±0.04^c	147.501±5.98^b	2.03±0.08^b
T_6	5.01±0.24	15.16±0.71	10.15±0.83^b	1.84±0.13^b	203.096±23.57^a	1.49±0.18^a
T_7	5.2±0.56	14.12±0.46	8.91±0.94^c	1.67±0.23^b	174.358±37.14^b	1.77±0.4^b
T_8	5.2±0.31	16.85±0.27	11.65±0.1^a	1.96±0.08^a	224.359±15.11^a	1.34±0.08^a

Values in the same column with different superscripts (a, b, c) differ significantly (P<0.05).
Data expressed as mean±SD, n = 3 (each replicate had 10 fish).

Proximate compositions of fish muscles (on wet weight basis) are specified in Table 3. The protein content in fish muscle was observed as 14.9% (T_0), 14.56% (T_1), 14.72% (T_2), 15.58 (T_3) and 15.91% (T_4). The lipid content ranged between 3.56% (T_0) to 3.93% (T_4). The results of the present study revealed that the protein and lipid contents did not vary significantly (P>0.05) among the fishes fed on T_0, T_1, T_2 and T_3 diets whereas, only protein content was observed to vary significantly for T_4. In case of Spirulina fortified diets, a significant difference was noticed for protein content. Which increased from 14.9% (T_0) to 16.89% (for T_7) and 16.93% (for both T_6 and T_8). An increase in carotenoid source in the diet, significantly affected the proximate composition of the fish when spirulina was the source, while marigold did not have any significant impact on carcass composition except T_4 (10% marigold). The findings are in concurrence with others (Boonyaratpalin *et al.* 2001 and Gurung *et al.* 2005) who reported that different sources of carotenoids

Table 3. Effect of marigold flower and spirulina meal on body composition (% wet weight basis) of *B. bendelisis* fingerlings

Treatment	Moisture	Dry matter	Ash	Crude protein	Crude fat
T_0	79.23±0.28	20.76±0.28	2.5±0.07	14.9±0.12^d	3.56±0.35^b
T_1	78.27±0.26	21.72±0.26	2.53±0.06	14.56±0.07^d	3.06±0.06^b
T_2	78.23±0.25	21.76±0.25	2.53±0.06	14.72±0.03^d	3.14±0.04^b
T_3	78.9±0.71	21.09±0.71	2.34±0.05	15.58±0.04^d	3.65±0.08^b
T_4	77.99±0.28	22±0.28	2.49±0.11	15.91±0.03^c	3.93±0.04^b
T_5	75.99±0.14	24±0.14	2.17±0.11	16.57±0.13^b	4.29±0.46^a
T_6	76.57±0.16	23.43±0.16	2.22±0.14	16.93±0.10^a	4.13±0.30^b
T_7	76.31±0.21	23.68±0.22	2.2±0.11	16.89±0.20^a	4.27±0.30^b
T_8	76.24±0.21	23.75±0.21	2.24±0.15	16.93±0.24^a	4.11±0.12^b

Values in the same column with different superscripts (a, b, c) differ significantly (P<0.05); Data expressed as Mean±SD; n = 3 (each replicate had 10 fish).

might have different impact on proximate composition of fish and prawn.

The total carotenoids and mineral composition of fishes exists in Table 4. Marigold improved carotenoids in the fish tissue from 2.09 µg g^{-1} (T_0) to 4.58µg g^{-1} (T_4). Matsuno *et al.* (1980) reported that dietary preference of different species plays an important role in their colouration. Hata and Hata (1972) also suggested that the occurrence of hydroxyl group within molecular configuration of carotenoids enhances its absorption by the digestive epithelium. Animals can not synthesize carotenoids by their own, so they depend on plants and algae to obtain these pigments (Britton *et al.* 1995). The pigments found in fishes are gained from these sources or from their prey. These absorbed carotenoids are transported through the blood to the muscles and skin where they are deposited. Due to higher concentration of carotenoids in the experimental diets, fish, utilized experimental diets better than the control one, which may be a ground for better pigmentation. The outcome of the analysis are also in agreement with those of Boonyaratpalin and Lovell (1977) who experimented with marigold petal meal on tiger barb (*Puntius tetrazona*) and colour was found improved significantly than the control fed fish and with Ezhil *et al.* (2008) also used marigold as a carotenoid source on pigmentation and growth of red sword tail (*Xiphophorus helleri*).

Table 4. Effect of marigold flower and Spirulina meal on total carotenoids (µg g-1) and mineral composition (mg 100^{-1} g) of muscles of *B. bendelisis* fingerlings.

Treatments	Total Carotenoids	Na	K	Ca
T_0	2.09±0.041^d	236±10^b	1115±13.4^d	419±28.5^a
T_1	2.42±0.160^d	256±7^b	1093±14.5^d	376±12.0^b
T_2	3.11±0.198^c	243±4^b	1049±14.5^e	395±7.6^a
T_3	3.62±0.251^b	204±4^b	1014±19.5^f	381±28.4^a
T_4	4.58±0.317^a	225±19^b	1024±7.6^f	387±18.1^a
T_5	2.53±0.096^d	242±7^b	1196±8.1^c	346±4.0^b
T_6	3.73±0.175^b	244±9^b	1276±11.5^a	365±5.6^b
T_7	3.72±0.420^b	277±11^a	1256±11.0^a	395±5.0^a
T_8	3.94±0.270^b	266±7^b	1216±23.2^b	451±6.5^a

Values in the same column with different superscripts (a, b, c) differ significantly (P<0.05).

Data expressed as Mean±SD, n = 3 (each replicate had 10 fish).

On the other hand, spirulina fortified diet, improved carotenoids content in the muscles from 2.09 µg g^{-1} (T_0) to 3.73 µg g^{-1} (T_6) and 3.94 µg g^{-1} (T_8). It is to be noted here that total carotenoid levels in the skin of rainbow trout, *Oncorhynchus mykiss* increased significantly with increase in green algae, *Haematococcus pluvialis* in their diet (Sommer *et al.* 1992), while, Choubert (1979) reported that the quantity of total pigments in the skin was greater in trout receiving the feed supplemented with increased amount of spirulina.

Sodium, potassium and calcium were also significantly affected even with increase in either marigold (potassium was negatively significant in marigold added diets) or Spirulina content in the diets. However, our results showed that the fishes fed with spirulina improved the potassium content from 1115 mg 100^{-1}g for T_0 to 1276 mg 100^{-1}g for T_6.

It can be concluded from the study that Spirulina fortified diet has more impact on growth and nutrient profile with moderately less impact on pigmentation, while marigold has superior impact on pigmentation with less impact on the growth and nutrient profile of the fishes.

ACKNOWLEDGEMENTS

The authors express gratitude to Dr N O Singh, Senior Scientist, Directorate of Coldwater Fisheries Research for his lot of help in statistical analysis, Dr P C Mahanta, Director, D C F R, Bhimtal, Dr K Borana, Barkatullah University, Bhopal, for their help and support of present work.

REFERENCES

Alagappan M, Vijila K and Archana S. 2004. Utilization of *Spirulina* algae as a source of carotenoid pigment for blue gouramis

(*Trichogaster trichopterus* P.). *Journal of Aquaculture and Aquatic Science* **10** (1): 1–11.

Amar E C, Kiran V, Satoh S and Watanabe T. 2001. Influence of various dietary synthetic carotenoids on bio defense mechanism in rainbow trout (*Oncorhynchus mykiss* Walboum). *Aquaculture Research* **32** (1): 162–63.

AOAC. 1995. *Official Methods of Analysis*. Association of Official Analytical Chemists 16th edn. USA.

APHA. 1995. Official methods for the examining of water and wastewater. American Public Health Association, Washington DC.

Boonyaratpalin M and Lovell R T. 1977. Diet preparation for aquarium fish. *Aquaculture* **12**: 53–62.

Boonyaratpalin M, Thongrod S, Supamattaya K, Britton G and Schlipalius L E. 2001. Effects of â-carotene source, *Dunaliella salina*, and astaxanthin on pigmentation, growth, survival and health of *Penaeus monodon*. *Aquaculture Research* **32** (1): 182–90.

Buyukcapar H M, Yanar M and Yanar Y. 2007. Pigmentation of Rainbow trout (*Oncorhynchus mykiss*) with carotenoids from Marigold flower (*Tagetes erecta*) and Red pepper (*Capsicum annum*). Turk. *Journal of Veterinary and Animal Science* **31**(1): 7–12.

Chatzifotis S, Pavlidis M, Jimeno C D, Vardanis A S and Divanach P. 2005. The effect of different carotenoid sources on skin coloration of cultured red progy (*Pagrus pagrus*). *Aquaculture Research* **36**: 1517–25.

Chien Y H and Jeng S C. 1992. Pigmentation of Kuruma prawn, *Penaeus japonicus* bate by various pigment sources and levels of feeding regimes. Aquaculture **102**: 333–46.

Choubert G. 1979. Tentative utilization of *Spirulina* algae as a source of carotenoid pigments for rainbow trout. *Aquaculture* **18**: 135–43.

Christiansen R, Lie Q and Torrissen O J. 1995. Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. First feeding fry. *Aquaculture Nutrition* **1**(3): 189–98.

Ezhil J, Jeyanthi C and Narayanan M. 2008. Marigold as a carotenoid source on pigmentation and growth of red Swordtail, *Xiphophorus helleri*. *Turkish Journal of Fisheries and Aquatic Sciences* **8**: 99–102.

Gupta S K, Jha A K, Pal A K and Venkateshwarlu G. 2007. Use of natural carotenoids for pigmentation in fishes. *Natural Product Radiance* **6**(1): 46–49.

Gurung T B, Wagle S K, Bista J D, Joshi P L, Batajoo R, Adhikari P and Rai A K. 2005. Participatory fisheries management for livelihood improvement of fishers in Phewa Lake, Pokhara, Nepal. *Himalayan Journal of Sciences* **3**(5): 47–52.

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 Fisheries Society* **26**: 91–101.

Halten B, Arnmesan A, Jobling M and Bjerkeng B. 1997. Carotenoid pigmentation in relation to feed intake, growth and social integration in Arctic char, *Salvelinus aipinus* (L.), from two anadromous strains. *Aquaculture Nutrition* **3**: 189–99.

Harpaz S, Rise M, Arad S and Gur N. 1998. The effect of three carotenoid sources on growth and pigmentation of juvenile freshwater crayfish *Cherax quadricarinatus*. *Aquaculture Nutrition* **4**: 201–08.

Hata M and Hata M. 1972. Carotenoids pigmentation of Gold fish: Carotenoid metabolism. *Bulletin of Japanese Society for the Science of Fish* **38**: 331–38.

Huner J V and Meyers S P. 1979. Dietary protein requirements of the red Cray fish, *Procambarus clarkii*, grown in a closed system. *Proceedings of World Mariculture Society* **10**: 751–60.

Jha G N, Sarma D and Qureshi T A. 2010. Natural Sources of Carotenoids and the Beauty of Coldwater Fish. *Coldwater Fisheries Management* Pp. 333–44. (Eds) Mahanta P C and Sarma D. DCFR Publication, Bhimtal.

Matsuno T, Katsuyama M, Lwahashi M, Koike T and Okada M. 1980. Intensification of colour of red Tilapia with lutein, rhodoxanthin and *Spirulina*. *Bulletin of Japanese Society for the Science of Fish* **46** (4): 479–82.

Olson A. 1979. A simple dual assay for vit. A and carotenoids in human liver. *Nutrition Report International* **19**: 807–13.

Peimin H, Yinjiang Z and Wenhui H. 1999. Effect of *Spirulina* feed on the body colour of Crucian carp. *Journal of Fish China/ Suichan Xuebao* **23**: 408–14.

Rema P and Gouveia L. 2005. Effect of various sources of carotenoids on survival and growth of goldfish (*Carassius auratus*) larvae and juveniles. *Journal of Animal and Veterinary Advances* **4**(7): 654–58.

Sahoo P K, Saikia S K and Das D N. 2009. Natural food resources and niche breadth of *Barilius bendelisis* (Hamilton) in river Dikrong, an upland riverine ecosystem in India. *Pan-American Journal of Aquatic Science* **4**(1): 12–16.

Shearer K D. 1984. Changes in the elemental composition of hatchery reared rainbow trout (*Salmo gairdnerri*) associated with growth and reproduction. *Canadian Journal of Fisheries and Aquatic Science* **41**: 1592–1600.

Sommer T R, D'souza F M L and Morrissey N M. 1992. Pigmentation of adult rainbow trout (*Oncorhynchus mykiss*) using the green algae *(Haematococcus pluvialis)*. *Aquaculture* **106**: 63–74.

Tacon A G. 1981. Speculative review of possible carotenoids function. *Progressive fish Culturist* **43**: 205–08.

Yamada S, Tanaka Y, Sameshima M and Ito Y. 1990. Pigmentation of prawn (*Penaeus japonicus*) with carotenoids. Effect of dietary astaxanthin, b-carotene and canthaxanthin on pigmentation. Aquaculture **87**: 323–30.