The Pharma Innovation Journal 2022; SP-11(11): 1943-1946

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 1943-1946

www.thepharmajournal.com
Received: 28-09-2022
Accepted: 30-08-2022

Gamit SA
Department of Aquatic Biology, VNSGU, Surat, Gujarat, India

Kapila Manoj
Department of Aquatic Biology, VNSGU, Surat, Gujarat, India

Bengani R
Department of Aquatic Biology, VNSGU, Surat, Gujarat, India

Gamit DN
Department of Aquatic Biology, VNSGU, Surat, Gujarat, India

Lende SR
Department of Aquatic Biology, VNSGU, Surat, Gujarat, India

Vasava RJ
Centre of Excellence in Aquaculture, Kamdhenu University, Ukai, Tapi, Gujarat, India

Solanki VK
Centre of Excellence in Aquaculture, Kamdhenu University, Ukai, Tapi, Gujarat, India

Vadher DR
Centre of Excellence in Aquaculture, Kamdhenu University, Ukai, Tapi, Gujarat, India

Mevada JN
Centre of Excellence in Aquaculture, Kamdhenu University, Ukai, Tapi, Gujarat, India

Corresponding Author:
Gamit SA
Department of Aquatic Biology, VNSGU, Surat, Gujarat, India

Comparision of growth and colour enhancement in *Poecilia sphenops* using different carotenoid supplements

Gamit SA, Kapila Manoj, Bengani R, Gamit DN, Lende SR, Vasava RJ, Solanki VK, Vadher DR and Mevada JN

Abstract
Colour is the most important indicators of quality in ornamental fish, making the exploration to find new sources of pigments in ornamental fish is growing. The experiment was aimed to study the impact of natural β- carotene vegetable sources *viz*., carrot (*Daucus carota*) and three flowers (rose, marigold and palash) for inducing colouration in an ornamental fish molly. The feeding trial was conducted for 60 days in plastic tanks. The study was planned using Completely Randomized Design with five treatments and its triplicates. Each tank was stocked with 5 fish of uniform size. One control and four treatments setup were organized for colour enhancement test. The result of the experiment showed difference in ABWG (Absolute weight gain) and SGR (Specific growth rate) of the fish. However, carrot fed fishes showed higher absolute weight gain (5.4567±0.89 gm) than the other treatment groups. 100% survival of fish was recorded in control diet (T1) and carrot diet (T5), while 86% in palash (T2), 93% in rose (T3) and 80% in marigold (T4). Water quality during the research were in optimal condition. Total body length of molly (3.5052 cm) was higher in rose petal meal diet as compared to other treatment and control. The body colouration and total carotenoid concentration of muscle tissue (48.4 µg/g) was higher in fish fed with rose diet. The study showed that incorporation of rose in diet was found to provide better growth performance and colouration in molly. The study concluded that the easily available natural sources incorporated feed can be used as a colour enhancer of ornamental fish which can be prepared at a lower cost that can be boon for ornamental fish farmers.

Keywords: Natural carotenoid, ornamental fish, carotenoid supplements

Introduction
One of the most popular hobbies in the world is ornamental fish keeping. "Ornamental Aquaculture, India's rapidly rising 'Aquatic Rainbow Sector,' requires real innovations and the employment of new technology in order to move to the next level and compete in the worldwide market" In the aquarium fish market, attractive colouration of ornamental fishes is an essential quality factor. Under rigorous cultivation conditions, fish that are colourful in nature frequently display faded pigmentation. India ranks 31st in ornamental fish production and contributes just 1.6 million USD to the worldwide ornamental fish trade. (Raja *et al.*, 2019) [10].

Aquarium fish keeping has a rich history that dates back many centuries. One of the most visually appealing aspects of ornamental fishes is the beauty of their colours, which determines their economic worth, which is further determined by the intensity and pattern of pigmentation. Colour is one of the most important criteria influencing the price of ornamental fish on the global market. (Saxena., 1994) [12]. Pigmentation is one of the important quality attributes of the aquatic animal for consumer acceptability.

Carotenoids are a kind of naturally occurring lipid-soluble organic pigment that is responsible for the red, orange and yellow colouration of aquatic species' skin, flesh, shell, and exoskeleton. Carotenoids are in high demand in the international market due to their diverse uses in the pharmaceutical business. Plant based natural carotenoids are mainly derived from flowers, fruits, vegetables, algae, yeast, etc. (Tsushima and Matsuno, 1998; Chapman, 2000) [13]. Which are not only inexpensive, but also a potential source of mixed carotenoids.

The drab silvery colour of this variant of the common or short-finned molly is accented by distributed black spot on its skin and a brilliant yellow fringe on the tips of its rounded dorsal and caudal fins, individuals of this species have a broad range of colours diversity, mostly consisting of silver, black and yellow-orange.

Materials and Methods

The experiment was conducted for a period of 60 days to study the comparative growth performance and survival of molly (*Poecilia sphenops*) fry reared in the freshwater tank. The material and methods used for the current research endeavor are explained below.

Experimental Fish

The molly (*Poecilia sphenops*) fry was used as experimental fishes. The active and healthy fry of molly for the experiment were collected from the Centre of excellence (COE) in aquaculture, research Centre Ukai. During the acclimatization period the 75 Nos uniformly sized fry fishes were stocked in 30 litres plastic aquarium tanks of water with continuous aeration prior to start of the experiment. The experiment was carried out for 60 days.

Experimental Feed Preparation

The experimental feed was prepared with basic ingredients such as one vegetable source of β- carotene i.e., carrot (*Daucus carota)*. Carrot was procured from market, washed, grated and dried under shade and grinded to prepare the powdered meal and rose flowers were procured from market, marigold was obtained from temple. Petals were separated from pods, dried under shade and grounded to prepare the meal. All the feed ingredients were powdered, mixed thoroughly and pelletised. Palash was collected and dried in darkroom to avoid denaturing of carotenoids.

The natural sources dried for 48 hours and then the contents were powdered and sieved in particle size of 0.1 to 0.2 mm and immediately stored in an airtight zipper bag. The experimental diets were prepared using carrot, marigold and palash. Five diets with same levels of dietary protein (approximately 28%) for Poecilia sphenops were formulated: commercial feed (control) and 3% supplement ingredients to prepare formulated diet for the experiment. All ingredients were ground thoroughly mixed and dough was prepared by adding required amount of water and 3% cod liver oil were added and mixed thoroughly.

The dough was taken into hand operated pelletiser to make 2.0 mm pellets for the preparation of pellet diet the pellets were sun dried, grinded in grinding machine and kept in plastic bottles at room temperature until further use diets were designated as T1 to T5. Five treatment diets namely T1, T2, T3, T4 and T5 comprising 28% protein content were formulated using the Latin square method (Hardy, 1980).

Table 1: Formation of Experimental Diets, (Ingredients in gm/100 gm)

Ingredients	T1	T2	T3	T4	T5
Fish meal	48	48	48	48	48
Wheat bran	1	1	1	1	1
Rice flour	17	14	14	14	14
Wheat flour	25	25	25	25	25
Carboxymethylcellulose	2	2	2	2	2
Sunflower oil	3	3	3	3	3
Fish oil	3	3	3	3	3
Vit mix	1	1	1	1	1
Palash powder	0	3	0	0	0
Rose powder	0	0	3	0	0
Marigold powder	0	0	0	3	0
Carrot powder	0	0	0	0	3

Feeding Experimental diet

Fish were fed at the rate of 10% of their body weight initially and after 10 days it was fed *ad libitum* till the end of experiment. The feed was broadcasted over the surface of water. The total daily feed ratio was divided into four equal portions delivered at 9:00 AM, 12:00 PM, 3:00 PM and 6:00 PM. After each sampling, the quantity of feed given was re-adjusted based on the increased weight of fish initially and after 10 days it was fed *ad libitum* till the end of experiment. The daily ration was divided into four equal parts and was given at 09:00 AM, 12:00 PM, 3:00 PM and 6:00 PM.

Pigment Extraction in fish tissue

Total carotenoid concentration (TCC) in the fish muscle tissue was analysed immediately after the completion of experiment following the pigment extraction method as described in Olson. (1979) [7].

The method used for pigment extraction from the *Poecilia sphenops* tissue was as described. One gram of entire molly body tissue (without head and alimentary canal) was taken in a 10 ml screw capped clear glass vials and 2.5 g of anhydrous sodium sulphate was added.

The sample was 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 ºC. when the chloroform formed a clear 1-2 cm layer above the caked residue, the optical density was read at (380, 450, 475 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 was used for the calculation.

Formula used for calculation

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

$$\text{Total carotenoid content} = \frac{\text{Absorption at maximum wavelength}}{0.25 \times \text{sample weight}}$$

Where, Dilution Factor= 10
Extinction coefficient = 0.25

Statistical Analysis

The experimental results were all data on growth, performance, survival and carotenoid content in skin of the fish are expressed in the form of mean and standard error (Mean±S.E.). Data from each treatment diet for each sampling period were analysed by non-parametric Kruskal Wallis test.

Results

Colour enhancing agent as feed additive from plant origin have been successfully incorporated in ornamental fishes.

Table 2: Physio-chemical parameters of water recorded in experimental tanks

Parameter	Minimum	Maximum
Temperature (°C)	22.4	26.6
Dissolved oxygen (ppm)	6.8	7
pH	7.7	8.5
Alkalinity (ppm)	135	150

Specific growth rate (SGR)

The specific growth rate (SGR) of *Poecilia sphenops* fry in different treatments is given in Table-3 Highest SGR was

found in T3 diet treatment (0.58±0.33) followed by Tl, T2, T4 and T5 and the lowest SGR (0.26±0.15) was observed in T2.

Table 3: SGR of *Poecilia sphenops* advance fry recorded in different treatments during culture period (Mean±SE)

Treatment	SGR-15 days	SGR-30 days	SGR-45 days	SGR-60 days
T1	2.0552±0.15	0.54418±0.31	0.37864±0.21	0.32910±0.19
T2	2.1424±0.05	0.06179±0.03	0.12062±0.06	0.26516±0.15
T3	2.1287±0.12	0.35227±0.20	0.50183±0.28	0.58686±0.33
T4	2.1353±0.14	0.33822±0.19	0.23895±0.13	0.34166±0.19
T5	1.7947±0.11	0.18103±0.10	0.35310±0.20	0.41436±0.23

Average body weight gain and Survival rate

The higher fish growth in terms of Average body weight gain was observed in T-5 (5.4567±0.89 gm) and lowest weight (gm) was observed in T4 treatment. Increased trend of body weight was observed in T5 group after fifteen days and which was continued throughout the experimental period Likewise fishes fed with rose petal supplemented diet showed marginally increased values in net weight gain (5.3567±1.25 gm).

The survival rate of molly fish at the end of research conducted for 60 days, the highest result was achieved by treatment 5 (3% carrot meal addition on commercial feed) and control that was 100% and decreased by the same value on treatment 3 (93.3333±6.66) and treatment 2 (3% palash meal addition to commercial feed) that was 86.6 6, while the lowest survival rate in treatment 4 (3% marigold addition to commercial feed) was 80.0000±11.54.

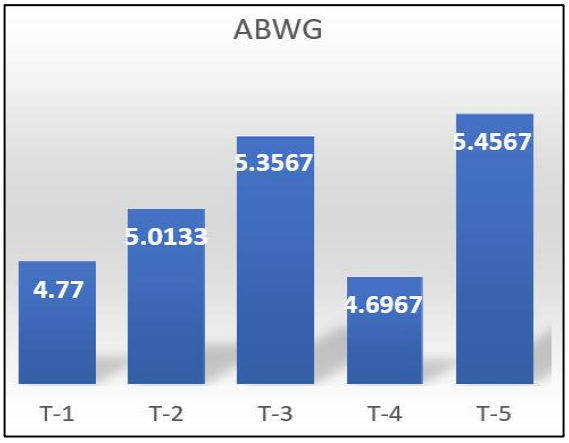

Fig 1: Average body weight gain of *Poecilia sphenops*

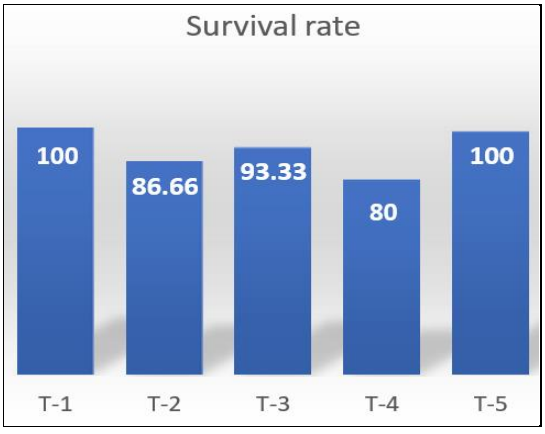

Fig 2: Survival rate of *Poecilia sphenops*

Total body length (cm) and Total Carotenoid in Fish Tissue

The average length (cm) of *Poecilia sphenops* observed at particular intervals is shown in Table: 4. The final average length recorded were 3.5333 cm, 3.4498 cm, 3.5052 cm, and 3.3154 cm and 2.8227 cm in treatment Tl, T2, T3, T4 and T5 respectively. The highest final length was observed in T2 (8.57±0.09 cm) and lowest in T5 (2.8227 cm) treatments. The Tl treatment showed higher length gain as compared to all the other treatments. During the end of experiment.

Carotenoid analysis of fish muscle and skin was done by following method of Olson (1979) [7] at end of the experiment. The results clearly showed the maximum carotenoid content was present in the fish fed with the rose petal feed. For instance, the highest total carotenoid content in the T3 group was found to be 48.4 µg/g wet weight and for the control group (T1) it was found to be 26.4 µg/g wet weight which was lower.

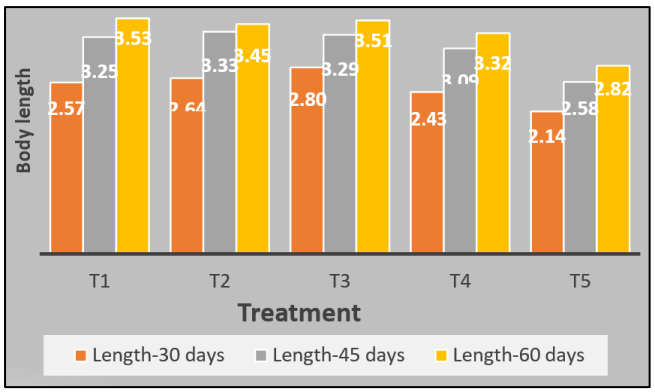

Fig 3: Total body length of *Poecilia sphenops*

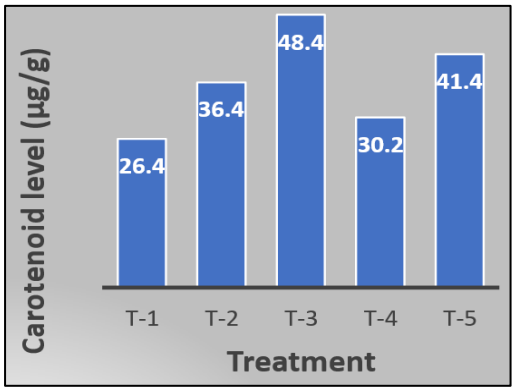

Fig 4: Total Carotenoid in Fish Tissue of *Poecilia sphenops*

Statistically strong significant difference was recorded between different treatment group with respect to specific growth rate and total body length (p<0.05).

Table 4: Kruskal Wallis Test

Ranks			
	Days	N	Mean Rank
SGR	30 days	5	13.00
	45 days	5	5.00
	60 days	5	6.00
	Total	15	
Total body length	30 days	5	3.40
	45 days	5	8.60
	60 days	5	12.00
	Total	15	

Test Statistics

	SGR	Total body length
Chi-Square	9.500	9.380
df	2	2
Asymp. Sig.	.009	.009

Discussion

Ornamental fishes are known for their vibrant, dazzling, and stunning colouration and form. The colouration is caused by skin pigments, while the backdrop might be caused by underlying tissue and body fluid. To keep the colouration of these fishes. A high-quality dietary supplement is essential. Not only should the diet include enough quantities of oil, protein, energy, vitamins, and minerals, but it should also provide a consistent supply of carotenoids in the proper proportion. This is best accomplished by using high - quality formulated feeds and supplements containing a well-balanced blend of natural colour- enhancing substances.

In the current study, it is obvious that carotenoid do have a role in fish growth, since rose petal meal resulted in beneficial growth impacts in terms of enhanced weight gain. Similar results were observed by Joseph *et al.* (2011) [4] in orange sword tail (*X. helleri*), where 1 percent of *Rosa indica* out of three levels (1, 3 and 5%) led in the greatest daily weight growth. Pailan *et al.* (2012 a.b) [10] found that Dwarf gourami (*Colisa lalia*) and rosy barb (*Puntius conchonius)* had the highest absolute growth rate (g), percent weight increase, and lowest FCR (1.66) when fed 6 and 4 percent rose petal meal, respectively. Ramamoorthy *et al.* (2010) [11] discovered that natural pigment sources such as carrot, *Daucus carota*; marigold meal, *Tagetes erecta*; China rose petal, *Hibiscus rosa sinensis*; and rose petal, *Rosa chinensis* enhanced the growth of marine ornamental fish, *Amphiprion ocellaris* when compared to non-pigmented diet. The study by Hata and Hata, 1972 [3]; Matsuno *et al.*, 1981 [5]; Ohkubo *et al.*, 1999 [6]; Paripatanont *et al.*, 1999 [9] and Ezhil *et al.*, 2008 [2] also revealed the enhancement of colouration of fish due to natural carotenoid supplements in the feed.

Conclusion

It was concluded that the dried carrot, rose petal meal, palash and marigold powder could be included in the diet of Molly (*Poecilia sphenops*.) as colour enhancer as well as growth promoter. synthetic carotenoids are pricey, cheap and readily accessible natural carotenoid sources can be incorporated into the diet to enhance better colouration in *Poecilia sphenops*. This will help ornamental fish sellers to get superior cost in trading of this fish.

Acknowledgments

The authors are extremely grateful to Dr. Kapila Manoj for providing the facilities to conduct the research work. Thanks are due to Dr. S. R. Lende Assistant Professor and in charge of Centre of Excellence (COE) in Aquaculture, Ukai, Kamdhenu university for introducing me to this work and that had been so kind in providing a place and some materials for this research.

References

1 Aquatic Animal Health: Program and Abstracts. University of California, School of Veterinary Medicine, Davis, CA; p. 94 (Abstr.).
2 Chapman, F.A. 2000. Ornamental fish culture, freshwater. Page 602-610 in Encyclopedia of Aquacult. Stickney, R.R. Ed., John Wiley & Sons, New York.
3 Ezhil J, Jeyanthi C, Narayanan M. Marigold as a Carotenoid Source on Pigment and Growth of Red Swordtail, *Xiphophorus helleri*. Turkish Journal of Fisheries and Aquatic Sci. 2008;8:99-102.
4 Hata M, Hata M. Carotenoid pigments in goldfish-IV. Carotenoid metabolism. Bulletin of Japanese Society of Science Fisheries. 1972;38: 331-338.
5 Hardy R. Fish feed formulation, In: Fish feed Technology, Agriculture development coordination programme. FAO/ADCP/Rep. 1980;80(1-1):233-239.
6 Joseph B, Sujatha S, Shalin JJ, Palavesam A. Infleunce of Four ornamental flowers on the growth and colouration of orange swordtail Chicilidae fish (*Xiphophorous helleri*, Heckel, 1940). International Journal of Biological Medical Research. 2011;2(3):621-626.
7 Matsuno T, Matsutaka H, Nagata S. Metabolism of lutein and zeaxanthin to ketocarotenoids in goldfish, *Carassius auratus*. Nippon Suisan Gakkaish. 1981;47:605-611.
8 Ohkubo M, Tsushima M, Makoa T, Matsuno T. Carotenoids and their metabolism in the goldfish *Carassius auratus* (Hibuna). Comparative Biochemistry and Physiology. 1999;24B:333-340.
9 Olson A. A simple dual assay for vitamin A and carotenoids in human and liver. Nutrition report international. 1979;19:807-813.
10 Pailan GH, Sinha A, Borkar A. a. Rose petals meal as a natural carotenoid source for pigmentation and growth of dwarf gourami (*Colisa lalia*). Animal Nutrition and Feed Technology. 2012;12:199-207.
11 Paripatananont T, Tangtrongpairoj J, Sailasuta A, Chansue N. Effect of astaxanthin on the pigmentation of goldfish Carassius auratus. Journal of the World Aquaculture Society. 1999;30:454-460.
12 Raja K, Aanand P, Padmavathy S, Sampath Kumar JS. Present and future market trends of India ornamental fish sector. International journal of fisheries and aquatic studies. 2019;7(2):6-15.
13 Ramamoorthy K, Bhuvaneswari S, Sankar G, Sakkaravarthi K. Proximate composition and Carotenoid Content of Natural Carotenoid Sources and its Colour Enhancement on Marine Ornamental Fish *Amphiprion ocellaris* (Cuvier 1880). World Journal of Fish and Marine Science. 2010;2(6):545-550.
14 Saxena A. Health; colouration of fish. Proceedings of International Symposium; c1994.
15 Tsushima M, Matsuno T. Carotenoid composition and two new 4-ketolutein isomers in the integuments of the red filefish, *Branchiostegus japonicas*. Fisheries Science; c1998. p. 464-468.