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Marigold as a Carotenoid Source on Pigmentation and Growth of Red Swordtail, *Xiphophorus helleri*

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Abstract

Colouration is one of the major factors deciding the market value of the ornamental fish. In captive conditions the fish show fade colouration and fail to get attracted by the buyers. To overcome the problem we have planned an experiment with the cheap pigmenting source namely the marigold petal meal. The results are encouraging so that marigold petal meal could be used as a pigmenting source at a concentration of 15 g / 100 g feed of dry weight. Leaching of pigmenting source is also a problem. Hence, experiment was conducted with marigold petal enriched gelatine based diet. From the present investigation, it is considered that a gelatine based diet enriched with both marigold petal powder and protein ingredients might give a better result of growth and pigmentation.

Key words: pigmentation, carotenoids, marigold petal meal, *Xiphophorus helleri.*

Introduction

The art of rearing and keeping fish in an aquarium is an age-old practice. At the dawn of the 21st century fish keeping is reflected in *ubiquitous aquaria* that feature as an integral part of modern interior decoration (Katia Oliver, 2001). Most of the fish are small in size, having attractive colours. Their movements are gentle and quiet without causing any sound. They have adaptability to live within a confined space in captivity. The aquarium fish are thus of a rapidly growing importance not only because of their aesthetic value but also of their immense commercial value in the export trade world over.

Colour is one of the major factors, which determines the price of aquarium fish in the world market (Saxena, 1994). Fish are coloured in nature often show faded colouration under intensive culture conditions. Fish, like other animals do not synthesize carotenoid and depend on dietary carotenoid content for the colouration. Hence, a direct relationship between dietary carotenoids and pigmentation exists in them (Halten *et al*., 1997).

The body colours of fish are predominantly dependent on the presence of special cells in the tissue, called chromatophores. Carotenoids are a group of over 600 natural lipid soluble pigments that are primarily produced in phytoplankton, algae and plants. Carotenoids are absorbed in animal diets, sometimes transformed into other carotenoids, and incorporated into various tissues. According to Anderson (2000), carotenoids also have excellent antioxidative characteristics.

If enhancement of colouration can be done by administering pigment enriched feed, it will definitely improve the quality and cost of the fish. However, detailed studies on colour enrichment in ornamental fish are lacking. Plant sources have also been utilized for inducing pigmentation in fish. For example, *Spirulina* have been used as a source of carotenoid pigments for rainbow trout and fancy carp (Choubert, 1979; Boonyaratpalin and Phromkunthony, 1986; Alagappan *et al*., 2004) and marigold petal meal was used for the tiger barb (Boonyaratpalin and Lovell, 1977).

To evaluate these strategies, the present work was carried out to know whether marigold petal meal could be used as a cheaper pigmenting source in order to enhance the bright red colouration of the experimental fish, *Xiphophorus helleri.*

Materials and Methods

Red swordtails (*Xiphophorus helleri*, Cyprinidae) of uniform size group (0.6 g) were purchased from a commercial aquarium fish farm and were acclimatized to laboratory conditions for one week before the start of the experiment. The experiments were conducted for a period of 28 days and were carried out in 21 plastic troughs of 20 litre capacity. These 21 troughs were grouped into seven sets and each set consists of 3 troughs. The fish were weekly weighed and recorded. For the present investigation, the feed was prepared in the form of dry pellets. The experimental diet composed of the basic ingredients like wheat flour, rice bran, tapioca flour, chicken meal and fish meal (Table: 1). Using the ingredients, diet with 40% protein was prepared using the square method (Hardy, 1980). They were mixed with different quantities of marigold petal meal content in the diet. Marigold flowers (*Tagestes erecta*

Table 1. Proximate and biochemical composition of the experimental diets (dry weight basis)

Ingredients	Quantity (g/100 g feed)	Protein (%)	Lipid (%)	Carbohydrate (%)
Chicken meal	13.04	46.0	14.0	5.2
Soyabean	15.22	70.6	9.7	8.7
Fish meal	15.22	60.0	5.2	3.7
Groundnut oilcake	13.04	43.6	40.9	16.7
Wheat flour	13.04	11.0	3.5	44.0
Tapioca flour	13.04	2.2	76.3	56.0
Rice bran	15.22	12	1.0	62.5
Vitamins and mineral mix (Supradyn tablet)	5	-	-	-

L., Asteracea) were purchased from the market and they were sun-dried and powdered. Marigold petal meal was added to the diets just before pelletization with respective concentration of 0, 3, 4, 6, 8, 15 g /100 g of basal diet. A gelatine based diet was also prepared by mixing marigold petal meal with the agar (China grass).

Formulas Used for Calculations

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

Total carotenoid content = [Absorption at maximum wave length /(0.25 x sample weight (g))] x 10

Where, 10 = Dilution factor; 0.25 = Extinction coefficient

Absolute growth rate (g) = Final mean weight – Initial mean weight

Relative growth rate (%) = [(Final mean weight– Initial mean weight)/Initial mean weight]× 100

Specific growth rate (%) = [(In final weight – In initial weight) / Rearing period (days)] × 100

Weight gain (mg/g/day) = [(Final mean weight – Initial mean weight)/(Initial mean weight / Rearing period (days)] × 100

The experiment was done in triplicates and was carried out in plastic troughs of 24.0 L capacity. The duration of the experiment was 21 days. The fish were fed with 5% of their body weight daily. Water in the experimental troughs was changed everyday for the sufficient supply of oxygen to the fish. At the end of the experiment, all fish were starved for one day to take the final wet weight.

Analysis of total carotenoids in the fish tissue was carried out prior to the start of the experiment and after the termination of the experiments following Olson (1979).

Pigment Extraction in Fish Tissue

The method used for pigment extraction from the red sword-tail tissue was as described in Olson (1979). One 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 5 ml 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 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.

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.

Results

The present work was carried out to determine whether carotenoids of marigold petal meal could induce pigmentation to make a red swordtail more colourful.

Total Carotenoids in Red Swordtail Muscle Tissue

Marigold petal meal was found to be an effective colour enhancer at a cheaper price. The results clearly showed that the maximum carotenoid content was present in the fish fed with 15/100 g dry weight feed. For instance, the total carotenoid content in the 15 g fed group was found to be 28.48±0.38 µg/g wet weight and for the control group it was found to be 2.76±0.34 µg/g wet weight (Figure 1).

Similarly, the carotenoid content in different

experimental diet fed fish also increased significantly except the 6 g/100 g dry weight fed experimental diet and analysis of variance showed that the carotene content between treatments differed significantly. Diet containing 6 g marigold petal meal incorporated into gel could better convert into colour than the diet containing similar (6 g/100 g) marigold petal meal. For example, the total carotenoid content in the 6 g pellet feed, fed group was 8.24±0.36 µg/g wet weight and the 6 g gel feed was 11.2±0.36 µg/g wet weight (Figure 1). The carotenoid content among the variation was found to be significant (P<0.001; Table 2).

Nutritional Evaluation of Experimental Diets

Fish fed with control diet always showed higher absolute growth rate, SGR, RGR and weight gain than fish fed with other diets. Fish fed with gel showed negative growth. Maximum SGR obtained by the fish fed with control diet was 1.60±0.19% and the minimum SGR value obtained by the fish fed with 6 g gel feed was -0.70±0.12%.

When fish were fed with 3 g, 4 g, 6 g, Gel (6 g), 8 g and 15 g of marigold petal feed, the growth rate to the tune of 12.2±0.2, 17.2±2.6, 18.5±1.1, -10.5±1.5, 17.4±0.6, 08.6±1.1 mg/g/wet weight, respectively were recorded (Table 3).

Discussion

Carotenoids are the primary source of pigmentation in ornamental tropical fish, responsible for various colours like yellow, red and other related colours. Normally, these are obtained through organisms rich in carotenoid content organisms in the aquatic food chain. But commercial feed ingredients such as yellow corn, corn gluten meal, and alfalfa are used as sources of carotenoids such as zeaxanthin and lutein (Lovell, 1992). Other carotenoid-rich ingredients used are marigold meal (lutein), red pepper (*Capsicum* sp.) extract (capsanthin) and krill or crustacean meals (astaxanthin) (Boonyaratpalin *et al.*, 1977 and 1989).

The maximum carotenoid content found in 15 g fed group could be directly related to the enhanced

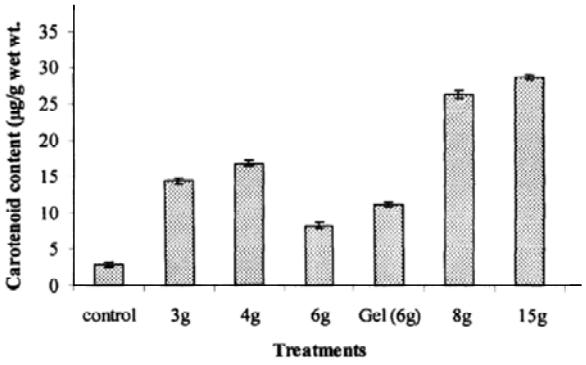

Figure 1. Carotenoid content of *Xiphophorus helleri* fed with different experimental diets.

Table 2. One way ANOVA for total carotenoid content of *Xiphophorus helleri* fed with different experimental diets

Source of variation	SS	df	MS	F	P-value
Between Groups	1573.878	6	262.313	1564.58	<0.001
Within Groups	2.3472	14	0.167657		
Total	1576.225	20			

Table 3. Shows SGR, AGR, RGR and weight gain in *Xiphophorus helleri* fed with different experimental diets

S. No	Treatments	Initial wt. (g)	Final wt. (g)	SGR (%)	RGR (%)	AGR (g)	Growth rate (mg/g/day)
1.	Control	0.46±0.03	0.72±0.02	1.60±0.19	56.5±7.0	0.26±0.01	30.6±3.3
2.	3 g	0.50±0.02	0.63±0.03	$0.80±0.02^{b}$	$26.0±0.5^{b}$	$0.13±0.01^{b}$	$12.2±0.2^{b}$
3.	4 g	0.50±0.04	0.70±0.02	$1.20±0.06^{c}$	$40.4±1.9^{c}$	0.20±0.01	$17.2±2.6^{c}$
4.	6 g	0.53±0.03	0.73±0.03	1.10±0.07	37.7±2.4	$0.20±0.01^{d}$	$18.5±1.1^{c}$
5.	Gel (6 g)	0.53±0.04	0.43±0.03	-0.70±0.12	-18.8±3.1	-0.11±0.01	-10.5±1.5
6.	8 g	0.56±0.03	0.76±0.04	$1.03±0.03^{bc}$	$35.7±1.2^{c}$	$0.20±0.01^{d}$	$17.4±0.6^{b}$
7.	15 g	0.60±0.01	0.72±0.03	$0.60±0.07^{b}$	$20.1±2.1^{b}$	$0.12±0.02^{b}$	$08.6±1.1^{b}$

Values in the same column sharing a common superscript are not significant (P<0.05).

level of carotenoid content in the particular ingredients. Alagappan *et al.,* (2004) have also obtained higher carotenoid level in the fish blue gourami, *Trichogaster trichopterus* with 4 g spirulina/ kg feed compared with the 0 g, 1 g, 2 g and 3 g.

The lower carotenoid content in the 6 g feed, might be due to the leaching of the pigmenting source that is incorporated in the feed. To overcome the leaching problem, gelatine based diet was tried. However, there was no significant increase in the carotenoid content in the fish fed with gelatine based diet. Per se, the fish had an edge over the fish fed with 6 g pellet feed. It might be due to the absence of energy dense materials in the gelatine based diet so that might have forced the fish to consume more of marigold powder resulting in more pigmentation.

Nutritional Evaluation of Experimental Diet

The development of manufactured feed could be considered as one of the contributing factors to the tremendous growth of this hobby's widespread popularity over the past 50 years (Earle, 1995). The increased acceptability of reliance upon manufactured feed for ornamental fish has focused the attention on the nutritional requirements of these beautiful aquatic vertebrates. In the present investigation, maximum SGR was obtained in control experimental diet. Kruger *et al.* (2001) stated that it would appear that a diet with at least 45% protein at 6% lipid level is needed for the best specific growth rate and feed conversion of growing (6–8 weeks of age) sword tails. The diet we have prepared contains only 35% protein. It is opined that the experimental period may have been too short to distinguish possible differences in SGR between the groups (Mazur *et al.*, 1993). Yew-Huchien and Shu-Ching-Jeng (1992) have worked in the pigmentation of kuruma prawn, *Penaeus japonicus* have got higher weight gains in the pigmented diets than those fed with the control diet.

The gelatine based diet showed a negative growth rate. This might be due to the fact that the diet was only a carbohydrate source. However, carbohydrates present a cheap energy source that would "spare" the catabolism of other components such as protein and lipids to energy (Sales and Janssens, 2003). Regarding the nutritional evaluation in the experimental diets, it was found that marigold petal meal has limited nutritional value and serves only as a pigmentation source. From the present investigations it is inferred that marigold petal served better as a pigmentation source rather than as a nutritional source. Continuous feeding of fish with marigold might increase pigmentation at least in *Xiphophorus helleri*.

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References

Alagappan, M., Vijila, K. and Archana, S. 2004. Utilization of spirulina algae as a source of carotenoid pigment for blue gouramis (*Trichogaster trichopterus* Pallas). Journal of Aquaricult. and Aquatic Sci., 10(1): 1-11.

Anderson, S. 2000. Salmon color and the consumer. IIFET Proceedings. 1–4.

Boonyaratpalin, M. and Lovell, R.T. 1977. Diet preparation for aquarium fish. Aquaculture, 12: 53-62.

Boonyaratpalin, M. and Phromkunthong, W. 1986. Effects of carotenoid pigments from different sources on colour changes of fancy carp, *Cyprinus carpio* Linn. J. Sci. Technol., 8(1): 11-20.

Boonyaratpalin, M. and Unprasert, N. 1989. Effects of pigments from different sources on colour changes and growth of red *Oreochromis niloticus.* Aquaculture, 79(1-4): 375-380.

Choubert, G. 1979. Tentative utilization of spirulina algae as a source of carotenoid pigments for rainbow trout. Aquaculture, 18: 135-143.

Cyanotech. 2002. Analysis of Beta-carotene and Total carotenoids from spirulina (Spectrophotometric method). *Spirulina pacifica*. Technical Bulletin, #003b, Cyanotech corporation. www. cyanotech.com.

Earle, K.E. 1995. The nutritional requirements of ornamental fish. Vet. Q., 17(1): 53–55.

Halten, B., Arnmesan, A., Jobling, M. and Bjerkeng, B. 1997. Carotenoid pigmentation in relation to feed intake, growth and social integration in Arctic char, *Salvelinus aipinus* (L.), from two anadromous strains. Aquacult. Nutr., 3: 189–199.

Hardy, R. 1980. Fish feed formulation, In: ADCP (Eds.), Fish feed Technology, Agriculture development co-ordination programme. FAO/ADCP/Rep., 80(1-1): 233-239.

Katia O., 2001. Ornamental fish trade – overview. INFOFISH International, 3: 14–17.

Kruger, D.P., Britz, P.J. and Sales, J. 2001. Influence of varying dietary protein content at three lipid concentrations on growth characteristics of juvenile swordtails (*Xiphophorus helleri* Heckel, 1848). Aquar. Sci. Cons., 3: 275-280.

Lovell, R.T. 1992. Dietary enhancement of colour in ornamental fish. Aquaculture Magazine, 18: 77-79.

Mazur, C.F., Tillapaugh, D., Brett, J.R. and Iwama, G.K. 1993. The effect of feeding level and rearing density on growth, feed conversion and survival in chinook salmon (*Onchorhynchus tsawytscha*) reared in salt water. Aquaculture, 117: 129-140.

Olson, A. 1979. A simple dual assay for Vit. A and carotenoids in human and liver. Nutrition report international, 19: 807-813.

Sales, J. and Janssens, G.P.J. 2003. Nutrient requirements of ornamental fish. Aquat. Living Resour., 16: 533-540.

Saxena, A. 1994. Health; colouration of fish. International Symposium on Aquatic Animal Health: Program and Abstracts. Univ. of California, School of Veterinary Medicine, Davis, CA, U.S.A., 94 pp.

Yew-Hu, C. and Shu-Ching, J. 1992. Pigmentation of kuruma prawn, *Penaeus japonicus* Bate, by various pigment sources and levels and feeding regimes. Aquaculture, 102: 333-346.