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ORIGINAL PAPER

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Utilization of natural and synthetic sources of carotenoids in the skin pigmentation of gilthead seabream (*Sparus aurata*)

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Abstract This work was undertaken to investigate the effects of various dietary carotenoid sources on the skin pigmentation in a marine teleost, the gilthead seabream (*Sparus aurata*). In a first trial, homogenous groups of seabream (initial mean body weight: 150 g) were fed, for 9 weeks, one of three diets containing 40 mg/kg of astaxanthin in three different forms: as biomass of laboratory produced *Haematococcus pluvialis* (LHP), as a commercial source of *H. pluvialis* (NR) and as synthetic astaxanthin (AST). A control diet (C) without added astaxanthin was also tested. In a second trial, fish (initial mean body weight: 97 g) were fed diets containing 40 mg/kg of each of the following synthetic carotenoids: astaxanthin (Ast), canthaxanthin (Cant), apocarotenoic acid ethyl ester (Z) and lutein (L). At the beginning and every 3 weeks until the end of the experiments, samples of skin from three different skin body locations (forefront, operculum, and along the dorsal fin), and of dorsal muscle and plasma, were withdrawn from five fish per group for subsequent analysis of total carotenoid content and carotenoid composition. Neither growth nor feed efficiency were significantly affected by dietary treatments. In both trials, the dietary carotenoid supplementation increased total carotenoid content in all skin zones sampled, but had no effect on the muscle pigment content. The carotenoid concentration was highest in the skin at the forefront area of the fish, irrespective of the carotenoid source fed. In trial I, the total carotenoid content in the plasma was not affected by the various dietary carotenoid sources, but the plasma astaxanthin content in fish fed diet AST was significantly higher than in fish fed any other experimental diets. In trial II, after 6 weeks of feeding, there were no major differences in the skin carotenoid concentration between fish fed the various diets, but the highest values were observed in fish fed diets Cant and L. Lutein esters and epilutein esters represented almost the totality of the pigments present in the skin samples of seabream, irrespective of the sampling zone. The plasma carotenoid composition was closely related to the carotenoid composition of the diets, irrespective of the dietary carotenoid source fed. However, given that the total carotenoid concentration of the skin and its composition were not significantly affected by the various dietary pigment sources within the constraints tested, it is still difficult to ascertain with absolute certainty the effectiveness of modulating the skin pigmentation in gilthead seabream by dietary means.

Keywords Carotenoids · *Sparus aurata* · Skin pigmentation · Astaxanthin

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Introduction

Fish, like other animals, cannot synthesise carotenoids *de novo* [1]. Wild fish obtain most of their carotenoids by feeding on small crustaceans and other invertebrates. However, under intensive rearing conditions where fish are normally deprived of their natural sources of food, to achieve their natural flesh pigmentation fish depend entirely on their dietary carotenoid intake. If carotenoids that are normally present in the diet, or possibly substitutes, are not included in the feed, then coloration of the skin, gonads and muscle tends to fade. Carotenoid pigments such as astaxanthin (3,3'-dihydroxy-4,4'-diketo-β,β-carotene) and canthaxanthin (4,4'-diketo-β,β-carotene) are

widely used as dietary supplements in diets for salmonids as a method for inducing the typical red colour of the flesh, which is perceived as an important quality criterion [2, 3]. Research on the pigmentation of fish has mainly focused on fish species of economic interest, namely salmonids. Numerous trials on dietary carotenoid supplementation for salmonid fish pigmentation have been reviewed by various authors [4, 5, 6]. In contrast, knowledge of the skin pigmentation of non-salmonid species is scarce, and few feeding trials with carotenoids in diets for white-flesh fish species have been reported [7, 8].

The gilthead seabream (*Sparus aurata*) is a high-value species in the Mediterranean region and consumers prefer a golden colour on its forefront, a red operculum and a colored lateral line, all characteristics that are closely associated with protein-carotenoid supramolecular assemblies. The general aim of this study was to investigate the colour enhancement of the skin and dorsal muscle of the gilthead seabream by a dietary carotenoid supplementation. Therefore, the potential of the freshwater unicellular green algae *Haematococcus pluvialis* (commercially and laboratory produced) as a carotenoid source for gilthead seabream was compared with synthetic astaxanthin (Trial I). In a second trial, the utilization of other synthetic pigment sources: astaxanthin, canthaxanthin, lutein and apocarotenoic acid ethyl ester on the skin pigmentation of seabream was tested (Trial II).

Materials and methods

Experimental diets and carotenoid sources

Trial I. A control diet (diet C) was formulated to contain 42% crude protein and 13% fat, without added astaxanthin (Table 1). With this basal mixture, three other diets were formulated to contain 40 mg/kg of astaxanthin in three different forms: as biomass of laboratory produced *H. pluvialis* (diet LHP), as a commercial source of *H. pluvialis* (diet NR) and as synthetic astaxanthin (diet AST) (Table 1). The laboratory microalgae *H. pluvialis* strain INETI 33 was cultivated in Bold Basal Medium [9] in airlifts at low light conditions (150 $\mu E\ m^{-2}\ s^{-1}$). Carotenogenesis was induced by nutrient starvation and sodium chloride addition (2%) at high light conditions (1000 $\mu E\ m^{-2}\ s^{-1}$). Harvesting was made without any flocculation by removing agitation. The total amount of carotenoid pigments was 1.9%. The commercial source of *H. pluvialis* was NatuRose (Cyanotech, Hawaii, USA), containing 1.5% astaxanthin. Synthetic astaxanthin was used as water-dispersable beadlets (Carophyll Pink, Hoffmann-La Roche, Lisbon, Portugal), containing 8% astaxanthin.

Trial II. Four isoproteic (46% crude protein) and isolipidic (9%) diets were formulated to contain 40 mg/kg of different synthetic carotenoids (Table 2). The pigments tested were synthetic astaxanthin (Carophyll Pink, Hoffmann-La Roche, Lisbon, Portugal) with 8% astaxanthin content (diet Ast), synthetic canthaxanthin beadlets (Carophyll Red, Hoffmann-La Roche, Lisbon, Portugal) with 10% canthaxanthin (diet Cant), synthetic apocarotenoic acid ethyl ester 10% (Carophyll Yellow, Hoffmann-La Roche, Lisbon, Portugal) (diet Z) and Flora-Glo with 10% lutein (diet L). Data on detailed carotenoid compositions of the various experimental diets is reported in Table 3. The pigment composition of the experimental diets reflected closely that of the dietary carotenoid supplements.

In all experimental diets, ingredients were finely ground, mixed in a horizontal helix ribbon mixer (model Mano, 100 l capacity, CPM, San Francisco, USA) for 15 min and pelleted dry using a steamless pelleting machine (model 3000, CPM, San Francisco, USA) fitted with a die of 4.5 mm diameter. During the pelleting process, the temperature varied from 50 to 60 °C. Diets were subsequently stored at 4 °C and light-protected throughout utilization.

Fish and experimental conditions

Trial I. The feeding trial was performed in a fish farm located at Olhão on the South coast of Portugal. Eight homogenous groups (duplicates) of 30 seabream (mean initial body weight, 150±5 g)

Table 1 Ingredients and chemical composition of the experimental diets fed to gilthead seabream (Trial I). *C* Control diet, *AST* synthetic astaxanthin diet, *NR* commercial source of *H. pluvialis* astaxanthin diet, *LHP* biomass laboratory produced *Haematococcus pluvialis* astaxanthin diet, *CPSP* fish protein concentrate, *DM* dry matter

Basal mixture (g/kg)	C	AST	NR	LHP
Fishmeal	480			
Wheat meal	360			
CPSP	100			
Fish oil	45			
Vitamin mix[a]	10			
Mineral mix[b]	5			
Choline chloride	3			
Astaxanthin source (mg/kg)	**Control**	**Synthetic astaxanthin**	**Commercial *H. pluvialis***	**Laboratory *H. pluvialis***
Synthetic (Carophyll Pink)[c]		40.0		
NatuRose[c]			40.0	
Laboratorial *H. pluvialis*[c]				40.0
Proximate composition				
DM (%)	91.8	91.6	91.7	91.1
Crude protein (% DM)	48.6	47.7	48.5	48.1
Crude fat (% DM)	12.8	12.7	13.2	12.9
Ash (% DM)	10.0	10.2	9.8	10.0

[a] Amounts of the vitamins in milligrams per kilogram diet: E, 20; K, 35; B1, 5; B2, 5; B3, 10; B5, 100; B6, 5; B9, 2; B12, 0.05; H (biotin), 0.5; ascorbic acid, 200; *p*-aminobenzoic acid, 50; inositol, 500; A: 10,000 UI kg^{-1} diet; vit. D3, 2,000 UI kg^{-1} diet

[b] Amounts of the minerals in milligrams per kilogram diet: Co^{2+}, 0.4; Cu^{2+}, 5.0; Fe^{2+}, 40; F^{2+}, 1.0; I^{2+}, 0.6, Mg^{2+}, 100; Mn^{2+}, 10

[c] On astaxanthin basis

Table 2 Ingredient and chemical composition of the experimental diets fed to gilthead seabream in Trial II

Basal mixture (g/kg)	Ast	Cant	L	Z
Fishmeal	480			
Wheat meal	360			
CPSP	100			
Fish oil	45			
Vitamin Mix[a]	10			
Mineral mix[b]	5			
Choline chloride	3			
Experimental diets (%)	**C. Pink**	**C. Red**	**C. Yellow**	**Flora Glo**
Basal mixture	98.60	98.60	98.60	98.60
Fish oil	1.00	1.00	1.00	1.00
Astaxanthin (Carophyll Pink) (mg/kg)[c]	40.0			
Canthaxanthin (Carophyll Red) (mg/kg)[c]		40.0		
Lutein (Flora-Glo) (mg/kg)[c]			40.0	
Apo-β-carotene (Carophyll Yellow) (mg/kg) [c]				40.0
Proximate composition				
DM (%)	87.47	87.98	87.57	87.69
Crude Protein (%DM)	46.47	46.94	46.28	45.80
Fat (%DM)	9.15	9.51	9.07	8.94
Ash (%DM)	12.82	13.06	12.92	12.71
Total carotenoids (μg/g)	46.39	51.25	42.50	41.24

[a] Amounts of the vitamins in milligrams per kilogram diet: E, 20; K, 35; B1, 5; B2, 5; B3, 10; B5, 100; B6, 5; B9, 2; B12, 0.05; H (biotin), 0.5; ascorbic acid, 200; *p*-aminobenzoic acid, 50; inositol, 500; A: 10,000 UI kg^{-1} diet; vit. D3, 2,000 UI kg^{-1} diet

[b] Amounts of the minerals in milligrams per kilogram diet: Co^{2+}, 0.4; Cu^{2+}, 5.0; Fe^{2+}, 40; F^{2+}, 1.0; I^{2+}, 0.6, Mg^{2+}, 100; Mn^{2+}, 10

[c] On astaxanthin basis

Table 3 Carotenoid composition of the various experimental diets used in Trial II with gilthead seabream

Diet	Carotenoid composition	Total carotenoids (%)
AST (Carophyll Pink)	Free, all-trans astaxanthin	73.47±1.17
	Free, cis astaxanthin	18.28±0.18
	Astacene	4.26±0.41
	Canthaxanthin	2.13±0.03
	Lutein	1.86±0.07
CANT (Carophyll Red)	Canthaxanthin	97.96±0.35
	Astaxanthin	0.86±0.04
	Lutein	1.26±0.13
L (Carophyll Yellow)	8'-apo-β-caroten-8'-al	92.59±0.54
	Unknown	2.55±0.03
	Lutein	3.79±0.25
	Zeaxanthin	1.07±0.13
Z (Flora-Glo)	Astaxanthin	1.60±0.10
	β-carotene	12.74±0.34
	All-trans lutein	79.35±0.44
	Cis lutein	2.51±0.28
	Zeaxanthin	3.80±0.03

Values are means±SE (*n*=3)

were grown in square glass-fiber tanks (volume, 600 l) supplied with a continuous flow of gravel-filtered seawater (flow rate, 10 l min^{-1}; salinity, 38‰; temperature, 21–25 °C) over 9 weeks. Fish were hand-fed each diet twice a day, at 2% body weight day^{-1} feeding rate.

Trial II. The feeding trial was carried out in the semi-closed recirculating water system at the Faculty of Sciences of the University of Porto, Portugal. Eight homogenous groups (duplicates) of 20 gilthead seabream (mean initial body weight, 97±2 g) were grown in cylindrical glass-fiber tanks (volume, 200 l) supplied with a continuous flow of gravel-filtered seawater (flow rate, 8 l min^{-1}; salinity, 35‰; temperature, 19–22 °C) over 6 weeks. Fish were hand-fed each diet twice a day, to visual satiety.

At the beginning of each trial and every 3 weeks thereafter, samples of skin from three different body locations, the forefront (Z1), the operculum (Z2) and along the dorsal fin (Z3) (Fig. 1), and samples of dorsal muscle in trial I were withdrawn from five fish per group. Samples were stored at –20 °C for subsequent analysis of total carotenoid content and carotenoid composition. From all sampled fish, blood was collected from the caudal vein with a heparinized syringe. Plasma was recovered after centrifugation and immediately stored at –20 °C for analysis of total carotenoid content and of carotenoid composition.

Analytical methods

Chemical composition analysis of the diets was made using the following procedures: dry matter after drying at 105 °C for 24 h; ash by combustion at 550 °C for 12 h, protein (N×6.25) by the Kjeldahl method after acid digestion and lipid after petroleum ether (40–60 °C) extraction in a Soxhlet apparatus [10].

Total carotenoid content in diets, skin and muscle was determined spectrophometrically after extraction with acetone [2]. Carotenoid concentration was expressed using extinction coefficients ($E^{1\%}_{1cm}$) of for astaxanthin and 2150 for algal pigments at their absorption maximum in dichloromethane [11] for the samples of the trial I, and 2550 for lutein at their absorption in ethanol for trial II samples. The plasma and skin samples of trial II were analyzed by HPLC. The isocratic HPLC system was used based on a normal phase system developed by Hoffmann La Roche for astaxanthin analysis. This system consisted of a 50×4.6 mm Silica Si60 Lichrosrob column and pre-column (5×4.6 mm), using 14%

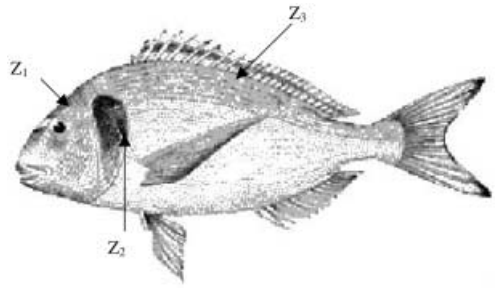

Fig. 1 Skin zones sampled for carotenoid analysis throughout the various experimental trials conducted with gilthead seabream and blackspot seabream

acetone in hexane as the mobile phase and a flow rate of 1.2 ml min^{-1}. The detection wavelength was set to 470 nm.

Statistical analysis

Data were submitted to a one-way analysis of variance (ANOVA I), in which the dietary pigment source was taken as independent variable. When appropriate, means were compared by Duncan's multiple range test. Statistical significance was tested at a 0.05 probability level.

Results and discussion

In both trials, fish grew within the range normally found in the literature for this species [12, 13]. Neither growth nor feed efficiency was significantly affected by dietary treatments. No mortality was associated with experimental treatments.

Trial I

Results on the skin pigmentation of gilthead sea bream in trial I showed that the various dietary astaxanthin supplements increased the total carotenoid content in all skin zones sampled when compared to the pigment content of the initial fish, but had no effect on the muscle pigmentation (Table 4). However, it is worth mentioning that this increase in the carotenoid concentration in the skin was similar to that observed in fish fed the control diet (diet C). This suggests that diet C also had a carotenoid content high enough to promote an increase in the skin pigmentation. The laboratory-produced *H. pluvialis* dry biomass, when used as a dietary carotenoid supplement, originated a total carotenoid content similar to the one obtained with both synthetic and commercially available *H. pluvialis* astaxanthin. A lower efficacy of *H. pluvialis* astaxanthin deposition in rainbow trout (*Oncorhychus mykiss*) flesh when compared to synthetic astaxanthin [14] has been in some cases associated with a non- or incomplete breakage of the cell wall and/or to the high percentage of esterified carotenoid. In spite of not having applied any prior treatment to *H. pluvialis* cells, our results indicate a high availability of pigments inside the spore.

The highest carotenoid content (40–75 mg/kg wet weight) was found in the skin at the forefront area (Z1) of fish, irrespective of the dietary astaxanthin source fed to gilthead seabream. In the other two skin areas analyzed (operculum and along the dorsal fin) it ranged from 15 to 35 mg/kg wet weight. The total carotenoid content in the dorsal muscle varied between 1 and 3 mg/kg wet weight, values which are much lower than those found in skin samples, but well in accordance with the market image of gilthead seabream white muscle. The use of the different astaxanthin sources as dietary supplements had no significant effect ($P>0.05$) on the total carotenoid content in any of the sampled skin areas or dorsal muscle of gilthead seabream. In a similar trial, Gouveia et al. (unpublished data) tested the use of the freshwater microalgae, *Chlorella vulgaris*, as a dietary pigment source in gilthead seabream. Their results, in terms of the total carotenoid content found in skin zones and muscle, are of the same magnitude as those found in the present study.

In red seabream fed a 100 mg/kg free astaxanthin, the carotenoid content of the skin increased after 1 month, but reached an apparent saturation point and no further increase was observed with feeding. On the other hand,

Table 4 Total carotenoid content (mg/kg wet weight) of the skin (*Z1* forefront, *Z2* operculum, *Z3* along the dorsal fin) and dorsal muscle of seabream fed the experimental diets for 3, 6 and 9 weeks (trial I). Values are means±SE, Total carotenoid content (mg/kg wet weight) of initial fish: 15.9 (Z1); 15.5 (Z2); 10.0 (Z3); 1.2 (muscle)

Duration	Area	Dietary treatments			
		C	LHP	NR	AST
Week 3	Z1	32.7±8.0	43.0±14.3	35.5±12.9	59.2±14.5
	Z2	18.8±2.7	21.2±1.1	25.6±1.2	24.9±0.2
	Z3	15.9±6.1	15.1±1.4	21.2±0.4	30.1±0.5
	Muscle	2.5±0.6	2.3±1.1	2.7±0.7	2.8±0.2
Week 6	Z1	40.9±2.4	54.1±21.7	47.7±4.8	71.8±8.5
	Z2	18.8±0.1	20.9±6.9	27.1±4.1	19.1±1.0
	Z3	20.0±0.9	9.9±0.1	23.3±7.5	26.3±1.3
	Muscle	1.6±0.2	1.5±0.6	1.5±0.2	2.0±0.1
Week 9	Z1	40.7±12.9	41.8±15.0	44.8±10.1	75.3±8.1
	Z2	16.3±2.0	20.3±2.0	21.6±4.6	25.6±2.9
	Z3	26.8±7.8	15.4±4.7	20.9±6.5	34.3±4.1
	Muscle	2.1±0.3	0.9±0.2	1.3±0.2	1.5±0.3

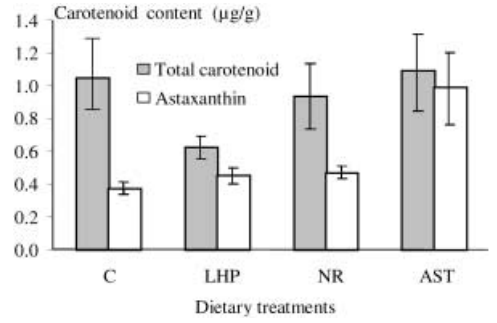

Fig. 2 Total carotenoid and astaxanthin contents of plasma samples from seabream fed the different experimental diets for 9 weeks. *C* Control diet, *LHP* biomass laboratory produced *Haematococcus pluvialis* astaxanthin diet, *NR* commercial source of *H. pluvialis* astaxanthin diet, *AST* synthetic astaxanthin diet. Values are means±SE (*n*=4)

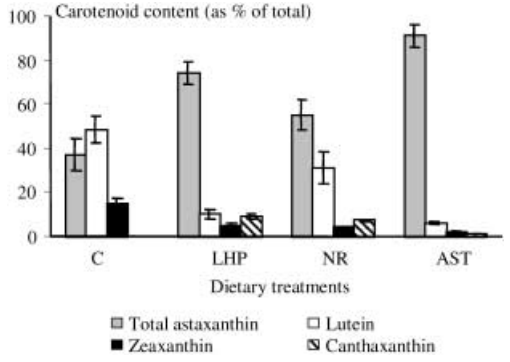

Fig. 3 Carotenoid composition of plasma samples from seabream fed the different experimental diets for 9 weeks. Values are means±SE (*n*=4)

fish fed 100 mg/kg of astaxanthin esters presented a significantly higher carotenoid content in the skin after 1 month of feeding, and a 1.7-fold higher astaxanthin content after 2 months [15]. Synthetic astaxanthin promoted a higher flesh pigmentation in rainbow trout than that obtained with *H. pluvialis* algal carotenoids [14, 16], supporting the idea of a better pigmenting efficacy of free astaxanthin in comparison with esterified astaxanthin, and a low availability of the pigment inside the spore of the algae [17, 18]. However, similar results were obtained with synthetic astaxanthin and *C. vulgaris* microalgal carotenoids in flesh pigmentation of rainbow trout [11, 19, 20].

According to [21], the relative plasma concentrations of carotenoids and astaxanthin can be used as an indicator of pigment absorption. At the end of the experimental period, the total carotenoid content in the plasma of gilthead seabream was not affected by the various dietary carotenoid sources. However, the total astaxanthin content in the plasma of seabream fed the synthetic astaxanthin diet (diet AST) was significantly higher than in fish fed any other experimental diet (Fig. 2). In a previous study with rainbow trout, [22] it was observed that under low dietary lipid levels (9%) the astaxanthin serum concentration in fish fed diets supplemented with algae biomass (*H. pluvialis*) was higher than in those fed a synthetic astaxanthin supplemented diet. However, no differences in astaxanthin serum concentrations were found in trout fed diets supplemented with either algal or synthetic astaxanthin when the dietary lipid level was raised to 24%.

The plasma carotenoid composition of fish fed the experimental diets is presented in Fig. 3. Fish fed diets NR, LHP or AST showed higher plasma levels of astaxanthin and lower levels of lutein and zeaxanthin than fish fed the control diet (diet C). Moreover, while canthaxanthin represented 2–8% of total carotenoids in the plasma of fish fed diets AST, NR and LHP, this carotenoid could not be found in the plasma of fish fed the control diet.

Despite the high plasma levels of astaxanthin, showing that dietary astaxanthin can be effectively bio-absorbed by seabream, the absence of a significant effect of the various astaxanthin sources on enhancing the skin pigmentation in the gilthead seabream suggests that astaxanthin is probably not a pigment of major importance to the pigmentation of this species. The role of other carotenoids, such as canthaxanthin, apocarotenoic acid ethyl ester and lutein, as dietary modulators of skin pigmentation in gilthead seabream was therefore investigated in trial II.

Trial II

The results on the skin pigmentation of gilthead seabream after 6 weeks of feeding in trial II showed that the various dietary carotenoid supplements significantly increased the total carotenoid content in all skin zones sampled, when compared to the pigment content of the initial fish (Table 5). The increased carotenoid concentration found in the skin after 6 weeks of feeding demonstrated that gilthead seabream was able to utilize efficiently the dietary carotenoids. However, in contrast to what had been observed in trial I, no saturation plateau of carotenoid deposition in the skin was attained after 3 weeks of feeding. Identical results have been reported in salmonid species, such as rainbow trout [5, 23, 24] and Arctic charr (*Salvelinus alpinus*) [25, 26].

At the end of the 6 weeks experimental period and irrespective of the dietary carotenoid source fed to gilthead seabream, the total carotenoid content in skin ranged between 14 and 24 mg/kg, wet weight. At this time the major carotenoid content was found in fish fed diets Cant and L, in the skin at the forefront area (Z1) and along the dorsal fin (Z3).

Data on the detailed carotenoid composition of the different skin areas of gilthead seabream fed the various experimental diets for 6 weeks is reported in Table 6. Overall, it was found that lutein esters and epilutein esters represented almost the totality of the pigments presented in the skin samples, irrespective of the body location. In fish fed the astaxanthin-rich diet, this pigment was present (14% of isolated carotenoids) in the form of astaxanthin esters, but only at the operculum skin zone. Similarly, fish fed the canthaxanthin-supplemented diet

Table 5 Total carotenoid content (mg/g wet weight) of the skin (Z1 forefront, Z2 operculum, Z3 along the dorsal fin) of gilthead seabream fed the experimental diets for 3 and 6 weeks. Values are means±SE (n=10), total carotenoid content (mg/kg wet weight) of initial fish: 5.0 (Z1); 0.7 (Z2); 1.1 (Z3)

Duration	Area	Dietary treatments			
		C. Pink	C. Red	C. Yellow	Flora Glo
Week 3	Z1	12.2±0.4	11.9±0.5	6.1±0.9	2.4±0.7
	Z2	6.0±0.6	5.1±0.6	3.4±0.5	4.0±0.2
	Z3	9.8±0.2	7.7±1.2	8.8±1.1	6.5±0.7
Week 6	Z1	17.1±2.4	23.1±1.4	14.0±0.6	23.7±0.0
	Z2	16.5±0.9	17.2±0.1	14.0±0.7	16.7±0.0
	Z3	19.8±2.0	21.7±0.0	20.0±0.8	15.6±1.2

Table 6 Detailed carotenoid composition of gilthead seabream as affected by the different dietary carotenoid sources after 6 weeks of feeding. Values are means±SE (n=10)

Skin area	Carotenoid (% isolated carotenoids)	Dietary treatments			
		C. Pink	C. Red	C. Yellow	Flora Glo
Z1	Free astaxanthin	0.1±0.0			
	Astaxanthin esters	1.0±0.4			
	Canthaxanthin				
	Lutein esters	60.3±0.2	61.0±0.4	100.0±0.0	100.0±0.0
	Epilutein esters	38.4±4.0	38.9±4.1		
Z2	Free astaxanthin				
	Astaxanthin esters	13.9±1.0	5.1±0.9		
	Canthaxanthin		8.7±1.1		
	Lutein esters	59.7±0.2	59.7±1.8	100.0±0.0	100.0±0.0
	Epilutein esters	26.4±0.6	26.4±2.3		
Z3	Free astaxanthin	0.1±0.0			
	Astaxanthin esters	3.3±0.2	1.0±0.4		
	Canthaxanthin				
	Lutein esters	60.1±0.3	60.4±2.6	100.0±0.0	100.0±0.0
	Epilutein esters	38.3±1.0	38.5±1.2		

also showed the presence of astaxanthin esters (5%) and canthaxanthin (9%) in this skin zone. Previous studies had shown that most of the hydroxy-carotenoids found in the fish skin occur as fatty acid esters [27, 28].

In the gilthead seabream, the carotenoid conversion pathway is not presently known, and so, in our experimental approach, we can only speculate about it. 3'-Epilutein appears to be generally predominant in the fish skin and presumably a product of epimerization of (3*R*, 3'*R*, 6'*R*)-lutein [29, 30, 31]. The lutein is indicated as a putative reductive metabolite of astaxanthin in rainbow trout, based on a minute recovery of radioactivity in lutein after feeding of labelled (3*S*, 3'S)-astaxanthin [29]. An analogous reductive pathway might also be occurring with gilthead seabream.

Carotenoids are lipid soluble and follow the same absorptive pathways as other dietary lipids. The high lutein content in the skin of gilthead seabream can also be related to a possible existence of interactions between carotenoids at the intestinal level, which may increase the lutein absorption. However, after 6 weeks of experimental feeding, the plasma carotenoid composition and the dietary carotenoid supply were closely related (Fig. 4). This high correlation between plasma carotenoid and dietary carotenoid compositions found in this work for seabream is in accordance with previous results [32, 33, 34]. Nevertheless, the plasma lutein concentration cannot be used as an indicator of lutein availability for skin pigmentation, as the correlation between plasma and skin lutein content is

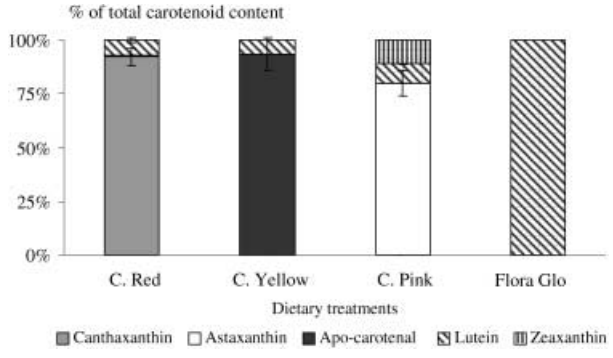

Fig. 4 Plasma carotenoid composition of gilthead seabream as affected by the different dietary carotenoid sources. *C. Red*, *C. Yellow* and *C. Pink* are Carophyll Red, Yellow and Pink

low. The plasma carotenoid levels observed in this work suggests that the ester synthesis takes place in the skin, because carotenoids are transported in the blood as their free forms. These results are in agreement with findings by other authors [4, 27]. Another type of competition that may occur between carotenoids, is at the level of their blood transport. Carotenoids are transported in the plasma of salmonid fish associated within lipoproteins [35, 36, 37, 38, 39]. In humans, the distribution of carotenoids among different lipoprotein classes has been reported to depend upon the physical properties, presumably the polarity, of the carotenoids: carotenes were found predomi-

nantly in the low-density lipoprotein fraction and hydroxy-carotenoids predominantly in the high-density lipoprotein [40, 41]. One may speculate that lipoproteins in the gilthead seabream might have a greater affinity for lutein than for the other carotenoids, resulting in a faster blood transport and therefore a more efficient deposition.

Although the conversion of lutein into vitamin A remains unconfirmed [31], it has been reported that astaxanthin and canthaxanthin might be vitamin A precursors in lower vertebrates such as fish [31, 42]. The gilthead seabream might reductively transform these carotenoids into vitamin A, thereby decreasing their deposition in the skin.

As main conclusions of this work, one can say that a dietary supplementation of 40 mg/kg of several carotenoid sources for 6 weeks tends to increase the total carotenoid content of gilthead seabream skin. However, such an increase was not significantly affected by the various dietary carotenoid sources tested. The strong relationship found between the plasma carotenoid composition and the dietary carotenoid supply suggests that all of them, algal and synthetic astaxanthin, canthaxanthin, apo-β-carotenal and lutein, are efficiently absorbed by seabream. However, irrespective of the dietary carotenoid source, lutein esters and epilutein esters represented almost the totality of pigments found in the skin of seabream. Knowledge of the carotenoid conversion pathway in gilthead seabream is extremely scarce and the experimental approach only allows some speculation about it. A reductive metabolic pathway for the conversion of astaxanthin into lutein, differences in the lipoprotein affinity with the various carotenoids, or even a preferential use of astaxanthin and canthaxanthin as vitamin A precursors, are hypotheses which require further investigation, but might be associated with the high content of lutein and epilutein esters found in the skin of gilthead seabream. It is therefore, still difficult to ascertain with absolute certainty the effectiveness of dietary carotenoid supplements as a tool to tailor the skin pigmentation in gilthead seabream.

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