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Research Article

Effect of different concentrations of feed enriched with carrot, beetroot and Spirulina powder on growth performance and survival rate of Albino Tiger barb, *Puntius tetrazona*. Bleeker, 1855 (Cypriniformes: Cyprinidae)

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Abstract

In the present study the fingerlings of *Puntius tetrazona* was exposed in the various concentrations and their impact on pigmentation was assayed in the laboratory. The feed was determined against *P. tetrazona* to various concentrations. Four types of feed was prepared by adding carrot (C-10, C-20, C-30, C-40 and C-50%), beet root (B-10, B-20, B-30, B-40 and B-50%), *Spirulina* (S-10, S-20, S-30, S-40 and S-50%) and mixed diet 1:1:1 (Carrot + Beet root + *Spirulina*) ratio which replaced of the feed ingredients. The impacts of feed on pigmentation were assessed 60 days. After 60 days, the feeding habits and color change in treated and control were recorded. The four different feed formulations tested were found to be most significant. From the result, it can be concluded the feed formulation of *Spirulina* added formulated feed is an excellent potential for controlling pigmentation. Fish length and weight was significantly enhanced (p<0.05) by the dietary supplements.

Keywords: Carrot, Beetroot, *Spirulina*, Growth parameters, Carotenoid, *Puntius tetrazona*.

1. Introduction

In the aquaculture, keeping colorful and fancy fish is known as ornamental fishes, is one of the oldest and popular hobbies dating back to many centuries. The growing interest in aquarium fishes has resulted in a steady increase in the aquarium fish trade globally. The value of international trade has increased steadily, touching US$ 342 million in 2010. The wholesale value of the global ornamental fish trade is estimated to be US$ 1 Billion. The entire industry, including accessories and fish feed, is estimated to be worth around US $18-20 billion. Even though, more than 2,500 species are traded, 30-35 species of freshwater fish dominate the market.

Proximate composition of fishes is an important ecological measure of condition that integrates both feeding condition and habitat quality. The biochemical composition of fish-flesh may vary within the same species of fish depending upon the fishing season, age, sex and habitat (1). The variation is also found within the different region of the water body (2). Protein is the main constituent of the fish's body, thus sufficient dietary supply is needed for optimum growth. Protein is the most expensive micronutrient in fish diet. So, the amount of protein in the diet should be just enough for fish growth where the excess protein in fish diets may be wasteful and cause diets to be unnecessarily expensive.

The *Spirulina* algae are rich in protein and vitamins, and it can be used to improve the immunity capacity of the animals which consume it. Consumption of *Spirulina* also increases the ability to absorb nutrients. When *Spirulina* algae are used as feed for young prawns and fingerlings, they exhibit good coloring, as well as maintains a low death rate and a high growth rate (3). The color of fishes is mainly because of chromatophore consists of chromatin, which is usually found on the skin. Four groups of main chromatin, which are (melanin, purin, preidum and carotenoid), produce color in the tissues and skins of animals and plants. Carotenoids that are dissolved in fat produce the colored amplitude of yellow to red in the skin. Also, they can produce orange and green colors in semen, skin and viand of fishes (4). Carotenoids that are naturally produced by plants and phytoplankton are divided into two groups: carotene and xanthophylls. More than 600 kinds of carotenoid are found in the nature, but few of them are used in animal food, medications, color of food, polish chemicals (5).

Fish like other animals cannot synthesize these pigments and in natural conditions, they acquire carotenoid pigments from various sources, including micro algae, crustaceans and insects, but in artificial conditions, supplementing or complementing diets with carotenoid pigments obtains this pigmentation. Hence, a direct relationship between dietary carotenoids and pigmentation exists in them (6). Therefore, skin color is the most important criterion used for their selection, although body shapes, behavioral patterns, and other environmental adaptations are important as well (7).

In one of the popular ornamental fish species are Albino tiger barb (*P. tetrazona*) its natives South East Asia. It has 4 vertical silver bands and a reddish body. The fish *P. tetrazona* is an active fish and this behavior coupled with their brightly colored body, is a much desired aquarium fish all over the world. There is no enough study about the effect of natural pigments in the color of Albino tiger barb. So this research was carried on the effect of natural pigment and growth performances of the carrot, Beetroot and *Spirulina* powder.

2. Materials and Methods

2.1. Collection of experimental fish

Healthy male and female *Puntius tetrazona* were collected from the commercial seller, Southern India Aquarium, Thiyagaraya Nagar, Chennai, Tamilnadu, India. They were stocked in recirculation rectangular glass tanks 25 liter capacity and acclimatized to laboratory condition for 10 days. They were fed with dried blood (*Glycera dibranchiata*) worms with well aerated water. A control feed was prepared using the fish meal as a protein source along with other ingredients exclusive of carotenoids (Type I feed). The second, third and fourth types of feed constituted inclusion of beetroot, carrot and *Spirulina* separately in 10,20,30,40 and 50% by replacing the fish meal of the control diets. The type IV feed was prepared by adding beet-root (10%) carrot (10%) and *Spirulina* (10%) in 1:1:1 ratio which replaced 30% of the fish meal of the control diet.

2.2. Preparation of experimental fish feed

The ingredients were collected from the local market. They were powdered using electric grinder and sieve through a fine mesh of 0.5 mm sieve. After taking appropriate weight of the ingredients, they were mixed thoroughly to get a homogeneous by adding the required amount of warm water and then made as dough. The dough was then placed in a pressure cooker for 15 minutes (12-15*1b*) cooked and then sufficient amount of vitamin and mineral mix were added and then pressed through a hand palletized. The wet noodles were collected in plastic sheets and dried in hot air oven for a day or two at 50°C. After drying, they were stored in plastic containers for further use.

After sufficient acclimatization period, fish of same weight (0.4±0.04) were recruited from the stock and divided into five batches, each batch was divided into five groups; each group in turn was divided into 3 sets, each containing 5 test individuals. The first group of fished were fed with control feed. The second group of fishes was fed with Carrot (C1 to C5), the third group with feed Beetroot (B1 to B5) and the fourth group with Spirulina (S1 to S5) respectively, whereas the fifth group were fed with mixed diet.

Formulae Used in Calculations

Average Growth rate (AGR)

Average Growth rate = Final mean weight - Initial mean weight

Percentage weight gain

$$\text{Percentage weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} X100$$

Food conversion ratio (FCR)

$$\text{Food conversion ratio} = \frac{\text{Feed give (dry weight)}}{\text{Body weight gain (wet weight)}} X100$$

Specific growth rate (SGR)

$$\text{Specific growth rate (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Experimental period in days}} X100$$

Survival percentage

Survival percentage was calculated at the end of the experiment by counting the number of fishes in each fiber tank and is calculated as follows:

$$\text{Survival (\%)} = \frac{\text{Total number of animal harvested}}{\text{Total number of animals stocked}} X100$$

Total carotenoid content

The total carotenoid content was calculated as µg per wet weight of tissue as follows:

$$\text{Total arotenoid content} = \frac{\text{Absorption at maximum wave length}}{0.25 X \text{ Sample weight (g)}} X100$$

Where, 10 = Dilution factor;
0.25 = Extinction coefficient

Triplicate set was maintained for each feeding experiment. The duration of experiment lasted for 60 days. During this period the fish were fed with 3% initial body weight twice a day (10 AM and 4 PM). The unfed and faeces were collected daily by manual siphoning. The physic-chemical parameters of water *viz*., DO (6.5±0.2 g/l), pH (6.8±0.2), temperature (26±1.0°C), hardness (358.7± 7.0 ppm), and alkalinity (316±4.2 ppm) were maintained throughout the experiment period. The assessment of water quality was done according to method (8).

The growth performance was assessed using the formulae and, the spectrophotometric estimation of carotenoids. For feed and fish is to find out any change in the body colouration of the fishes the method followed by the (9). The biochemical analysis of the fish and fish feeds were analyzed using the methods (8).

3. Results

Different formulated feed (C-1, C-2, C-3, C-4, C-5), (B-1, B-2, B-3, B-4, B-5), (S-1, S-2, S-3, S-4, S-5) and mixed diet were given to the experimental fish, *Puntius tetrazona*. Remarkable variations were observed in the length of the fish fed with different formulated feed. It was observed that remarkable variation in length gain was observed during 60 days of the experimental period. The increasing trend of length gain was noticed in *Puntius tetrozona* as a C-20 % (37.67) > C-10% (28.76) > mixed (28.57) > C-30% (25.33) > C-40% (20.50) > and C-50% (17.86 % coc). As well as data pertaining to the experiment clearly revealed that experimental fish *Puntius tetrozona* fed with C-20% formulated feed gained the remarkable weight gain Table 2.

Similarly, in beet root formulated feed diet showed the variation in length gain was observed during 60 days of the experimental period. The increasing trend of length gain was noticed in *Puntius tetrazona* as B-50% (29.79) > mixed (28.57) > B-30% (27.34) > B-40% (26.17) > B-20% (24.31) >and B-10% (22.07 % coc). The maximum weight gain was noticed in B-50% beet root formulated feed diet fed by *Puntius tetrozona* during 60 days of the experimental period.

Although it was observed that the *Spirulina* formulated feed showed the remarkable length gain in the end of the experimental period. The increasing trend of length gain was noticed in *Puntius tetrazona* as S-30% (54.03) > S-20% (44.44) > S-40% (39.61) > S-50% (34.57) > S-10% (33.33) >and mixed feed (28.57 % coc). The maximum weight gain was noticed in S4 spirulina formulated diet fed by *Puntius tetrazona* during 60 days of the experimental period (table 4). The perusal of the data pertaining to the above experiment clearly indicated that the Average Growth Rate of carrot treated group was found in the increased in the C-20 % (0.56±0.10) followed by a C-10 % (0.41±0.10) and the least AGR were observed in C-50% (0.30±0.17). Whereas in beet root treated group was founded in the increased in B-50% (0.41±0.12) followed by B-40% (0.39±0.12) and the least AGR was observed in B-10% (0.29±0.13) (Table: 5.7). Similarly, in *Spirulina* group were found in the increased in S-30% (0.58±0.06) followed by S-40% (0.53±0.17) and the least AGR was observed in S-10% (0.41±0.15) (table 5).

The perusal of the data pertaining to the above experiment clearly indicated that the Percentage Weight Gain (PWG) of carrot treated group where found to the increased in C-20% (172.37±34.17), Whereas in beet root treated group where found to the increased in B-50% (125.97±55.45) and the *Spirulina* formulated feed group where found to the increased in S-40% (170.09±83.53) followed by S-30% (157.96±55.58) and the least PWG was observed in S-5(104.70±76.26).The perusal of the data pertaining to the above experiment clearly indicated that the Food Conversion Ratio (FCR) of carrot treated group were found to the increased in C-50% (0.29±0.18), Whereas in beet root treated group were found to the increased in B-10% (0.21±0.13) and *Spirulina* group were found to the increased in S-50% (0.21±0.13).

The perusal of the data pertaining to the above experiment clearly indicated that the Specific Growth Rate (SGR) of carrot treated group where found to the increased in C-20% (0.93±0.16), Whereas in beet root treated group where found to the increased in B-50% (0.68±0.19) and *Spirulina* group where found to the increased in S-30% (0.96±0.10) followed by S-40% (0.88±0.28) and the least SGR was observed in S-10% (0.68±0.24).In nutshell it was observed that all the experiment group showed 100 % Survival Rate (SR).

The perusal of the data pertaining to the above experiment clearly indicated that the carotenoids of all the three species like *Puntius tetrazona*, were shown the carrot based formulated feed were found in the increased in the C-50 % (55.04±0.57), beet root formulated diet B-40% (55.16±1.04) and *Spirulina* based formulated feed were found to the increased in S-50% (67.36±0.53) proximate composition values are presented in the table 1.

Table: 1. Proximate Composition (%) of Experimental Diets

Ingredients	Control	Feed type					Mixed
		10%	20%	30%	40%	50%	
Fish meal (FM)	42	38	38	34	30	28	28
Rice Bran	16	16	14	12	10	8	8
Ground nut oil cake	18	16	12	12	12	10	10
Soybean meal	12	12	12	12	12	12	12
Tapioca Flour	8	8	8	8	8	8	8
Vitamin mineral mix	4	4	4	4	4	4	4
Beetroot powder(BP)	-	6	12	18	24	30	B : C : SP 10 : 10 : 10
Carrot powder(CP)	-	6	12	18	24	30	B : C : SP 10 : 10 : 10
Spirulina powder(SP)	-	6	12	18	24	30	B : C : SP 10 : 10 : 10

Multivitamin and multiminerals tablets (Ritalvit), Dietary supplement. Vitamin A- 5000 I.U, Vitamin D3 - 400 I.U, Vitamin E - 25 I.U, Thiamine mononitrate-10 mg, Riboflavine-10 mg, Pyridoxine HCl-3 mg, Methylcobalamine-500 mcg, Niacinamide-50 mg, Calcium pantothenate-12.5 mg, Ascorbic acid-100 mg, Folic acid- 1500 mcg, Biotin -6o mcg, L-Glutamic acid- 100 mg, DL Methionine- 25 mg, Magnesium Oxide- 30 mg, Manganese sulphate eq. to Elemental manganese-2.5 mg, Copper sulphate pentahydrate eq. to Elemental Copper- 2.5 mg, Zinc Sulphate monohydrate eq. to Elemental Zinc-22.5 mg, Chromium picolinate eq. to Elemental chromium -100 mcg, Sodium Selenite eq. to Elemental Selenium - 60 mcg., Other ingredient:-, Microcrystaline cellulose, Dibasic calcium phosphate, sodium starch Glycolate, Colloidal Silicon dioxide, magnesium stearate, Hydroxy propyl methyl cellulose,purified Talc, propylene glycol.

Table .2: Length and weight gained by the, *Puntius tetrazona* fed by different formulated feed (n=5).

No. of days	Length of the fish (mm)						
	Control	C-10%	C-20%	C-30%	C-40%	C-50%	Mixed*
Initial	2.96±0.05	3.06±0.19	2.92±0.04	3±0.14	3.22±0.11	3.36±0.11	2.8±0.25
15 days	3.14±0.30 (6.08)	3.24±0.05 (5.88)	3.18±0.16 (8.90)	3.16±0.29 (5.33)	3.3±0.10 (2.48)	3.42±0.13 (1.79)	3.02±0.28 (7.86)
30 days	3.4±0.27 (14.86)	3.48±0.56 (13.73)	3.42±0.11 (17.12)	3.52±0.51 (17.33)	3.68±0.33 (14.29)	3.66±0.31 (8.93)	3.32±0.04 18.57
45 days	3.64±0.30 (22.97)	3.62±0.23 (18.30)	3.78±0.19 (29.45)	3.66±0.23 (22.00)	3.74±0.34 (16.15)	3.82±0.20 (13.69)	3.52±0.33 (25.71)
60 days	3.78±0.13 (27.70)	3.940.19 (28.76)	4.02±0.30 (37.67)	3.76±0.36 (25.33)	3.88±0.08 (20.50)	3.96±0.21 (17.86)	3.6±0.16 (28.57)
Weight of the fish (g)							
Initial	0.348±0.09	0.36±0.02	0.328±0.05	0.378±0.09	0.363±0.01	0.421±0.01	0.296±0.04
15 days	0.476±0.07 (36.78)	0.528±0.07 (46.67)	0.542±0.06 (65.24)	0.478±0.10 (26.46)	0.434±0.09 (19.56)	0.486±0.06 (15.44)	0.346±0.05 (16.89)
30 days	0.572±0.12 (64.37)	0.598±0.06 (66.11)	0.758±0.07 (131.10)	0.640±0.08 (69.31)	0.516±0.04 (42.15)	0.566±0.07 (34.44)	0.426±0.08 (43.92)
45 days	0.686±0.12 (97.13)	0.694±0.08 (92.78)	0.812±0.10 (147.56)	0.69±0.10 (82.54)	0.666±0.12 (83.47)	0.634±0.18 (50.59)	0.552±0.16 (86.49)
60 days	0.738±0.16 (112.07)	0.768±0.09 (113.33)	0.886±0.12 (170.12)	0.764±0.11 (102.12)	0.729±0.12 (100.83)	0.724±0.17 (71.97)	0.676±0.06 (128.38)

All the values determined mean ± S. D of five replications. Values in parentheses holds percentage change over control. C1=Carrot 10%; C2=Carrot 20%; C3=Carrot 30%; C4=Carrot 40%; C5=Carrot 50%; *mixed = Carrot 10%+Beetroot 10%+Spirulina10%.

Table .3: Length and weight gained by the, *Puntius tetrazona* fed by different formulated feed (n=5).

No. of days	Length of the fish (mm)						
	Control	B-10%	B-20%	B-30%	B-40%	B-50%	Mixed*
Initial	2.96±0.05	2.9±0.12	2.88±0.19	2.89±0.10	2.98±0.26	2.82±0.18	2.8±0.25
15 days	3.14±0.30 (6.08)	3.04±0.18 (4.83)	2.94±0.32 (2.08)	3.24±0.11 (12.11)	3.11±0.11 (4.36)	2.98±0.21 (5.67)	3.02±0.28 (7.86)
30 days	3.4±0.27 (14.86)	3.12±0.42 (7.59)	3.16±0.33 (9.72)	3.48±0.41 (20.42)	3.23±0.11 (8.39)	3.14±0.30 (11.35)	3.32±0.04 18.57
45 days	3.64±0.30 (22.97)	3.32±0.04 (14.48)	3.34±0.27 (15.97)	3.52±0.37 (21.80)	3.4±0.44 (14.09)	3.38±0.33 (19.86)	3.52±0.33 (25.71)
60 days	3.78±0.13 (27.70)	3.54±0.29 (22.07)	3.58±0.40 (24.31)	3.68±0.48 (27.34)	3.76±0.40 (26.17)	3.66±0.40 (29.79)	3.6±0.16 (28.57)
Weight of the fish (g)							
Initial	0.348±0.09	0.332±0.05	0.314±0.04	0.341±0.06	0.328±0.05	0.344±0.07	0.296±0.04
15 days	0.476±0.07 (36.78)	0.430±0.08 (29.52)	0.370±0.03 (17.83)	0.376±0.07 (10.26)	0.430±0.12 (31.10)	0.428±0.05 (24.42)	0.346±0.08 (16.89)
30 days	0.572±0.12 (64.37)	0.550±0.13 (65.66)	0.47±0.15 (49.68)	0.478±0.10 (40.18)	0.561±0.15 (71.04)	0.524±0.16 (52.33)	0.426±0.07 (43.92)
45 days	0.686±0.12 (97.13)	0.596±0.06 (79.52)	0.548±0.12 (74.52)	0.650±0.17 (90.03)	0.614±0.13 (87.20)	0.635±0.13 (84.59)	0.552±0.16 (86.49)
60 days	0.738±0.16 (112.07)	0.622±0.10 (87.35)	0.632±0.07 (101.27)	0.708±0.14 (107.62)	0.714±0.09 (117.68)	0.753±0.11 (118.90)	0.676±0.06 (128.38)

All the values determined mean ± S. D of five replications. Values in parentheses holds percentage change over control. C1=Carrot 10%; C2=Carrot 20%; C3=Carrot 30%; C4=Carrot 40%; C5=Carrot 50%; *mixed = Carrot 10%+Beetroot 10%+Spirulina10%.

Table .4: Length and weight gained by the, *Puntius tetrazona* fed by different formulated feed (n=5)

No. of days	Length of the fish (mm)						
	Control	S-10%	S-20%	S-30%	S-40%	S-50%	Mixed*
Initial	2.96±0.05	2.88±0.24	2.7±0.28	2.48±0.29	3.08±0.29	3.24±0.25	2.8±0.25
15 days	3.14±0.30 (6.08)	3.08±0.22 (6.94)	2.92±0.31 (8.15)	2.76±0.23 (11.29)	3.34±0.21 (8.44)	3.3±0.17 (1.85)	3.02±0.28 (7.86)
30 days	3.4±0.27 (14.86)	3.46±0.19 (20.14)	3.3±0.16 (22.22)	3.34±0.13 (34.68)	3.77±0.19 (22.40)	3.64±0.21 (12.35)	3.32±0.04 18.57
45 days	3.64±0.30 (22.97)	3.72±0.13 (29.17)	3.56±0.18 (31.85)	3.72±0.16 (50.00)	3.85±0.19 (25.00)	3.98±0.13 (22.84)	3.52±0.33 (25.71)
60 days	3.78±0.13 (27.70)	3.84±0.30 (33.33)	3.9±0.10 (44.44)	3.82±0.29 (54.03)	4.3±0.18 (39.61)	4.36±0.24 (34.57)	3.6±0.16 (28.57)
Weight of the fish (g)							
Initial	0.348±0.09	0.35±0.05	0.382±0.11	0.388±0.09	0.344±0.09	0.49±0.14	0.296±0.04
15 days	0.476±0.07 (36.78)	0.422±0.10 (20.57)	0.522±0.14 (36.65)	0.528±0.15 (36.08)	0.494±0.15 (43.60)	0.616±0.13 (25.71)	0.346±0.05 (16.89)
30 days	0.572±0.12 (64.37)	0.512±0.11 (46.29)	0.608±0.15 (59.16)	0.796±0.10 (105.15)	0.584±0.12 (69.77)	0.802±0.16 (63.67)	0.426±0.08 (43.92)
45 days	0.686±0.12 (97.13)	0.622±0.12 (77.71)	0.706±0.11 (84.82)	0.848±0.09 (118.56)	0.748±0.12 (117.44)	0.862±0.08 (75.92)	0.552±0.16 (86.49)
60 days	0.738±0.16 (112.07)	0.758±0.11 (116.57)	0.824±0.06 (115.71)	0.964±0.11 (148.45)	0.872±0.09 (153.49)	0.924±0.11 (88.57)	0.676±0.06 (128.38)

All the values determined mean ± S. D of five replications. Values in parentheses holds percentage change over control. C1=Carrot 10%; C2=Carrot 20%; C3=Carrot 30%; C4=Carrot 40%; C5=Carrot 50%; *mixed = Carrot 10%+Beetroot 10%+Spirulina10%.

Table .5: Growth performance and Survival (%) of Albino tiger barb (*Puntius tetrazona*) fed in experimental diets. Each value is the mean ±SD of three observations.

Experimental diets	AGR	PWG	FCR	SGR (%)	Survival rate
Control	$0.39±0.2^{b}$	$123.46±74.9^{g}$	$0.21±0.23^{b}$	$0.65±0.34^{b}$	100
C-10%	$0.41±0.10^{b}$	$114.79±31.45^{f}$	$0.14±0.05^{a}$	$0.68±0.17^{c}$	100
C-20%	$0.56±0.10^{c}$	$172.37±34.17^{k}$	$0.09±0.02^{a}$	$0.93±0.16^{e}$	100
C-30%	$0.39±0.15^{b}$	$112.02±58.84^{e}$	$0.20±0.18^{b}$	$0.64±0.26^{b}$	100
C-40%	$0.37±0.12^{b}$	$101.08±34.56^{c}$	$0.16±0.06^{a}$	$0.61±0.21^{b}$	100
C-50%	$0.30±0.17^{a}$	$71.97±40.88^{a}$	$0.29±0.18^{c}$	$0.51±0.29^{a}$	100
B-10%	$0.29±0.13^{a}$	$93.24±51.81^{b}$	$0.21±0.13^{b}$	$0.48±0.21^{a}$	100
B-20%	$0.32±0.09^{a}$	$103.7±34.65^{d}$	$0.16±0.07^{a}$	$0.53±0.15^{a}$	100
B-30%	$0.37±0.14^{b}$	$111.37±47.2^{e}$	$0.16±0.07^{a}$	$0.61±0.23^{b}$	100
B-40%	$0.39±0.12^{b}$	$123.45±53.15^{g}$	$0.14±0.06^{a}$	$0.64±0.19^{b}$	100
B-50%	$0.41±0.12^{b}$	$125.97±55.45^{h}$	$0.14±0.05^{a}$	$0.68±0.19^{c}$	100
S-10%	$0.41±0.15^{b}$	$122.29±57.34^{g}$	$0.15±0.07^{a}$	$0.68±0.24^{c}$	100
S-20%	$0.44±0.12^{b}$	$133.31±83.22^{i}$	$0.14±0.07^{a}$	$0.74±0.20^{c}$	100
S-30%	$0.58±0.06^{c}$	$157.96±55.58^{j}$	$0.10±0.03^{a}$	$0.96±0.10^{e}$	100
S-40%	$0.53±0.17^{c}$	$170.09±83.53^{k}$	$0.12±0.08^{a}$	$0.88±0.28^{d}$	100
S-50%	$0.43±0.16^{b}$	$104.70±76.26^{d}$	$0.21±0.13^{b}$	$0.72±0.27^{c}$	100
Mixed	$0.38±0.06^{b}$	$130.94±31.44^{h}$	$0.12±0.03^{a}$	$0.63±0.10^{b}$	100

Table .6. Availability of carotenoids in the formulated diets and in the body tissue of *Puntius tetrazona*

Feed Types	Caritenoids(μg/g) in feed	Caritenoids(μg/g) in *Puntius tetrazona*
Control	31.84±1.05	39.92±1.07
C-10%	43±0.70	40.6±0.70
C-20%	44.32±1.14	45±1.07
C-30%	45.2±0.75	47.92±0.53
C-40%	47.68±1.12	49.84±0.54
C-50%	48.44±1.07	55.04±0.57
B-10%	40.08±0.61	40.96±0.76
B-20%	43.64±1.01	45.84±0.79
B-30%	46.92±0.71	50.72±0.80
B-40%	48.84±0.91	55.16±1.04
B-50%	52.12±0.99	60.72±0.66
S-10%	48.64±1.03	45.92±0.75
S-20%	58.84±0.94	51.88±0.68
S-30%	62.72±0.79	55.44±0.74
S-40%	66.8±0.44	59.44±0.75
S-50%	75.4±0.72	67.36±0.53
mixed	62.96±0.54	54.68±1.26

Table .7: Biochemical composition of different feed concentrations.

Parametes	Control	Carrot					Beet root					Spirulina					Mixed
		10%	20%	30%	40%	50%	10%	20%	30%	40%	50%	10%	20%	30%	40%	50%	
Protein	32.60	29.93	32.53	32.14	33.68	33.98	30.75	31.23	31.56	32.4	33.2	33.55	35.18	38.01	38.91	42.16	32.42
Lipid	5.51	3.41	3.63	3.56	3.79	3.96	3.36	4.03	4.11	4.16	4.23	3.30	3.18	3.33	3.74	4.10	3.72
Ash	11.61	9.33	10.79	9.99	7.89	8.90	10.87	10.1	10.71	9.32	9.8	8.59	8.7	10.83	11.57	11.89	12.89
Moister	9.95	9.86	9.82	9.67	9.77	9.84	9.45	9.31	9.47	9.54	9.77	9.73	9.85	9.06	9.76	9.82	9.65
NFE	38.22	40.06	40.89	41.29	41.68	41.64	41.09	41.02	41.45	40.91	41.14	37.08	37.40	37.55	37.76	37.86	36.69
Fiber	6.06	6.27	6.16	6.02	6.81	6.42	5.53	4.62	5.17	5.61	5.03	6.48	5.04	4.08	4.52	3.69	4.28

Table: 8. Physico-chemical parameters recorded in experimental tanks during the experimental periods.

S.No	Parameters	*Puntius tetrazona*
1	Temperature(C^o)	26
2	Dissolved oxygen	6.4
3	pH	6.8
4	Hardness(ppm)	352
5	Alkalinity(ppm)	312

4. Discussion

Fish, like other animals such as birds, absorb dietary carotenoids through the intestinal mucosa (10), transport them through the blood via serum lipoproteins (12), metabolically oxidize them to other forms (13) and deposit them into specialized skin cells called chromatophores (14). Goldfish and Koi carp can convert the carotenoids lutein and zeaxanthin to astaxanthin.

Carrot to a greater extent influenced the weight of the fish. Perusal of the data pertaining to the present investigations revealed that the weight was apparently increased among the different formulated feed with different ratio in *Puntius tetrazona*. These findings are attributed to the earlier findings of several authors who have been reported that carrot induced more than 20% weight in *Carassius auratus* (15) in *X. helleri* (16) and Red Swordtail (17). In beet-root diet showed the remarkable variation in length and weight in B-50%.

Similarly fish fed with 30 % spirulina formulated feed were shown the maximum growth parameters apparently increased in the following trend *P. tetrazona* . Our findings are in agreeing with the earlier findings of (18); *Poecilia reticulata*, (19); (*Xiphophorus maculates*) and (20); (Gold fish) (21); Female guppies, *P. reticulata*, (22 and 23) ; *X. helleri*). Our results correspond well with earlier studies by (24) and (25); (*C.carpio*). (26) reported a higher growth of *Rhabodosargus sorba* fed with spirulina up to 50% inclusion of feed. (27) they observed a positive growth in *Haliotismidae* fed with balanced feed with 25% spirulina incorporated meal. Gold Fish *Carassius* *auratus* reared in pond water showed a higher SGR fed with commercial feed ZP (3.16) and BK (3.55) than rearing in well water because of the presences of carotenoid (28). Among all the three carotenoid based formulating feed spirulina-incorporated diets produced better SGR and FCR than the carrot and beet-root. Similar results were also reported by (29) in *Labeo rohita*, (30) in *Fenneropenaeus indicus.*

The coloration of ornamental fishes gives the market value of the fish. Many of the brightly colored fishes showed fading in coloration due to various factors like exposure to sunlight, chlorine, starvation, pH, hardness of water, turbidity of water, stress, toxicants and pollutants in water, low quality of water, poor husbandry techniques, poor quality feeds, nutrient-deficient live feeds, etc. The resultant ornamental fishes have decreased market rates when compared to the brightly colored ones. An attempt was made on supplementation of microbial cell mass (rich in carotenoids) incorporated feed to the fishes which were affected either biologically or chemically and the recoveries of coloration of the fishes were studied.

In our study *Spirulina* fed fish showed the remarkable variety compare to other feed ingredients. This result proved that fishes fed with carotenoid enriched feed showed faster recovery of carotenoids when compared to those provided with only the control feed. The main reason for the color recovery in stressed fishes could be attributed to the presence of the carotenoids in the lyophilized fermented broth (31). Color enhancement through the use of carotenoids in feed has been

confirmed by a number of authors. Reports on the intense coloration of freshwater red velvet swordtails (*Xiphophorus helleri*), rainbow fish (*Pseudomugil furcatus*) and topaz cichlids (*Cichlasoma myrnae*) when fed diets containing carotenoid rich strains of *Spirulina platensis* and *Haematococcus Pluvialis* (32), *Carassius auratus* (33), carotenoid that is readily metabolized from the yellow pigment zeaxanthin are reported earlier (34).

Besides the effectiveness of carotenoids in pigmentation of fish skin a positive effect on the nutrition of larval fish and survival rates of young fry was discussed (35; 36). The addition of algae to the rearing tank of marine fish larvae (green water culture) has been shown to enhance growth and survival as well as the quality of the fry (37; 38). One of the beneficial effects attributed to adding algae is an increase in ingestion rates of food by marine fish larvae (39). The carotenoids level of 45 mg kg^{-1} diet used in this work for survival and growth purposes was due to the high pigmentation attained with this concentration (40; 41).

5. Conclusion

Carotenoids are widespread and important pigment classes of the organisms as well as contributing characteristic quality criterion for marketing and consumer demands of aquaculture products. The appearance of an animal product, especially color plays an important role in the marketing. Color, nutritional value, healthy appearance, freshness and sensory test components are the subconscious elements to choose the product. Choosing a product is affected by the educational condition, environment and customs as well. Traditional aquaculture is being replaced with the modern manner. This situation needs to harvest the most amount of product in higher quality. This has been accompanied by some problems regarding the product. Fish in the nature have special skin and flesh color. In the diets of fish for which pigmentation is important, synthetic and natural carotenoid source is included in order to eliminate the problem.

References

1. C.V.L. Srivastava. A textbook of Fishery and Indian fisheries, Kitab Mohal, Allahbad, India. (1985), 47-86.
2. R. Jacquot. “Organic constituents fish and other aquatic animals”, *In Fish as Food Ed.* Borgstrom, Academic Press, New York and London. (1961), 145-209.
3. A. Sermwattanakul and B. Bamrungtham. Institute for Research of Beautiful Water Animals and Exhibition Places, Bangkok, Thailand. Feed for Beautiful Fish. (2000), 16–19.
4. R. Fujii. Chromatophores and pigments. In: Hoar, W.S. and D.J. Randall (Eds.). Fish Physiology. (1969), 3: 307-353.
5. A. Bricaud., A. Morel., M. Babin., K. Allali., H. Claustre. Variations of light absorption by suspended particles with chlorophyll a concentration in oceanic (case 1) waters: analysis and implications for bio optical models. Journal of Geophysical Research. (1998), 103 (13): 31033–31044.
6. B. Halten., G.H. Aas., E. H. Jorgensen., T. Storebakken and U.C. Goswami. Pigmentation of 1, 2 and 3 years old Arctic charr (*Salvelinus alpinus*) fed two different dietary astaxanthin concentrations. Aquaculture. (1995),138: 303- 312.
7. F. A. Chapman. Ornamental fish culture, freshwater. In: Encyclopedia of aquaculture. Stickney R. R. (ed), John Wiley & Sons, Inc., New York. (2000), 602-610.
8. AOAC. “Official methods of Analysis”, 17th Edition, Association of Official Analytical Chemist, Washington DC. (2000), 2200.
9. A. Olson. A simple dual assay for Vit. A and carotenoids in human and liver. Nutrition report International, 1979), 19: 807-813.
10. H.C. Furr and R. M. Clark. Intestinal absorption and tissue distribution of carotenoids.Nutritional Biochemistry. (1997), 8: 364-377.
11. J.Bowen., C. Soutar., R.D. Serwata., S. Lagocki., D.A. White., S.J. Davies and A.J. Young. Utilization of (3S, 3´S)-astaxanthin acyl esters in pigmentation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition. (2002), 8: 59-68.
12. T., Katayama, Shintani, K. and Chichester, C.O. The biosynthesis of astaxanthin. Comparative Biochemistry Physiology. (1973), 448:253-257.
13. S. Chatzifotis., M. Pavlidis., C. Donate Jimenso., G.Vardanis., A. Sterioti and P. Divanach. The effect of different carotenoid sources on skin coloration of cultured red porgy (*Pagrus pagrus*). Aquaculture. Res. (2005), 36:1517-1525.
14. C.M. Aravindan., S. Preethi and K.M. Abraham. Effect of increased bio-availability of betacarotene on the pigmentation of gold fish *Carassius auratus*. Journal of Inland Fisheries Society of India. (2001), 33: 49-53.
15. R. James and K. Sampath. Effect of animal and plant protein diets on growth and reproductive performance in an ornamental fish, *Xiphophorus helleri*. Indian J. Fish., (2004a), 51:75-86.
16 J. Ezhil., C. Jeyanthi., N. Narayanan. Marigold as a carotenoid source on pigmentation and growth of red swordtail, *Xiphophorus helleri*. Turkey Jounal of Fish Aquatic Science. (2008), 8: 99–102.
17. T.Daniel and R. Kumuthakalavalli. The use of Spirulina, a blue green alga, as a substitute for fish meal in diets for *Cirrhinus mrigala* fingerlings. Indian Zool. (1991), 15:5-7.
18. S. Okada., W.L. Liao., T. Mori., K. Yamaguchi and T. Watanabe. Pigmentation of cultured striped jack reared on diets supplemented with the blue green alga, *Spirulina maxima*. c., (1991), 57:1403-1406.
19. R. James., I. Vasudhevan and K. Sampath. Interaction of spirulina with different levels of vitamin E on growth, reproduction, and coloration in goldfish (*Carassius auratus*). Israeli Journal of Aquaculture. – Bamidgeh, (2009), 61(4):330-338.
20. B.T. Dahlgren. The effects of population density on fecundity and fertility in the guppy, *Poecilia reticulata* (paten). Journal of Fish Biology. (1979), 15:71-91.
21. R. James and K. Sampath. Effect of animal and plant protein diets on growth and reproductive performance in an ornamental fish, *Xiphophorus helleri*. Indian Journal of Fishery Biology, (2004a), 51:75-86.
22. R. James and K. Sampath. Effect of feed type on growth and fertility in ornamental fish, *Xiphophorus helleri*. Israeli J. Aquacult. - Bamidgeh, (2004b), 56:264-273.
23. M.C. Nandeesha., S.S. De Silva and D. Krishnamurthy. Evaluation of mixed feeding schedules in two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*). (1993), 753-765.
24. Manohar M. Probiotic and Spirulina as a Source of Immunostimulants and Growth in Common Carp. Ph.D. thesis, Manonmaniam Sundaranar Univ., Tamilnadu, India, (2005).
25. A.F. M. El-Sayed, “Evaluation of soybean meal, Spirulina meal and chicken offal meal as feed additive on growth, feed efficiency, and body

composition in silver seabream", Fishery Sciences (1994), *61*, 5-28.

26. J. Britz. The suitability of selected protein sources for inclusion in formulated diets for the south African abalone, *Haliotis midae*, Aquaculture (1996), 140:63-73.
27. G.K. Wallat., A.M. Lazur and F.A. Chapman, Carotenoids of different types and concentrations in commercial formulated fish diets affect color and its development in the skin of the Red Oranda variety of Goldfish. North American Journal Aquaculture, (2005), 67:42-51.
28. K. Ghosh., S.K. Sen and A.Kay. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, *Labeo rohita*, fingerlings. Israeli Journal Aquaculture - Bamidgeh, (2003).,55:13-21.
29. S. Ziaei-Nejad., M.H. Rezaei., G.A. Takami., D.L. Lovett., A.R. Mirva-Ghefi and M. Shakour. The effect of Bacillus spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquaculture, (2006), 252:516-524.
30. S. Dharmaraj., B. Ashokkumar and K. Dhevendaran. Food-grade pigments from *Streptomyces* sp. isolated from the marine sponge *Callyspongia diffusa*. Food. Res. International journal, (2009), 42: 487-492.
31. H. Ako., C.S. Tamaru., L. Asano., B. Yuen and M. Yamamoto. Achieving natural colouration in fish under culture. UJNR Technical Report, (2000), 28.
32. Wallat G. K., Lazur A. M., Chapman F. A. Concentrations in commercial formulated fish diets affect color and its development in the skin of the red oranda variety of goldfish. North American Journal of Aquaculture, (2005), 67:42-51.
33. M. Hata and M. Hata. Carotenoid pigments in gold fish-IV. Carotenoid metabolism. Bulletin of the Japanese Society of Scientific Fisheries, (1972), 38: 331-338.
34. F. Shahidi., B. Metusalach and J.A. Brown. Carotenoids pigments in seafoods and aquaculture, Critical Reviews in Food Science and Nutrition, (1998), 38,1-67.
35. M. Planas and I.Cunha. Larviculture of marine fish: problems and perspectives. Aquaculture, 1999.177:171-190.
36. Reitain, K., J.R. Rainuzzo, G. Oie and Y. Olsen, 1997. A review of the nutritional effects of algae in marine fish larvae, Aquaculture, 155:207-221.
38. P. Lazo., M.T.Dinis., J. Holt., C. Faulk and C. Arnold. Co-feeding micro particulate diets with algae: Toward eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus*). Aquaculture, (2000), 188: 339-351.
39. K. Naas., T. Naess and T. Harboe. Enhance first feeding of halibut larvae (*Hippolossus hippoglossus*) in green water. Aquaculture, (1992),105: 143-156.
40. L. Gouveia., P. Rema., O. Pereira and J. Empis. Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. Aquaculture Nutrition, (2003),9:123- 129.
41. Tippawan Paripatananont, Jirasak Tangtrongpairoj, Achariya Sailasuta and Nantarika Chansue. Effect of Astaxanthin on the Pigmentation of Goldfish *Carassius auratus*. Journal of the World Aquaculture Society, (1999), 30 (4): 454–460.