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EFFECTS OF SUPPLEMENTATION OF MARIGOLD (*TAGETES ERECTA*) OLEORESIN ON GROWTH, SURVIVAL AND PIGMENTATION OF ROSY BARB, *PUNTIUS CONCHONIUS* (HAMILTON)

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ABSTRACT
The study was carried out for a period of 45 days to elucidate the effect of marigold oleoresin on growth, survival and pigmentation of rosy barb, *Puntius conchonius*. Fish with initial weights ranging from 0.44 g to 0.47 g were fed with test diets T_0, T_1, T_2 and T_3 supplemented with marigold oleoresin at levels 0, 60, 120 and 180 ppm respectively. At the end of the feeding trial the total carotenoid concentration in fish muscle found significantly higher ($p < 0.05$) in both male (3.92 ± 0.01 μgg^{-1}) and female (3.35 ± 0.02 μgg^{-1}) fishes fed diet incorporated with 120 ppm marigold oleoresin. Survival rate of fish significantly increased ($p < 0.05$) with the supplementation of marigold oleoresin in the diet, highest being in 120 ppm level (96.66 ± 3.33 %). However, there was no significant effect observed in growth, feed conversion ratio and specific growth rate of the fishes. Carotenoid content analysis from whole fish revealed that incorporation of dietary carotenoid resulted in a significant increase in total carotenoid concentration. The study concluded that cheaper and natural colour enhancer diets for rosy barb can be prepared using marigold oleoresin at 120 ppm level.

INTRODUCTION

Ornamental fish sector plays a vital role as a component of international fish trade, fisheries, aquaculture and development (FAO, 2014). Fish skin pigmentation, fin shape and size of the fish are the major factors which determine the market value of the ornamental fishes (Paripatananont *et al.,* 1999). Intensive culture of ornamental fishes under captivity for a long duration results in faded or degraded colouration of fishes (Saxena, 1994). Since fish cannot biosynthesize carotenoids *de novo* like other animals and they rely on aquatic vegetation in natural environment to meet their carotenoid requirements. So it is essential to supplement carotenoids in their diets (Chatzifotis *et al.,* 2005). These carotenoids are a group of naturally occurring lipid soluble organic pigments that are responsible for the red, orange and yellow colour in the skin, flesh, shell and exoskeleton of aquatic animal (Pailan *et al.,* 2012). Dietary carotenoids play an important role in the regulation of skin and muscle colour in fish (Ahilan *et al.,* 2008). Various synthetic pigments like á-carotene, canthaxanthin, zeaxanthin, and astaxanthin have been used as dietary supplements to enhance the pigmentation of fish and crustaceans (Shahidi *et al.,* 1998). The increasing cost of synthetic pigments and the public concerns on the use of synthetic additives, many alternative natural carotenoid sources have also been studied (Jha *et al.,* 2012). Various dietary trials have been conducted on fishes by supplementing different sources of natural carotenoid pigments, such as yeast (*Rhodotorula sanneii*) (Savolainen & Gyllenberg, 1970), chesnut flowers (Neamtu *et al.,* 1976), dried flowers (Torrissen *et al.,* 1989), paprika (*Capsicum annum*) (Tsushima *et al.,* 1998), carrot (*Daucus carota*), marigold petal (*Tagetes erecta*), China rose petal (*Hibiscus rosasinensis*), rose petal (*Rosa chinensis*) (Ramamoorthy *et al.,* 2010; Pailan *et al.,* 2012), *Rosa indica, Ixora coccinea, Crossandra infundibuliformiss* (Joseph *et al.,* 2011), Spirulina (Guroy *et al.,* 2012) and red pepper (*Capsicum annum*) (Maede *et al.,* 2013) can be potentially incorporated as a carotenoid sources in the fish diet. Among these marigold (*Tagetes erecta*) is a hardly annual branching herb grown in tropical regions of India as loose flower and landscape plant but also as a source of natural carotenoid pigment (Usha Bharathi *et al.,* 2014). Marigold (*Tagetes erecta*), a bright orange tropical flower are a significant source of xanthophylls (Verghese, 1998) and contains lutein as a principal component (Bolanos *et al.,* 2005). Marigold petal meal was used for the tiger barb (*Puntius*

tetrazona), red swordtail (*Xiphophorus helleri*) and gold fish (*Carassius auratus* L.) (Boonyarapatin and Lovell, 1977; Ezhil *et al.*, 2008; Alma *et al.*, 2013) to enhance pigmentation. Marigold flowers contain free fatty acids, waxes, sterols and esterified lutein and its oleoresin is the hexane extract of the dehydrated flowers.

With these in background, the present study was conducted to evaluate the effect of supplementation of marigold oleoresin on the growth and coloration of the rosy barb, *Puntius conchonius* is one of the candidate species for tropical aquariums.

MATERIALS AND METHODS

Feed ingredients and diet formulations

The ingredients used in the formulation of experimental diets and proximate composition of diets are presented in Table 1 and 2 respectively. The analysis of proximate composition of all the ingredients and the test diets was carried out by employing standard methods (AOAC, 2005). A suspension of marigold oleoresin was made in soybean oil by heating it up to 55°C and was mixed with experimental diets on a slow mixer. Three test diets namely T_1, T_2 and T_3 comprising 30% protein content were formulated using the square method (Hardy, 1980). Diet T_1 had 60 ppm, T_2 had 120 ppm and T_3 had 180 ppm marigold oleoresin, and a control diet (T_0) was formulated without marigold oleoresin. The carotenoid quantity incorporated in the different diets was adjusted with the quantity of rice bran.

Experimental protocol

The dietary trials were conducted in 12 fibre glass aquarium tanks (20 L capacity) at the College of Fisheries, Mangalore, Karnataka, India. Rosy barb seedlings of uniform size (0.44 g to 0.47 g) which were bred and reared in the fish farm of College of Fisheries were used for the study. The study was carried out in triplicate for a period of 45 days. The stocking density was maintained at 15 fishes per tank. The fishes were fed at the rate of 5% of their body weight during the entire period of the experiment. The feed was provided twice daily at 08.00 h and 17.00 h. The fishes were sampled once in a fortnight and measured individually to assess the growth. The feeding rate was adjusted based on the weight of fish after each sampling. Fecal matter and uneaten food was removed daily. Water in the experimental tanks was replaced with fresh and clean water every day to maintain optimal water quality. The physico-chemical parameters of water were estimated following standard methods (APHA, 1998). At the end of the experimental period survival rate and wet weight of the fishes were recorded. The growth parameters such as weight gain, Relative growth rate (RGR), Specific growth rate (SGR) and Feed conversion ratio (FCR) were calculated using the following formulae.

Net weight gain (g) = Final mean weight – Initial mean weight

Relative growth rate (%) = [(Final mean weight – Initial mean weight) / Initial mean weight] x100

Specific growth rate (%) = [(ln final weight – ln initial weight) / Rearing period (day)] x100

Feed conversion ratio = Total dry feed offered (g) / Total wet weight gain (g)

Carotenoid analysis

Total carotenoid concentration (TCC) in the muscle tissue of both male and female fishes was analysed immediately after the completion of experiment following the pigment extraction method as described by Olson (1979). One gram body tissue of *Puntius conchonius* separated from head and alimentary canal was taken in a 10 ml screw caped clear glass vial and added with 2.5 g of anhydrous sodium sulphate. The sample was gently mashed and then 5 ml of chloroform was added and left overnight at 0°C. When the chloroform formed a clear layer of 1-2 cm above the caked residue, the optical density was read at 380, 450, 470 and 500 nm, in spectrophotometer (Systronics Visiscan-167) 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 wave length at which maximum absorption, was used for the calculation.

Total carotenoid concentration (μg/g wet wt.) = [Absorption at maximum wave length / (0.25 X sample weight (g)] x 10

Where, 10 = dilution factor; 0.25 = Extinction coefficient

Statistical analysis

The experimental results were tabulated and analyzed statistically by using one-way analysis of variance (ANOVA). Duncan's multiple range test was used for mean separation

RESULTS

Water quality parameters

The physico-chemical parameters of water in the experimental tanks during the experimental period are presented in Table 4. The water quality parameters were maintained within the normal range required for tropical fishes *viz.*, temperature 24 to 30ºC, dissolved oxygen $>$ 5 mg l^{-1}, free carbon dioxide $<$ 5 mg l^{-1}, ammonia-nitrogen $<$ 0.1 mg l^{-1}, pH 7 to 8.5 and total alkalinity 50 to 300 mg l^{-1} (Santhosh and Singh, 2007) throughout the experimental period.

Growth and survival

The proximate composition of all the experimental diets was almost similar and the protein content in the diets was approximately 30 % (Table 2). The survival and growth rates of the fishes at end of the experimental period are shown in Table 3. There were no significant effects ($p > 0.05$) of marigold oleoresin supplementation on growth parameters of rosy barb. However, fishes fed with 120 ppm marigold oleoresin supplemented diet showed marginally increased values in net weight gain (0.68 ± 0.07 g), relative growth rate (145.46%), specific growth rate (1.99%) and low feed conversion ratio (1.43) than the other treatments and control group. Dietary supplementation of marigold oleoresin significantly ($p < 0.05$) affected the survival rate of the rosy barb. The survival rate of fishes fed with marigold oleoresin incorporated diets and control diet ranged from 76.66 % to 96.66 % (Table 3). Fishes fed with diet containing 120 ppm marigold oleoresin had the highest survival rate of 96.66 %.

Total carotenoid concentration (TCC)

Total carotenoid concentration of both male and female fishes fed with experimental diets and control diet is given in Fig. 1.

Table 1: Ingredients and their proportion in experimental diets

Diet ingredients(g/1000 g feed)	T_0	T_1	T_2	T_3
Fish meal	209.10	209.10	209.10	209.10
Soybean meal	209.10	209.10	209.10	209.10
Wheat flour	209.10	209.10	209.10	209.10
Groundnut cake	114.10	114.10	114.10	114.10
Rice bran	114.14	114.04	113.98	113.92
Tapioca flour	114.10	114.10	114.10	114.10
Soyabean oil	20.00	20.00	20.00	20.00
[1]Vitamins and mineral mix (Agrimin forte)	10.00	10.00	10.00	10.00
Marigold oleoresin	-	0.06	0.12	0.18

[1]Vitamins and mineral mix (mg kg^{-1} feed) (Vitamin A 700000 IU; Vitamin D3 70000 IU; Vitamin E 250 mg; Nicotinamide 1000 mg; Cobalt 150 mg; Copper 1200 mg; Iodine 325 mg; Iron 1500 mg; Magnesium 6000 mg; Potassium 100 mg; Sodium 5.9 mg; Manganese 1500 mg; Sulphur 0.72%; Zinc 9600 mg; Calcium 25.5%; Phosphorus 12.75%)

Table 2: Proximate composition (%) of different experimental diets

Treatments	Dry matter	Moisture	Protein	Crude lipid	Ash	Crude fibre	NFE*
T_0 (Control)	92.18±0.21	7.82±0.21	30.23±0.29	7.76±0.14	10.62±0.08	6.23±0.06	37.34±0.14
T_1 (60 ppm)	92.31±0.16	7.69±0.13	30.31±0.11	8.16±0.12	11.15±0.17	6.54±0.08	36.15±0.21
T_2 (120 ppm)	92.37±0.21	7.63±0.31	30.02±0.02	7.92±0.07	11.26±0.20	6.14±0.18	37.03±0.21
T_3 (180 ppm)	92.64±0.04	7.36±0.11	30.13±0.12	8.13±0.05	10.72±0.29	6.36±0.11	37.10±0.10

Nitrogen free extract = 100 - (% moisture + % crude protein + % crude lipid + % crude fibre + % ash)

Table 3: Growth performance, feed conversion ratio and survival rate of rosy barb fed different experimental diets

	Diets T_0	T_1	T_2	T_3
Initial mean wt. (g)	0.44±0.01	0.45±0.01	0.47±0.01	0.45±0.02
Final mean wt. (g)	0.91±0.02	1.10±0.03	1.16±0.08	1.07±0.05
Net Weight Gain (g)	0.47±0.01	0.65±0.02	0.68±0.07	0.62±0.07
Relative Growth Rate (%)	106.81±0.15	144.94±10.16	145.46±12.86	137.30±9.45
SGR (%)	1.53±0.07	1.96±0.08	1.99±0.12	1.92±0.08
FCR	1.62±0.05	1.43±0.01	1.43±0.08	1.55±0.10
Survival rate (%)	76.66 ± 3.33^{c}	89.99 ± 3.34^{ab}	96.66 ± 3.33^{a}	83.33 ± 3.33^{bc}

The means with different superscript in each row indicate a significant difference ($p < 0.05$). Each value is a Mean ± SE ($n = 3$).

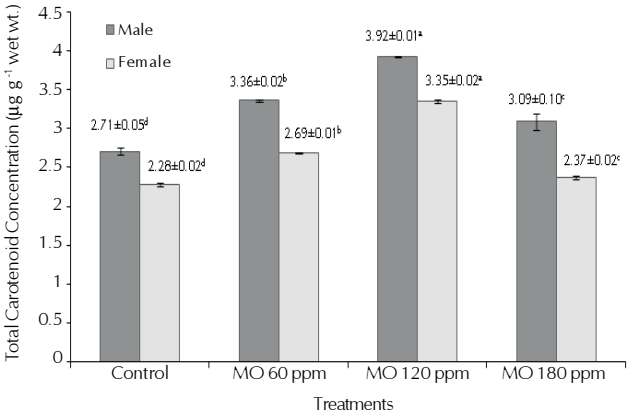
Table 4: Physico-chemical parameters of water in the experimental tanks during experimental period

Parameters	Minimum	Maximum
Temperature (°C)	27.0°C	30.5°C
Dissolved Oxygen (mg l^{-1})	6.20	7.14
pH	6.4	7.2
Free Carbon dioxide (mg l^{-1})	1.49	2.23
Total alkalinity (mg l^{-1} $CaCO_3$)	71	96
Ammonia- Nitrogen (μg l^{-1})	0.04	0.52

In general female fishes are lighter in colouration and fetches low price in the market. In this study both female and male fishes were evaluated separately for their carotenoid concentration in muscle tissue after the end of feeding trial. The fishes fed with diet having 120 ppm marigold oleoresin contained the highest total carotenoid concentration of 3.92 µg g^{-1} and 3.35 µg g^{-1} wet weight of muscle tissue in male and female fishes respectively and which are significantly higher than the other treatments ($p < 0.05$). In addition, the fishes fed with 120 ppm marigold oleoresin incorporated diet showed bright yellow to orange colouration than the other treatments as shown in Fig. 2. The colour of fishes fed with 60 ppm marigold oleoresin diet slightly resembled the colour of fishes fed with 120 ppm incorporated diet. The fishes showed faded colouration when fed with diets having higher inclusion levels of marigold oleoresin.

DISCUSSION

The effects of carotenoids on growth and survival of aquatic animals are controversial (Jha *et al.,* 2012). Many studies on the effect of incorporation of carotenoid in the diets of fishes like Atlantic salmon fry (*Salmo salar*), rainbow trout (*Oncorhyncus mykiss*), goldfish (*Carassius auratus*) and characins (*Hyphessobrycon callistus*) have shown positive effect on growth and survival of the fishes. (Christiansen *et al.,* 1995; De la Mora *et al.,* 2006; Sinha and Asimi, 2007; Pan *et al.,* 2010). Where as, the studies conducted to evaluate the effects of dietary carotenoids on fishes like porgy (*Pagrus auratus* & *Pagrus pagrus*) (Chatzifotis *et al.,* 2005; Kalinowsky *et al.,* 2005), characins (*Hyphessobrycon callistus*) (Wang *et al.,* 2006), *Cichlasoma severum* (Kop *et al.,* 2010), marine ornamental fish (*Amphiprion ocellaris*) (Ramamoorthy *et al.,* 2010) and goldfish (*Carassius auratus* L.) (Alma *et al.,* 2013) have not shown any significant effects on growth and survival when compared with fish fed with control diet. In our previous study, diet containing 60 ppm marigold oleoresin improved the growth of orange chromide (*Etroplus maculatus*) with respect to specific growth rate, relative growth rate, and absolute growth rate. But there was no significant effect found in FCR and survival rate of fish (Jagadeesh *et al.,* 2014). The results obtained in the present study are comparable with the recent studies conducted by Ramamoorthy *et al.* (2010) and Alma



Means with different superscript indicate significant difference ($p < 0.05$). Each value is a Mean ± SE (n = 3)

Figure 1: Total Carotenoid Concentration (µg/g wet wt.) of male and female fishes fed with diets containing marigold oleoresin at different incorporation levels

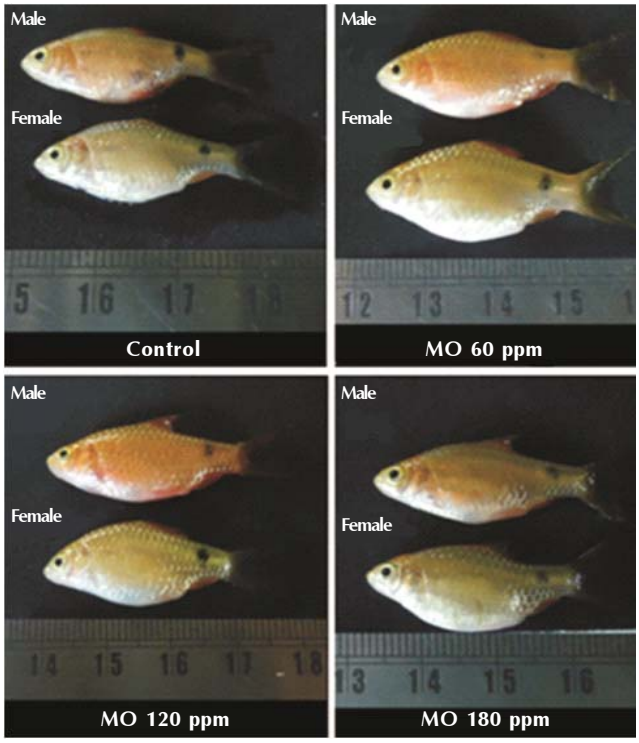


Figure 2: Differences in colour of male and female fishes fed with different experimental diets

et al. (2013) where they reported that incorporation of marigold meal in fish diet do not promote the growth of fish. Similar to our previous study on *Etroplus maculatus* the fishes fed with diet having marigold oleoresin contained the highest total carotenoid concentration (Jagadeesh *et al.,* 2014). Jha *et al.* (2012) observed that dietary supplementation of marigold flower meal at 10% produced the highest carotenoid deposition in the flesh of Snow Trout (*Schizothorax richardsonii*). Adnankhan *et al.* (2015) tested dietary inclusion of plant sources such as marigold and Ixora at 5% as pigment enhancers in the diet of sword tail (*Xiphophorus helleri*) and observed the maximum carotenoid content in treatment groups fed with Marigold diet followed by Ixora diet. The carotenoid source and its effectiveness on deposition of pigments in fish body is species specific (Ha *et al.,* 1993). Also sexual dichromatism is often common in several fish species. In general males are more brightly coloured than their female counterparts (Rinku Gogoi *et al.,* 2013). In the present study even female fishes fed with carotenoid diets also exhibit the remarkable pigmentation due to increased total carotenoid concentration in their body. Fish species may exhibit varying pathways for carotenoid metabolism (Matsuno, 2001). Alma *et al.* (2013) reported that 200 mg of carotenoids from marigold meal is optimum to increase the pigmentation in skin of goldfish and over that level they have not found any additional accumulation of carotenoids. Similarly, in the present study, fish fed with 120 ppm marigold oleoresin diet showed increased level of carotenoids in their muscle tissue than other treatment fishes. The extended levels of marigold oleoresin did not show any increase in the colour of the fishes. The dietary supplementation of marigold oleoresin did not have any remarkable effects on growth of rosy barb. The results of this study thus showed increased survival rate and carotenoid deposition in the muscle tissue of both male and female fishes with the incorporation of marigold oleoresin at an optimal level (120ppm) in diet. So, marigold oleoresin, a cheap source of carotenoids is easily available and could be effectively utilised to increase colouration of rosy barb. Hence the findings of this investigation could be taken advantage of in the rearing of the ornamental rosy barb, *Puntius conchonius*.

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