

## The effect of different carotenoid sources on skin coloration of cultured red porgy (*Pagrus pagrus*)

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### Abstract

This study presents data on the effect of carotenoid sources on skin coloration of red porgy (*Pagrus pagrus*). Three experiments were conducted: in the first, fish were fed an astaxanthin (Naturose<sup>®</sup>)-supplemented diet, while the second fish received diets supplemented with  $\beta$ -carotene (Rovimix  $\beta$ -caroten<sup>®</sup>) or lycopene (Lyc-O-Mato<sup>®</sup>): Carotenoids were added to the level of 100 ppm in each diet, while a non-carotenoid-supplemented diet served as a control. In the third experiment, the effect of dietary protein/carbohydrate ratio on melanin content in the skin was investigated. For this experimentation, four diets were formulated to contain 50/23, 40/32, 30/48 and 20/59 protein/carbohydrate ratio. Naturose<sup>®</sup> astaxanthin increased total carotenoid content in the dorsal skin area while  $\beta$ -carotene and lycopene seem to have had no significant effect. Naturose<sup>®</sup> was the only carotenoid source that had a significant effect on skin hue, promoting a reddish coloration to the dorsal skin area and a ventral hue similar to wild red porgy. No apparent effect of carotenoid source on skin melanin content was observed. In contrast, dietary protein/carbohydrate ratio affected melanin content in the skin. The fish fed the 50/23 diet showed significantly higher values. Farmed red porgy had eight times higher dorsal-skin melanin content than wild ones.

**Keywords:** carotenoids, red porgy, coloration, melanin

### Introduction

Fish skin colour is primarily dependent on the presence of chromatophores (melanophores, xantho-

phores, erythrophores, iridophores, leucophores and cyanophores), containing pigments such as melanins, carotenoids (e.g. astaxanthin, canthaxanthin, lutein and zeaxanthin), pteridines and purines. Further, fish can exhibit different coloration patterns as a result of the dispersion or aggregation of chromatosomes and the distribution of chromatophores in the skin (Withers 1992). A combination of environmental, neural, endocrine and husbandry-related factors influences chromatophore mobility and pigment deposition in cultured fish (Fujii 2000). The interactions between the above factors are complex and only partially understood by fish physiologists and fish farmers.

Dietary carotenoids play an important role in the regulation of skin and muscle colour in fish. Carotenoids are synthesized from geranylgeranyl diphosphate by all photosynthetic organisms (Giuliano, Aquilani & Dharmapuri 2000). In their biosynthetic pathway, lycopene is converted to  $\beta$ -carotene, which in turn is further metabolized to astaxanthin. Photosynthetic plants can synthesize lycopene and  $\beta$ -carotene while astaxanthin is a non-plant carotenoid. In common with other animals, fish do not possess the ability 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 carotenoid-rich feed and, therefore, the necessary carotenoids must be added to the diet. The effectiveness of carotenoid source in terms of deposition and pigmentation is species specific (Ha, Kang, Kim, Choi & Ryu 1993). In addition, not all fish species possess the same pathways for the metabolism of carotenoids and, consequently, there is no universal transformation of carotenoids in fish tissues. Goldfish

(Dutch lion head) convert the yellow xanthophyll pigment zeaxanthin to the red carotenoid pigment astaxanthin through 4-ketozeaxanthin (Hata & Hata 1972). Conversely, trout (*Oncorhynchus mykiss*) performs the opposite transformation that is the conversion of astaxanthin to zeaxanthin (Katsuyama, Komori & Matsumo 1987). Salmon and red sea bream (*Pagrus major*) do not convert xanthophylls to canthaxanthin or astaxanthin (Storebakken & No 1992; Torrissen 2000). Shrimps (*Penaeus monodon*) have the ability to convert  $\beta$ -carotene to astaxanthin (Boonyaratpalin, Thongrod, Supamattaya, Britton & Schlipalius 2001; Linan-Cabello, Paniagua-Michel & Hopkins 2002). In salmonids, astaxanthin is found in its unesterified form in white muscle while esterified astaxanthin is the predominant form in the skin (Bowen, Soutar, Serwata, Lagocki, White, Davies & Young 2002; White, Moody, Serwata, Bowen, Soutar, Young & Davies 2003). Similar to salmonids red sea bream (*P. major*) deposits esterified astaxanthin in its skin (Ha *et al.* 1993; Lorenz 1998).

There have been many attempts to induce pink or red skin coloration to cultured red sparides. As it was perceived that the red colour of wild fish is mainly because of astaxanthin, cultured fish were fed diets supplemented with free astaxanthin or sources of astaxanthin or even with astaxanthin precursors. In Australian snapper (*Pagrus auratus*) both esterified (NatuRose<sup>®</sup>) and unesterified (Carophyll Pink<sup>®</sup>, Hofmann-La Roche, Basel, Switzerland) astaxanthin improved skin redness (Booth, Warner-Smith, Allan & Glencross 2004), while in red porgy (*Pagrus pagrus*) a shrimp meal supplement, *Pleisonika* sp., containing a mixture of natural carotenoids with an apparent predominance of astaxanthin (Cejas, Almansa, Tejera, Jerez, Bolannos & Lorenzo 2003), a krill meal containing 20 mg kg<sup>-1</sup> astaxanthin (Chebbaki, Robaina, Vergara, Izquierdo, Fernandez-Palacios & Chatzifotis 2002) or a shrimp shell meal containing 40 mg kg<sup>-1</sup> astaxanthin (Kalinowski, Robaina, Fernández-palacios, Schuchardt & Izquierdo 2005) improved red body coloration. Similarly, in red sea bream (*P. major*) astaxanthin ester was effective in providing red coloration while other carotenoids such as  $\beta$ -carotene, zeaxanthin, lutein and canthaxanthin did not have marked effects (Nakazoe, Ishii, Kamimoto & Takeuchi 1984; Ha *et al.* 1993).

Although the addition of carotenoid in diet promotes a reddish coloration, the cultured red porgy still displays a darker colour unlike wild specimens, which have a pink and silver colour on the dorsolateral surface of their skin. This might be because of the

high melanin content and/or the low or wrong type of carotenoids in the skin. Melanin, which is synthesized from tyrosine and whose main function is photoprotection, could be overproduced as a physiological response of fish to culture conditions as a result of the exposure to high-intensity sunlight or stress imposed by aquaculture practices. Cultured red porgy apart from being exposed to ultraviolet (UV) light received protein-rich diets, which may promote melanogenesis because of their high tyrosine content. In addition, the lack of dietary carotenoids may further enhance melanogenesis, as carotenoids are known to prevent oxidative stress. To get a better understanding on the nutritional regulation of skin colour in red porgy, towards the development of a natural hue in the reared population, the present study aims (a) to investigate the effectiveness of three carotenoids, namely astaxanthin,  $\beta$ -carotene and lycopene in improving red skin coloration, and (b) to identify whether the protein/carbohydrate ratio of the diet affects melanin formation in the skin.

## Materials and methods

The red porgies used for the experiments were obtained from a genetically homogenous stock and raised on a non-carotenoid-supplemented commercial gilthead sea bream diet. Fish were housed in 500 L circular polyester tanks (triplicate tanks per treatment; 20 fish per tank), which had a black background and were supplied with biologically filtered seawater of 40 ppt salinity, oxygenated to saturation by air supply. The rearing tanks were under natural photoperiod. Fish had constant access to feed supplied by self-feeders. Wild specimens as well as fish raised at a local fish farm were also obtained for comparison with the experimental fish. The farmed red porgies were caught by net and killed in icy water while the wild fish were caught by a local fisherman using long lines. Specimens were placed on ice until they were transported to the laboratory and preserved at  $-80^{\circ}\text{C}$ .

Three experiments were conducted: in the first one, fish were fed on an astaxanthin (1.58% astaxanthin content; Naturose<sup>®</sup> – Cyanotech Corporation, Kailua-Kona, HI, USA) supplemented diet and in the second one, with a diet supplemented with  $\beta$ -carotene (10%  $\beta$ -carotene content; Rovimix  $\beta$ -caroten<sup>®</sup> – Roche, Hellas, SA, Australia) or lycopene (0.92% lycopene content; Lyc-O-Mato<sup>®</sup> – Natural Products Industries, Beer-Sheva, Israel). Diets were supplemented

**Table 1** Diet formulation (g kg<sup>-1</sup> diet) and growth performance of fish fed experimental diets (mean ± SEM, n = 3)

Ingredient	Experiment 1		Experiment 2		Experiment 3				
	Astaxanthin	Control	β-carotene	Lycopene	Control	50/23*	40/32*	30/48*	20/59*
Fish meal	650	650	750	750	750	714	571	428	286
Wheat starch	120	120	80	80	80	150	250	350	450
Fish oil	80	80	75	75	75	54	73	92	107
Mineral+vitamin†	20	20	25	25	25	20	20	20	20
Choline chloride	4	4	4	4	4	4	4	4	4
Alginate	20	20	20	20	20	20	20	20	20
Celulose	100	106	45	36	46	32	56	80	107
NatuRose®	6	0	0	0	0	6	6	6	6
Lyc-O-Mato®	0	0	0	10	0	0	0	0	0
Rovimix-β-caroten®	0	0	1	0	0	0	0	0	0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Chemical analysis (% dry weight)									
Protein	45.5	45.3	52.2	52.8	52.5	50.2	42.6	29.2	19.8
Fat	15.4	15.3	16.1	16.1	16.2	13.6	13.8	14.1	13.9
Growth performance‡									
Water temperature range (°C)	15–17	15–17	22–25	22–25	22–25	21–25	21–25	21–25	21–25
Duration (weeks)	10	10	10	10	10	10	10	10	10
Initial fish weight (g)	82.0 ± 8.5	82.0 ± 5.2	149.7 ± 20.2	144.6 ± 15.5	151.6 ± 22.8	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1
Final fish weight (g)	98.0 ± 9.6	105.9 ± 10.5	170.9 ± 25.8	179.5 ± 18.6	173.1 ± 25.3	23.4 ± 3.2*	19.5 ± 1.4*	12.3 ± 0.40*	8.2 ± 0.6*

\*Protein/CHO ratio. CHO = total carbohydrates (fibre inclusive) = 100 – protein–lipid–ash.

†Mineral and vitamin mix for aquaculture (No. 5024), Roche Vitamin Hellas.

‡In the same line values with asterics (\*) were statistically significant, P < 0.05.

with 100 ppm of each carotenoid, while a non-carotenoid-supplemented diet served as a control (Table 1). In a third experiment, the effect of dietary protein/carbohydrate ratio on melanin formation in the skin was investigated. For this experimentation four diets (Table 1) were formulated to contain the following protein/carbohydrate ratios: 50/23, 40/32, 30/48 and 20/59 (total CHO = total carbohydrate (inclusive fibre) = 100 – protein–lipid–ash). All ingredients were thoroughly mixed and moistened by the addition of 75% (w/v) water, and then made into pellets using a mincer. The diets were dried overnight at 35 °C and stored at –18 °C until used. Before starting the experiments, fish were acclimated to control diets for 1 week. Each experiment lasted for 10 weeks.

Skin colour was measured in the dorsal (D) and ventral (V) skin area of five randomly selected fish from each tank immediately after capturing the fish, using a portable spectrophotometer (Miniscan™ XE, HunterLab, Reston, VA, USA) and employing the CIE L\* (lightness; dark = 0, white = 100), a\* (red = +values, green = –values) and b\* (yellow = +values, blue = –value) colour scale. The three-dimensional characteristics of colour appearance, i.e. the lightness

attribute ('Value', L\*), and the two chromatic attributes hue ( $H_{ab}^* = \arctan(b^*/a^*)$ ) and chroma ( $C_{ab}^* = (a^{*2} + b^{*2})^{0.5}$ ) were calculated. Colour measurements were not taken for the third experiment because the small size of the fish was not compatible with the spectrophotometer specifications.

The skin was dissected from the dorsal area for melanin and carotenoid content determination. Skin melanin concentration was determined using a modified procedure of Wilson and Dodd (1973), as described in Szisch, van der Salm, Wendelaar Bonga and Pavlidis (2002). Total carotenoids were extracted with acetone and quantified spectrophotometrically using the extinction coefficient  $E1_{1\text{cm}}^{1\%} = 2100$  for astaxanthin in acetone.

The effect of astaxanthin on the red porgy's pigment cells was studied in 10 anterior dorsal innervated scales by employing a video-image processing apparatus adapted to a stereoscope (Leica MZ 125, Leica Microsystems AG, Wetzlar, Germany) without any further treatment. The system consisted of a colour camera (DDC-IRIS, Sony, Tokyo, Japan) and a processing unit image (Image Analysis, Image Pro-plus 2.0, Media Cybernetics, Silver Spring, MD, USA) monitor.

Crude protein and crude fat in diets were determined using the procedure of Kjeldahl after acid digestion and using diethyl ether extraction respectively (AOAC 1975).

### Statistical analysis

The statistical errors are expressed as the standard error of the mean (SEM). Data were analysed for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Bartlett's test), and when necessary, they were log transformed before being treated statistically. Statistical comparisons of skin melanin concentration and carotenoid content between experimental groups were made using one-way analysis of variance (ANOVA). Two-way ANOVA was applied to check for significant changes between experimental groups and time (initial vs. final sampling) within a single parameter ( $L^*$ , Chroma). If significant ( $P < 0.05$ ), Tukey's significant means test was applied to identify groups that were significantly different. Statistical comparison of the hue variable among experimental groups was performed according to Zar (1996). Differences between groups were tested by the Watson–Williams test.

### Results

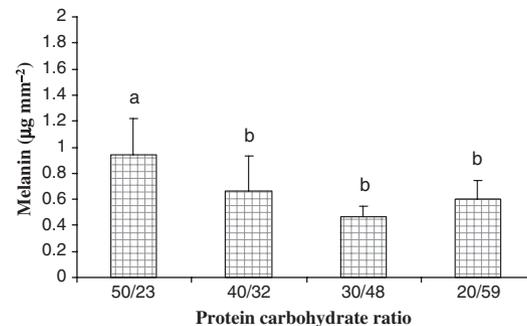
All experimental diets were equally accepted by fish. The carotenoid-supplemented diets did not appear to have any effect on red porgy's growth rate. Only the protein to carbohydrate ratio significantly affected fish growth (experiment 3). As the dietary starch increased in expense of protein, fish exhibited reduced growth; fish fed the 50/23 diet showed the highest final weight, while the 20/59, the lowest (Table 1).

In terms of total carotenoid deposition, only astaxanthin remarkably increased the total carotenoid content of the dorsal skin area, which approaches the values of wild specimens (astaxanthin-fed fish:  $27.7 \pm 4.1 \mu\text{g}$  total carotenoids  $\text{g}^{-1}$  skin; wild fish:  $38.4 \pm 3.2 \mu\text{g}$  total carotenoids  $\text{g}^{-1}$  skin), while  $\beta$ -carotene and lycopene seem to have no significant effect (Table 2). The wild red porgy had astaxanthin diester as its predominant carotenoid followed by unidentified xanthophylls (unpublished data of our laboratory). On the contrary, farmed red porgy fed the sea-bream (non-carotenoid) diet had non-detectable levels of carotenoids and had six times higher dorsal-skin melanin content ( $8.0 \pm 0.8 \mu\text{g}$

**Table 2** Dorsal skin melanin and carotenoid concentrations (mean  $\pm$  SEM,  $n = 5$ ) of red porgy fed diets supplemented with different carotenoids

	Total carotenoids ( $\mu\text{g g}^{-1}$ skin)	Melanin ( $\mu\text{g mm}^{-2}$ skin)
Experiment 1		
Astaxanthin	$27.7 \pm 4.1^a$	$3.30 \pm 0.4$
Control	$4.33 \pm 0.5^b$	$2.13 \pm 0.3$
Experiment 2		
$\beta$ -carotene	$8.6 \pm 0.8$	$6.2 \pm 0.6$
Lycopene	$13.5 \pm 2.2$	$5.8 \pm 0.7$
Control	$7.6 \pm 1.5$	$5.8 \pm 0.6$
Farmed red porgy (non-carotenoid diet)	$0.0 \pm 0.0^a$	$8.0 \pm 0.8^a$
Wild fish (cretan origin)	$38.4 \pm 3.2^b$	$1.4 \pm 0.3^b$

Statistical differences between groups within each experiment are indicated by different superscripts ( $P < 0.05$ ).



**Figure 1** Effect of dietary protein/carbohydrate ratio on melanin content in the skin of red porgy (mean  $\pm$  SEM,  $n = 5$ ). Statistical differences between groups are indicated by different letters ( $P < 0.05$ ).

melanin  $\text{mm}^{-2}$  skin) than wild red porgies ( $1.4 \pm 0.3 \mu\text{g}$  melanin  $\text{mm}^{-2}$  skin). No apparent effect of astaxanthin,  $\beta$ -carotene or lycopene on skin melanin content was observed (Table 2). In contrast, the protein/carbohydrate ratio of the diet affected melanin deposition; fish fed the highest protein/carbohydrate ratio (50/23) showed the highest melanin content (Fig. 1).

A marked dorsoventral gradient was observed in all colour attributes, with the ventral area being lighter than the dorsal one (Table 3). In all experiments and in both the dorsal and ventral skin area, there was a statistically significant difference in  $L^*$  value (lightness) between the initial and the final samplings within each respective group, except for the  $\beta$ -carotene group (Table 3). However, there was no significant difference between the experimental groups and no treatment provided a skin lightness value similar to wild fish.

**Table 3** Lightness, hue and chroma values (mean  $\pm$  SEM,  $n = 5$ ) of the dorsal and ventral skin area of red porgy fed diets supplemented with different carotenoids

Group/skin area	Dorsal		Ventral	
	Initial	Final	Initial	Final
<i>L*</i>				
Experiment 1				
Astaxanthin	41.84 $\pm$ 2.41 <sup>a</sup>	50.70 $\pm$ 0.87 <sup>b</sup>	52.65 $\pm$ 0.45 <sup>a</sup>	58.46 $\pm$ 0.67 <sup>b</sup>
Control	44.34 $\pm$ 1.38 <sup>a</sup>	49.95 $\pm$ 1.65 <sup>b</sup>	53.67 $\pm$ 0.97 <sup>a</sup>	62.83 $\pm$ 0.76 <sup>b</sup>
Experiment 2				
$\beta$ -carotene	49.66 $\pm$ 1.43 <sup>a</sup>	51.29 $\pm$ 1.40 <sup>ab</sup>	55.02 $\pm$ 1.31 <sup>a</sup>	56.35 $\pm$ 1.15 <sup>ab</sup>
Lycopene	46.88 $\pm$ 1.19 <sup>a</sup>	55.10 $\pm$ 2.72 <sup>b</sup>	53.20 $\pm$ 1.13 <sup>a</sup>	58.20 $\pm$ 1.69 <sup>b</sup>
Control	48.31 $\pm$ 1.06 <sup>a</sup>	54.35 $\pm$ 1.05 <sup>b</sup>	54.31 $\pm$ 1.42 <sup>a</sup>	57.09 $\pm$ 1.15 <sup>b</sup>
Farmed red porgy (non-carotenoid diet)		33.87 $\pm$ 4.50 <sup>a</sup>		53.40 $\pm$ 3.24 <sup>c</sup>
Wild fish (Cretan origin)		60.75 $\pm$ 1.22 <sup>b</sup>		80.30 $\pm$ 4.26 <sup>d</sup>
<i>Hue</i>				
Experiment 1				
Astaxanthin	1.25 $\pm$ 0.32 <sup>a</sup>	0.81 $\pm$ 0.04 <sup>b</sup>	1.26 $\pm$ 0.05 <sup>a</sup>	0.86 $\pm$ 0.02 <sup>b</sup>
Control	1.42 $\pm$ 0.21 <sup>a</sup>	1.04 $\pm$ 0.13 <sup>a</sup>	1.34 $\pm$ 0.06 <sup>a</sup>	1.25 $\pm$ 0.04 <sup>a</sup>
Experiment 2				
$\beta$ -carotene	0.86 $\pm$ 0.05 <sup>a</sup>	1.04 $\pm$ 0.03 <sup>b</sup>	0.99 $\pm$ 0.03 <sup>a</sup>	1.12 $\pm$ 0.03 <sup>b</sup>
Lycopene	0.81 $\pm$ 0.03 <sup>a</sup>	1.00 $\pm$ 0.04 <sup>b</sup>	0.91 $\pm$ 0.02 <sup>a</sup>	1.07 $\pm$ 0.02 <sup>b</sup>
Control	0.79 $\pm$ 0.04 <sup>a</sup>	0.95 $\pm$ 0.05 <sup>b</sup>	0.90 $\pm$ 0.02 <sup>a</sup>	1.06 $\pm$ 0.03 <sup>b</sup>
Farmed red porgy (non-carotenoid diet)		0.58 $\pm$ 0.05 <sup>a</sup>		1.11 $\pm$ 0.06 <sup>c</sup>
Wild fish (Cretan origin)		1.10 $\pm$ 0.06 <sup>b</sup>		0.99 $\pm$ 0.04 <sup>d</sup>
<i>Chroma</i>				
Experiment 1				
Astaxanthin	5.08 $\pm$ 1.00 <sup>a</sup>	10.19 $\pm$ 0.48 <sup>b</sup>	8.05 $\pm$ 0.23 <sup>a</sup>	11.26 $\pm$ 0.61 <sup>b</sup>
Control	5.14 $\pm$ 0.54 <sup>a</sup>	4.16 $\pm$ 0.53 <sup>a</sup>	7.16 $\pm$ 0.57 <sup>a</sup>	5.93 $\pm$ 0.25 <sup>a</sup>
Experiment 2				
$\beta$ -carotene	8.29 $\pm$ 0.52 <sup>b</sup>	9.92 $\pm$ 0.54 <sup>d</sup>	13.11 $\pm$ 0.52 <sup>b</sup>	11.72 $\pm$ 0.42 <sup>b</sup>
Lycopene	8.66 $\pm$ 0.53 <sup>b</sup>	9.30 $\pm$ 0.58 <sup>d</sup>	12.50 $\pm$ 0.53 <sup>b</sup>	11.90 $\pm$ 0.66 <sup>b</sup>
Control	6.82 $\pm$ 0.61 <sup>a</sup>	7.57 $\pm$ 0.48 <sup>c</sup>	10.46 $\pm$ 0.47 <sup>a</sup>	9.92 $\pm$ 0.39 <sup>a</sup>
Farmed red porgy (non-carotenoid diet)		3.45 $\pm$ 0.53 <sup>a</sup>		8.51 $\pm$ 1.42 <sup>c</sup>
Wild fish (Cretan origin)		10.86 $\pm$ 0.97 <sup>b</sup>		5.31 $\pm$ 1.32 <sup>d</sup>

Statistical differences between groups as well as initial vs. final measurements within each experiment are indicated by different superscripts ( $P < 0.05$ ).

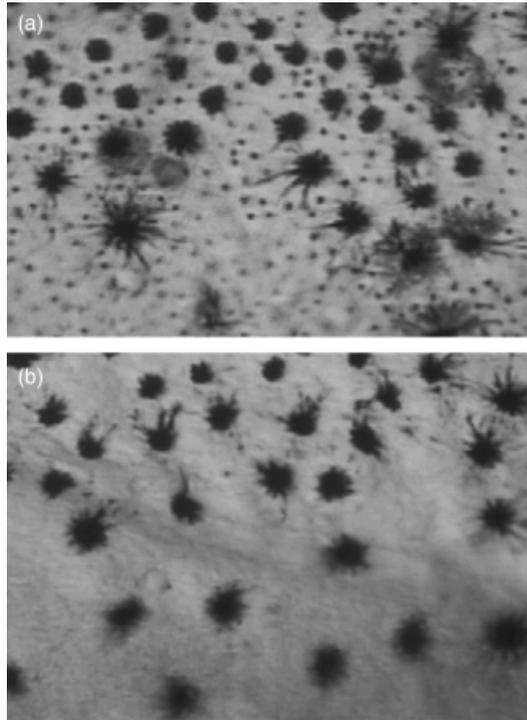
Astaxanthin was the only carotenoid source that had a significant effect on the hue of the dorsal skin (Table 3) and further significantly increased the chroma close to values of wild fish. Overall astaxanthin promoted a reddish coloration of the dorsal skin area and a ventral hue similar to that of the wild red porgy. Lycopene and  $\beta$ -carotene had no significant effect on either dorsal or ventral hue.

Two types of pigment cells were observed in the skin on the scales of fish fed the hematococcus supplemented diet, the bigger as melanophores and the smaller as granules coloured red or yellow xanthophores/erythrophores (Fig. 2). Melanophores and xanthophores/erythrophores were observed at the stages ranging from full pigment dispersion to full pigment aggregation. In contrast, mainly melanophores and very rarely xanthophores/erythrophores

were observed on the scales of the control, lycopene- and  $\beta$ -carotene-fed fish.

## Discussion

In nature, carotenoids have been implicated in diverse functions such as pigmentation, antioxidant activity, immunostimulation and reproduction, and they play a positive role in intermediary metabolism (Segner, Arend, Poeppinghausen & Schmidt 1989; Torrissen, Hardy & Shearer 1989; Anstey 2002; McGraw & Ardia 2003; Watanabe and Vassallo-Aguis 2003). Further, there are also reports (Torrissen 1984; Christiansen, Lie & Torrissen 1995) that link carotenoids to growth enhancement in Atlantic salmon fry (*Salmo salar*), or to improvement of survival rate in kuruma prawn (*P. japonicus*) (Chien & Jeng 1992).



**Figure 2** Pigment cells in the skin covering the scales of red porgy (a) fish fed the astaxanthin supplemented diet. Melanophores and erythrophores/xanthophores are present from full pigment dispersion to full pigment aggregation. (b) Fish fed the control, lycopene or  $\beta$ -carotene diet. Melanophores and very rare erythrophores/xanthophores are present.

Yet there is no unequivocal agreement on this issue, as in species like sea bream (*Sparus aurata*), red porgy (*P. pagrus*) (Chebbaki *et al.* 2002; Kalinowski *et al.* 2005) or rainbow trout (*Oncorhynchus mykiss*) (Sommer, Potts & Morrissy 1991; Nickell & Bromage 1998; White *et al.* 2003), carotenoids do not cause any notable increase in growth. In agreement with the latter studies, our results showed that esterified astaxanthin,  $\beta$ -carotene or lycopene do not promote the growth of red porgy.

As red sea bream cannot convert other carotenoids ( $\beta$ -carotene, zeaxanthin, lutein and canthaxanthin) to astaxanthin (Tanaka, Katayama, Simpson & Chichester 1976; Nakazoe *et al.* 1984; Ha *et al.* 1993), it is evident that the reddish pigmentation of cultured red sea bream can be obtained only after a supply of dietary astaxanthin (Katayama, Shintani, Shimaya, Imai & Chichester 1972; Tanaka *et al.* 1976; Fujita, Satake, Watanabe, Kitajima, Miki, Yamaguchi & Konosu 1983; Nakazoe *et al.* 1984). Our data with red porgy verify this observation. From the three carotenoid

sources studied, only NatuRose<sup>®</sup>, predominately containing esterified astaxanthin (Lorenz 2001), increased total carotenoid content of the skin and indeed enhanced colour redness. In addition to red porgy, haematococcus algae provided reddish coloration also in rainbow trout (Sommer *et al.* 1991; Sommer, D'Sousa & Morrissy 1992; Choubert & Heinrich 1993). In goldfish (*Carassius auratus*), red coloration was also exhibited by the microalga *Chlorella vulgaris* (Gouveia, Rema, Pereira & Empis 2003), which contained astaxanthin as the dominant carotenoid.

Early studies in red sea bream (Nakazoe *et al.* 1984; Ito, Kamata, Tanaka & Sameshima 1986; Ha *et al.* 1993) reported that esterified astaxanthin is superior to free astaxanthin in providing red coloration to the skin. These results are in apparent contradiction with later studies in coho salmon (*Onchorhynchus kisutch*), Australian snapper (*P. auratus*) or gilthead sea bream (*S. aurata*) (Mori, Makabe, Yamaguchi, Konosu & Arai 1989; Gomes, Dias, Silva, Valente, Empis, Gouveia, Bowen & Young 2002; Booth *et al.* 2004) in which fish free astaxanthin was as effective as esterified astaxanthin and even superior to esterified astaxanthin (Sommer *et al.* 1991) when algae *Haematococcus pluviatilis* was used as a source of esterified astaxanthin. It is worth mentioning that as far as salmonids are concerned, astaxanthin is absorbed in the digestive tract in its free form after ester hydrolysis, and is found in the plasma in its free state and deposited in the skin after re-esterification (Torrissen *et al.* 1989; Storebakken & No 1992 and reference therein). Therefore, the differences in the effectiveness of astaxanthin forms in providing red coloration may be because of poor digestibility, absorption and tissue deposition of different astaxanthin sources either because fish have limited ability to rupture algal cells or to metabolize different optical or geometrical isomers of astaxanthin (Choubert & Luquet 1983; Foss, Storebakken, Austreng & Liaaen Jensen 1987; Sommer *et al.* 1991; Bjerkeng, Følling, Lagocki, Storebakken, Olli & Alsted 1997; White *et al.* 2003). Further, the efficacy of carotenoid sources is dependent on other nutrients in the diet such as the lipid content (Barcosa, Morais & Choubert 1999), the amount of polyunsaturated fatty acids (Bjerkeng, Hatlen & Wathne 1999) or the level of  $\alpha$ -tocopheryl acetate (Bjerkeng, Hamre, Hatlen & Wathne 1999).

As was the case with red sea bream (Nakazoe *et al.* 1984),  $\beta$ -carotene failed to have an effect on coloration on red porgy, and judging from the low carotenoid content of the skin in fish fed the  $\beta$ -carotene-supplemented diets, it is also likely that the

accumulation of  $\beta$ -carotene in the skin is minimal as well as its conversion (if any) to astaxanthin. Unlike red porgy, other species such as goldfish (Dutch Lionhead) and shrimps (*P. monodon*) can convert  $\beta$ -carotene to astaxanthin (Hata & Hata 1972; Boonyaratpalin *et al.* 2001).

Because of advances in plant sciences (Sandmann 2001), lycopene can be produced relatively inexpensively and it was thought worthwhile to investigate its effect on coloration of red porgy. Lycopene is a red carotenoid in tomatoes. In our trial, lycopene was not effective in providing red coloration nor did it influence total carotenoid content of the skin. Indeed, this observation is not surprising as in the biosynthetic pathway of carotenoids, lycopene should be converted to  $\beta$ -carotene before its ketolation to astaxanthin (Giuliano *et al.* 2000). Unpublished observations of our laboratory also indicated that red porgy is unable to deposit lycopene on its skin.

We conclude that natural astaxanthin – predominantly in its esterified form – of NatuRose<sup>®</sup> is the most effective in providing red coloration in red porgy among the carotenoids tested. The development of a natural hue of farmed red porgy, however, is a complex procedure that can be broken up into two main components, i.e. the supply of the right carotenoids in the feed and the prevention of hypermelanosis in the skin. Under open-cage cultured conditions the UV light as well as other husbandry stressors may induce both melanin synthesis and proliferation of melanophores. Indeed, cultured red porgy have considerably higher melanin content compared with wild fish (Table 2) causing a severe reduction of skin lightness (Table 3). Under our experimental conditions, red porgy were held in indoor tanks under natural light and certainly did not experience the illumination conditions of open cages.

Melanins, which are produced via synthetic pathways that convert the amino acid tyrosine to eumelanins (and to phaeomelanins in higher vertebrates), are the main pigments responsible for the dark coloration of cultured fish (Hearing 2005). This is because of an overproduction of melanin and/or the change in the number, type and distribution of melanophores (physiological colour change). Curiously little attention has been given to mechanisms of melanin production in cultured fish, which is the main cause for the dark coloration. Even if fish receive the right mixture of carotenoids, they will not get the right hue because of the dark colour of melanin.

Melanin deposition was not affected by the carotenoid sources tested but only by the protein to carbo-

hydrate ratio of the diet. Fishmeal is a rich source of phenylalanine and tyrosine, the precursors of melanin. It is plausible that reduction in dietary protein may limit the availability of the above amino acids for melanin synthesis. In cats, low levels of dietary tyrosine not only resulted in low concentrations of tyrosine in plasma but also decreased total melanin concentration in hair (Yu, Rogers & Morris 2001).

In conclusion, this study showed that esterified astaxanthin is effective in giving a reddish coloration to red porgy. However, for the development of colour identical to wild fish many other factors such as melanin production or distribution of chromatophores in the skin need to be considered.

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