Fishery Technology 51 (2014) : 25 - 30

Effect of Marigold Oleoresin on Growth, Survival and Pigmentation in Orange Chromide, *Etroplus maculatus* (Bloch, 1795)

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Abstract

The study was conducted to evaluate the effect of marigold oleoresin on growth, survival and total carotenoid content in muscle tissue of *Etroplus maculatus*. Fish with size group ranging from 0.60 g to 0.62 g were used for the study. The study was carried out in triplicate groups for a period of 45 days. Fishes were stocked at the rate of 15 per tank. Three test diets namely T_1, T_2 and T_3 with 30% protein level were formulated. Diet T_1 had 60 ppm, T_2 had 120 ppm and T_3 had 180 ppm marigold oleoresin, and diet without marigold oleoresin supplementation served as control (T_0). There was no significant effect in survival rate and feed conversion ratio of the fish ($p>0.05$). However, 60 ppm marigold oleoresin fed fishes showed increased weight of 1.04±0.08 g, lower feed conversion ratio of 1.53±0.05 and higher survival rate (82.14%) than the other treatment groups. The fishes fed with 60 ppm marigold oleoresin incorporated diet showed significantly higher specific growth rate of 1.36±0.21%, relative growth rate of 69.10±3.4% and the absolute growth rate of 0.42±0.02 g. The body colouration and total carotenoid concentration of muscle tissue (4.62±0.02 μg g^{-1}) was significantly higher in fish fed with 60 ppm marigold oleoresin diet. Among the tested doses, 60 ppm marigold oleoresin showed better results than 120 ppm and 180 ppm marigold oleoresin inclusion in the fish diet.

Keywords: Marigold oleoresin, *Etroplus maculatus*, carotenoid, feed convertion ratio

Received 01 June 2013; Revised 13 September 2013; Accepted 19 November 2013

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Introduction

Etroplus maculatus, also known as orange chromide, belongs to the genus Etroplus, which is the only genus endemic to India. The fish is found to be widely distributed in almost all rivers and backwaters of Peninsular India and Sri Lanka (Jayaram, 1999). Due to its calm nature, small size with attractive bright yellow to orange colour and round black blotches on flanks *E. maculatus* became one of the candidate species for tropical aquariums (Bindu & Padmakumar, 2012). Dietary carotenoids play an important role in the regulation of skin and muscle colour in fish (Ahilan et al., 2008). Culture of ornamental fishes under high density in captive condition without supplementation of the dietary carotenoids leads to faded colouration and decreased commercial value of fish (Harpaz & Padowicz, 2007). In natural environment, fishes depend on aquatic vegetation to meet their carotenoid requirements. Since fish cannot biosynthesize carotenoids de novo like other animals it is essential to provide them in their diets (Chatzifotis et al., 2005). Various synthetic pigments like α-carotene, canthaxanthin, zeaxanthin, and astaxanthin and also natural sources such as yeast, bacteria, algae, higher plants, and crustacean meal have been used as dietary supplements to enhance the pigmentation of fish and crustaceans (Kalinowski et al., 2005; Shahidi et al., 1998). The increasing cost of synthetic pigments has made researchers to evaluate the natural compounds such as yeast, marine bacteria, green algae and even plant extracts as pigment sources. Spirulina have been used as a source of carotenoid pigment for rainbow trout, fancy carp and yellow tail cichlid *Pseudotropheus acei* (Guroy et al., 2012). Marigold petal meal was used for the tiger barb and red swordtail (Boonyarapatin & Lovell,

1977; Ezhil et al., 2008) to enhance their pigmentation. Ramamoorthy et al. (2010) have used natural carotenoid sources such as carrot (*Daucus carota*), marigold petal (*Tagetes erecta*), China rose petal (*Hibiscus rosasinensis*) and rose petal (*Rosa chinensis*) and found that they can enhance colour of marine ornamental fish *Amphiprion ocellaris.* Similarly *Hibiscus rosasinensis, Rosa indica, Ixora coccinea* and *Crossandra infundibuliformiss* have been utilized to enhance the growth and body colouration of red sword tail, *Xiphophorus hellerei* (Joseph et al., 2011). The orange chromide is recently gaining popularity in the world of ornamental fishes due to its brilliant colours.

There is limited information in the literature on the colour enhancement studies of the orange chromide. Marigold oleoresin, which is the hexane extract of the dehydrated marigold flowers (*Tagetes erecta*) contain free fatty acids, waxes, sterols and the esterified lutein. Therefore, the study was conducted to evaluate the effect of marigold oleoresin as a carotenoid substrate on the growth and coloration of orange chromide, *Etroplus maculatus.*

Materials and Methods

The study was conducted in 12 fibre glass aquarium tanks (20 l capacity) at the College of Fisheries, Mangalore to evaluate the effect of Marigold oleoresin on growth, survival and total carotenoid content in muscle tissue of *Etroplus maculatus*. Uniform size group (0.60 g to 0.62 g) of orange chromides (*Etroplus maculatus*) were procured from a commercial aquaculture fish farm and were acclimatized to experimental conditions for a week. Fish were fed with dry pellet diet at the rate of 5% of their body weight. The study was carried out in triplicate for a period of 45 days. Fishes were stocked at the rate of 15 per tank. The water quality parameters were maintained within the normal range required for tropical fishes viz., temperature 24 to 30°C, 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} as per Santhosh & Singh (2007) throughout the experimental period by replacing 50% of the water in experimental tanks with fresh and clean water every day.

The ingredients used in the formulation of different experimental diets were fishmeal, soybean meal, groundnut oil cake, wheat flour, rice bran, tapioca flour, vitamin and mineral premix. All the ingredients were procured from the local market and were analyzed for proximate composition prior to formulation of the test diets employing standard methods (AOAC, 2005). Moisture content was estimated by hot air oven method. The samples were heated at 105°C for 16 h and then cooled and weighed to a constant weight. Crude protein was analyzed using Kjeltec system (Tecater 1002 Distilling Unit), fat content by Soxtech system (Tecater 1043 Extraction Unit), fibre content by using Fibretech System (Tecater 1017 Hot Extractor). Ash content was determined by first drying the sample and then heating it in a muffle furnace at 550±10°C for 6 h. Carbohydrate content was calculated as nitrogen free extract (NFE) by the difference method (Hastings, 1976). Marigold oleoresin was the hexane extract of the dehydrated marigold flowers (*Tagetes erecta*) which was procured from M/S Avesthagen Ltd, Bangalore. A suspension of marigold oleoresin was made in soybean oil by heating it upto 55°C and was mixed with experimental diets on a slow mixer. Three test diets namely T_1, T_2 and T_3 with 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 diet without marigold oleoresin supplementation served as control (T_0). Proximate composition of the experimental diets is given in Table 1.

Fish were fed at the rate of 5% of their body weight till the end of the experiment. The feed was dispersed over the surface of water twice daily at 08.00 h and 17.00 h. After each sampling, the quantity of feed given was re-adjusted based on the increased weight of fish. The fishes were sampled once in a fortnight to assess the growth. The stocked fish were collected during each sampling and measured individually for growth parameters. After the experimental period of 45 days all the survived fishes were collected and their weight and survival data were recorded. The other growth parameters such as Absolute growth rate (AGR), Relative growth rate (RGR), Specific growth rate (SGR) and Feed conversion ratio (FCR) were calculated by using the following formulae.

$$\text{Absolute growth rate (g)} = \text{Final mean weight} - \text{Initial mean weight}$$

$$\text{Relative growth rate (\%)} = \frac{(\text{Final mean weight} - \text{Initial mean weight})}{\text{Initial mean weight}} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Rearing period (day)}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Total dry feed offered (g)}}{\text{Total wet weight gain (g)}}$$

The physico-chemical parameters of water such as temperature, carbon dioxide, dissolved oxygen, ammonia and pH in all the experimental tanks were estimated (APHA, 1998). Total carotenoid concentration (TCC) in fish muscle tissue was analysed immediately after the completion of experiment following the pigment extraction method as described in Olson (1979). One gram body tissue of *Etroplus maculatus* separated was taken in a 10 ml screw capped clear glass vials and added with 2.5 g of anhydrous sodium sulphate. The sample was gently mixed 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 layer of 1-2 cm 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 wave length, at which there was maximum absorption, was used for the calculation.

$$\text{Total carotenoid concentration } (\mu g\ g^{-1} \text{ wet wt}) = \frac{\text{Absorption at maximum wave length}}{(0.25 \times \text{sample weight (g)})} \times 10$$

Where, 10 = dilution factor; 0.25 = Extinction coefficient

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

Table 1. Ingredients and chemical composition of experimental diets

Diet ingredients (g 1000 g^{-1} feed)	T_0 (Control)	T_1 (60 ppm marigold oleoresin)	T_2 (120 ppm marigold oleoresin)	T_3 (180 ppm marigold oleoresin)
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	114.10	114.10	114.10	114.10
Tapioca flour	114.10	114.10	114.10	114.10
Soyabean oil	20.00	20.00	20.00	20.00
Vitamins and mineral mix[1] (Agrimin forte)	10.00	10.00	10.00	10.00
Marigold oleoresin	-	60 mg	120 mg	180 mg
Proximate composition (%)				
Dry matter	92.18±0.21	92.31±0.16	92.37±0.21	92.64±0.04
Moisture	7.82±0.21	7.69±0.13	7.63±0.31	7.36±0.11
Protein	30.23±0.29	30.31±0.11	29.72±0.02	29.23±0.12
Crude lipid	7.76±0.14	8.16±0.12	7.92±0.07	8.13±0.05
Ash	10.62±0.08	11.15±0.17	11.26±0.20	10.72±0.29
Crude fibre	6.23±0.06	6.54±0.08	6.14±0.18	6.36±0.11
NFE[2]	37.34±0.14	36.15±0.21	37.33±0.21	38.20±0.10

[1] Vitamins and mineral mix (mg kg^{-1} feed) (Vitamin A 700000 IU; Vitamin D_3 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%)

[2] Nitrogen Free Extract = 100 - (% Moisture + % Crude protein + % Crude lipid + % Crude fibre + % Ash)

Results and Discussion

The changes in growth performance during the 45 days of feeding period are shown in Table 2 and Fig. 1. The feed conversion ratio values were not significantly ($p>0.05$) affected by the diets supplemented with different concentrations of marigold oleoresin. However, the lowest and best feed conversion ratio (1.53±0.05) was observed in fishes fed with 60 ppm marigold oleoresin incorporated diet. Increased final mean weight (1.04±0.01 g) compared to other treatments was also recorded in fishes fed with 60 ppm marigold oleoresin incorporated diet. Significantly higher specific growth rate (1.36 %), relative growth rate (69.10 %) and absolute growth rate (0.42 %) were also found in fish fed with 60 ppm marigold oleoresin diet than other treatments ($p < 0.05$). The survival rate of fishes fed with marigold oleoresin supplemented diets and control diet ranged from 67.85 % to 82.14 % (Table 2). Fishes fed with diet containing 60 ppm marigold oleoresin had the highest survival rate of 82.14 %, although there was no significant difference in the survival rate ($p > 0.05$).

Total carotenoid concentration of the fishes fed with experimental diets and control diet is shown in Fig. 1. The fishes fed with diet having 60 ppm marigold oleoresin contained the highest total carotenoid concentration of 4.62 μg g^{-1} wet weight of muscle tissue which is significantly higher than the other treatments ($p< 0.05$). In addition, the fishes fed with 60 ppm marigold oleoresin incorporated diet showed darker yellow spots on the body than the other treatments as shown in Fig. 2. The colour of fishes fed with control diet slightly resembled but was less than the colour of fishes fed with 60 ppm marigold oleoresin incorporated diet. But the fishes showed faded colouration when fed with diets having higher inclusion levels of marigold oleoresin.

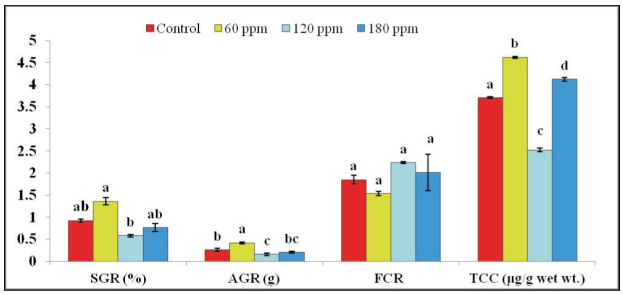

Fig. 1. Specific Growth Rate, Absolute Growth Rate, Feed Conversion Ratio and Total Carotenoid Concentration of *Etroplus maculatus* fed with diets containing marigold oleoresin

Means with different superscript indicate significant difference ($p < 0.05$)

Each value is a Mean ± SE ($n = 3$)

Many commercial products extracted from marigold flower (*Tagetes erecta*) are being used throughout the world by poultry and nutraceutical industries. These products are rich in lutein and zeaxanthin, which play an important role as precursors of astaxanthin and enhance pigmentation of animals (Del Villar-Martínez et al., 2007). In the present study the improved growth performance with respect to final mean weight, specific growth rate, absolute growth rate and relative growth rate was higher in fish fed with 60 ppm marigold oleoresin than the higher inclusion levels such as 120 ppm and 180 ppm. Also the fish fed with 60 ppm marigold oleoresin supplemented diets showed highest survival rate. The results of the present experiment clearly indicate that the fish fed with marigold oleoresin incorporate at lower level (60 ppm) showed bright yellow colouration with significantly higher carotenoid accumulation in the body. The results obtained in the current study are supported by Harpaz & Padowicz (2007), who examined the

Table 2. Changes in growth performance and Survival rate of *Etroplus maculatus* fed with different diets

Treatments	Initial mean wt. (g)	Final mean wt. (g)	Relative Growth Rate (%)	Survival rate (%)
T_0	0.62±0.01	0.89±0.08	$42.60±4.2^{b}$	$71.42±7.14^{a}$
T_1	0.61±0.01	1.04±0.11	$69.10±3.4^{a}$	$82.14±3.87^{a}$
T_2	0.62±0.01	0.78±0.03	$25.80±4.4^{c}$	$67.85±3.57^{a}$
T_3	0.60±0.02	0.81±0.05	$35.00±1.5^{bc}$	$71.42±6.14^{a}$

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

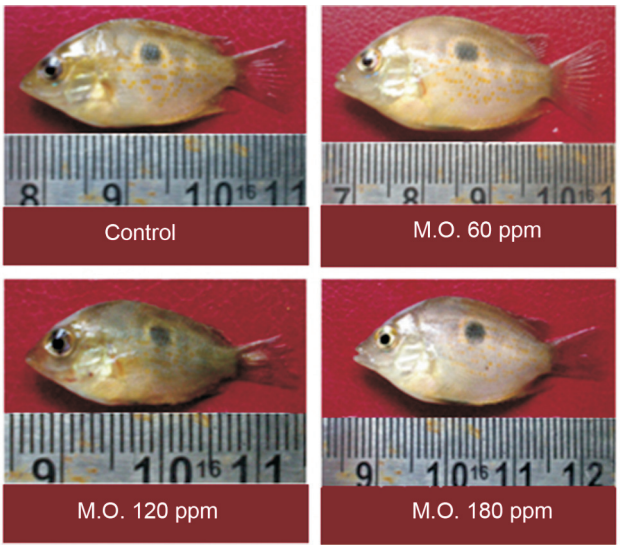

Fig. 2. Effect of marigold oleoresin on colour development in *Etroplus maculatus*

effects of adding carotenoids from oleoresin paprika at 60, 120, and 240 mg kg^{-1} to fish feeds for ornamental dwarf cichlid, *Microgeophagus ramirezi* for 45 days. They found that there was no significant effect on overall growth among treatments and the addition of 60 mg carotenoid per kg diet resulted in slightly better growth and they opined that addition of 60 mg oleoresin paprika per kg diet is sufficient to obtain good coloration in *M. ramirezi*. Wang et al. (2006) found no differences in growth and survival of the ornamental fish *Hyphessobrycon callistus* (neon serape tetra) when fed with diets containing two types of carotenoids, astaxanthin and â-carotene at three concentrations (10, 20, and 40 mg kg^{-1}) for 8 weeks. They also reported that the body content for astaxanthin and â-carotene increased with increased dietary carotenoid concentration. Vernon-Carter et al. (1996) have found that the total carotenoid concentration in the body of *Litopenaeus vannamei* has increased when shrimp fed with diets supplemented with oleoresin from marigold flowers. Ahilan et al. (2008) demonstrated the effect of three additives such as coriander, mint and amaranth leaves at 1 to 5 % on the growth and body colouration of goldfish. They observed that the significant difference between 1 per cent level and control in mint fed groups, also the groups fed with higher concentrations (3 and 5 per cent levels) showed lesser growth than the control. Feed incorporated with mint at 3 per cent level improved the colouration and 1 per cent level enhanced the growth. They concluded that, amaranth and mint at

Table 3. Physico-chemical parameters of water in the experimental tanks during experimental period

Parameters	Recorded values
Temperature (°C)	27.5 – 29°C
Dissolved Oxygen (mg l^{-1})	5.34 – 6.40
pH	6.9 - 7.5
Free Carbon dioxide (mg l^{-1})	1.30 – 2.77
Total alkalinity (mg l^{-1})	64 - 91
Ammonia- Nitrogen (µg l^{-1})	0.04 - 0.41

1% level enhanced the growth and colouration in adult goldfish. While reduced growth in gold fish fed with excess levels of carotenoid reduced the growth. Similarly in the present study the reduced growth performance was observed in fishes fed diets incorporated with 120 ppm and 180 ppm marigold oleoresin.

The water quality of the experimental tanks during the 45 days of experimental period is presented in Table 3. There was not much variation in water quality parameters among the different treatments due to daily exchange of water. In the present study, important water quality parameters such as temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and ammonia-nitrogen measured in different treatments throughout the experimental period were found to be well within the acceptable range. The present study indicated that *Etroplus maculatus* fed with diet containing 60 ppm marigold oleoresin showed improved growth, survival and total carotenoid concentration of muscle tissue than the fish fed with diets containing 120 ppm and 180 ppm marigold oleoresin.

Acknowledgement

Authors are thankful to M/S Avesthagen Ltd, Bangalore, for supplying carotenoids to conduct research.

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