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Original Article

Influence of Four ornamental flowers on the growth and colouration of orange sword tail Chicilidae fish (*Xiphophorus hellerei*, Heckel, 1940)

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ABSTRACT

The present research was designed to study the effect four botanical additives (*H. rosasinensis, Rosa indica, Ixora coccinea and Crossandra infundibuliformiss*) on the growth and body colouration of an ornamental fish of red sword tail Xiphophorus hellerei (Heckel). This experiment was conducted in adult female fish for a period of 75 days. The carotenoid pigment sources were added to the supplementary diet at 1.5, 2.5 and 3.5 percent levels of concentrations respectively. The fishes were treated with at the rate of 5 to ten percent level of body weight. Three fold increases in growth was observed in H. rosasinensis fed fishes followed by R. indica. Furthermore, the similar weight gain has been observed rest of the two flower petals (I. coccinea and C. infundibuliformiss). The percentage of colour pigments obtained in adult fish were maximum in I. coccinea one percent level then remaining flowers peal showed another highest pigment production was R. indica, H. rosasinensis and C. infundibuliformiss. Consequently a significant difference was found between individuals fed by natural pigment material and those by unpigmented feeds ($p \leq 0.05$). It was demonstrated that natural pigment substances have an impact on coloration of cichlid and the groups did not exhibit any distinctions in feed conversion and growth rates. Therefore, it was determined that these pigment sources have an effect on the colour of cichlid fish.

1. Introduction

Ornamental fishes are nowadays rapidly gaining importance because their aesthetic value and also due to their immense commercial value in the export trade world over. Attractive colouration determines the commercial value of ornamental fish pigmentation in the skin is responsible for colouration in the fish [1].Carotenoids are the primary source of the pigmentation on the skin of fishes [2]. In natural environment, the fishes meet their carotenoid requirements by ingesting aquatic plants or through their food chain. But fishes can not synthesis the carotenoid denovo [3,4,5]. Carotenoids are absorbed in animal diets, sometimes transformed into other carotenoids, and incorporated into various tissues. The molecular properties of rainbow trout (Oncorhynchus mykiss) retinol-binding protein (rtRBP), the specific retinol carrier in vertebrate plasma, were studied to elucidate its role in transporting retinols to developing fish oocy[6,7]. The red colouration of salmonids, crustaceans and some aquarium fish have become of interest in the cultivation. Dietary carotenoids play significant part in the regulation of skin and muscle color in fish [8]. Astaxanthin is the main carotenoid pigment of red-pink coloured aquatic animals, being widely used in aqua-cultural processes because it is a standardized and chemically stable product with a high carotenoid concentration [9]. The colour enhancing diets should contain additional natural pigments to enhance the colour of ornamental fish[10]. In earlier days Ali and Salim [11] established the fish do not possessed that fish do not possess the ability to synthesize carotenoids. Hence the carotenoid pigmentation of fish results depends upon the supplementary feed contains the carotenoid amount. Several

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authors have been proved that the fish can be pigmented by including processing wastes and botanical plant source[12,13,1]. Since, there is no more works has been conducted this kind of orange word tail fish with these four kinds of flower petals carotenoid pigments. Hence the present study were undertaken to find out the influence of four botanical additives on growth and colouration of orange sword tail.

2. Materials and Methods

The experimental fish of Orange swordtails (Xiphophorus helleri) (H.) of uniform size group (1g) were purchased from a commercial aquarium fish farm from Nagercoil from Kanyakumari District, Tamilnadu and were acclimatized to laboratory conditions for one week before the start of the experiment. The experiments were conducted for a period of 21 days and were carried out in 5 plastic troughs of 20 litre capacity. The fish were weekly weighed and recorded. For the present investigation, the feed was prepared in the form of dry pellets [4].The experimental diet composed of the basic ingredients like flower petals of (H. rosasinensis, R. indica, I. coccinea and C. infundibuliformiss) (Table-1). Using the four ingredients, diet with 40% protein was prepared using the square method. They were mixed with different quantities of four types of flower petal meal content in the diet. These flowers were purchased from the local area of in and around the college campus. Four flower petal meals were added to the diets just before pelletization with respective concentration of 0,2,3,4 mg /100 g of basal diet. The prepared foods were provided two times daily to the experimental fishes (X. helleri) to satiation. The control food for control tank feed 1, feed 2, feed 3 for experimental demand. The unfed was collected from the tank; the feeding experiment was continued for a period of 21 days. For the present study, four different tanks of orange sword tail were cultured in glass tanks. Among the four tanks, one was used as control tank remaining three tanks were experimental tank F1, F2 and F3 respectively. The experimental was carried out at seven days interval for 21 days. During the experimental period the fishes were fed on control and experimental diets added with carotenoid at the rate of two times a day. Water quality parameters were maintained by aeration. The physicochemical parameters such as temperature, dissolved oxygen, ammonia pH in all the experimental tanks were estimated. Standard methods were employed for the analysis of the water quality parameters

2.1.Pigment Extraction in Fish Tissue

The method used for pigment extraction from the red sword-tail tissue was as described in Five gram of entire red sword-tail 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 5ml of Chloroform was added and left overnight at 0°C. When the chloroform formed a clear 1-2 cm layer above the caked residue, the optical density was read at 380, 450, 470 and 500 nm in a spectrophotometer. A blank prepared in a similar manner was used for comparison. The wavelength at which maximum absorption, was used for the calculation.

2.2.Qualitative estimation of total Carotenoids:

The Qualitative analysis of carotenoid was carried out by the Thin Layer Chromatography (TLC) method [15].The identification of compounds carried by comparing the Rf values of samples.

2.3.TLC Plate Preparation

The TLC plates are made by spreading of silica gel G for TLC over the glass plates of size 20cm x 20 and the thickness of 250µ.The plates were allowed to dry at 100◦c in the oven. After drying, the plates were ready to use. Before using the plates for separation it should be activated at 120°C for2-3hrs for obtaining better results and clear separation.

2.4. Developing of TLC

Saturation of the developing chamber has a strong influence on separation and reproducibility hence recommended in carotenoid work. The saturation chamber was rapidly charged with the Mobile phase (Acetone/Methanol 9:1) described by Hector et al. [16].

2.5.Detection of Spots In TLC

A Smaller amount of samples and reference compounds were spotted at the level of minimum quantities (2-5 µl).Then the TLC plate was placed on the chamber with mobile phase (Acetone/Methanol) to develop the color spots. After mobile solvent searching the upper limit, plates were taken out and dried for few minutes. The developed and dried chromatogram was stained by 5gms iodine vapours the distance traveled by each spot in the experimental samples was measured from the base line and relative Rf values were calculated .By comparing the standard Rf values for the chosen mobile phase, the number of carotenoids present in samples were identified.

$$\mathbf{Rf=\frac{Distance\ traveled\ by\ the\ substance}{Distance\ traveled\ by\ the\ solvent}}$$

2.6.Method of TLC

Thin-layer chromatography consists of a stationary phase immobilized on a glass or plastic plate, and an organic solvent. The sample, either liquid or dissolved in a volatile solvent, is deposited as a spot on the stationary phase. The constituents of a sample can be identified by simultaneously running standards with the unknown. The bottom edge of the plate is placed in a solvent reservoir, and the solvent moves up the plate by capillary action. When the solvent front reaches the other edge of the stationary phase, the plate is removed from the solvent reservoir. The separated spots are visualized with ultraviolet light or by placing the plate in iodine vapor. The different components in the mixture move up the plate at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

2.7. Statistical Analysis

Sigmastat 3.5 was used for statistical analysis. A one way ANOVA was applied to find out the significant differences among average values of total carotenoid content and the difference between the mean treatments were tested with Tukey test. A two

way ANOVA was applied to establish significant differences between the values of nutritional parameters and the difference between the mean treatments were tested with Tukey test.

3. Result

The present work was carried out to determine whether carotenoids of Hibiscus rosa-sinensis, Rosa indica, Ixora Coccinea, Crossandra infundibuliformiss petals could induce pigmentation to make orange swordtail more color. The amount of total caroteniod was higher in C. infundibuliformiss-1.802 mg/gm followed by Hibiscus rosasinensis, Ixora Coccinea, Rosa indica contains 1.52, 1.014 and 0.47 respectively. An ornamental fish does not have the capacity of synthesizing the carotenoid itself hence it must be supplied through diet.

Table: 1. List of Ingredients used for carotenoid based food preparation

Ingredients	Percentage of contents (included food stuff)			
	Control	Experimental diet -1 (1%)	Experimental Diet-2 (3%)	Experimental diet -3 (5%)
Fish meal (gm)	10	20	25	30
Casein (gm)	10	20	25	30
Soya meal (gm)	10	20	25	30
Tapioca powder (gm)	10	20	25	30
Wheat flour (gm)	10	20	25	30
Rice bran (gm)	15.5	20	25	30
Vitamin and Mineral Mixture	0.05	0.05	0.05	0.05

Table-2. The growth of adult orange sword tail with four different botanical additives at three different concentrations

Treatment	Concentration (%)	Mean Weight				Mean wt	Wt gain / (day in g)	Specific growth rate	P-value
		7^{th}	14^{th}	21^{st}	28^{th}				
H. rosasinensis	1	3.80	3.91	4.67	5.10	1.55	0.522	0.46	
	3	4.22	4.97	4.98	5.18	1.84	0.781	0.51	$4.21x10^{-5}$
	5	4.11	5.22	5.36	5.40	2.34	0.235	0.61	
	1	4.35	4.41	4.58	4.72	3.54	0.542	0.54	
I. coccinea	3	4.65	4.69	4.84	4.98	3.84	0.165	0.35	0.0035
	5	5.12	5.21	5.46	5.68	3.97	0.321	0.35	
	1	4.03	4.14	4.25	4.57	3.45	0.310	0.52	
	3	4.21	4.34	4.67	4.61	3.56	0.256	0.54	0.0018
C. infundibuliformiss	5	4.58	4.48	4.73	4.89	2.97	0.455	0.67	
	1	3.10	3.45	3.89	4.10	2.11	2.013	0.59	0.0057
R. indica	3	3.54	3.58	3.93	4.10	1.38	0.254	0.73	
	5	3.85	3.91	4.11	4.35	2.58	0.041	0.56	
Control	optimum	5.12	5.49	5.49	5.60	4.48	0.054	0.45	

The data regarding the growth of adult orange sword tail with four different botanical additives at three different concentrations are presented in Table 2. The mean weight of the adult orange sword tail fish on the first day of stocking in the control was 4. 48g. The initial mean weight of the *H. rosasinensis* fed fish group at three different concentrations viz. 1, 3 and 5 percent were 1.55, 1.84 and 2.34g and the initial mean weight in the I. coccinea were recorded to be 2.54, 2.84 and 2.97g respectively. Furthermore, the mean weight gains of the fish fed with *C. infundibuliformiss* were found to be 3.45, 3.56 and 2.07mg. Moreover, R. indica showed optimum level of mean weight gain observed in this flower fed fish such as 2.11, 1.38 and 2.58 on 1, 3 and 5% respectively. Among the four flowers treated diet, I. coccinea fed fishes having maximum mean weight 4.72, 4.98 and 5.68mg in 1, 2 and 5% respectively. Though, the four different kinds of diet revealed better specific growth rate was observed on two flowers such as *C. infundibuliformiss* and *R. indica.*

3.1.Column Chromatography

The extracts obtained from chloroform and benzene mixture (525: 225ml) was subjected to column chromatographic study which eluted successively with chloroform, glacial acetic acid, diethyl ether and methanol. Totally twenty five (each of 10ml) fractions were eluded from the column. Various fractions were grouped individually by monitoring TLC behaviour and their Rf value is presented in (Table. 3) the prepared supplementary feed. A light greenish yellow elution obtained from the botanical based carotenoid feed. Of these various colour fractions only 15-18 fractions was selected for further purification. However, fraction was obtained while Glacial acetic acid and Chloroform (70:30) at 30-50 C. From this fraction yields a 0.78mg of dark green colored product. It was again subjected to column chromatography eluted successively Benzene and Methanol with different proportion. In order to that 19 fractions were obtained from these compounds. For instance, the various groups of other compounds are also subjected in TLC assay were determined based upon the RF values. Apart from these RF values of peculiar compounds assessed with the help of TLC chromatography and qualitative phytochemical analysis presented in table (Table 5). The RF value recorded for the fraction of 9 to 13 (Table 4).

Table 3. TLC Pattern of Recolumn (15-18) fractions of botanical based carotenoid supplementary feed for Red sword tail *Xiphophorus hellerii*

Fraction from carotenoid food	RF values	
	I	II
	0.540	-
1	0.540	-
2	0.510	-
3	0.320	-
4	0.380	0.67
5	0.401	0.64
6	0.405	0.66
7	0.425	0.67
8	0.35	0.57
9	0.34	0.55
10	0.39	0.34
11	0.39	-
12	0.28	-
13	0.26	-

Table 4. Preliminary phytochemical screening of fraction on carotenoid supplementary feed 15-18 with Recolumn chromatography

Compounds	Solvents		
Steroids	Distilled water	Acetone	Methanol
Triterpenoids	+	+	+
Reducing sugar	-	-	-
Carbohydrate	-	-	-
Alkaloids	+	+	+
Phenolic compounds	+	+	+
Saponin	-	+	+
Xantho protein	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+

+ means for presence of the compound

- means Absence of the compound

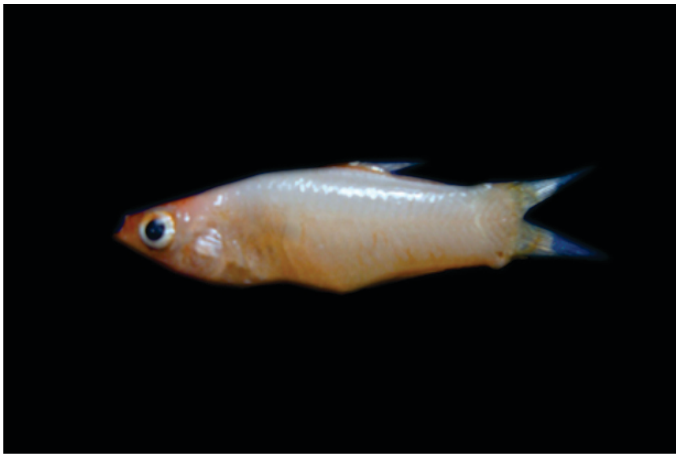
Fig.1. External morphology of orange sword tail (Xiphophorus helleri) after treated with three concentration of flower based carotenoid based supplementary feed

CONTROL

F1 (a)

F2 (b)

F3 (c)



4. Discussion

Carotenoids are also vital nutrients for healthy growth, metabolism, and reproduction [17].Further work is needed to determine the optimum types, concentrations and combinations of carotenoids (specifically astaxanthin, zeaxanthin, and lutein) that are required to produce acceptable levels of the natural orange-red skin coloration in juvenile Red Oranda gold fish by Use of practical or semi purified diets in this type of study is imperative for limiting the effects of dietary deficiencies or energy difference on pigment assimilation that may occur with commercial diet formulations. These types of studies would allow investigators and feed manufacturers to further refine which diet formulation will produce the optimal level of orange-red skin coloration. Investigation of color-enhancing diets as finishing feeds for goldfish in well-water culture systems is also warranted as is done in the trout and salmon culture industries [19].

Overall results for natural orange-red skin coloration development, speed of skin color change, feed formulation carotenoid profile, survival and growth demonstrated that the VibraGro feed should be a suitable diet for juvenile Red Oranda goldfish reared in a well-water culture system [20,21].The carotenoid astaxanthin appears to impart the orange-red coloration in this variety of goldfish. Other feeds that demonstrated positive results for speed of skin color change may be suitable for use in a pond-water culture system based on our overall results for goldfish reared in pond water and given a feed that contained pigments (Zeigler Tropical) (carotenoid based referred only commercial feeds are continually modified, as they are subject to least-cost formulation, new research, and updates on nutrient requirements of fish [22].In this study, we demonstrated how feed formulations dramatically affect color development in the skin of fancy goldfish, and how the type and amount of pigment in a feed formulation may impart yellow, orange and red skin coloration.

The protective function of carotenoids associated with the reaction centers and antenna complex are so critical those an inability to form cyclic carotenoid due to a block in carotenoid biosynthesis [23].We also observed that inhibition of carotenoid denaturation by NF does not lead to phytoene accumulation in the dark. This can be explained by a reduced flux into the carotenogenic pathway and would be in line with the above mentioned hypothesis that a strongly reduced carotenoid synthesis occurs in darkness [24,25].

According to our knowledge, the hypolipidemic and hypocholesterdemic effects of carotenoids have not previously been investigated. Dietary carotenoids source and concentration had no effect on the growth and survival of red devil agree with the following studies '[26] and] also found that there were no differences in growth and feeding rates among rainbow trout fed AX and BC. Neither growth nor feed efficiency of gilthead sea bream was affected by diets supplemented with synthetic AX or H. pluvialis or no carotenoids [28]. Furthermore, , , suggested when rainbow trout fed with *Dunaliella salina,* which contains BC, and P. rhodozyma, which contains free AX, and found no difference in growth rate between the treatment groups and control group.

This study presents data on the effect of carotenoid sources on skin coloration of Orange sword tail (X. hellerei). The physical and chemical characteristic of the diet plays an important role on species. Similarly the result from the experiment shows that the artificial diets definitely imparted different skin coloration, growth and biochemical changes in orange sword tail. The carotenoid based artificial feeds reduced the best orange red skin colouration in orange sword tail. The quantity of biochemical, growth and carotenoid analysis objectively demonstrated the different concentrated diet source imparted large differences in skin color types and amounts. More studies were reported only carotenoid source that had a significant effect on skin hue, promoting a reddish coloration to the dorsal skin area and a ventral hue similar to wild red porgy (Fig 1). No apparent effect of carotenoid source on skin melanin content was observed. In contrast, dietary protein/carbohydrate ratio affected melanin content in the skin. Finally these results indicate that the efficiency of carotenoid assimilation by orange sword tail is not age dependent; some individuals will begin carotenoid assimilation sooner than others depending on their concentration of diet.

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