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EFFECTS OF NATURAL AND SYNTHETIC PIGMENTS IN DIETS ON FLESH COLORATION AND GROWTH OF RAINBOW TROUT *(ONCORHYNCHUS MYKISS* W.)

Ibrahim Diler[1], Belgin Hossu[2], Kamil Dilek[3], Yilmaz Emre[1] and Huseyin Sevgili[1]

[1] *Fisheries Research Institute of Mediterranean, Ministry of Agriculture & Rural Affairs, P.O. Box 190, Antalya, Turkey*

[2] *Department of Aquaculture, Fisheries Faculty, University of Ege, 35100, Bornova, Izmir, Turkey*

[3] *Department of Fisheries, Ihsaniye Technical College, University of Kocaeli, Ihsaniye, Kocaeli, Turkey*

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Abstract

The desired pink to red color of rainbow trout flesh (*Oncorhynchus mykiss* W.) can be obtained by adding carotenoids to the fish diet. This study was conducted to determine the effects on growth and color retention of natural pigments (30 ppm red pepper meal, 60 ppm red pepper meal, 30 ppm shrimp by-products meal, 60 ppm shrimp by-products meal), synthetic carotenoids (30 ppm astaxanthin, 60 ppm astaxanthin), and a control group (no added pigment). Duplicates of each of the seven treatments were reared for three months. The best specific growth rates were obtained with 30 ppm astaxanthin (0.83%) and 60 ppm red pepper meal (0.84%); the lowest was in the control (0.54%). The lowest food conversion ratio was obtained with 30 ppm astaxanthin (1.38) and highest in the control (2.23; $p<0.05$). Visual coloration values ranged from 14.46±0.23 in the 30 ppm astaxanthin group to 11.55±0.25 in the control. Retention coefficients ranged from 6.63 in the 30 ppm astaxanthin group to 1.79 in the 60 ppm shrimp by-products meal ($p<0.05$). Tristimulus chromometer *a* values ranged from 0.87±0.47 in the control to 6.96±0.47 in the 60 ppm astaxanthin treatment, *b* values from 10.94±0.27 in the control to 12.90±0.27 in the 60 ppm red pepper meal treatment, and *L* values from 46.81±0.50 in the 60 ppm astaxanthin group to 54.57±0.26 in the control ($p<0.05$).

* Corresponding author. Fisheries Research Institute of Mediterranean, Ministry of Agriculture and Rural Affairs, P.O. Box 190, Antalya, Turkey, tel.: +90-242-2510585, fax: +90-242-2510584, mobile phone: +90-533-7717213, e-mail: idiler@yahoo.com

Introduction

Rainbow trout production in Turkey has greatly increased over the last ten years and surpassed all expectations (DIE, 2003). Quality factors such as appearance, color, and freshness meet customer demands. In flesh of salmonids (*Salmo* spp., *Oncorhynchus* spp., *Salvelinus* spp.), pink color is highly valued. Carotenoids are common in live organisms and some 600 have been identified (Olson, 1989, cited in Torrisen, 1989a).

Synthetic or natural pigments are added to cultivated rainbow trout diets to obtain a color similar to that of wild strains. Products such as astaxanthin, relatively low amounts of canthaxanthin, β-caroten, lutein, tunaxanthin, echineon, and zeaxanthin carotenoids can be found in fish (Torrisen et al., 1989), krill, crab, shrimp, copepods, etc., and their by-products, and in plants such as red pepper, alfalfa meal, velvet flower, and single cell algae including *Spirulina* spp., *Chlamydomonas* spp., *Haematococcus* spp., and the yeast-like *Phaffia rhodozyma*. In addition to natural carotenoids, chemical synthesized sources are widely used in fish, especially trout, feeds. Canthaxanthin, which was intensively explored until the 1980s, has been replaced by astaxanthin that can be detected in its free form in fish flesh and in its mono or diester forms in skin. Astaxanthin is 1.3 times more effective than canthaxanthin (Foss et al., 1984; Schiedt et al., 1985; Torrisen, 1986, 1989b; Choubert and Storebakken, 1989).

In wild salmonids, astaxanthin and its sterochemical or chiral derivatives (3S, 3'S; 3R, 3'S; 3R, 3'R) are produced during the zooplankton digestion process. Ando and Hatano (1986) found these three isomers were retained equally in coho salmon (*Oncorhynchus kisutsch*) flesh whereas Schiedt et al. (1985) and Foss et al. (1987) found that the most retained form in trout flesh was 3R, 3'R. In growing fish, 90% of the carotenoids are localized in the tissue and flesh, while in adults they are present mainly in the skin and ovarium (Kitahara, 1983). About 10% of the carotenoids are concentrated over the lateral line of trout larvae, fry, and immature fish (Torrisen et al., 1989; No and Storebakken, 1991a). During maturation, the carotenoids move to the skin of males and eggs of females (Torrisen, 1984; Torrisen et al., 1989). In nature, they migrate into eggs that constitute 18% of the total body content (Sivtseva, 1982). A low portion is transferred to sperm (Czeczuga, 1975).

This experiment was conducted to improve rainbow trout color by making it similar in color to its wild counterpart. Synthetic and natural carotenoid sources where added to the diet and the effects of pigment on growth, feed conversion ratio, and carcass color were determined.

Materials and Methods

Fish and experimental design. The feeding trial was conducted in a private trout farm with rainbow trout of an average initial weight of 95 g. Twelve batches of 66 individuals, each, were stocked into four ponds (8 x 2 x 1 m) divided by nets into three sections, each. Two control batches of 100 fish, each, were stocked in a similar pond divided into two sections. All fish were fed the control diet during the first two weeks for adaption. Afterwards, the fish were fed experimental diets for three months. Average water temperature was 9.0ºC, oxygen level 8.2 mg/l, and pH 7.5. Nitrite and nitrate levels were within normal ranges for trout.

The fish were weighed and total lengths were measured biweekly. To assess color, five fish were taken from each replicate every month, skinned, flattened, packed with transparent nylon and aluminium foil, and kept in a deep freezer (-20ºC) until analysis.

Feed and proximate analyses. Diets were isocaloric (13.5 MJ/kg digestible energy) and isonitrogenous (47% crude protein; Table 1). Feedstuffs were obtained from the market, powdered synthetic astaxanthin (Carophyll pink®) from Hoffman La-Roche, Basle, Switzerland, and red pepper meal from a spice seller. Shrimp by-products were obtained from a shrimp processing firm, dried in the shade, and ground into meal. The feed was pelleted with a meat mincer with a diameter of 4.5 mm, adjusted to 55-60ºC, mixed, and formed into a dough (Hasting and Higgs, 1978; Korkut and Hossu, 1998). The moist

Table 1. Ingredients (g/kg) and chemical analysis (%) of experimental diets.

	Diet						
	Control	*30 ppm astaxanthin*	*60 ppm astaxanthin*	*30 ppm red pepper*	*60 ppm red pepper*	*30 ppm shrimp by-products*	*60 ppm shrimp by-products*
Ingredient							
Fishmeal	450	450	450	450	450	450	450
Soybean meal	100	100	100	100	100	100	100
Full fat soybean meal	100	100	100	100	82	58	-
Meat-bone meal	200	200	200	200	200	200	158
Wheat meal	58	57.6	57.2	20	-	-	-
Vitamin premix [1]	20	20	20	20	20	20	20
Mineral premix [2]	10	10	10	10	10	10	10
Fish oil	60	60	60	60	60	60	60
Antioxidant [3]	2	2	2	2	2	2	2
Carophyll pink powder [4]	-	0.4	0.8	-	-	-	-
Red pepper meal	-	-	-	38	76	-	-
Shrimp by-product meal	-	-	-	-	-	100	200
Chemical analysis							
Dry matter	92.0	92.2	92.3	92.5	92.5	92.2	92.2
Crude protein	46.6	46.6	46.6	46.7	46.5	48.5	48.7
Crude fat	14.7	14.7	14.7	15.0	15.13	14.3	13.7
Crude fiber	2.3	2.3	2.3	3.0	3.6	2.0	1.6
Crude ash	14.4	14.4	14.4	14.5	14.6	16.3	18.3
Calcium	4.2	4.2	4.2	4.2	4.1	5.1	5.5
Phosphorus	2.5	2.5	2.5	2.5	2.4	2.6	2.5
Total xantophyll (mg/kg)	-	30	60	30	60	30	60
Digestible energy (MJ/kg)	13.3	13.3	13.3	13.1	13.1	13.1	13.1

[1] Vitamin mixture (per kg feed): A 18.0 IU, D3 2.0 IU, E 200 IU, K 12 mg, C 150 mg, B2 30 mg, B1 20 mg, B12 0.05 mg, pyridoxine 20 mg, panthotenic acid 10 mg, niacin 220 mg, inositol 210 mg, folic acid 5 mg, biotine 0.5 mg, coline 2.0 mg.
[2] Mineral mixture (per kg feed): zinc 70 mg, manganese 60 mg, magnesium 60 mg, ferro 4 mg, copper 2 mg, iode 1.5 mg, cobalt 0.5 mg, selenium 0.05 mg.
[3] Buthylhydroxitoluene, liquid form
[4] 8% astaxanthin, Hoffman La-Roche, Basle, Switzerland

feed was dried in an oven and stored in plastic bags at room temperature until use. Feed allowances were calculated according to water temperature and percent live weight. Feed was given twice a day (at 09:00 and 16:00).

Proximate analysis of feedstuffs, diets, and flesh was determined as follows: dry matter after drying at 105ºC in an oven, ash by incineration at 550ºC, protein by the Kjeldahl method after acid digestion, fat after extraction with petroleum ether by the Soxhlet method, flesh according to Bligh and Dyer (1959), crude fiber, Ca, and P according to New (1987), and total pigment analyses according to AOAC (1990).

Visual absorbance evaluation. The Roche Color Card for salmonids with a scale of 11-18 (from pink to red) was used for visual evaluation. Fillets were placed on a white setting under natural light. Readings were performed monthly by 11 volunteers. A visual score of 13 or higher is considered appropriate for marketed trout (Johnson and Wathne, 1989; Smith et al., 1992).

Chemical analysis. Spectrophotometric absorbance values were taken at 474 nm according to Bjerkeng (1990, 1992). For standard absorbance values, $E_{1\%, 1\ cm} = 1900$ was used (Foss et al., 1984, 1987; No and Storebakken, 1991a). Retention of coefficient (Rc) values in the flesh were calculated as suggested by Smith et al. (1992).

Physical analysis (colorimeter). Flesh color was measured with a Hunterlab Data Absorbance D-65 Reflectance Colorimeter under a 1.5 cm light source. Average readings were obtained by considering mean values from three readings from three parts of the fillet (posterior dorsal, anterior of adipose fin, and anterior tail). The colorimeter was first calibrated to white and then *L*, *a*, and *b* values were measured in the flesh and skin of the trout where *L* = lightness, *a* = redness (as opposed to greenness), and *b* = yellowness (as opposed to blueness; Skrede and Storebakken, 1986a).

Statistical analysis. Data were subjected to analysis of variance through Microsoft Excel 7.0 and differences between means were tested by Duncan's multiple range test according to Duzgunes et al. (1987).

Results

Growth performance. In all groups, mortality was less than 1% and fish grew normally (Table 2, Fig. 1). The highest and lowest weight gains were in the 30 ppm astaxanthin and control groups, respectively.

Color evaluation. Visual evaluations of flesh color according to the Roche salmon color card are shown in Table 3. Monthly changes in carotenoid concentrations in the flesh are given in Table 4. Hunter values (Hunter and Harold, 1987) are shown in Table 5. After 90 days, the highest and lowest brightness (*L*) values were in the control and 60 ppm astaxanthin groups, respectively. Effects of pigment sources on flesh redness (*a*) were evident by the first month of the study, when groups fed astaxanthin had redder flesh than most other groups. After 90 days, the best *b* results (yellowness) were in the 60 ppm red pepper group and the poorest in the control.

Discussion

Growth parameters. There is a linear relationship between fish size and pigment retained in tissues (Choubert and Store-bakken, 1989; Torrisen, 1989a; No and Storebakken, 1992). Torrisen (1986, 1989a) stressed that pigmentation for fish smaller than 50 g would not be economical. In the present study, fish with an initial weight of 95 g grew to 173.0-218.5 g. Other than for trout, little information exists on the effect of pigment sources on fish growth. Some authors hypothesized that carotenoids could possess additional benefits to fish such as enhanced resistance to detrimental environmental factors, cancer-preventive effects, strengthening of the immune system, and enhancement of survival during larval and postlarval stages (Tacon, 1981; Torrisen, 1984; Schiedt et al., 1985). Protein efficiency ratio was highest in groups fed red pepper and shrimp by-product meal and lowest in the control and those fed astaxanthin. This may be due to the different protein content in the pigment sources.

Table 2. Growth (means±SE) of trout raised on feed containing natural and synthetic cartenoids.

	Diet						
	Control	*30 ppm astaxanthin*	*60 ppm astaxanthin*	*30 ppm red pepper*	*60 ppm red pepper*	*30 ppm shrimp*	*60 ppm shrimp*
Initial wt (g)	101.4±0.55[a]	95.3±0.62[a]	104.5±0.42[a]	101.8±0.68[a]	89.0±0.71[a]	95.2±0.49[a]	95.5±0.57[a]
Final wt (g)	173.0±1.45[a]	218.5±2.12[b]	215.7±3.12[b]	188.2±2.05[b]	206.0±3.45[b]	177.2±2.81[b]	195.0±3.56[b]
Growth rate (%) [1]	70.9±0.95[a]	129.3±3.85[b]	106.4±2.47[b]	96.0±4.21[b]	131.5±3.33[b]	86.1±2.06[a]	104.0±2.78[b]
SGR [2]	0.54±0.05[a]	0.83±0.08[b]	0.71±0.03[b]	0.71±0.03[b]	0.84±0.08[b]	0.62±0.06[b]	0.71±0.04[b]
FCR [3]	2.23±0.11[a]	1.38±0.06[b]	1.54±0.08[b]	1.50±0.06[b]	1.44±0.03[b]	1.94±0.08[b]	1.53±0.04[b]
PER [4]	0.95±0.02[a]	1.53±0.04[b]	1.35±0.03[b]	2.7±0.04[b]	2.6±0.06[b]	2.36±0.05[b]	2.6±0.06[b]

Means in a row with different subscripts are significantly different (p<0.05).
[1] Growth rate = 100[(final body weight – initial body weight)/initial body weight]
[2] Specific growth rate = 100[(Ln final body weight – Ln initial body weight)/days]
[3] Feed conversion ratio = dry feed intake/wt gain
[4] Protein efficiency ratio = wt gain/protein intake

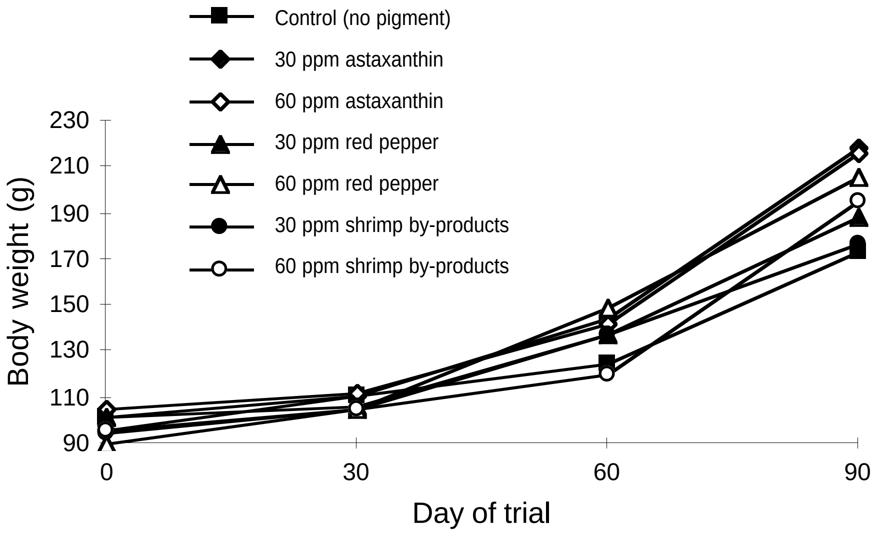

Fig. 1. Growth of trout fed diets containing different color enhancers.

Table 3. Initial and final color values (means±SE) of trout fillets according to salmon color card (Hoffman La-Roche, Basle, Switzerland).

	Initial	*Final*
Control	11.25±0.27[a]	11.55±0.25[a]
30 ppm astaxanthin	11.06±0.27[a]	13.40±0.21[c]
60 ppm astaxanthin	11.40±0.27[a]	14.46±0.23[d]
30 ppm red pepper	11.34±0.25[a]	12.86±0.02[b]
60 ppm red pepper	11.59±0.25[a]	12.93±0.18[b]
30 ppm shrimp by-products	11.50±0.26[a]	11.93±0.03[a]
60 ppm shrimp by-products	11.45±0.28[a]	12.73±0.22[b]

Means with different superscripts are significantly different ($p<0.05$).

Visual evaluation of flesh. According to Smith et al. (1992), marketable fish with visual values of 13-14 on the Roche color card are optimal in terms of quality and attractiveness. These values were attained by the astaxanthin groups but only approached by those fed red pepper.

Chemical evaluation of flesh. In March and McMillan (1996), carotenoid concentrations and carotenoid accumulation coefficients in

Table 4. Monthly changes in retention of coefficients (Rc) in fillet.

	30 days	*60 days*	*90 days*
Control	0.21	1.68	1.73
30 ppm astaxanthin	0.72	4.80	6.63
60 ppm astaxanthin	1.34	2.56	4.66
30 ppm red pepper	0.21	1.72	3.93
60 ppm red pepper	0.57	3.19	3.06
30 ppm shrimp by-products	0.24	1.77	1.92
60 ppm shrimp by-products	0.27	0.80	1.79

Rc = 100[∑n(CPT final – CPT initial)(TW final – TW initial)/(TFI)(PCF)], where ∑n = number of fish, CPT = concentration of pigment of tissue in mg/kg, TW = tissue weight in g = average live weight x tissue weight as a % of body weight (for fillets, 0.588), TFI = total feed intake in g, PCF = pigment concentration of feed in ppm (Smith et al., 1992)

the flesh were calculated by spectrophotometric absorbance values read on a 474 nm scale (Smith et al., 1992). In the formula, 1900 was considered an extinction coefficient (Foss et al., 1984, 1987; Skrede and Storebakken, 1986a; Bjerkeng et al., 1990; No and Storebakken, 1991a).

The retention coefficient value (Rc) for flesh was highest in fish fed the 30 ppm astaxanthin diet (6.63) and poorest in fish fed the shrimp by-product diet (1.79). Unexpectedly, results for both red pepper diets were similar (3.06 and 3.93). Smith et al. (1992) reported values of 3.20, 3.39, 1.62, and 1.84 for fish fed 15, 30, 45, and 60 ppm astaxanthin, respectively, while No and Storebakken (1991a) reported values for fish fed 57 ppm astaxanthin as 7.84 at 15ºC and 6.99 at 5ºC. Similar results were obtained in other studies (Storebakken et al., 1986; Choubert and Storebakken, 1989; Bjerkeng et al., 1990; Storebakken and Choubert, 1991). Concentrations of 4-6 mg/kg carotenoid concentration in fish flesh can be easily distinguished (Foss et al., 1984; Skrede et al., 1990; Smith et al., 1992). Rc values in this study were within this range.

A relationship between pigment accumulation and fish weight has been established (Abdul-Malik et al., 1975, cited in Torrisen et al., 1989). Carotenoids retained in flesh of fish over 1 kg range 20-25 mg/kg (Storebakken et al., 1986; Skrede et al., 1990) and 6 mg/kg for fish weighing around 100-200 g (Foss et al., 1987; Torrisen et al., 1989; Bjerkeng et al., 1990). Arai et al. (1987) reported that while pigment accumulation did not differ for 50-90 g fish, it gradually increased in larger fish. This could be due to their higher digestion activity.

In a study of 5 kg salmon, the carotenoid concentration was 11.3 mg/kg for wild salmon and 6.5 mg/kg for cultured salmon (Skrede and Storebakken, 1986b). Peterson et al. (1986, cited in Torrisen, 1989b) found 3.2 mg/kg pigment retained in flesh of brown trout when the fish were fed diets including 50 ppm red pepper meal. Likewise, in this study, 3.39 and 5.50 mg/kg pigment was retained in fish fed 30 and 60 ppm red pepper meal, respectively. Choubert and Luquet (1982) stated that red pepper provided redness in salmon due to its capsantin and capsorubin contents that improved feed intake through its capsanthin and bitterness. The carotenoid concentration from the diets containing shrimp by-product meal was comparable to the results of Choubert and Luquet (1982; 2 mg/kg), obtained

Table 5. Hunter color values (means±SE) for rainbow trout fed different carotenoids.

	Initial	*30 days*	*60 days*	*90 days*
L value (brightness)				
Control	50.25±0.05	51.31±1.02^{b}	53.60±0.68^{b}	54.57±0.26^{d}
30 ppm astaxanthin	51.90±0.05	51.11±1.02^{b}	50.13±0.68^{a}	48.91±0.37^{b}
60 ppm astaxanthin	50.65±0.05	49.50±1.02^{a}	49.00±0.68^{a}	46.81±0.50^{a}
30 ppm red pepper	53.90±0.05	52.20±1.02^{c}	52.62±0.68^{b}	50.84±0.37^{c}
60 ppm red pepper	53.75±0.05	54.30±1.02^{d}	52.42±0.68^{b}	50.29±0.45^{c}
30 ppm shrimp by-products	51.70±0.05	51.38±1.02^{b}	50.16±0.68^{a}	50.11±0.46^{c}
60 ppm shrimp by-products	52.09±0.05	52.40±1.02^{c}	51.90±0.68^{a}	49.50±0.56^{bc}
a value (redness)				
Control	0.85±0.05	0.80±0.5^{a}	0.79±0.48^{a}	0.87±0.47^{a}
30 ppm astaxanthin	0.77±0.05	2.49±0.5^{b}	4.71±0.48^{c}	5.49±0.47^{c}
60 ppm astaxanthin	0.86±0.05	2.65±0.5^{b}	4.32±0.48^{c}	6.96±0.47^{d}
30 ppm red pepper	0.72±0.05	1.33±0.5^{a}	1.65±0.48^{b}	3.40±0.47^{b}
60 ppm red pepper	0.75±0.05	3.03±0.5^{c}	2.16±0.48^{b}	3.84±0.47^{b}
30 ppm shrimp by-products	0.78±0.05	1.32±0.5^{a}	1.35±0.48^{ab}	1.6±0.47^{ab}
60 ppm shrimp by-products	0.82±0.05	1.76±0.5^{ab}	2.29±0.48^{b}	3.32±0.47^{b}
b value (yellowness)				
Control	10.22±0.05	10.40±0.57^{a}	10.64±0.45^{a}	10.94±0.27^{a}
30 ppm astaxanthin	9.52±0.05	10.77±0.57^{a}	11.29±0.45^{b}	11.60±0.27^{b}
60 ppm astaxanthin	9.82±0.05	10.85±0.57^{b}	11.60±0.45^{bc}	11.90±0.27^{b}
30 ppm red pepper	10.25±0.05	10.53±0.57^{a}	11.37±0.45^{b}	12.49±0.27^{c}
60 ppm red pepper	10.11±0.05	10.30±0.57^{a}	11.49±0.45^{b}	12.90±0.27^{d}
30 ppm shrimp by-products	9.87±0.05	10.30±0.57^{a}	11.55±0.45^{bc}	12.40±0.27^{c}
60 ppm shrimp by-products	9.28±0.05	10.47±0.57^{a}	11.91±0.45^{c}	12.80±0.27^{cd}

Means in a column with different superscripts are significantly different ($p<0.05$).

when shrimp by-products were included at 11% in a diet for 200 g rainbow trout.

Physical evaluation of flesh. Reflectometers are used in research on food and fishery sciences for measuring color (Skrede and Storebakken, 1986a,b; King, 1996). D-65 and C (lightness) are commonly employed. In this study, D-65, giving light like sunlight, was used.

As the concentration of carotenoid in the diets and tissues increased, *L*, *a*, and *b* values in the flesh dropped. *B* values were highest in 60 ppm pepper and shrimp by-product groups. No and Storebakken (1991b) reported that as the carotenoid concentration increased, *a*, and *b* increased but not *L*. They found that *L, a*, and *b* were 1.67, 9.27, and 52.1, respectively. Similar trends were observed in this study. Skrede and Storebakken (1986a) obtained *L, a,* and *b* values of 42.9, 8.3, and 10.1 in cultured and 42.9, 10.1, and 10.0 in wild salmon. There is a close relationship between instrumental color measurements and carotenoid concentration (Skrede and Storebakken, 1986a,b). No and Storebakken (1992) compared effects of sea water and fresh water on pigmentation and observed no differences among *L, a*, and *b*.

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