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# Color Enhancement in the Ornamental Dwarf Cichlid *Microgeophagus ramirezi* by Addition of Plant Carotenoids to the Fish Diet

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Key words: color enhancement, carotenoids, cichlid, *Microgeophagus ramirezi,* growth

**Abstract**

The present research examined the effects of adding carotenoids from oleoresin paprika to fish feeds for ornamental dwarf cichlid, *Microgeophagus ramirezi*. The growth rate, survival, carotenoid accumulation level, and color intensity were evaluated. Post larvae and near-adult (three months old) fish were tested to determine when carotenoids are better assimilated. The addition of carotenoids had no effect on the growth rate or survival in either life stage, however, they had a clear effect on color enhancement. After 45 days, near-adult fish that consumed carotenoid-supplemented diets at 60, 120, or 240 mg/kg had significantly higher levels of carotenoids (72.19±4.55, 84.81±5.29, and 86.55±4.50 µg/g dry matter, respectively) than control fish (33.69±1.06 µg/g), with no significant differences between treatments. After 75 days, post larvae that consumed 240 mg/kg carotenoids accumulated significantly more carotenoids in their body (59.34±3.93 µg/g dry matter) than fish that consumed only 60 mg/kg carotenoids (40.53±2.37 µg/g dry matter) or no supplemental carotenoids (29.18 µg/g dry matter). Visual examination revealed a strong correlation between level of pigment accumulation and color appearance in the fish. Results indicate that addition of 60 mg oleoresin paprika per kg diet is sufficient to obtain good coloration in *M. ramirezi*.

## Introduction

The dwarf cichlid *Microgeophagus ramirezi* (Myers and Harry 1948), also known as *Apistogramma ramirezi* or the Ram cichlid of South America, is popular among ornamental fish hobbyists. Its vibrant blue, black, and red colors make it an attractive addition to freshwater aquariums. Various cichlid species are raised in intensive production conditions on fish farms in Israel. High density culture of ornamental fish in artificial conditions without the dietary addition of carotenoids (pigments) leads to dull color performance, subsequent difficulties in marketing, and decreased profitability.

Like other animals, fish cannot synthesize carotenoids, and they must obtain them via

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food (Sommer et al., 1991, 1992). Therefore, carotenoid supplementation is needed to enhance color performance. An abundance of studies on the addition of carotenoids to fish diets have been conducted on salmonids and involved the use of different carotenoid sources and inclusion levels (e.g., Sommer et al., 1991; No and Storebakken, 1992; Smith et al., 1992; Storebakken and No, 1992; Meyers, 1994; Halten et al., 1995, 1997; Diler et al., 2005; Ingle de la Mora et al., 2006; Yanar et al., 2007). In contrast, a limited number of studies have been conducted on carotenoid addition to diets of ornamental fish, e.g., goldfish and koi carp (Hancz et al., 2003; Gouveia and Rema, 2005), neon serpae tetra (Wang et al., 2006), and guppies (Grether et al., (1999).

Fish feeds are usually enhanced with relatively expensive astaxanthin or canthaxanthin carotenoids. Therefore, there is a growing need to find cheaper carotenoid substitutes. Carotenoids extracted from paprika (oleoresin paprika) contain mainly capsanthin and may be a cheaper substitute for the currently used feed additions. The present research examined the effects of carotenoids from paprika (oleoresin paprika) added to *M. ramirezi* fish feeds on growth rate, survival, carotenoid accumulation level, and color intensity of the fish.

Carotenoid deposition can vary according to fish size or age (Halten et al., 1995). It is commonly assumed that carotenoids are better assimilated when the fish approach sexual maturity. This assumption was examined by providing carotenoid-supplemented feed at varying levels to fish approaching the adult stage (three months old at the beginning of the experiment) and to post larvae.

## Materials and Methods

*Fish and stocking. Microgeophagus ramirezi* were brought to the laboratory from a commercial farm. After one week of adaptation to laboratory conditions, they were stocked in experimental aquaria. For experiment I, several hundred three-month-old fish nearing maturity were stocked at a density of twelve per aquarium with five replicates of each treatment. For experiment II, several hundred post larvae were stocked at a density of fifteen per aquarium with six replicates of each treatment.

The fish in each aquarium were weighed as a batch with an analytic balance to the nearest 0.01 g at the onset of the experiment and every fortnight. The fish were counted and bulk weighed after brief drying on tissue paper. This periodic weighing was carried out, despite disturbance to the fish, to update the feed rate.

*Experimental system*. The fish were reared in an indoor closed experimental system under controlled laboratory conditions. The system consisted of 20 glass aquaria (19 x 45 x 22 cm), each containing 16 liters of water. Each aquarium contained an airstone that provided constant aeration. The aquaria were connected to a central biofilter and a settling unit of 100 l through which water was circulated by a submersible pump at a rate equivalent to one total tank replacement every hour.

The biofilter contained small plastic cylinders, 1.5 cm long with a diameter of 1 cm, inside plastic net bags. A heating element (1 kW) connected to a thermostat maintained the temperature at 26.7±0.6°C, checked daily with a submersed maximum/minimum thermometer. The natural photoperiod was enhanced by a florescent light that provided light at an intensity of 1200 lux during daylight hours.

Dead fish (if present) were removed and the settling tank was cleaned, allowing new water to compensate for the water loss (approximately 2% of the total water volume) during this brief cleaning. Every 3-4 days, the settling tank was cleaned more thoroughly and organic particles were flushed from the biofilter. The amount of water replaced during this procedure was roughly 5%.

Ammonia ($NH_4^+$) was measured with Merck kit #11117 and did not exceed 0 mg/l. Nitrite ($NO_2$) was measured with Merck kit #11118 and ranged from 0 to 0.25 mg/l. Oxygen levels were at saturation throughout the experiment and pH, measured with pH-test strips (Merck 09535), ranged from 8 to 8.5.

*Feed.* The fish were fed a diet containing 54% protein and 14% fat (RMC Ltd., Israel). The feed was given in powdered form and the particle size was adapted to the mouth orifice

of the fish as they grew. Carotenoids were added to the diet in the form of oleoresin derived from the paprika pepper (capsicum). In experiment I, three levels of carotenoid addition were tested: 60, 120, and 240 mg/kg. In experiment II, two levels were tested: 60 and 240 mg/kg. Control fish received no added carotenoids. The carotenoid concentration in the pepper oleoresin extract was tested using the method of Weissenberg et al. (1997) and found to be 10%. Fish were fed twice a day (morning and late afternoon) with a total daily feed portion equal to 10% of their body weight.

*Growth rate*. The specific growth rate (SGR) of the fish was calculated using the formula: $SGR = (\ln W_f - \ln W_i)/T \times 100$, where $W_f$ = final avg wt, $W_i$ = initial avg wt, and T = time in days.

*Carotenoid accumulation.* A random sample of six fish was removed from each aquarium. Each of the six sampled fish was placed into a container, frozen at -20°C, and freeze dried using an Ilshin lyophilizer (model FD8518). Two days later, the dried fish were cut and homogenized with a HG-300 homogenizer (Hsiangtai Machinery Industry). Three ml of acetone were added to each fish sample. The samples were covered with parafilm, kept overnight in a dark room, and then centrifuged in a Dupont GLC-2 model centrifuge for 5 min at 6000 rpm. Color intensity was measured at a wavelength of 474 nanometer using an Ultrospec®2000 spectrophotometer (Biochrom Ltd.). The amount of carotenoids in each sample was calculated according to the dry weight of that sample.

*Statistical analysis.* Statistical analysis was carried out using ANOVA with JMP and Excel software. Differences were considered significant when $p<0.05$.

## Results

The addition of dietary carotenoids had no effect on the growth rate or survival of the mature fish or the post larvae (Table 1, Fig. 1). On the other hand, the carotenoid levels in near-adult fish that received the supplemented diet were 72.19±4.55, 84.81±5.29, and 86.55±4.50 µg/g dry matter, significantly higher than in the control, 33.69±1.06 µg/g dry matter (Fig. 2). In the post larvae, carotenoid

Table 1. Growth and survival of adult and post larvae *Microgeophagus ramirezi* fed diets containing oleoresin paprika carotenoids. There were no significant differences among treatments.

| | *Final wt (g)* | *Survival (%)* |
|---|---|---|
| *Adult*[1] | | |
| Control (no added carotenoids) | 0.77±0.06 | 82.5±14.25 |
| 60 mg/kg | 0.81±0.06 | 85.0±10.45 |
| 120 mg/kg | 0.74±0.03 | 90.0±6.25 |
| 240 mg/kg | 0.79±0.09 | 85.0±10.45 |
| *Post larvae*[2] | | |
| Control (no added carotenoids) | 0.45±0.06 | 80.0±7.02 |
| 60 mg/kg | 0.48±0.07 | 90.0±6.99 |
| 240 mg/kg | 0.43±0.07 | 80.0±7.30 |

[1] fed supplemented diet for 45 days
[2] fed supplemented diet for 75 days

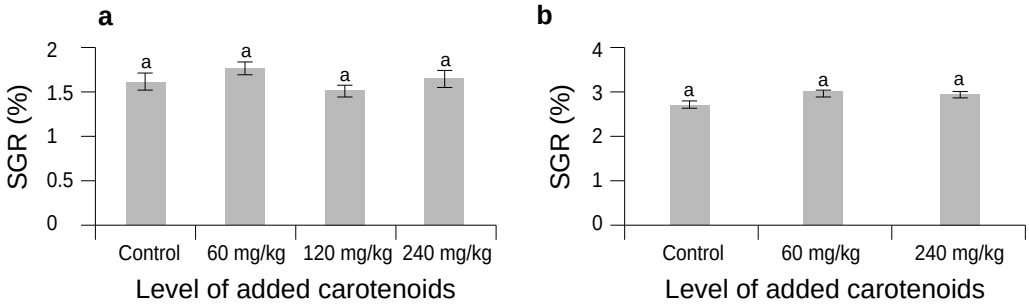


Fig. 1. Specific growth rates (SGR) of (a) adult dwarf cichlid *Microgeophagus ramirezi* fed diets containing 60, 120, or 240 mg/kg oleoresin paprika carotenoids for 45 days and (b) post larvae fed diets containing 60 or 240 mg/kg for 75 days.

accumulation was significantly higher in fish that received the 240 mg/kg diet (59.34±3.93 µg/g dry matter) than in fish that received the 60 mg/kg diet (40.53±2.37 µg/g dry matter) and both were significantly higher than in the control (29.18 µg/g dry matter). Despite receiving the supplementation for 75 days, the amount of carotenoids that accumulated in the post larvae was much lower than the amount that accumulated in the adult fish fed the supplement for only 45 days.

Visual examination of the fish showed a strong correlation between pigment accumulation level and color appearance in the fish.

## Discussion

The results show that it is possible to use extracts of pepper (oleoresin paprika), a natural source of carotenoids, as a food additive for color enhancement in dwarf cichlid ornamental fish. Even at a low concentration of 60 mg/kg, a saturation point in the accumulation

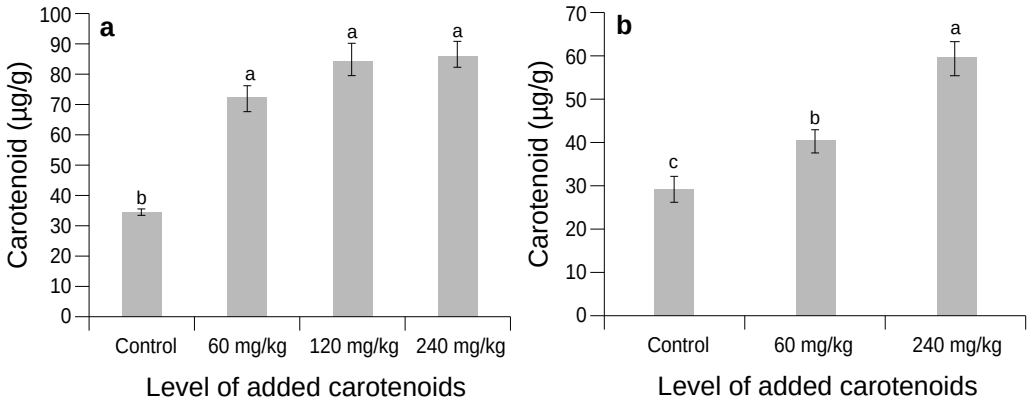


Fig. 2. Carotenoid accumulation in (a) adult dwarf cichlid *Microgeophagus ramirezi* fed diets containing 60, 120, or 240 mg/kg oleoresin paprika carotenoids for 45 days and (b) post larvae fed diets containing 60 or 240 mg/kg for 75 days.

of carotenoids was reached in near-adults. In post larvae, the dietary carotenoid concentration influenced the level of accumulation in the body, causing a stronger graded expression of color in comparison with control fish.

Besides their use for color enrichment in fish, carotenoids are excellent antioxidants (Matsufuji et al., 1998). Thus, although no such effect could be detected in the present study, the added carotenoids might have had an additional effect on color enhancement by contributing to better health or growth.

Our results are similar to those found by Wang et al. (2006) who used two types of carotenoids, astaxanthin and β-carotene, in pigmented diets for the ornamental fish *Hyphessobrycon callistus* (common name: neon serape tetra) at three concentrations (10, 20, and 40 mg/kg). They found no differences in growth and survival of the fish after eight weeks of rearing. They also found that the body content of astaxanthin and β-carotene increased with increased dietary carotenoid concentration, similar to our results.

Sommer et al. (1992) demonstrated that the total carotenoid level in trout skin was significantly related to the level of the pigment in the diet and that addition of pigment to the diet had a near significant effect on growth ($p$<0.07). In the present experiment, although the overall growth did not significantly differ among treatments, in both experiments the addition of 60 mg carotenoid per kg diet resulted in slightly better growth.

Determining the optimal concentration of pigment to be given to fish has economic importance as finding the lowest amount of pigment that will still produce a high vibrancy of colors will reduce expenses. Indeed, we found that adult market size *M. ramirezi* reached the saturation point in the accumulation of carotenoids when fed the low concentration of 60 mg pigment per kg feed.

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