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Effect of natural β-carotene sources-Carrot (*Daucus carota*) and Spinach (*Spinacia oleracea*) on the growth of an ornamental fish - sword tail (*Xiphophorus hellerii*)

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Abstract
The present study was aimed to assess the impact of dietary natural β-carotene sources *viz*., Carrot and Spinach on the growth of an ornamental fish swordtail (*Xiphophorus hellerii*). Experimental swordtails were cultured for 35 days in aquarium tanks. They were fed with experimental diets prepared with two natural vegetable sources *viz.* Carrot (*Daucus carota*) and Spinach (*Spinacia oleracea*) to supply 20, 25 and 30 mg of β-carotene individually per 100 g of diet. There were 6 experimental diets for each β-carotene sources and 1 common control diet (devoid of β-carotene). The proximate analysis of the experimental diet was also carried out. The water quality in the entire aquarium was maintained for assessing the growth. The fish were fed once a day at the rate of 3% of their body weight and the diets were readjusted after assessment of increase in fish weight every week. The net weight gain in experimental duration was highest in fish fed with *Spinacia oleracea* (0.73 g) followed by *Daucus carota* (0.54 g) where the fish were fed with 30 mg of β-carotene per 100 g of basal diet and lowest in control tank (0.29 g). The maximum specific growth rate (SGR) and gross conversion efficiency (GCE) were noticed in T_6 i.e. 1.11 and 0.88 respectively. From this study, it could be concluded that the natural β-carotene incorporated feed as growth enhancer can be prepared at a lower cost using the cheaply available sources.

Keywords: β-carotene, Growth, *Daucus carota*, *Spinacia oleracea*, Aquarium

1. Introduction
It is the one of the oldest hobby to keep colorful and fancy fishes well-known as ornamental fishes, aquarium fishes, or live jewels in the world. The growing interest in aquarium fishes has resulted in a sensible rise in the ornamental fish trade globally. These colorful and attractive fishes are nowadays rapidly gaining importance in aquaculture because of their aesthetic and immense commercial value in the export trade world over. The ornamental fish trade with a turnover of 6 Billion US dollar and an annual growth rate of 8% offers a lot of scope for development in India [12]. Better feed conversion and faster growth in farmed ornamental fish mean lesser expenditure on feed which otherwise contributes a sizeable input cost. The efficient feed also curtails the culture period giving higher fish production and consequently increased profit. Growth promoter administered through fish feed; result in measurable growth, weight gain and better feed conversion. Several such substances including anabolic steroids and protein hormones have been used as growth enhancers. Similarly, certain non-hormonal substances such as herbs and herbal products having fish growth promoting properties can be utilized in fish feed, without incurring an additional cost of the feed. Many herbal preparations are used for promoting human growth as well as for treatment of ailments as mentioned in the traditional medication methods of Ayurveda. However the use of herbal feed in aquaculture as a growth promoter is a recent advent. Satavari and Ashwagandha effectively used as a growth promoter in fish feed [10]. Later several researchers have tried herbal products as a growth promoter in the fish diet [13, 8, 4, 15]. The present study was conducted to evaluate growth promoting effect of natural sources of β-carotene i.e. the readily available vegetables Carrot (*Daucus carota*) and Spinach (*Spinacia oleracea*) in powder form were added in the supplementary feed of commonly cultured live bearer aquarium fish swordtail (*Xiphophorus hellerii*).

2. Materials and Methods

2.1 Experimental Fish

Experimental ornamental fish swordtails (*Xiphophorus hellerii*) were obtained from the local ornamental fish dealer and were acclimatized to laboratory condition in glass aquaria. The average length and weight of experimental fish 2.8 to 3.6 cm and 0.46 to 0.54 g respectively. The fish were conditioned and fed with control diet (devoid of β-carotene source) for 3 weeks to equalize their body carotenoid content in indoor conditions. The water exchange and aeration were given sufficiently. Three FRP tanks of 200 litres were used for conditioning the fish and 100 fish were placed in each tank prior to administrating the experimental diets.

2.2 Carotenoid Analysis in natural sources

The carotenoid content of natural β-carotene sources i.e. Carrot and Spinach were analyzed [19]. 200 mg samples of dried ground powdered form were transferred into 10 ml pre-weight glass tube. The samples were mixed thoroughly in about 5 ml acetone containing 1.5 gm of a hydrous sodium sulphate with the help of homogenizer. The volume of extraction was made up to 10 ml of acetone. The samples were stored at 4°C in the refrigerator for 3 days and then extracted 3 to 4 times until no more color would be obtained. The solution was centrifuged at 5000 rpm for 5 minutes and then absorption was measured in a spectrophotometer (wavelength (λ) 470, 662 and 645). After detecting β-carotene content in Carrot and Spinach i.e. 1.10 and 1.58 per cent respectively, proportional quantity of dried and powdered natural β-carotene source was mixed in basal diet, replacing equivalent amount of rice bran (Table. 1), for achieving 20, 25 and 30 mg/100 g β-carotene in experimental diet for both the selected sources.

2.3 Experimental Feed Preparation

The experimental feed were prepared with basic ingredients such as Wheat flour, Rice bran flour, Tapioca flour, Soya bean flour, Fish flour, Groundnut oil cake and Vitamin mixture [14]. They were split into 7 equal portions. The two vegetable sources of β-carotene such as Carrot (*Daucus carota*) and Spinach (*Spinacia oleracea*) were collected, air-dried in dark room to avoid denaturing of carotenoids. After oven drying the natural sources at 45°C for 48 hours, the sources were powdered and sieved in particle size of 0.1 to 0.2 mm then stored in the refrigerator at 4°C. The experimental diets were prepared using Carrot and Spinach powder mixed in the basal diet to supply 20, 25 and 30 mg of β-carotene per 100 g of diet. For the purpose dried Carrot powder was added in the diet at the rate of 1.82, 1.27 and 1.72 g per 100 g of diet replacing the rice bran. Similarly, Spinach powder was added in the diet at the rate of 1.33, 1.67 and 1.99 g per 100 g of diet (Table 1).

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

Ingredients	Treatments/ in gram						
	T_1	T_2	T_3	T_4	T_5	T_6	T_7 (Control)
Soya bean	17	17	17	17	17	17	17
Fish meal	15	15	15	15	15	15	15
Groundnut oil cake	15	15	15	15	15	15	15
Wheat flour	14.5	14.5	14.5	14.5	14.5	14.5	14.5
Tapioca flour	14	14	14	14	14	14	14
Rice bran*	12.68	12.23	11.77	13.17	12.83	12.51	14.5
Vegetable oil	5%	5%	5%	5%	5%	5%	5%
Vitamins	5%	5%	5%	5%	5%	5%	5%
Dried Carrot powder **	1.82	2.27	2.73	---	---	---	---
Dried Spinach powder **	---	---	---	1.33	1.67	1.99	---
Total	100	100	100	100	100	100	100
Final β-carotene content mg/100 gm	20	25	30	20	25	30	0.00

***** Adjusted according to the quantity of powdered carrot/spinach.

****** The quantity of natural carotenoid will be proportionate to supply 20, 25 and 30 mg β carotene/100 gm diet. The dried Carrot powder used contains 1.1033% β- carotene and Dried Spinach powder contains 1.5837% β- carotene.

2.4 Feeding Experimental diet

This study was carried out in the indoor system for 1 control and 6 treatments in 21 experimental glass aquaria of 50 litres capacity. There were 3 replications assigned for each experimental diet. All fishes were fed once a day at the rate of 3 per cent of their body weight with the experimental diet. During the experiment, the major physico-chemical parameters were maintained at stable conditions. The experiment was carried out for 35 days. The observations for growth parameter of the fishes were measured initially and then at every 7 days interval and accordingly the diets were readjusted every week in each aquaria. The water quality in test aquaria was maintained by aeration, removal of faecal matter and replacement of at least 20 per cent of water every week.

2.5 Analytical Methodology

Water quality parameter *viz*., air and water temperature, pH, electrical conductivity, dissolved oxygen, free carbon dioxide, total alkalinity, N-nitrogen, orthophosphate and total hardness were analyzed initially and weekly [2, 20]. The experimental diets were analyzed for the proximate composition viz., moisture, crude protein, lipid, and carbohydrate and ash contents as per standard methods [1]. The growth parameters of experimental fish *viz.* weight gain, specific growth rate, feed conversion ratio, gross conversion efficiency, were analyzed [6]. The data obtained for growth parameter of swordtail fed with β-carotene fortified diets were statistically analyzed by applying complete randomized design (CRD) and analysis of variance (ANOVA) for checking the significance ($P < 0.05$) level of treatments [18].

3. Results and discussion

No considerable fluctuation in water temperature was observed (23.20 to 23.50 °C). The pH of the experimental water was in the range 7.7 to 8.2, whereas, a range of electrical conductivity was 900 to 1030 µS/cm. A narrow fluctuation in dissolved oxygen was recorded and the value

ranged between 5.5 to 7.2 mg/l. The free carbon dioxide in all the treatments was absent. The total alkalinity of experimental water ranged between 300 to 330 mg/l and hardness between 450 to 480 mg/l. The value of orthophosphate 0.03 to 0.05 mg/l and nitrate-N 0.32 to 0.58 mg/l were also found congenial in experimental waters.

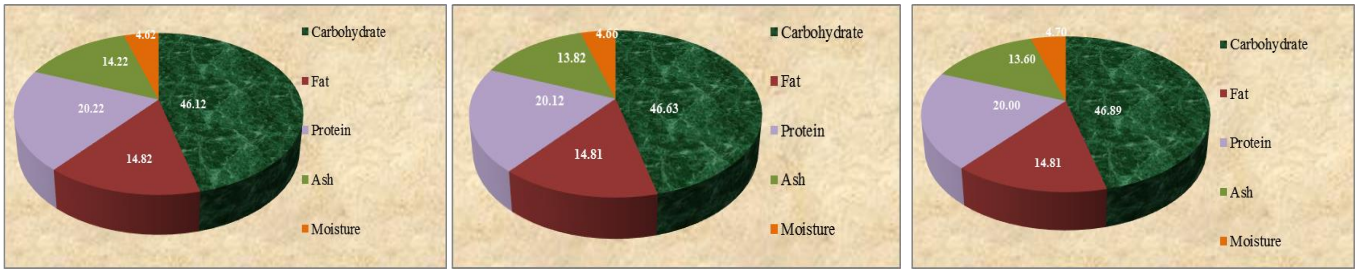
The proximate composition of Spinach and Carrot mixed diet and control diet used in present study with their maximum values were carbohydrates (46.10 to 46.89 per cent), lipid (14.79 to 14.82 per cent), protein (20.00 to 20.22 per cent), ash (13.60 to 14.22 per cent) and moisture (4.62 to 4.70 per cent) (Figure 1).

The weight gain of swordtail was significantly increased with the use of natural β-carotene sources Carrot and Spinach ($P< 0.05$, CD = 0.0219). The maximum weight gain (0.73 g, 146%) was noted in T_6 i.e. in Spinach mixed diet. Whereas, in Carrot mixed diet the maximum weight gain was noted in T_3 i.e. (0.54 g, 108%) (Table 2). Similarly, in Carrot and Spinach mixed diets, the maximum values of other growth parameters were, specific growth rate (0.91 and 1.11), Feed conversion ratio (1.37 and 1.14), gross conversion efficiency (0.73 and 0.88) respectively.

The results of the growth parameters of experimental fishes are given in Table 2. The results indicate a better growth performance in fish fed with Spinach mixed diet (146 per cent increase – T_6) followed by Carrot mixed diet (108 per cent increase – T_3). The same diets indicated better specific growth rate (SGR) 1.11 and 0.91 in T_6 and T_3 respectively. The feed conversion ratio (FCR) was maximum in control compared to 1.14 to 1.68 in Carrot and Spinach mixed diet respectively. The results also reveal better gross conversion efficiency (GCE) (0.88) of *Xiphophorus hellerii* when fish were fed with a maximum dose of Spinach (T_6) i.e. to supply 30 mg β-carotene in 100 g of diet. As such the result in the present study reveals better growth performance and feed conversion when fish were fed with Spinach mixed diet followed by Carrot mixed diet compared to the control diet.

The results in present investigation supported by several researchers who found herbal ingredients as growth promoter in supplementary diet that include the herb Ashwagandha (*Withania somnifera* L. Dunal) [10], Safed musli (*Asparagus adscendens* Roxb.) [11], Kali musli (*Circuligo orchioides Gaertn.*) and mulethi (*Glycyrrhiza glabra* Linn.) [13], Bala (*Sida cordifolia* Linn.) [8] and some authors successfully used Makhana (*Euryale ferox* Salisb) [16], Satavari (*Asparagus racemous* Wild) and Ashwagandha (*Withania somnifera* L. Dunal) [9] as herbal growth promoter in fish diet. The dietary carotenoid supplementation positively affects the growth of fish as also found in the present study. The present study showed that various sources of dietary carotenoid increased the growth and survival of fish. This agrees with the study of authors who reported that feeding with marigold petal 15 g/100 g increased the growth rate of Red Swordtail (*Xiphophorus hellerii)* reared for 60 days [5]. The results obtained in the current study are supported by the researcher who used the vegetable product as a dietary carotenoid source and observed improved final weight, weight gain and specific growth rate of juvenile goldfish *Carassius auratus* [7]. Similarly, there was improved in a specific growth rate of *Silurus glanis* when fed with carotenoid-rich microalgal biomass [21]. The growth rate of fishes in the group fed with China rose petal feed was the highest in terms of weight [17]. However, the author reported an insignificant effect of a dietary supplement of - Astaxanthin 70 mg/kg on the growth of Atlantic salmon (*Salmo salar*) reared for 22 weeks [3].

In a study on the effect of various natural carotenoids on the fish growth and found that the synthetic Astaxanthin was more effective than red pepper and marigold flower although they contained equal amounts of carotenoids. In this study the carrot and marigold were absorbed better compare to the others carotenoid such as rose and hibiscus. As found in the present study, among the four different carotenoids used, these authors found carrot as an effective supplement for net wet weight gain (7.681 mg/kg) in *Amphiprion ocellasris* (Cuveir 1880), a marine ornamental fish [14].



Carrot mixed diet in% **Spinach mixed diet in%** **Control diet in%**

Fig 1: Proximate composition of experimental fish diet

Table 2: Growth parameters of *Xiphophorus hellerii* fed with feed additives mixed diet during the experimental period of 35 days

S. No.	Treatments	Initial individual weight of fish (g)	Weight gain (g)	Specific growth rate (SGR)	Feed given	Feed conversion ratio (FCR)	Gross conversion efficiency (GCE)
1	T_1	0.5	0.41	0.56	0.69	1.68	0.59
2	T_2	0.5	0.45	0.80	0.71	1.58	0.63
3	T_3	0.5	0.54	0.91	0.74	1.37	0.73
4	T_4	0.5	0.60	0.98	0.77	1.28	0.78
5	T_5	0.5	0.67	1.05	0.81	1.21	0.83
6	T_6	0.5	0.73	1.11	0.83	1.14	0.88
7	T_7 (Control)	0.5	0.29	0.56	0.65	2.24	0.45
	CD	---	0.0219	0.0342	0.0287	0.0617	0.0276
	CV	---	2.03	1.96	1.89	2.01	1.94
	SEm±	---	0.0072	0.0113	0.0095	0.0203	0.0091

4. Conclusions

It could be concluded from the present experiment that dietary supplementation of carrot at the rate 2.73 g/100 g of diet and Spinach at the rate of 1.99 g/100 g of diet can enhance the growth of swordtail (*Xiphophorus. hellerii*). Because synthetic carotenoids are costly, inexpensive and readily accessible natural carotenoid sources such as carrot and spinach can be incorporated into a swordtail diet to obtain better growth, feed utilization and additional market value. This will help fish keepers and ornamental fish sellers to get superior cost in trading of this fish.

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