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ISSN 0792 - 156X

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PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -  
Kibbutz Ein Hamifratz, Mobile Post 25210,  
ISRAEL

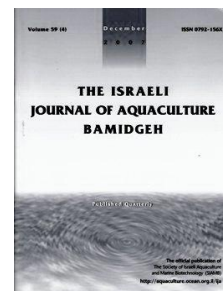
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## Enhancement of Growth Performance and Pigmentation in Red *Oreochromis mossambicus* Associated with Dietary Intake of Astaxanthin, Paprika, or Capsicum

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(Received 27.3.12, Accepted 22.5.12)

Key words: tilapia, *Oreochromis mossambicus*, astaxanthin, paprika, capsicum, pigmentation, growth

### Abstract

Diets for tilapia, *Oreochromis mossambicus*, were supplemented with astaxanthin, paprika, or capsicum to determine their effects on growth performance and pigmentation. Seven isonitrogenous (37% crude protein) and isoenergetic (18 kJ/g) diets were formulated to contain 40 or 60 mg carotenoid per kg diet. Triplicate groups of 210 fish were stocked in 21-l plastic boxes and fed one of the three experimental diets or the control diet for 45 days. Significantly better final weight, weight gain, specific growth rate, and food conversion ratio were obtained with the diets containing 40 or 60 mg/kg paprika than with control diet ( $p < 0.05$ ). In general, the carotenoid-supplemented diets significantly increased the values of redness ( $a^*$ ), yellowness ( $b^*$ ), and chroma ( $C^*$ ), and decreased the values of lightness ( $L^*$ ) and hue ( $H^0_{ab}$ ) on the tail, body, and head areas ( $p < 0.05$ ). Results show that paprika or capsicum oleoresin can be used as an alternative natural carotenoid source in *O. mossambicus* diets to ensure good pigmentation, better growth, and feed utilization.

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## Introduction

Hundreds of exotic fish species including guppies, mollies, platies, and tilapias are popular among ornamental fish hobbyists throughout the world (Whittington and Chong, 2007; Çelik et al., 2010). The most important characteristic of ornamental fish is the color intensity of the skin. This criterion generally determines the market value of ornamental fish.

Natural and synthetic carotenoids are used in fish diets to enhance skin and fillet coloration. However, synthetic carotenoids are expensive and comprise 10-15% of the total feed cost while natural carotenoids could serve as cheaper alternatives (Buttle et al., 2001). Oral administration of red pepper meal, shrimp by-product meal, and astaxanthin in *Oncorhynchus mykiss* (Yanar et al., 1997; Diler et al., 2005), red pepper meal in *Labidochromis caeruleus* (Yılmaz and Ergün, 2011), astaxanthin, canthaxanthin, and *Gammarus* spp. in *Carassius auratus* (Yeşilayer et al., 2011), and beetroot and marigold in *Schizothorax richardsonii* (Jha et al., 2012) was successfully used for coloration. In addition, paprika oleoresin (Scabini et al., 2011; Yeşilayer et al., 2011) and capsicum oleoresin (Harpaz and Padowicz, 2007) also enhance coloration in fish.

The aim of this study was to determine the effect of astaxanthin (Lucantin® Pink), paprika, and capsicum on the skin color and growth performance of red *Oreochromis mossambicus*.

## Materials and Methods

**Fish and experimental conditions.** Healthy cultured red tilapia, *Oreochromis mossambicus* (~5 g), were used in the experiment. Water quality parameters were temperature  $28.4 \pm 0.2^\circ\text{C}$ , pH  $7.3 \pm 0.2$ , dissolved oxygen  $7.0 \pm 0.3$  mg/l, and conductivity  $560 \pm 10$  uS. The parameters were measured daily with a YSI 85 oxygen, conductivity, temperature hand-held meter, and a HANNA C 200 (HI 83200) photometer.

**Experimental diets.** Astaxanthin (Lucantin® Pink, 10% formulation, BASF, Ludwigshafen, Germany), paprika oleoresin (carotenoids 40 g/kg, Kutluer, Turkey), and capsicum oleoresin (carotenoids 6 g/kg, Kutluer, Turkey) were added to the feed to reach carotenoid concentrations of 40 and 60 mg/kg (Table 1). The control group was fed an unsupplemented diet. The ingredients were mixed in a blender and pressed through a 1-mm die pelleting machine. The pellets were dried in a drying cabinet and stored in bags frozen at  $-20^\circ\text{C}$  until use. The pellets were crushed into desirable particle sizes before feeding.

**Experimental design.** The experiment was conducted in triplicate for each diet. Before the start of the experiment, the fish were fed a feed containing 37% protein and 10% fat. After conditioning for 15 days, the fish were randomly allocated to 21-l plastic boxes at 10 fish per box. The recirculating system consisted of a water heater, a sump, filters, and a biofilter containing bioballs. Water was

Table 1. Proximate composition and chemical analysis of tilapia diets supplemented with carotenoids.

	Astaxanthin (mg)			Capsicum (mg)		Paprika (mg)	
	Control	40	60	40	60	40	60
<b>Ingredients (g/kg dry matter)</b>							
Fishmeal	310	310	310	310	310	310	310
Fish oil	65	65	65	65	65	65	65
Soybean meal	320	320	320	320	320	320	320
Wheat flour	132	132	132	132	132	132	132
Starch	133	132.6	132.4	126	119	132	131.5
Vitamin-mineral mix	40	40	40	40	40	40	40
Astaxanthin <sup>1</sup>	0	0.40	0.60	0	0	0	0
Capsicum oleoresin	0	0	0	7	14	0	0
Paprika oleoresin	0	0	0	0	0	1.0	1.5
<b>Chemical analysis</b>							
Dry matter	91.53	91.49	91.47	90.93	90.32	91.44	91.39
Protein (% dry matter)	37.05	37.05	37.05	37.05	37.05	37.05	37.05
Fat (% dry matter)	10.04	10.04	10.04	10.04	10.04	10.04	10.04
Ash (% dry matter)	9.49	9.49	9.49	9.49	9.49	9.49	9.49
NFE <sup>2</sup>	34.95	34.92	34.90	34.35	33.75	34.86	34.82
Energy (kJ/g) <sup>3</sup>	18.65	18.65	18.64	18.55	18.45	18.64	18.63

<sup>1</sup> Lucanthin® Pink

<sup>2</sup> Nitrogen-free extracts = matter - (crude protein + crude lipid + crude ash)

<sup>3</sup> calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid, 17.0 kJ/g NFE.

exchanged at a daily rate of ~10% of the total volume. The fish were fed the experimental diets three times daily at a rate of 3% body weight per day for 45 days.

**Proximate composition.** Proximate compositions of the diet ingredients were analyzed using standard methods (AOAC, 1998), dry matter by drying at 105°C in an oven to a constant weight, crude protein by the Kjeldahl method, crude fat by ether extraction, and crude ash by incineration at 525°C in a muffle furnace for 12 h.

**Growth trial.** Growth performance and feed utilization were calculated every 15 days according to the formulae: weight gain (%) = 100(final fish wt - initial fish wt)/initial fish wt, specific growth rate (%/d) = 100(ln final fish wt - ln initial fish wt)/no. of experimental days, feed conversion ratio = feed intake/wt gain.

**Color measurements.** The color of all fish was measured on the tail, at the center of the lateral body area, and on the dorsal side of the head (Fig. 1). Measurements were recorded at the end of the feeding trial using a Konica Minolta Chroma Meter CR-400 according to the color system of the International Commission on Illumination (CIE):  $L^*$  = lightness, where dark = 0 and white = 100,  $a^*$  = redness, where positive values = red and negative values = green, and  $b^*$  = yellowness, where positive values = yellow and negative values = blue. Hue ( $H^{\circ}_{ab}$ ) and chroma ( $C^*$ ) were calculated using the formulae: if  $a^* > 0$  then  $H^{\circ}_{ab} = \tan^{-1}(b^*/a^*)$  or if  $a^* < 0$  then  $H^{\circ}_{ab} = 180 + \tan^{-1}(b^*/a^*)$ ,  $C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$  (Sharma, 2003).

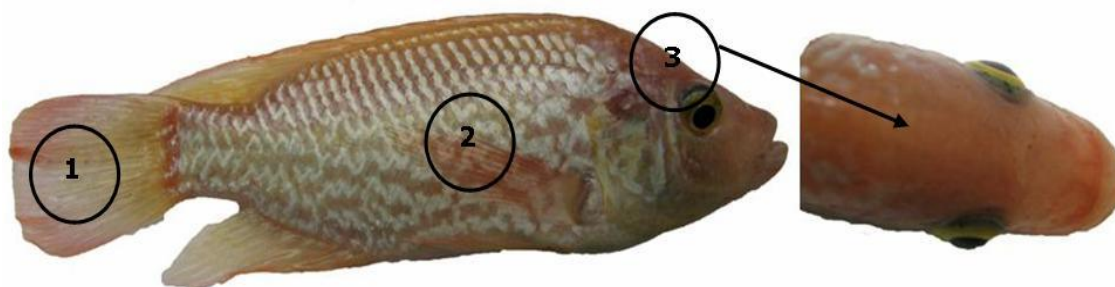


Fig. 1. Areas on the (1) tail, (2) body, and (3) head of tilapia where color was measured.

**Statistics.** Statistical computations were carried out with SPSS 17 for Windows and consisted of one-way ANOVA with a probability level of 0.05 for rejection of the null hypothesis. After ANOVA, significant differences among means were determined by Tukey's multiple range test. The relationships between carotenoid concentration and color parameters were established by linear regression analysis.

## Results

Fish growth is shown in Fig. 2. The best final weight, weight gain, SGR, and FCR were obtained with the 60 mg paprika diet (Table 2). The carotenoid-rich diets significantly decreased lightness ( $L^*$ ) on the tail, body, and head, except the 40 mg astaxanthin diet that significantly increased lightness on the body. Red chromaticity ( $a^*$ ) was significantly increased by the carotenoid-rich diets, except for the 40 mg astaxanthin diet on the body and head. Yellow chromaticity ( $b^*$ ) was significantly increased by most of the diets in the tail and body areas, and by some on the head. Generally, the carotenoid-rich diets significantly decreased  $H^{\circ}_{ab}$  on all three areas (tail, body, and head).  $C^*$  values of the carotenoid-supplemented groups were

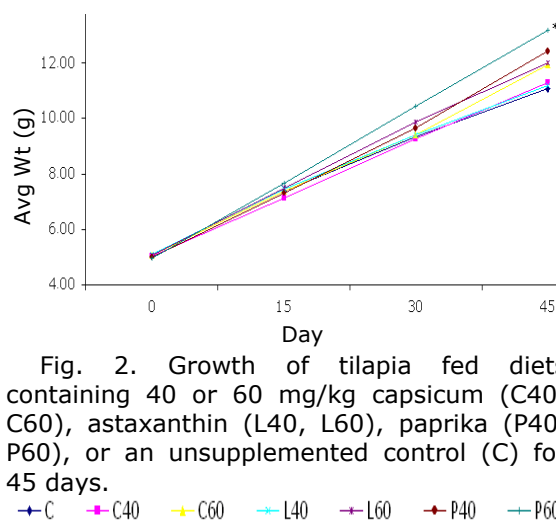


Fig. 2. Growth of tilapia fed diets containing 40 or 60 mg/kg capsicum (C40, C60), astaxanthin (L40, L60), paprika (P40, P60), or an unsupplemented control (C) for 45 days.

significantly higher than those of the control, except for the 40 mg astaxanthin diet and, on the tail area, the 40 mg capsicum diet.

Table 2. Growth and color of tilapia fed diets containing supplemental carotenoids for 45 days.

	<i>Astaxanthin</i>			<i>Capsicum</i>		<i>Paprika</i>	
	<i>Control</i>	<i>40 mg</i>	<i>60 mg</i>	<i>40 mg</i>	<i>60 mg</i>	<i>40 mg</i>	<i>60 mg</i>
Initial wt (g)	5.07±0.03	5.09±0.01	5.04±0.01	5.02±0.01	5.05±0.01	4.98±0.06	4.95±0.09
Final wt (g)	11.07±0.43 <sup>b</sup>	11.17±0.03 <sup>b</sup>	12.03±0.54 <sup>ab</sup>	11.30±0.18 <sup>b</sup>	11.93±0.25 <sup>ab</sup>	12.43±0.44 <sup>ab</sup>	13.19±0.17 <sup>a</sup>
Wt gain (g)	6.01±0.44 <sup>b</sup>	6.08±0.04 <sup>b</sup>	6.99±0.54 <sup>ab</sup>	6.27±0.17 <sup>b</sup>	6.88±0.27 <sup>ab</sup>	7.44±0.43 <sup>ab</sup>	8.24±0.26 <sup>a</sup>
SGR	1.30±0.07 <sup>b</sup>	1.31±0.01 <sup>b</sup>	1.45±0.08 <sup>ab</sup>	1.35±0.02 <sup>b</sup>	1.43±0.04 <sup>ab</sup>	1.52±0.06 <sup>ab</sup>	1.63±0.05 <sup>a</sup>
FCR	1.42±0.09 <sup>a</sup>	1.36±0.02 <sup>ab</sup>	1.16±0.09 <sup>abc</sup>	1.28±0.03 <sup>ab</sup>	1.18±0.05 <sup>abc</sup>	1.15±0.02 <sup>bc</sup>	0.97±0.03 <sup>c</sup>
<i>Effects on tail color</i>							
<i>L*</i>	77.52±0.26 <sup>a</sup>	76.85±0.30 <sup>a</sup>	70.12±0.35 <sup>b</sup>	71.00±0.51 <sup>b</sup>	69.77±0.56 <sup>b</sup>	66.35±1.14 <sup>c</sup>	63.92±0.68 <sup>c</sup>
<i>a*</i>	4.28±0.19 <sup>e</sup>	6.89±0.32 <sup>d</sup>	16.98±0.20 <sup>c</sup>	6.94±0.24 <sup>d</sup>	20.61±0.99 <sup>b</sup>	20.77±0.48 <sup>b</sup>	30.31±1.07 <sup>a</sup>
<i>b*</i>	7.05±0.14 <sup>d</sup>	8.56±0.32 <sup>d</sup>	13.36±0.28 <sup>c</sup>	8.36±0.18 <sup>d</sup>	13.75±0.36 <sup>c</sup>	20.47±1.00 <sup>b</sup>	24.17±1.27 <sup>a</sup>
<i>H<sub>ab</sub><sup>o</sup></i>	58.82±1.32 <sup>a</sup>	51.09±1.66 <sup>b</sup>	38.12±0.58 <sup>d</sup>	50.46±1.15 <sup>b</sup>	34.45±0.96 <sup>d</sup>	43.80±1.33 <sup>c</sup>	38.08±0.92 <sup>d</sup>
<i>C*</i>	8.31±0.14 <sup>d</sup>	11.12±0.31 <sup>d</sup>	21.64±0.27 <sup>c</sup>	10.93±0.20 <sup>d</sup>	24.86±0.98 <sup>c</sup>	29.39±0.88 <sup>b</sup>	38.91±1.53 <sup>a</sup>
<i>Effects on body color</i>							
<i>L*</i>	69.44±0.44 <sup>b</sup>	77.29±0.62 <sup>a</sup>	63.63±0.47 <sup>cde</sup>	61.23±1.39 <sup>de</sup>	59.57±0.97 <sup>e</sup>	64.43±1.59 <sup>cd</sup>	66.63±0.86 <sup>bc</sup>
<i>a*</i>	0.50±0.02 <sup>e</sup>	1.05±0.07 <sup>e</sup>	15.16±0.41 <sup>bc</sup>	6.08±0.38 <sup>d</sup>	17.62±0.59 <sup>ab</sup>	13.21±0.61 <sup>c</sup>	17.27±1.03 <sup>a</sup>
<i>b*</i>	11.71±0.75 <sup>c</sup>	10.33±0.43 <sup>c</sup>	17.39±0.49 <sup>b</sup>	24.61±1.02 <sup>a</sup>	21.70±0.74 <sup>a</sup>	15.55±1.19 <sup>b</sup>	17.26±0.81 <sup>b</sup>
<i>H<sub>ab</sub><sup>o</sup></i>	87.29±0.20 <sup>a</sup>	83.98±0.43 <sup>a</sup>	48.81±1.02 <sup>c</sup>	76.16±0.63 <sup>b</sup>	50.74±1.36 <sup>c</sup>	47.73±2.85 <sup>c</sup>	45.44±2.10 <sup>c</sup>
<i>C*</i>	11.73±0.75 <sup>d</sup>	10.39±0.43 <sup>d</sup>	23.18±0.48 <sup>bc</sup>	25.40±1.06 <sup>ab</sup>	28.17±0.68 <sup>a</sup>	20.99±0.97 <sup>c</sup>	24.91±0.93 <sup>ab</sup>
<i>Effects on head color</i>							
<i>L*</i>	69.91±0.39 <sup>a</sup>	64.16±0.44 <sup>b</sup>	59.44±0.50 <sup>c</sup>	64.16±0.76 <sup>b</sup>	62.32±0.51 <sup>b</sup>	62.08±0.63 <sup>b</sup>	58.78±0.58 <sup>c</sup>
<i>a*</i>	18.28±0.18 <sup>d</sup>	20.26±0.27 <sup>d</sup>	29.60±0.53 <sup>b</sup>	25.54±0.60 <sup>c</sup>	32.14±0.43 <sup>a</sup>	33.86±1.05 <sup>a</sup>	28.31±0.49 <sup>b</sup>
<i>b*</i>	27.97±0.37 <sup>b</sup>	23.73±0.77 <sup>c</sup>	29.53±0.98 <sup>ab</sup>	32.21±0.84 <sup>a</sup>	26.84±0.49 <sup>b</sup>	32.11±0.52 <sup>a</sup>	27.61±0.59 <sup>b</sup>
<i>H<sub>ab</sub><sup>o</sup></i>	56.77±0.42 <sup>a</sup>	49.04±1.21 <sup>b</sup>	42.21±0.50 <sup>c</sup>	48.85±0.88 <sup>b</sup>	44.94±0.25 <sup>c</sup>	43.70±0.74 <sup>c</sup>	44.21±0.30 <sup>c</sup>
<i>C*</i>	33.44±0.33 <sup>c</sup>	31.39±0.50 <sup>c</sup>	40.00±0.64 <sup>b</sup>	39.18±0.98 <sup>b</sup>	45.44±0.64 <sup>a</sup>	46.85±1.20 <sup>a</sup>	39.56±0.74 <sup>b</sup>

Values are means±SEM (n = 3). Different superscripts in a row indicate significant differences between groups ( $p < 0.05$ ).

Regression analysis between color parameters and total carotenoid concentrations show that, whatever the pigment source, color was enhanced (Table 3). Correlations between *a\**, *b\**, and *C\** were positive in fish fed diets containing carotenoids while *L\** and *H<sub>ab</sub><sup>o</sup>* negatively correlated. Yellow chromaticity negatively correlated only with astaxanthin in the head area. Differences in coloration are visible in Fig. 3.

Table 3. Regression analysis between color parameters and carotenoid concentration (C).

	<i>Lucanthin pink</i>	<i>R<sup>2</sup></i>	<i>Capsicum oleoresin</i>	<i>R<sup>2</sup></i>	<i>Paprika oleoresin</i>	<i>R<sup>2</sup></i>
Tail	<i>L*</i> = 78.4 - 0.1C	0.52	<i>L*</i> = 77.2 - 0.1C	0.63	<i>L*</i> = 77.1 - 0.2C	0.65
	<i>a*</i> = 3.0 + 0.2C	0.71	<i>a*</i> = 2.5 + 0.2C	0.59	<i>a*</i> = 4.1 + 0.4C	0.89
	<i>b*</i> = 6.5 + 0.1C	0.62	<i>b*</i> = 6.4 + 0.1C	0.61	<i>b*</i> = 7.5 + 0.3C	0.67
	<i>H<sub>ab</sub><sup>o</sup></i> = 60.1 - 0.3C	0.54	<i>H<sub>ab</sub><sup>o</sup></i> = 60.5 - 0.4C	0.63	<i>H<sub>ab</sub><sup>o</sup></i> = 58.6 - 0.4C	0.64
	<i>C*</i> = 7.0 + 0.2C	0.72	<i>C*</i> = 6.5 + 0.2C	0.60	<i>C*</i> = 8.5 + 0.5C	0.84
Body	<i>L*</i> = 72.0 - 0.1C	0.05	<i>L*</i> = 69.1 - 0.2C	0.37	<i>L*</i> = 68.8 - 0.1C	0.06
	<i>a*</i> = -1.5 + 0.2C	0.58	<i>a*</i> = 0.7 + 0.3C	0.78	<i>a*</i> = 0.8 + 0.3C	0.78
	<i>b*</i> = 10.6 + 0.1C	0.19	<i>b*</i> = 13.0 + 0.2C	0.43	<i>b*</i> = 11.7 + 0.1C	0.17
	<i>H<sub>ab</sub><sup>o</sup></i> = 92.1 - 0.6C	0.62	<i>H<sub>ab</sub><sup>o</sup></i> = 90.1 - 0.6C	0.77	<i>H<sub>ab</sub><sup>o</sup></i> = 84.8 - 0.7C	0.69
	<i>C*</i> = 9.8 + 0.2C	0.37	<i>C*</i> = 12.3 + 0.3C	0.69	<i>C*</i> = 11.8 + 0.2C	0.57
Head	<i>L*</i> = 70.2 - 0.2C	0.75	<i>L*</i> = 69.8 - 0.1C	0.52	<i>L*</i> = 69.8 - 0.2C	0.72
	<i>a*</i> = 17.1 + 0.2C	0.63	<i>a*</i> = 17.8 + 0.2C	0.83	<i>a*</i> = 20.2 + 0.2C	0.45
	<i>b*</i> = 27.2 - 0.03C	0.05	<i>b*</i> = 27.7 + 0.1C	0.16	<i>b*</i> = 28.9 + 0.01C	0.01
	<i>H<sub>ab</sub><sup>o</sup></i> = 57.2 - 0.2C	0.64	<i>H<sub>ab</sub><sup>o</sup></i> = 56.8 - 0.2C	0.71	<i>H<sub>ab</sub><sup>o</sup></i> = 55.8 - 0.2C	0.72
	<i>C*</i> = 32.1 + 0.1C	0.22	<i>C*</i> = 32.9 + 0.2C	0.60	<i>C*</i> = 35.4 + 0.1C	0.23





Fig. 3. Color of *Oreochromis mossambicus* fed an unsupplemented control (C) or experimental diets containing 40 or 60 mg/kg astaxanthin (L40, L60), capsicum (C40, C60), or paprika (P40, P60) for 45 days.

### Discussion

Hot red chili (capsicum) and sweet pepper (paprika) extracted from *Capsicum annum* are inexpensive and abundant natural carotenoid sources. In this study, growth performance was significantly improved in the paprika groups. Likewise, 30 to 60 mg/kg paprika improved growth performance of rainbow trout (Diler et al., 2005). However, growth was negatively affected by *C. annum* and its extracts when used in high doses for rainbow trout (Ingle de la Mora et al., 2006) and *Zacco platypus* (Lee et al., 2010). This was likely due to anti-nutritional factors such as excessive pungency (Lee et al., 2010), tannin (Esayas et al., 2011), or saponin (Rao et al., 2000).

In the present study,  $L^*$  and  $H^o_{ab}$  decreased as the dietary concentration of the carotenoid increased. Similar results were obtained when astaxanthin, *C. annum*, or its oleoresins were supplemented in diets for *Labidochromis caeruleus* (Yılmaz and Ergün 2011), *Zacco platypus* (Lee et al., 2010), and rainbow trout (Diler et al., 2005; Kouakou and Choubert, 2006; Yeşilayer and Erdem, 2011), demonstrating that the accumulation of carotenoids in fish skin lowers lightness and hue.

The diets containing carotenoids increased red ( $a^*$ ), yellow ( $b^*$ ), and chroma ( $C^*$ ) in the tilapia skin. Likewise, these carotenoids have successfully induced redness and yellowness in skin of koi carp (Hancz et al., 2003), *Pagrus auratus* (Booth et al. 2004), *Labidochromis caeruleus* (Yılmaz and Ergün, 2011), and goldfish (Hancz et al., 2003; Yeşilayer et al., 2011). Further, chroma ( $C^*$ ) was enhanced by the addition of a carotenoid source in red porgy (Kalinowski et al., 2007).

These studies show how well a much cheaper pigmentation affects skin or fillet color in fish within a short period, enabling the formulation of economically feasible diets. Red or gold tilapia (*O. mossambicus*) are evaluated by two concepts: a major advantage of red-gold tilapia for aquarium hobbyists is its attractive color while, for consumers, the red color of cultured salmonids, porgy, and snapper raise their market value and consumers often pay a higher price/kg for red tilapia than other tilapias of equivalent weight (Lovshin, 1998).

In conclusion, the present study suggests that paprika or capsicum can be used as alternative natural carotenoid sources in diets for red tilapia, *O. mossambicus*. A suitable dietary level ensures good pigmentation, better growth, and better feed utilization.

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