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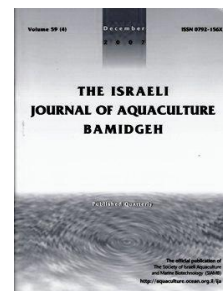
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Effect of Marigold Flower and Beetroot Meals on Growth Performance, Carcass Composition, and Total Carotenoids of Snow Trout (*Schizothorax richardsonii*)

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Key words: *Beta vulgaris*, carotenoids, FCR, growth, SGR, *Schizothorax richardsonii*, *Tagetes erecta*

Abstract

A 60-day experiment was carried out to elucidate the effect of marigold (*Tagetes erecta*) flower and beetroot (*Beta vulgaris*) meals on growth performance, carcass composition, and total carotenoids of snow trout, *Schizothorax richardsonii*. Two hundred and seventy fingerlings (9.19±0.29 g) were randomly distributed into nine treatments in triplicate (10 fish per tank). Nine isonitrogenous (35.25±0.9% crude protein) diets were prepared with graded levels (3%, 5%, 7%, 10%) of either marigold flower meal or beetroot meal; the control diet contained neither marigold flower nor beetroot meal. Weight gain and specific growth rate were significantly ($p<0.05$) higher in fish fed the diet containing 10% beetroot meal than in those fed the control diet. Body carotenoid was significantly enhanced ($p<0.05$) by the dietary supplements and increased linearly with the increase of marigold flower meal ($Y = 0.532x + 1.126$, $R^2 = 0.9803$) and beetroot meal ($Y = 0.491x + 1.341$, $R^2 = 0.9376$). Results indicate that inexpensive and readily available natural carotenoid sources such as marigold flower and beetroot meals can be incorporated into diets for *S. richardsonii* to enhance pigmentation and ornamental value.

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Introduction

Snow trout, *Schizothorax richardsonii*, is a small indigenous coldwater fish locally known as asela. It belongs to the family Cyprinidae, subfamily Schizothoracinae, that is widely distributed in Himalayan and sub-Himalayan regions. *Schizothorax richardsonii* thrives well in coldwater streams, lakes, and rivers and is commercially important due to its ornamental and food value. Hence, it is widely cultured in the hilly regions of Himalaya.

The food value of fish is determined by the quality and quantity of protein and other nutrients in the muscle while the ornamental value is associated with coloration due to carotenoids or pigment-bearing substances in the tissues (Halten et al., 1997). Carotenoids are most conspicuous in petals, pollen, fruit, tomatoes, citrus fruits, and some roots (Tacon, 1981). Higher animals, including fish, are unable to synthesize carotenoids *de novo* (Goodwin, 1984), and are dependent on dietary sources (Hata and Hata, 1972). Therefore, there is a direct relationship between dietary carotenoids and pigmentation in fish (Halten et al., 1997). Fishes in the wild obtain the food of the quality required for proper growth, pigmentation, and nutrient profile. But in captive conditions, a lack of nutrients and pigment-bearing substances can result in retarded growth, faded coloration, and a degraded nutrient profile of the fish. Diets suitable for pigmentation, nutrient quality, and growth have been determined for Atlantic salmon (Storebakken et al., 1987), rainbow trout (Choubert and Storebakken, 1989; Bjerkgeng et al., 1992), and ornamental dwarf cichlids (Harpaz, 2007).

Carotenoid pigments can be produced commercially and are commonly used for pigmentation of fish including salmonids (Yanar et al., 2007). However, because of public concerns about the use of synthetic additives, alternative natural carotenoid sources have also been studied. In the aquaculture industry, feed additives such as carrots, red peppers, marigold flowers, rose petals, China roses, chestnut flowers, spirulina, crustacean waste, yeast, synthetic astaxanthin, vitamin C, and vitamin E have long been used to obtain the desired quality of fish (Ellis, 1979; Kim et al., 1999; Ezhil et al., 2008; Yeşilayer et al. 2011; Yilmaz and Ergün, 2011). Red pepper and marigold flower can be used to enhance color in rainbow trout (Yanar et al., 2007). Marigold flower and beetroot are readily available sources of pigmentation in the lower stretches of the Himalayan region. Thus, in this experiment we elucidate the effects of marigold flower and beetroot, which are inexpensive, abundant, and rich in carotenoids, on growth performance, carcass composition, and total carotenoids in snow trout fingerlings.

Materials and Methods

Experimental fish. Fingerlings of *Schizothorax richardsonii* (9.19 ± 0.29 g) were collected from the local Nainital stream in Uttarakhand and transported in a 500-l circular container with sufficient aeration to the experimental facilities at the hatchery complex of the Directorate of Coldwater Fisheries Research in Bhimtal, India. Fish were acclimated to the experimental rearing conditions for one week. During acclimation, fish were fed the control diet.

Experimental design and feeding. Nine isonitrogenous diets ($35.25 \pm 0.9\%$ crude protein) were prepared with graded levels (3%, 5%, 7%, or 10%) of either marigold flower or beetroot meal; the control diet contained neither marigold flower or beetroot meal (Table 1). After acclimation, the 270 fingerlings were randomly distributed into twenty-seven 100-l fiberglass tanks (nine diet groups in triplicate) following a completely randomized design. Groups were fed their respective diets for 60 days at 5% of the body weight daily, divided into two doses; two thirds of the daily ration was given at 09:00 and one third at 18:00. Fecal matter was removed by siphoning. The flow-through system had a water flow of 2-3 l/min through an inlet at one and an outlet at the opposite end of each tank. Water quality was within optimum ranges throughout the experiment: dissolved oxygen 6.0-8.5 mg/l, pH 7.3-8.2, temperature 18-20°C.

Proximate analysis of feed. The proximate composition of the experimental diets was determined following standard methods of AOAC (1995). Moisture was determined by drying at 105°C to a constant weight. Ash was determined by incinerating the samples in a muffle furnace at 600°C for 6 h. Nitrogen was estimated by the Kjeldahl method (2200

Kjeltec Auto distillation, Foss Tecator, Sweden) and crude protein was estimated by multiplying the percent nitrogen by 6.25. Ether extract was measured by the solvent extraction method (1045 Soxtec extraction unit, Tecator, Sweden) using diethyl ether (boiling point 40-60°C) as a solvent.

Table 1. Diet composition (%) and proximate analysis (% dry matter basis), means±SE, n = 3.

	Diet									
	Control	Marigold flower meal (%)					Beetroot meal (%)			
		3	5	7	10	3	5	7	10	
Fishmeal	20.27	20.27	20.27	20.27	20.27	20.27	20.27	20.27	20.27	20.27
Soybean meal	20.27	20.27	20.27	20.27	20.27	20.27	20.27	20.27	20.27	20.27
Wheat bran	21.73	21.73	21.73	21.73	21.73	21.73	21.73	21.73	21.73	21.73
Rice bran	29.73	26.73	24.73	22.73	19.73	26.73	24.73	22.73	19.73	19.73
Fish oil ¹	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vegetable oil ²	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin-mineral mix ³	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sodium alginate ⁴	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Marigold flower meal	-	3.00	5.00	7.00	10.00	-	-	-	-	-
Beetroot meal	-	-	-	-	-	3.00	5.00	7.00	10.00	-
<i>Proximate analysis (%)</i>										
Moisture	13.28±0.02	12.89±0.03	12.50±0.01	12.85±0.05	12.69±0.02	11.85±0.02	12.15±0.02	12.54±0.03	11.90±0.02	11.90±0.02
Dry matter	86.72±0.25	87.11±0.41	87.50±0.23	87.15±0.33	87.31±0.54	88.15±0.02	87.85±0.02	87.46±0.03	88.1±0.02	88.1±0.02
Ash	10.85±0.05	12.05±0.02	12.96±0.04	13.89±0.03	14.90±0.05	10.05±0.07	10.70±0.03	10.25±0.05	10.18±0.04	10.18±0.04
Crude protein	35.21±0.08	35.40±0.05	35.95±0.02	36.25±0.01	36.70±0.02	34.72±0.04	34.51±0.03	34.35±0.04	34.09±0.01	34.09±0.01
Ether extract	6.65±0.02	6.23±0.01	6.45±0.02	6.15±0.03	6.20±0.02	5.96±0.02	6.05±0.01	6.27±0.03	6.33±0.03	6.33±0.03
Carotenoids (µg/g diet)	29.1±0.94	110.12±2.32	178.74±3.13	292.32±7.84	411.28±6.98	120.23±5.41	183.24±6.63	304.14±8.74	407.25±7.51	407.25±7.51

¹ Procured from local market

² Ruchi Soya Industries Ltd., Raigad, India

³ Agrimin Forte (Virbac Animal Health India Pvt. Ltd., Mumbai 59, India); contains (per kg): Vitamin A 700,000 IU, Vitamin D₃ 70,000 IU, Vitamin E 250 mg, nicotinamide 1000 mg, cobalt 150 mg, copper 1200 mg, iodine 325 mg, iron 1500 mg, magnesium 6000 mg, manganese 1500 mg, potassium 100 mg, selenium 10 mg, sodium 5.9 mg, sulfur 0.72%, zinc 9600 mg, calcium 25.5%, phosphorus 12.75%

⁴ Himedia Ltd., India

Total carotenoids. Total carotenoids were determined as described by Olsan (1979). Feed samples were gently mashed with a glass rod against the side of a vial, then 5 ml chloroform was added and the vial was left overnight at 0°C. When the chloroform formed a clear 1-2 cm layer above the caked residue, 0.3-ml aliquots of chloroform were diluted to 3 ml with absolute ethanol, and optical density was read in a spectrophotometer at 380, 450, 470, and 500 nm. A blank prepared in a similar fashion was used for comparison. The wavelength at which the maximum absorption was obtained was used to calculate total carotenoids, expressed as µg/g.

Growth study. Growth performance of the fish was measured in terms of weight gain (%), specific growth rate (SGR, %/day), and feed conversion ratio (FCR) using the following equations: wt gain = 100(final wt - initial wt)/initial wt, SGR = 100(Log_e final wt - Log_e initial wt)/no. experimental days, FCR = feed given/wet body wt gain.

Statistical analysis. Mean values of all parameters were subjected to one-way ANOVA to study the treatment effects and Duncan's Multiple Range Tests to determine the significance of differences between any two means. Comparisons were made at 5% probability. All data were analyzed using statistical package SPSS (Version 16.0).

Results

There were very few accidental mortalities during the 60-day trial. There were no significant effects of marigold meal supplementation on weight gain, specific growth rate, or feed conversion ratio (Table 2). Weight gain, specific growth rate, and feed conversion ratio were significantly improved in fish fed the 10% beetroot diet. There was a third order polynomial relationship between percent weight gain and marigold flower meal supplementation, $Y = 6.1258x^3 - 56.893x^2 + 158.35x - 0.546$, $R^2 = 0.9281$ and a second

order polynomial relationship between percent weight gain and beetroot meal supplementation, $Y = 4.5464x^2 - 12.718x + 119.15$, $R^2 = 0.9476$ (Fig. 1a). Crude protein increased with dietary supplementation of marigold flower as well as beetroot meal. There was an inverse correlation ($R^2 = 0.98$) between moisture content and crude fat. Body carotenoid significantly rose with dietary supplementation of marigold as well as beetroot (Fig. 1b). The highest carotenoid was obtained in fish fed the 10% marigold diet, followed by those fed the 10% beetroot diet, which did not significantly differ from fish fed the 7% beetroot diet. Body carotenoid increased linearly with the increasing supplementations.

Table 2. Growth performance and carcass nutrient composition (%wet weight) of *Schizothorax richardsonii* fingerlings fed diets with graded levels of marigold flower meal or beetroot meal, means \pm SE, n = 3 (10 fish per replicate).

Diet	Specific growth rate (%)	Feed conversion ratio	Moisture (%)	Dry matter (%)	Ash (%)	Crude protein (%)	Crude fat (%)
Control	1.22 \pm 0.05 ^a	2.80 \pm 0.18 ^b	76.98 \pm 0.10 ^c	23.02 \pm 0.10 ^a	2.74 \pm 0.06 ^e	14.86 \pm 0.05 ^a	3.39 \pm 0.06 ^b
Marigold flower (%)							
3	1.40 \pm 0.18 ^{ab}	2.41 \pm 0.49 ^{ab}	75.75 \pm 0.14 ^a	24.25 \pm 0.14 ^c	2.16 \pm 0.03 ^a	15.00 \pm 0.04 ^b	4.27 \pm 0.03 ^d
5	1.39 \pm 0.19 ^{ab}	2.44 \pm 0.47 ^{ab}	75.58 \pm 0.05 ^a	24.42 \pm 0.05 ^c	2.23 \pm 0.02 ^{ab}	15.16 \pm 0.02 ^c	4.28 \pm 0.02 ^d
7	1.25 \pm 0.03 ^a	2.71 \pm 0.08 ^b	75.66 \pm 0.11 ^a	24.34 \pm 0.11 ^c	2.28 \pm 0.05 ^{abc}	15.51 \pm 0.02 ^e	4.36 \pm 0.02 ^d
10	1.42 \pm 0.11 ^{ab}	2.27 \pm 0.27 ^{ab}	77.04 \pm 0.07 ^c	22.96 \pm 0.07 ^a	2.38 \pm 0.04 ^{cd}	15.58 \pm 0.04 ^e	3.19 \pm 0.05 ^a
Beetroot (%)							
3	1.29 \pm 0.01 ^a	2.56 \pm 0.04 ^{ab}	76.80 \pm 0.09 ^c	23.20 \pm 0.09 ^a	2.32 \pm 0.03 ^{bc}	15.27 \pm 0.04 ^d	3.41 \pm 0.06 ^b
5	1.35 \pm 0.02 ^a	2.42 \pm 0.05 ^{ab}	76.14 \pm 0.11 ^b	23.86 \pm 0.11 ^b	2.33 \pm 0.01 ^{bc}	15.49 \pm 0.05 ^e	3.98 \pm 0.02 ^c
7	1.40 \pm 0.03 ^{ab}	2.27 \pm 0.09 ^{ab}	77.02 \pm 0.09 ^c	22.98 \pm 0.09 ^a	2.35 \pm 0.05 ^{bc}	15.72 \pm 0.02 ^f	3.36 \pm 0.08 ^b
10	1.67 \pm 0.02 ^b	1.73 \pm 0.04 ^a	76.73 \pm 0.08 ^c	23.27 \pm 0.08 ^a	2.47 \pm 0.04 ^d	15.95 \pm 0.04 ^g	3.43 \pm 0.01 ^b

Values in a column with different superscripts differ significantly ($p < 0.05$).

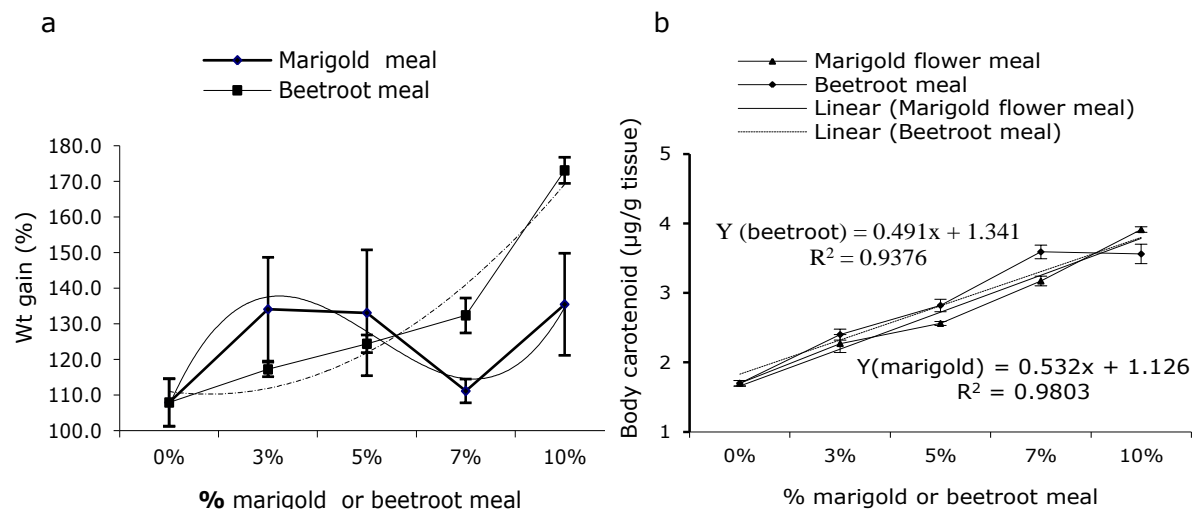


Fig. 1. (a) Growth and (b) total body carotenoids of snow trout (*Schizothorax richardsonii*) fingerlings fed diets containing marigold flower meal or beetroot meal for 60 days.

Discussion

Fish skin color is primarily dependent on chromatophores (melanophores, xanthophores, erythrophores, iridophores, leucophores, cyanophores) containing pigments such as melanins, carotenoids (e.g., astaxanthin, canthaxanthin, lutein, zeaxanthin), pteridines, and purines (Chatzifotis et al., 2005). In common with other animals, fish are unable to biosynthesize carotenoids *de novo*, but they can modify alimentary carotenoids and store them in the integument and other tissues. Farmed fish have no access to natural carotenoid-rich feeds and, therefore, the necessary carotenoids must be added to their

diet. The effectiveness of a carotenoid source in terms of deposition and pigmentation is species specific (Ha et al., 1993).

Marigold flower has color-enhancing effects on fishes (Vernon et al., 1996; Buyukcapar et al., 2007; Yanar et al., 2007; Ezhil et al., 2008). The present investigation shows that carotenoid contents of fish fed diets supplemented with the carotenoid sources (marigold flower or beetroot) were significantly higher than those of the control group at the end of the 60 days, similar to results in rainbow trout fed marigold flower meal and red pepper as carotenoid sources (Yanar et al., 2007). In our previous study we also observed a linear relationship between dietary supplementation of carotenoids and growth (Sarma and Jha, 2010). Red pepper also improves pigmentation in salmonids (Carter et al., 1994; Yanar et al., 1997).

The effects of carotenoids on growth and survival of aquatic organisms are controversial. The specific growth rate and skin coloration improved in *Silurus glanis* fed carotenoid-rich microalgal biomass (Začková et al., 2011). Likewise, the growth rate of rainbow trout improved by dietary supplementation of 3.2% marigold flower meal (Buyukcapar et al., 2007). Similarly, in our study, weight gain improved when beetroot was used as a source of carotenoids at higher levels. However, in a study of *Xiphophorus helleri*, the SGR was higher in the unsupplemented control groups than in fish fed carotenoid-supplemented diets (Ezhil et al., 2008). Some studies on crustaceans report non-significant effects of dietary carotenoid on both growth and survival (Yamada et al., 1990; Harpaz et al., 1998) and growth (Yilmaz and Ergün, 2011). In contrast, dietary supplementation of carotenoids resulted in no differences in growth or survival of characins, *Hyphessobrycon callistus* (Wang et al., 2006).

The increased crude protein content of fish fed supplementary marigold flower meal agrees with the significant increase in meat protein obtained in rainbow trout fed marigold flower meal (Buyukcapar et al., 2007). We are unable to find literature with which to compare the effects of beetroot meal supplementation on whole body composition.

In conclusion, this study shows that dietary supplementation of either marigold flower meal or beetroot meal can enhance growth of *S. richardsonii*. Dietary supplementation of marigold flower meal at 10% produced the highest carotenoid accumulation in the flesh. Since synthetic carotenoids are expensive, cheap and readily available natural carotenoid sources such as marigold flower and beetroot can be incorporated into snow trout diets to obtain greater pigmentation and market value. This will help farmers and other stakeholders realize greater profits in the culture of this species.

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