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Dietary Supplement of Natural Carotenoid Source and Commercial Feed on Growth and Colouration in Freshwater Ornamental Fish, *Poecilia sphenops*

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Abstract:
Ornamental fishery is a well-known commercial business and hobby around the world and to manage their healthy growth and bright colouration in captive conditions, a well designed aquarium, healthy feed and good water quality is must. Feed, its ingredients and feeding regime is important for the growth, colouration and general health of fish for aesthetic appearances. Thus, the dietary supplements and their management contribute to the sustainability and well-being of an aquaculture system and decide how well the income can be generated thereby also reducing major economic losses. In the present investigation effect of three types of diets viz., basic diet or customized formulated diet (D1), commercial diet (D2) and natural pigment source, *Lantana camara* (10g / 1000g)incorporated in formulated diet (D3) fed to ornamental live-bearer fish Red Eyed Orange Molly (*Poecilia sphenops*) for a period 60 days was observed. The results showed 100% survivability (0% mortality) with significant improvement in health conditions, growth performance and colouration in all the three group of fish. The growth parameters were found to be significantly high ($p < 0.05$) among all fish groups whereas, morphological indices such as condition factor, hepatosomatic index and viscerosomatic index showed significant difference between groups fed with three feed regimes (D1, D2 and D3) for 60 days ($p < 0.05$). The highest pigmentation range was obtained in fishes fed with D3 followed by D2 and D1 respectively. In conclusion, *L. camara* incorporated diet (D3) fed test fish, *P. sphenops* showed significantly positive improvement in their growth performance and colouration compared to commercial diet (D2).

Keywords: Ornamental fish, *Lantana camara,* Diet, Body Colouration, Growth Parameters.

INTRODUCTION

The ornamental fish species of genus *Xiphophorus* and *Poecilia* belonging to *Poeciliidae* family include Molly (*Poecilia sphenops, Poecilia latipinna*), Guppy (*Poecilia reticulata*), Swordtail (*Xiphophorus helleri*) and Platy (*Xiphophorus maculatus*) are among those produced in Singapore, Malaysia, Indonesia, Thailand, India and China. These fish have aesthetic value and their attractive colouration and swimming behaviour is a stress reliever. In addition, their resistance to captivity stress and

survivability in a restricted space of aquariums these fish serve employment opportunities to youth by taking up culture of their native varieties and their export. Its domestic keeping is a popular hobby which makes them commercially important. The market value of ornamental fish is directly associated with the colour, fin, size and shape of the fish body. The effect of nutrition on growth, colouration and reproduction of ornamental fishes was studied by several researchers. Chong et al, (2004) reported that nutritive requirement of the brood stock is an important factor in the growth and reproductive performance in most fish species since dietary protein level influences the body weight. These fish can regulate and maintain their food intake in wild and natural conditions and reduce any nutritional deficiencies but these problems are widely noted when they are kept in a confined environment. Smaller fish have high protein requirements but as they grow their protein requirement decreases. Pigmentation of skin is another mandatory feature which increases the market value of fish and is dependent solely on their diet and nutrition. Bright colouration is observed in fish collected from their natural habitat since they consume plant or animal consisting of natural source of pigment but when kept in captivity in pond or aquarium for a long time the skin colour gets faded which is a disadvantage in marketing of fish.

Lacustrine culture is a developing industry today. The customer prefers bright fish skin colour which is achieved in cultivable ornamental fish by providing them a reliable pigment source incorporated commercial or formulated diet since fish cannot synthesize their own carotenoids they obtain it from the food they consume. Many scientists (Gouveia and Rema, 2005b; Yousefian et al, 2012) have reported enhancement of fish colour when fed with any suitable pigment (as per required concentration) incorporated diet. This in turn resulted in better quality ornamental fish than those present in wild environment (Chapman and Miles, 2018).Biological pigments present within specialized skin cells called chromatophores gives ornamental fishes their brilliant hues. The chromatophore cells are located below the epidermis. Pigments are converted by cellular metabolism to other compounds which imparts colour to the skin and depending on the type of pigment the chromatophore gets its name such as melanophore cells (black or brown), cyanophore (blue), xanthophore (yellow), erythrophore (red), leucophore (white) and iridophore (Chapman and Miles, 2018). Carotenoids are an organic pigment and according to their chemical structure, it has carotenes and xanthophylls. They occur naturally in chloroplast and photosynthetic organisms; act as immune stimulants and antioxidants; functioning as the basis for pigmentation in animals. They are absorbed mainly in the intestine and due to their hydrophobic nature they are transported by high density lipoproteins (HDL) to peripheral tissues (Nakamura and Hata, 1985). Their absorption and distribution depends on the fish species, age, physiological state, feed type and environment inhabited (Czeczuga et al, 1991). There are factors that discriminate the nutritional requirements in fish such as absorption of minerals through the gills and requirement of dietary unsaturated fatty acids and vitamin C was confirmed by Lovell, (2000). Aucoin et al, (1990) observed reduction in mortality of alpha-terthienyl, a phototoxic phytochemical, treated *Manduca sexta* larvae with increase in dietary levels of certain antioxidants as β-carotene and vitamin E. Robinson et al, (2002) had reported that synthetic β-carotene was one of the effective dietary supplements which enabled the fish body to produce vitamin A for improving pigmentation in its skin and muscle. Carroll and Berenbaum, (2006) reported that carotenoid can protect vital cellular components from damage due to UV radiation exposure. Kop et al, (2010) reported that different types of pigmentation sources such as synthetic or natural carotenoids could be used at various levels efficiently for ornamental fishes. Kaur et al, (2016) suggested that natural β-carotene was more effective than synthetic one. However, maintenance of nutrition and pigmentation among fish which are subjected to confined conditions of aquarium is a problem (Lovell, 2000).

Lantana camara, belonging to family Verbenaceae commonly known as *Sleeper weed* or *wild red sage* has been tested as a natural source of pigment which can act as an antibiotic resistance modifier to be used against multi-resistant bacteria (Sousa et al, 2010). Thus among cheap and cost effective natural plant based pigment source, *L. camara* was selected as feed additive. It possesses bio-active compounds including carotenoid (β-carotene) and various medicinal properties such as antioxidant, anti-microbial, anti-fungicidal and anti-insecticidal properties. Its flower petals, oil and other parts are

used for treating various skin ailments, asthma, chicken pox, measles and many more related diseases.

The test fish, *Poeciliia sphenops* commonly called as Red eyed orange molly belonging to the family - *Poeciliidae* is a very demandable live-bearer, exotic and tropical Cyprinid fish. In their natural habitat they are found in streams, rivers and ponds of Mexico and the United States. They grow to a maximum size of 7cm and feed mainly on vegetable matter. The male poecilid fish is characterized by the presence of gonopodium (highly modified anal fin), which is a sex-hormone influenced structure (Cohen, 1946). Females are larger and plumper than males. The gender and fish's species can be easily identified by their colour.

The information regarding specific nutritional requirements, its digestibility, type of feed and feeding alternative is mainly specific to results obtained from farmed fish kept under controlled conditions and not ornamental fish. Scientists have shown that fish fed with desired pigment at the right concentration enhances fish colouration and produces fish of an ornamental quality that may equal or exceed the quality of fish coming from the wild (Kop et al, 2010; Nguyen et al, 2014). Thus, keeping the above mentioned review into account, an attempt was made to study the ornamental fish, *Poeciliia sphenops* with commercial interest so as to evaluate and compare the efficiency of diet formulation (D1, D2 and D3) containing carotenoids required for healthy and disease resistant fish with 100% survivability, good growth performance and improved skin and flesh pigmentation. Prevention of any outbreaks of diseases one of the primary key factors for successful development of aquaculture practices was also monitored during the study. This study is a pioneer work relating to the cost-effective carotenoid pigment source incorporated formulated diet fed live-bearer ornamental fish and is aimed to help the value of aquarist to achieve healthy growth with continuous bright colour and decreased maintenance costs of ornamental fish as well.

MATERIALS AND METHODS

Experimental fish and design:
The experiment was conducted with juveniles of test fish Red Eyed Orange Molly *P. sphenops,* obtained from its brood stock, Ornamental Fish Research Center, Hebbal, Bengaluru. The uniformly sized juveniles were brought to the laboratory with oxygenated habitat water. These juveniles were quarantined in potassium-per-manganate (1:1000ppt) and acclimatized in the laboratory in a fiberglass tank with 50 liters of water capacity for a period of two weeks. Daily 10-15% part of the water along with faecal matter was siphoned from the aquarium and replaced it with fresh dechlorinated tap water. During acclimatization, the juveniles were fed with a formulated diet (control). Healthy and disease free juveniles were selected for further experiment.

Collection and identification of *Lantana camara*
Lantana camara is an upright thorny shrub, a popular ornamental garden plant, reaching the height at 2-3 m. The inflorescence is a hemispherical head, axillary or terminal, yellow, pink or orange and red coloured, made up of many small tubular flowers of *L. camara,* were collected from the nursery garden of Bangalore university campus, Bangalore. These flowers were identified and authenticated by a Professor, Department of Botany, Bangalore University as *L. camara,* belonging to Phylum: Spermatophyta; Subphylum: Angiospermae; Class: Dicotyledonae; Order: Lamiales; Family: Verbenaceae. Various parts of the plant are used for treating skin ailments, asthma, chicken pox and measles etc. The proximate composition of the flower extract of *L. camara* taken as feed additive in the present study consisted of β- carotene along with other components.

Preparation of formulated diet
The customized formulated diet (D1) was prepared with the following ingredients; fish meal, groundnut oil cake, wheat flour, rice bran, vegetable oil, vitamin and mineral mix procured from local market at Bangalore. The ingredients were kneaded with a measured amount of water and oil to form pellets of 20µg by using Square method (Hardy, 1980). The *L. camara* flower and its petals (natural pigment carotenoid source) were dried, powdered and sieved along with other ingredients of

formulated diet (all in powder form) in a concentration of 10g/1000g of formulated diet (after standardizing).

Experimental procedures:
After acclimatization the healthy juveniles of test fish molly, selected for the experiment were divided into three groups (one control and 2 experimental group I & II) with three replicates each. Each tank was stocked with 15 juveniles (5male: 10female) in triplicate for a period of 60 days with mean initial body weight of 0.8± 0.00 g and length of 3.5 ± 0.00 cm. The juveniles were starved for 24 h prior to the experiment and their body length and weight was measured. They were fed twice a day (9.00 am & 18.00 pm) with customized formulated diets during the experiment. The control fish group were fed with customized formulated diet 1 (D1-without addition of flower petals of *L. camara*), experimental group I were fed with diet 2 (D2-commercial diet (CD) - Optimum micro pellet, Made in Thailand) and experimental group II with diet 3 (D3 = D1+10g/1000g of flower petals of *L. camara)*. Daily rations were adjusted every two weeks according to fish body weight in each of the tanks. It is to be noted that the literature on optimal daily feeding rates for *P. sphenops* may vary since protein requirements varies with the rearing environment as well as their genetic composition and feeding rates (% BW/day) which was determined based on the recommendations of different researchers.

Proximate composition of customized formulated diet D1, D2, D3 and *L. camara* flower and its petals was analysed employing standard methods (AOAC, 2005). Crude protein - Kjeldahl method (2100-Auto-analyzer, Foss, Hillerod, Denmark), Crude lipid - ether extraction method using a Soxtec System HT (Soxtec System HT6, Foss, Hillerod, Denmark), Moisture – oven drying at 105°C for 24hr and Total Ash by combustion at 550°C for 12 h.

Water quality parameters:
Water was stored for 12 hrs prior to the use for control and experimental groups under laboratory conditions. The physico -chemical characteristics of water were monitored weekly. The temperature of water was measured (mercury thermometer) and maintained constant at 26 ± 0.05 ºC, dissolved oxygen (DO) measured as 7.1 ± 0.08 mg/l, free ammonia 0.73 ± 0.02 mg/l, hardness 240 ± 0.07 ppm, alkalinity was 213.5 ± 0.02 mg/l, nitrite 3 ± 0.02 ppm (by standard method of APHA et al, 2005) and pH (digital pH meter, Eu-Tech) values 7.5 ± 0.04 respectively, in all the control experimental tanks throughout the experiment period.

Analysis of growth parameters:
The following growth parameters were analysed at the end of the experiment; weight gain (WG), specific growth rate (SGR), and survival rate were analyzed based on the standard formula followed by (Zhu et al, 2014a). The feed conversion ratio (FCR) and condition factor (K) was also analyzed Bailey et al, 2003). The calculations were assessed are as follows:

Survival rate (SR %) = (Final fish number - Initial fish number) × 100/Initial fish number
Body Weight gain (BWG %) = 100 X (final body weight - initial body weight) / Initial body weight

Specific growth rate (SGR% d^{-1}) = 100 × [$Log_n(W_2)$ - $Log_n(W_1)$] / time (days)
Where, W_1 and W_2 indicate the initial and final weight (g), respectively.

Feed conversion ratio (FCR) = Feed delivered to group (g) / Live biomass gain of that group (g)
Body Length gain (BLG%) (cm) = Av. final body length - Av. initial body length /Av. initial body lengthX100
Condition factor (CF%) = 100 X (W/L^3)
Where, W= wet body weight (g) and L = standard body length (cm)

Analysis of Morphological indices
Morphological indices such as Hepato - somatic index (HSI) and Visceral somatic index (VSI) were calculated as:
HSI (%) = 100 X weight of liver/weight of fish
VSI (%) = 100Xweight of visceral organs & associated fat tissue (g)/ weight of body (g)

Total Carotenoid Concentration (TCC)

The skin colour of fish was monitored by visual examination and the skin and muscle tissue was analyzed for the estimation of total carotenoid concentration at the end of 60 days by following the pigment extraction method (Olson, 1979). One gram of entire fish body tissue, excluding head and alimentary canal was taken, gently mashed in a glass homogenizer to which 2.5 g of anhydrous sodium sulphate was added. 5ml of chloroform was then added to it and kept overnight at 0^{o}C. 0.3ml of aliquots of separated chloroform was then diluted with 3ml of absolute ethanol. The optical density was read at 380, 450, 470 and 500 nm, in a Systronic spectrophotometer Model no.104 and maximum absorption was recorded at 470 nm wavelength. A blank was prepared in a similar manner without using the fish body tissue.

Total carotenoid concentration (TCC- µg/g wet wt.) = 10 X (absorbance at maximum wave length / 0.25 sample weight (g); (Where, 10 = dilution factor, 0.25 = Extinction coefficient).

Data sampling and Statistical Analysis

The statistical analysis of data experimental and control groups was in the form of Mean and standard error (± SEM) was analysed and tabulated by using One-way and two-way analysis of variance (ANOVA) and Tukey's multiple comparison post hoc test and the least significant differences was used to compare means at $P<0.05$. The linear relationship was assessed by using linear regression and Pearson correlation coefficient. All statistical analysis was done by using GraphPad Prism ver. 5.0

RESULTS

The customized formulated diet (D1) was prepared with the following ingredients as mentioned in table 1. Proximate composition of diets D1, D2, D3 and *L. camara* flower petal (natural pigment carotenoid source) analysis is depicted in table 2. The chemical analyses of its flower revealed presence of various medicinal properties, proteins, vitamins, minerals and carotenoid pigment such as Beta (β) -carotene a responsible pigment for colouration (Khan et al, 2013) and delphinidine monoglucoside (anthocyanidin-3-o-glycosides) (Ram and Mathur, 1984). Diet 3 shows the presence of β-carotene, Ascorbic acid and Zinc content which is absent in other diets D1 and D2. Crude fibre, crude protein, total ash and moisture content are highest in Diet 3. Diet 1 and D3 showed high levels of total fat, dry matter and Nitrogen Free Extract (NFE). Diet 2 is the commercial diet consisted of small quantity of moisture, crude protein, fibre and fat content. Spirulina (100mg) was the colouring ingredient as mentioned in the table 2.

Table 1: Ingredients of customized formulated feed in grams

Sl. No.	Formulated Diet (FD) Ingredients	(g $100g^{-1}$ feed)
1	Fish meal	32
2	Groundnut oil cake	20
3	Wheat flour	12
4	Rice bran	20
5	Vegetable oil (ml)	2
6	Vitamin and mineral mix* (Maxirich *forte*)	4

Note: *Vitamins and mineral mix (mg/100g feed): (Vitamin A 1600 IU; Vitamin D3 100 IU; Vitamin E Acetate 5 IU; Energy 7.457 Kcal; Protein 0.00253 g; Fat 0.824 g; Carbohydrate 0.01.4 g; Calcium 75 mg; Phosphorous 58 mg; Vitamin C 25 mg; Nicotinamide 15 mg; Magnesium 3 mg; Potassium 2 mg; Vitamin B1 1 mg; Vitamin B2 1mg; Calcium Pantothenate 1 mg; Vitamin B6 0.5mg; Manganese 0.5 mg; Zinc 0.5 mg; Folic acid 50 mcg; Vitamin B12 0.5 mcg; Copper 0.45 mg; molybdenum 0.1 mg; Iodine 0.075 mg).

Table 2: Proximate composition of D1, D2 and D3 on dry matter basis (%) (Mean ± SEM) (AOAC, 2005)

Sl. No.	Proximate composition	Diet 1 (FD)	Diet 2 (CD)	Diet 3 (D1+10g1000g^{1})	*L. camara* flower petal
1	Crude protein	32.2 ± 0.04	32.02 ± 0.039	33.82 ± 0.00	1.62 ± 0.31
2	Crude fibre	1.14 ± 0.02	4.1 ± 0.099	21.86 ± 0.00	20.73 ± 6.35
3	Total ash	17.28 ± 0.06	Nil	18.18 ± 0.005	0.84 ± 0.04
4	Moisture	11.18 ± 0.04	10.06 ± 0.036	68.87 ± 0.016	51.72 ± 0.23
5	Dry matter	88.51 ± 0.15	Nil	88.51 ± 0.15	Nil
6	Total fat	5.02 ± 0.01	4.16 ± 0.08	5.02 ± 0.01	Nil
7	Nitrogen Free Extract	50.03 ± 0.02	Nil	50.03 ± 0.02	Nil
8	Ether extract	9.23 ± 0.17	Nil	9.23 ± 0.17	Nil
9	β-carotene	Nil	Nil	20 ± 0.01	20 ± 0.01
10	Ascorbic acid	Nil	Nil	25.34 ± 0.76	25.34 ± 0.76
11	Zinc content	Nil	Nil	1.12 ± 0.32	1.12 ± 0.32

Note: FD- Formulated Diet; CD- Commercial Diet

Growth performance

Weekly growth of P. sphenops fed with diets D1, D2 and D3:

The juveniles of *P. sphenops* were fed with the three diets D1, D2 and D3 for 60 days and their weekly growth performance is presented in Fig 1. A continuous increase in growth was observed in fish fed with D3 from 0 to 60th day with a sudden boost in body weight and growth after 15th day and later after 30th day which continued till 60th day. Fish fed with D2 induced continuous growth till 30 days and later growth was slow with a steady increase recorded till 60th day. Fish fed with D1 showed a constant growth but significantly less than those fed with D3 from 0 to 60th day of experimental period. Thus significant growth was recorded in fish fed with D3 incorporated with natural pigment source when compared to those fed with D1 and commercial Diet (D2)

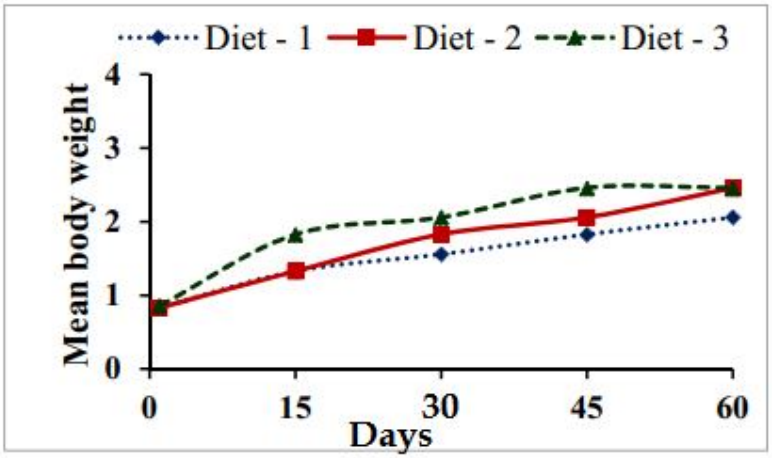


Figure 1: Weekly growth performance of *P. sphenops* fed with different diets *viz*., D1, D2 and D3.

The growth performance of the *P. sphenops* is represented according to the survivability, BWG, BLG, SGR, FCR, and CF for 60 days of the experiment (Fig 2A to 2E). The survival percentage showed 100% in all the control as well as in experimental groups. The fishes under experimental group II fed with D3 showed significantly higher final weight (3.69 ± 0.022), body weight gain (2.89 ± 0.011; one - way ANOVA; $df = 2$; $F = 734.6$; $p < 0.0001$; $N = 15$), body length gain (1.91 ± 0.014; one -way ANOVA; $df = 2$;

F = 1866; *p* < 0.0001; *N* = 15), SGR (1.084 ± 0.007; one -way ANOVA; *df* = 2; *F* = 128.4; *p* < 0.0001; *N* = 15) and CF (1.183 ± 0.006; one -way ANOVA; *df* = 2; *F* = 1089; *p* < 0.001; *N* = 15) when compared with experimental group I fishes fed with D2 followed by control group fed with D1 for a period of 60days. The values of the FCR showed a reduction in fishes of experimental group II fed with D3 (0.255 ± 0.0001) (one -way ANOVA; *df* = 2; *F* = 1089; *P*<0.0001; *N* = 15) when compared with experimental group I fed with D2 (0.264 ± 0.0005) and control group fed with D1 (0.269 ± 0.0002). This indicated that the diet 3 incorporated with 10g / 1000g of natural pigment *L. camara* showed a better feed utilization compared to Diet 1 or Diet 2. The flower of *L. camara* in Diet 3 consists of ascorbic acid (25.34 ± 0.76) and NFE (50.03 ± 0.02). Significant difference was observed in body length gain and condition factor in fish groups fed D1, D2 and D3.

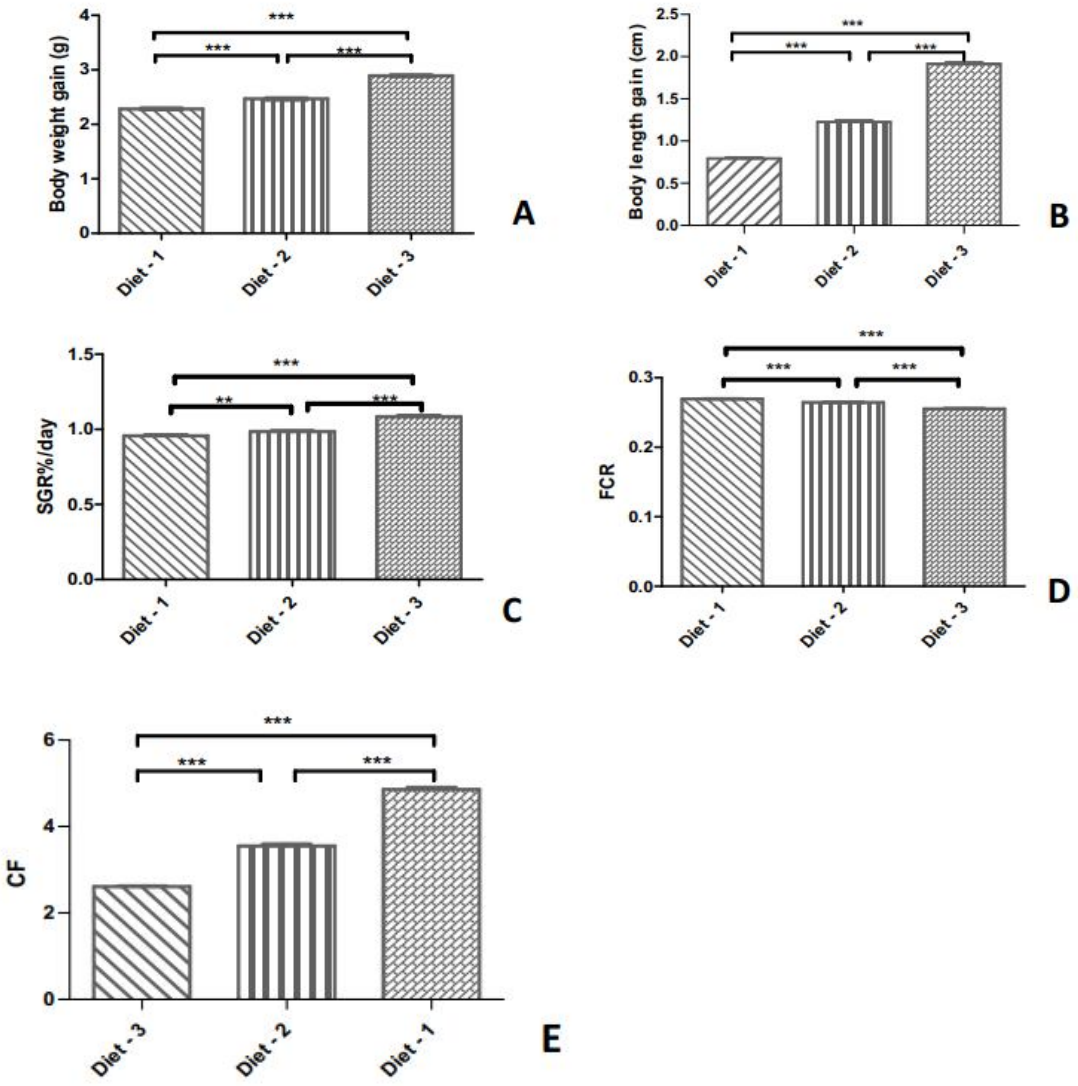


Figure 2A to 2E: Effect on growth parameters A) Body weight gain (BWG); B) Body length gain (BLG); C) Specific growth rate (SGR); D) Feed conversion ratio (FCR); E) Condition factor (CF) of *P. Sphenops* with three different diets viz., D1, D2 & D3 Data are shown as mean ± SE. Significance was calculated by one-way ANOVA and post-hoc test was done in accordance with Tukey's comparison using GraphPad Prism 5 and significance was represented as ***$p < 0.0001$; **$p < 0.001$; *$p < 0.05$.

The results of morphological indices, VSI and HSI in fish fed with D1, D2 and D3 for a period of 60 days are represented in Fig 3A & 3B. VSI and HSI of the fish fed with D3 showed a significantly

higher value (0.334 ± 0.001; one - way ANOVA; *df* = 2; *F* = 321.3; *p* < 0.0001; *N* = 15) and (0.086 ± 0.0003; one -way ANOVA; *df* = 2; *F* = 895.2; *p* < 0.0001; *N* = 15) respectively, when compared to those fed with D2 and D1.

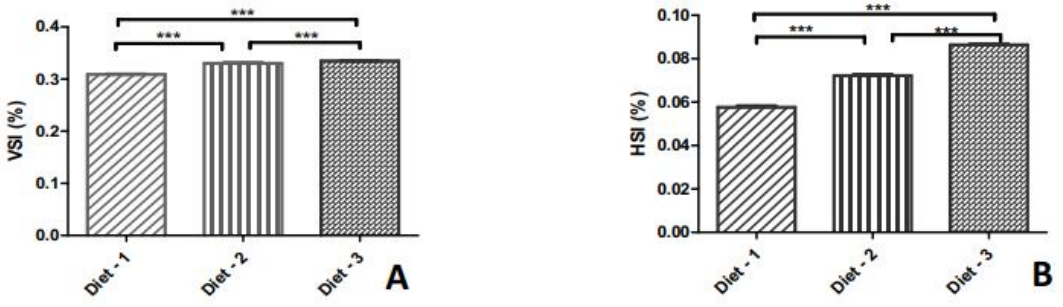
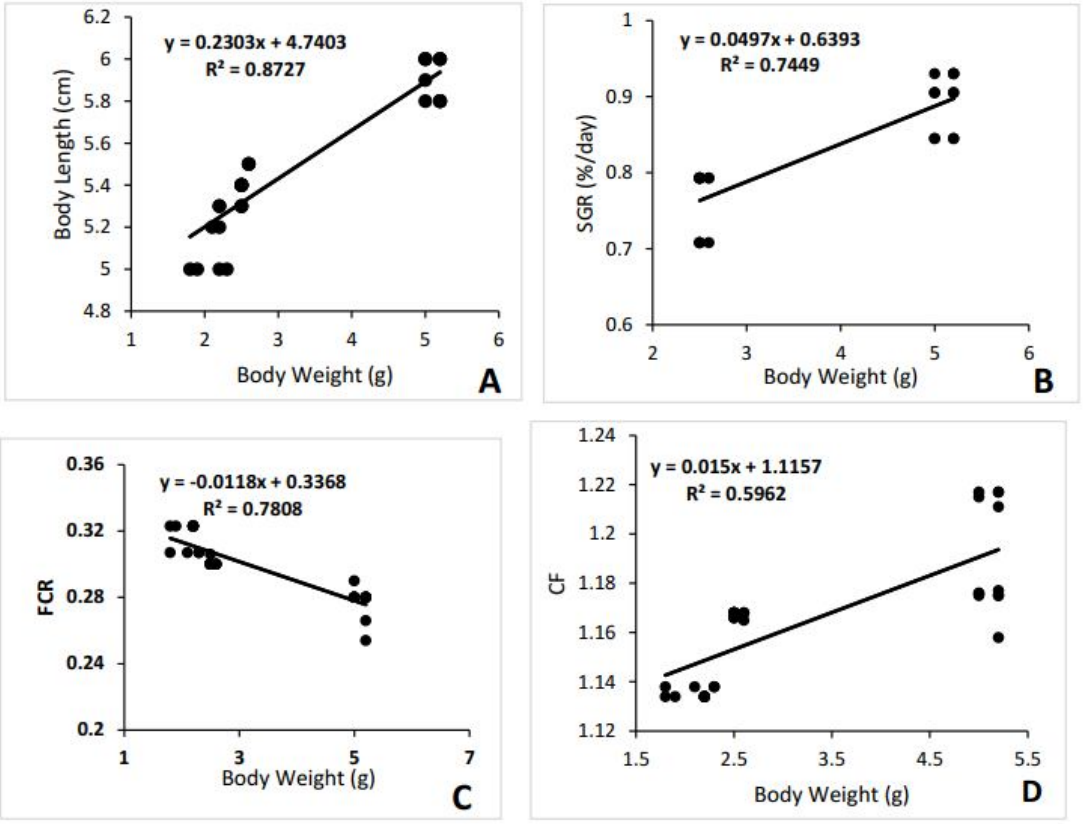


Figure 3A and 3B: Effect on morphological indices H) Visceral somatic index (VSI); I) Hepato-somatic index (HSI) of *P. sphenops* fed with three different diets viz., D1, D2 & D3. Data are shown as mean ± SE. Significance was calculated by one-way ANOVA and post-hoc test was done in accordance with Tukey's comparison using GraphPad Prism 5 and significance was represented as ***$p < 0.0001$; **$p < 0.001$; *$p < 0.05$.

The results of correlation test between the body weight and other growth parameter of the *P. sphenops* in the experiment is depicted in the Fig 4A to 4F. Body weight showed positive correlation with body length showing a slope of $R^2 = 0.8727$ (Fig 4A); SGR a slope of $R^2 = 0.7449$ (Fig 4B); CF a slope of $R^2 = 0.5969$ (Fig 4D); HSI a slope of $R^2 = 0.8183$ (Fig 4E); and VSI a slope of $R^2 = 0.8906$ (Fig 4F). FCR a slope of $R^2 = - 0.7808$ (Fig 4C); Body weight showed a negative correlation with feed conversion ratio. This further confirmed better utilization of diet D3 by the fish.



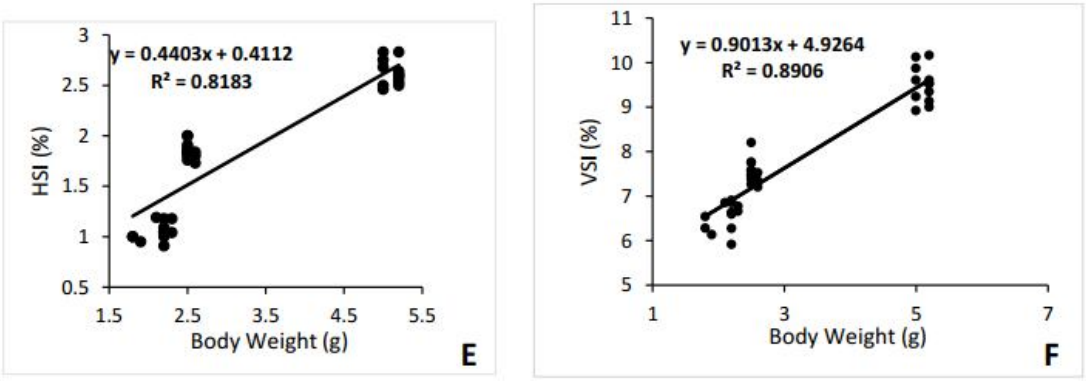


Figure 4A to 4F: Correlation between body weight with total length (A), BL (B), SGR (C), FCR (D), CF (E), HSI and with (F), VSI of *P. sphenops* fed with three diets D1, D2 and D3, degree of relatedness is represented as linear regression line. Two-tailed Pearson correlation was done using Graphpad Prism 5.

Total carotenoid concentration (β carotene) in fish scale and skin

Total carotenoid concentration (TCC) in scale and skin of fish, *P. sphenops* analysed after 60 days of feeding with diets D1, D2 and D3 is represented in Fig 5. The results showed statistically significant differences in TCC at level $p < 0.0001$ between all the three groups. Fishes fed with D3 (*Lantana* flower petal incorporated diet) showed significantly high levels of TCC levels (0.179 µg/g wet weights), when compared with those fed with D2 (0.123 µg/g wet weights) followed by control group fed with D1 (0.006 µg/g wet weights) ($P< 0.0001$)(one -way ANOVA, $df = 2$; $F = 1173$; $p < 0.0001$; $N = 15$) (Fig 5).

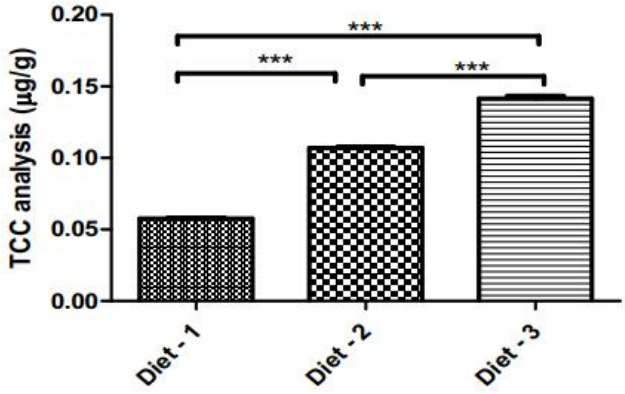


Figure 5: Spectrophotometer analysis (ug/g wet weight) of total body carotenoid concentration (TCC) showing significant difference ($p < 0.05$) in skin and muscle of *P. sphenops* between those fed with D1, D2 and D3 are denoted by ***$p < 0.0001$; **$p < 0.001$; * $p < 0.01$.

Correlation between total body carotenoid concentration (TCC) of Diet 1 (µg/g) with Diet 2 (µg/g) and Diet 3 (µg/g) of *P. sphenops,* degree of relatedness is represented as linear regression line. Two-tailed Pearson correlation was done using Graphpad Prism 5 (Fig. 6A & 6B). TCC of Diet 1 was positively related with TCC of Diet 2 showing a slope of $R^2 = 0.9999$ (Fig 6A); and with TCC of Diet - 3 a slope of $R^2 = 0.9504$ (Fig 6B).

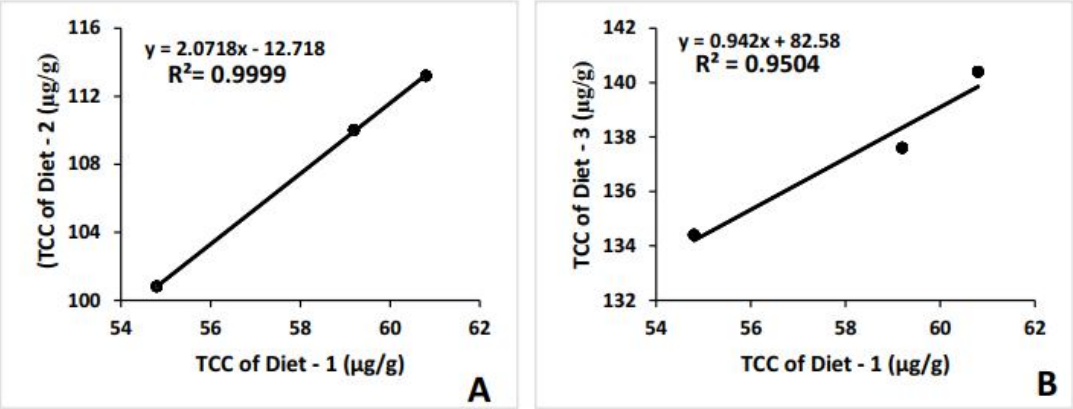


Figure 6A and 6B: Correlation between total body carotenoid concentration (TCC) in skin and muscle of *P. sphenops* fed with Diet 1 with Diet 2 (µg/g) (6A) and Diet 1 with Diet 3 (µg/g) (6B) and degree of relatedness is represented as linear regression line. Two-tailed Pearson correlation was done using Graph Pad Prism 5.

A comprehensive data showed statistical difference at level $p < 0.0001$ on growth and body colouration of fish fed with D1, D2 and D3 for 60 days as represented in fig 7. Fish fed with D3 showed significant increase in growth and TCC level which was followed by D2 and D1 (with D1 (two - way ANOVA, $df = 2$; $F = 582.70$; $p < 0.0001$; N= 15), with D2 (two – way ANOVA, $df = 1$; $F = 719.71$; $p < 0.0001$; $N = 15$) and with D3 (two - way ANOVA, $df = 2$; $F = 752.31$, $p < 0.0001$; $N = 15$) (Fig. 7).

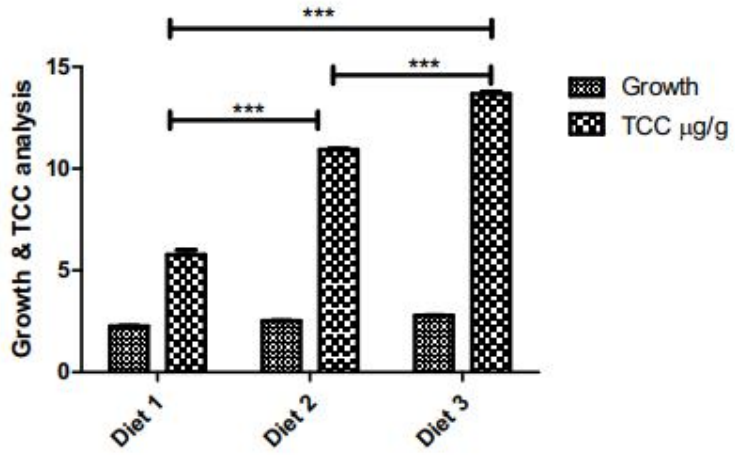


Figure 7: Analysis of growth performance and TCC in skin and muscle of *P. sphenops* fed with three different diets viz., D1, D2 & D3. Data are shown as mean ± SE. Significance was calculated by two-way ANOVA and post-hoc test was done in accordance with Tukey's comparison using Graph Pad Prism 5 and significance was represented as ***$p < 0.0001$.

DISCUSSION

In the Aquaculture sector ornamental fish culture is a profitable and beneficial business. Guppy, molly, swordtail and platy belonging to genus *Poecilia* and *Xiphophorous,* goldfish (*Carassius auratus*) have been successfully utilized as marketable fish. Although these ornamental fish are well-known worldwide but information regarding nutritional requirements for healthy survivability, good growth

and pigmentation of live bearers is inadequate or it is based on the information from species of food fish used in aquaculture has prompted us to study. Thus nutrition being an important factor in the reproductive performance in most fish high protein diet was recommended for increase in relative fecundity as well as fry production of *X. helleri* by Chong et al, (2004) The cost and carcinogenic effect of synthetic pigment and antioxidant has gained interest of scientist towards natural compounds and antioxidants which are green plant based such as *Chlorella vulgaris* which has been most effective to improve the skin colour intensity in ornamental fish (Gouveia et al, 2003a). In the present investigation survivability, growth performance and colouration was studied in red eyed orange molly, *P. sphenops* fed formulated diet (with and without flower petal incorporated) sources D1, D2 and D3. 100% survival rate with improvement in the health, physiology of fish, growth and colouration was observed in the fish groups after 60 days of feeding. Likewise, 100% survival was recorded in Koi carp fed with diets incorporated with 3, 5 and 7% carrot meal but 90% in 5 and 7% and 80% in control groups by Jain, (2015). Survival rate was 90-95% in rosy barb fed with rose petal meal incorporated diet whereas 85% in the control group was observed by Pailan et al, (2012). Jagdeesh et al, (2014) reported 67.85% to 82.14% of survival rate in *Etroplus maculates* fed with marigold oleoresin supplemented diets and control diet respectively. Arulvasu et al, (2013) obtained a survival rate of 76.6% in control group of *Xiphophorus helleri* when fed with diet without rose petal extract and 90%, 93.3% and 96.6% with rose petal extract (200mg/kg, 1000mg/kg and 2000mg/kg) respectively. Kumar et al, (2017) also reported 86.66 to 100% survivability of *Carassius auratus* when fed with three natural plant pigment sources (African tulip tree flower, red paprika, pomegranate peel) incorporated diet. Therefore, formulated diet with *L. camara* flower petal were found to be better than commercial or any other natural diet since 100% survivability of fishes was observed in fish fed with D1, D2 and D3. Carotenoids are synthesized by plants and phytoplankton (microalgae). Research has proved that carotenoid complemented feed is good antioxidant potential as in *Cyprinus carpio*(Patil and Thakare, 2017)and carotenoid-protein complexes are the main cause of skin and muscle pigmentation in fish (Sinha and Asimi, 2007); therefore, an optimum level of carotenoid should be added to their diet.

In the present study a marked improvement was observed in growth performance in terms of body length and weight in the fish, *P. sphenops* when fed with formulated diet incorporated with pigment source, powdered flower petal *L. camara* (D3, $P<0.05$). However, commercial diet D2 showed better results than control diet in terms of fish growth. Several authors have also studied the effects of different nutritional diets on the growth performance and colouration of different fish species. Earlier Amar et al, (2001) and Watanabe and Vassallo-Agius, (2003) had reported that carotenoids play a role in the intermediary metabolism of fish for improving utilization of nutrients resulting in improvement in its growth. This pigment source has been recognized as a protein source for animal feed due to its high protein content, essential vitamins, minerals and macro and micronutrients. In the present study a rapid increase in growth of red eyed molly fish was observed from 15th day onwards and a second boost after 30th day in those fed with D3 when compared to those fed with D2 and D1 which showed a constant growth till the end of the experiment. In similar lines with the present study, James and Sampath, (2004) reported increase in mean body length and weight in fish when fed with Artemia or mixed diet when compared to other individual diets and pelleted diet showed highest feeding rate but poor conversion rate

These results were in agreement with reports regarding enhancement in growth and colouration in *Puntius sophore* fed with 10% *Spirulina platensis* by Bagre et al, (2012) and for SGR in Koi carp fed with 180mg kg^{-1} of marigold meal as carotenoid supplement by Swian et al, (2014) Wassef et al, (2010) reported negative effect of dietary carotenoid on growth and feed efficiency on gilthead seabream, *Sparus aurata.* Such an effect might be due to difference in fish species, fish size, and feeding behaviour and the enhancement in growth performance of European seabass fed with carotenoid supplemented diet may be due to antioxidant property of carotenoid (Pan et al, 2010). Teimouri et al, (2013) and Zhu et al, (2016b) reported beneficial effects of macro and microalgae (in a range of 0.05-20%) in the diets of fish and shrimp species in terms of nutritional performance, enhancement of growth, health and coloration.

In the present investigationflower petals of *L. camara* incorporated D3 fed fish showed significant improvement in net weight gain (WG) and SGR with reduction in FCR levels indicating best utilization of the diet D3 when compared to non-*Lantana* based diet (D1 & D2). This can be attributed to higher fibre content in Diet 1 and Diet 2which reduced the digestibility of the diet whereas the flower of *L. camara* in Diet 3 containing NFE (50.03 ± 0.02) and ascorbic acid (25.34 ± 0.76) might have enhanced digestion efficiency of the formulated diet thereby increase in fish body weight and growth was recorded. The results were in agreement with Sivarama et al, (2004)for enhanced WG, SGR and positive FCR in greasy grouper juveniles, *Myristica fragrans* fed with diets incorporated with tulsi*, Ocimum sanctum* and *Ashwagandha;* Pandey et al, (2014)for increased SGR levels in *L. rohita* fed with prickly chaff-flower; Sahu et al, (2007)for positive SGR and FCR, in *L. rohita* fed with mango kernel; Rani et al, (2014)in *C. auratus* fingerlings fed with plant incorporated with animal source and Liang et al, (2012)for weight gain and SGR with significantly reduced FCR in red white koi carp fed with 150, 200 and 250 mg of astacin/kg of diet. Nguyen et al, (2014) reported increased growth and feed utilization in koi carp when fed with supplementation of astaxanthin @ 80 mg/kg. In contrast to the above results few earlier reports (Kop et al, 2010; Seyedi et al, 2013) had suggested the role of carotenoids only on colour enhancement without having much effect on the fish growth.

According to the studies conducted by Mizanur et al, (2013) on juvenile Korean rockfish, HSI and condition factor (CF) are involved with the growth, physical conditions, energy reserves and capability of fish to tolerate environmental stress (temperature). Fish have small liver when they are kept in poor and stressful environmental conditions i.e. energy levels reserved in the liver are low. Such morphological indices showed significant effect by feeding frequency and temperature. In the present studies HSI and VSI of molly fish showed an increasing trend when fed with *Lantana* based diet to commercial diet and non-*Lantana* based diet (D3>D2>D1). Ebeneezar et al, (2019)observed significant effects in HSI, VSI and crude protein content in silver pompano fish fed with low dietary lysine levels but high dietary lysine had no effect on the growth and feed utilization compared to the optimum dietary lysine levels in fish. Such morphological indices showed significant effect by feeding frequency and temperature. A significant increase in condition factor, HSI and VSI of Korean rockfish was noted when fed with two meals a day in 15°C and at 1 meal a day in 19°C (Mizanur and Bai, 2014). According to Oh et al, (2008) HSI is a good compensatory growth index in Juvenile black rockfish and this was in agreement with Cho, (2012) in olive flounder.

In the present study, maximum TCC levels were noted in fishes fed with D3 (*Lantana* flower petal) when compared with those fed commercial diet (D2) followed by the control group. Thus the colour intensity and prominence of dark orange colour increased throughout the skin of *P.sphenops* fedwith diets enriched with natural β-carotene sources viz D3 compared to commercial diet D2 and D1. Many authors reported that blue green algae and spirulina when used as feed influenced colour development of fish viz., in *Cyprinus carpio* and *Carassius auratus*, (Gouveia et al, 2003) and in showa koi (Sun et al, 2012). Few plant origin feed additives have been successfully used by researchers in ornamental fishes as colour enhancers. Current findings on enhancement of colour intensity of the skin of *P.sphenops* feddiets enriched with natural β-carotene sources are well supported by Hancz et al, (2003) in gold fish and koi carp fed paprika. Kop and Durmaz, (2008) also observed significant differences in colouration between individuals of cichlid (*Cichlasoma severum sp*. Heckel, 1840) fed by red pepper and carrot diets and those fed with unpigmented feeds. High TCC and astaxanthin content in the skin and muscle of rainbow trout, *Oncorhynchus mykiss* fed both marine bacteria and synthetic astaxanthin was reported by Kurnia et al, (2015).These findings also confirm the results of present study where prominent colouration was observed in fish fed with *L. camara* supplemented diet but it was not coefficient to commercial diet. Correlation regression analysis of literature data revealed that the relation between dietary 1 and diet 3 is significant as compared with diet 1 and diet 2. The skin colouration of aquarium fish depends on nutritional characteristics like lipid, various dietary protein, vitamins, feed additives or ingredients (Sefc et al, 2014; Karadal et al, 2017). According to Ha et al, (1993) carotenoid source for pigment deposition is species specific. In contrast to the above findings Chatzifotis et al, (2005) reported low carotenoid content of the skin in red porgy fed with β-carotene supplemented diet. However, very limited information is available on the rearing

and early nutritional requirements of ornamental fish for bright colouration and healthy growth performance.

CONCLUSION

From the present study it can be concluded that fish fed with formulated diet incorporated with dry flower petals of *L. camara* showed positive results with respect to growth performance and coloration of *P. sphenops* (TCC - 10g / 100g) and also for hepatosomatic index, condition factor and visceral somatic index. Further it can be inferred that appropriate colour of ornamental fish can be maintained by addition of suitable pigment which should form part of fish diet on a prolonged basis and with increase in feeding regimes. Thus it is suggested that for better growth performance, survivability and enhancement of colour, incorporation of aneasily available, compatible and affordable natural carotenoid source, flower petals of *L. camara*, in formulated diet will prove cost-effective for ornamental fish farmers as compared to commercial diet.

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Conflict of interest
The authors have no financial or conflict of interest to declare.

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