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Artificial pigmentation and flesh quality in red porgy (*Pagrus pagrus*)

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Abstract

Red porgy (*Pagrus pagrus*) is a highly valued species of increasing interest for the Mediterranean aquaculture, characterized by a natural red-pink skin coloration, which acquires a gray color under culture conditions. This study was carried out in order to define new diets to modify the pigmentation variability of the red porgy skin. Six thousand red porgy juveniles (29.21 ± 6.39 g) were randomly distributed in three tanks with a volume of 55 m^3 each and fed for 8 months with three extruded diets supplemented with graded levels of synthetic carotenoids (GFP0, GFP30, and GFP60). Color, carotenoid content, fatty acid composition of fish skin, chemical and fatty acid composition of fish flesh, and digestive enzymes and histological analysis of the intestine and liver of the fish were determined. The results showed that the supplementation of the synthetic carotenoids affected significantly the skin color indexes with the higher values of redness, yellowness, and chroma recorded in the fish fed with the GFP60 diet, while the other parameters investigated were unaffected except the intestinal protease activities of the GFP60 group, which had lower results. The present study showed encouraging results for developing a new research, but they are not satisfying to the application of this carotenoid supplement feed for this species in production farming.

Keywords: *Pagrus*, Carotenoid, Pigmentation, Chemical composition, Digestive enzymes

Background

In Europe, fish aquaculture in a marine environment developed following two successive waves: Atlantic salmon and then sea-bass and sea-bream. Rearing a new marine fish species is sustained for many complementary reasons. Among sparids, the snappers, *Pagrus major* and *Pagrus pagrus*, are two species that are in the mixed category FAO of the red sea bream, combining 3,000 to 5,000 tons of annual production in Europe (Suquet et al. 2009).

Ever since in commercial fisheries, red porgy, *P. pagrus*, is highly appreciated for its appearance and meat quality; this species is a candidate for aquaculture diversification in the Mediterranean area.

Red porgy is characterized by a natural red-pink skin coloration, which acquires a gray color under farming conditions (Kentouri et al. 1994; Stephanou et al. 1995; TECAM 1999; Cejas et al. 2003; Van der Salma et al. 2004).

Since fish, like other animals, are unable to synthesize carotenoids *de novo* (Goodwin 1986), their skin color is highly dependent on the carotenoids present in the diet (Chatzifotis et al. 2005; Kalinowski et al. 2005, 2007). Pavlidis et al. (2008) reported the possibility of an environmental control of the skin coloration of red porgy by integration of pigments in the diets, while García et al. (2010) investigated the use of marine and freshwater crab meals as a supplement in the diets for red porgy to obtain a pink skin.

Although the optimum duration of the carotenoid supplementation time for red porgy to achieve a skin coloration which is similar to wild specimens has not yet been established, most studies refer that 2 months are adequate, further than this gives a gray color due to excessive carotenoid accumulation.

Farmed fish have no access to carotenoid-rich feeds, and therefore, the necessary carotenoids must be added to the diet. The effectiveness of a carotenoid source in terms of deposition and pigmentation is species-specific (Ha et al. 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.

The *Pagrus* spp. has been selected because there are few studies on the reproduction in controlled conditions and quality of artificial diets (Kentouri et al. 1995).

Many papers showed data on the pigmentation of fish skin and fillet under a laboratory scale (Chatzifotis et al. 2005; Kalinowski et al. 2007; Tejera et al. 2007), whereas this study was performed on the production scale. The aim of the present study was to investigate the effects of new diets containing dietary carotenoid sources on the coloration of the red porgy skin and their effect on the fish digestive integrity.

Methods

Fish, diets, and experimental design

Six thousand red porgy (*P. pagrus*) juveniles, with an average weight of 29.21 ± 6.39 g, were randomly distributed in three tanks with a volume of 55 m^3 outdoors and cultured in a commercial farm (Acqua Azzurra, Pachino, Italy). Each tank was supplied with a seawater input of 200 L/min with a constant water temperature of 20°C and oxygenated to about 70% saturation.

Two fish groups were fed with commercial extruded diets (GoldFin Pagrus™, GFP), manufactured by Skretting Italia SpA, (Mozzecane, Italy), supplemented with 30 mg/kg of synthetic astaxanthin and 30 mg/kg of xanthophylls (GFP30), or 60 mg/kg of synthetic astaxanthin and 30 mg/kg of xanthophylls (GFP60), respectively. Those

Table 1 Chemical composition (% dry matter (DM)) and total carotenoid content (mg astaxanthin/kg) of the experimental diets

	GFP0	GFP30	GFP60
Moisture	2.08 ± 0.04	6.14 ± 0.35	5.44 ± 0.25
Crude protein	49.82 ± 0.87	50.44 ± 0.62	49.20 ± 0.44
Ether extract	16.76 ± 0.16	16.65 ± 0.08	16.88 ± 0.11
Ash	7.54 ± 0.16	7.66 ± 0.22	7.43 ± 0.34
Gross energy (MJ/kg DM)	22.53 ± 0.14	23.63 ± 0.10	23.44 ± 0.07
Carotenoid	4.82	12.97	17.61

Table 2 Lipid content of the fatty acids (mg/g) of the total lipid of the experimental diets

	GFP0	GFP30	GFP60
C14:0	51.40	48.08	47.89
C15:0	2.95	2.96	3.34
C16:0	76.46	100.37	123.98
C18:0	9.12	18.16	25.69
C20:0	-	1.62	2.91
C16:1 n-7	31.65	38.63	42.78
C18:1 n-9	36.00	58.68	153.90
C18:1 n-7	8.66	13.48	19.11
C20:1 n-11	11.83	8.89	6.76
C20:1 n-9	1.56	-	3.05
C22:1 n-11	12.55	-	2.89
C18:3 n-3	4.73	8.09	21.05
C18:4 n-3	11.46	6.62	5.56
C20:4 n-3	4.72	2.69	2.92
C20:5 n-3	35.39	44.27	32.34
C21:5 n-3	2.02	2.19	1.55
C22:5 n-3	4.51	6.66	6.53
C22:6 n-3	19.40	21.31	18.83
C18:2 n-6	14.59	51.32	112.62
C18:3 n-6	1.93	2.66	2.10
C20:2 n-6	-	1.03	1.26
C20:3 n-6	-	-	2.70
C20:4 n-6	2.19	3.84	4.06
C22:5 n-6	-	-	10.01
C16:4 n-1	8.65	9.33	4.58
C16:2 n-4	6.12	6.86	7.93
C16:3 n-4	5.39	6.44	7.24
SFA[a]	139.9	171.2	203.8
MUFA[b]	105.2	121.1	230.1
PUFA[c]	121.1	173.3	241.3
n-3	82.22	91.82	88.77
n-6	18.70	58.84	132.73
n-3/n-6	4.40	1.56	0.67

[a]Saturated fatty acids; [b]monounsaturated fatty acids; [c]polyunsaturated fatty acids.

experimental diets were tested against a control diet without any pigments (GFP0). The pigmenting pool used in the feeds is mainly constituted of astaxanthin derived from yeast (*Phaffia rhodozyma*) and a mixture of plant pigments, including alfalfa, red peppers, and seaweed extract.

All the diets met the nutritional requirements for sparids in terms of essential nutrients (Guillaume and Choubert 2001; Koshio 2002), and the proximate composition and the carotenoid content of the experimental diets are shown in Table 1, whereas Table 2 shows their fatty acid (FA) composition. The trial lasted 8 months, from November 2007 until July 2008.

Table 3 Mean values of the skin color indexes

	GFP0	GFP30	GFP60
Lightness	66.79 a	59.17 b	57.31 b
*a**	0.22 c	2.76 b	3.00 a
*b**	1.35 b	0.67 c	5.26 a
Chroma	1.93 c	3.18 b	6.07 a
Hue	0.91	0.15	1.05

In the rows, different letters indicate statistical differences $P \leq 0.05$.

Carotenoid analysis

Carotenoids in the diet and skin samples were determined according with the method reported by Sachindra et al. (2005). Samples were repeatedly extracted using acetone until no further pigment was extractable. The acetone extracts were pooled and phase-separated with petroleum ether (BP 40°C to 60°C). The petroleum ether extract was repeatedly washed with 0.1% NaCl to remove traces of acetone, dried with sodium sulfate, filtered, flushed with nitrogen, and then evaporated under vacuum at 40°C using a rotary flash evaporator. The resulting carotenoid concentrate was taken up in petroleum ether and made up to a known volume, and the absorbance of the appropriately diluted extract was measured at 468 nm using a spectrophotometer. The concentration of the carotenoids in the extract was quantified by a pure synthethic astaxanthin standard and expressed as mg/kg.

Color evaluation

Skin pigmentation was determined using a bench colorimeter Chroma Meter CR-400 Konica Minolta Sensing (Minolta Sensing Inc, Osaka, Japan) in the CIELAB color space (CIE 1976). A total of three readings were taken on the same *repere* point, approximately 1 cm behind the operculum between the lateral line and pectoral fin, always on the left side of the fish.

The lightness (L^*), redness (a^*), and yellowness (b^*) were recorded, and chroma and hue indices were calculated as chroma ($C^* = (a^*2 + b^*2)^{1/2}$) and hue ($H_0 = \tan^{-1}(b^* / a^*)$) (Boccard et al. 1981). Chroma is related to the quantity of pigments, and high values represent a more vivid color and

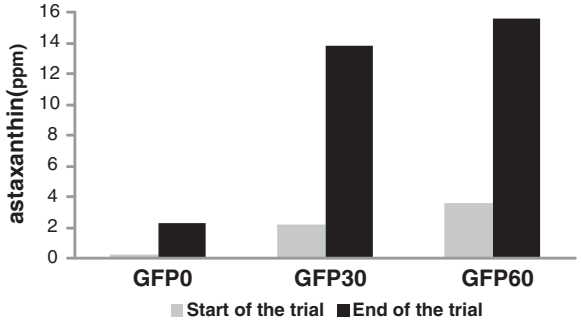

Figure 1 Skin total carotenoid content of red porgy at the start and end of the feeding trial.

Table 4 Main fatty acids (mg/g of total lipids) of fillets at beginning and end of experiment

	Initial	GFP0	GFP30	GFP60
C14:0	18.0 ± 2.6	25.6 ± 1.7	24.0 ± 4.5	25.1 ± 0.1
C16:0	76.7 ± 2.5	100.4 ± 14.3	130.1 ± 39.5	153.7 ± 15.0
C18:0	19.7 ± 2.3	25.3 ± 3.3	27.2 ± 10.6	53.6 ± 1.5
C20:0	0.8 ± 0.1	0.9 ± 0.1	1.9 ± 0.6	2.6 ± 0.4
C16:1 n-7	21.6 ± 2.5	30.7 ± 0.1	33.3 ± 7.7	36.1 ± 1.1
C18:1 n-9	64.4 ± 7.6	83.6 ± 4.3	122.4 ± 37.9	141.6 ± 22.2
C18:1 n-7	10.5 ± 0.4	14.4 ± 1.0	20.8 ± 6.0	25.9 ± 3.0
C20:1 n-11	5.1 ± 0.1	8.6 ± 3.0	13.7 ± 4.1	17.8 ± 3.1
C22:1 n-11	3.2 ± 0.1	7.6 ± 4.2	10.9 ± 3.6	15.5 ± 2.5
C22:1 n-9	1.0 ± 0.0	-	3.6 ± 0.6	4.4 ± 0.2
C18:3 n-3	7.2 ± 0.4	8.3 ± 0.4	11.5 ± 3.5	12.8 ± 3.7
C18:4 n-3	2.8 ± 0.7	5.3 ± 0.8	5.4 ± 0.8	5.9 ± 1.2
C20:5 n-3	16.2 ± 2.6	28.2 ± 0.4	42.5 ± 17.4	56.5 ± 0.3
C22:5 n-3	4.0 ± 0.2	6.7 ± 0.6	18.5 ± 9.9	19.0 ± 1.8
C22:6 n-3	18.7 ± 1.6	38.6 ± 1.0	76.2 ± 66.3	137.4 ± 57.8
C18:2 n-6	65.8 ± 12.4	76.2 ± 13.6	113.0 ± 45.4	138.6 ± 1.0
C18:3 n-6	1.7 ± 0.2	2.2 ± 0.2	3.5 ± 1.0	3.1 ± 0.6
C20:2 n-6	1.1 ± 0.2	1.4 ± 0.1	3.6 ± 1.2	3.5 ± 0.1
C20:4 n-6	1.9 ± 0.1	4.1 ± 0.4	5.8 ± 4.2	10.3 ± 2.8
C22:5 n-6	1.0 ± 0.2	2.2 ± 1.4	2.5 ± 1.4	4.1 ± 1.6
SFA[a]	118.3 ± 2.1	156.9 ± 16.2	206.7 ± 61.0	241.5 ± 16.4
MUFA[b]	108.2 ± 6.4	147.7 ± 2.2	216.5 ± 55.0	247.4 ± 18.2
PUFA[c]	130.3 ± 9.2	190.6 ± 11.5	304.7 ± 139.1	412.5 ± 55.0
PUFA n-3	51.1 ± 1.6	92.2 ± 1.6	162.7 ± 91.7	239.8 ± 55.0
PUFA n-6	72.2 ± 13.1	86.9 ± 12.2	132.0 ± 49.4	161.2 ± 2.4
n-3/n-6	0.7 ± 0.2	1.1 ± 0.1	1.2 ± 0.3	1.5 ± 0.1

[a]Saturated fatty acids; [b]monounsaturated fatty acids; [c]polyunsaturated fatty acids.

denote a lack of grayness. Hue is the attribute that permits colors to be classified as red, green, yellow, blue, and so on.

Fatty acid analysis

The efficacy of catorenoid sources is dependent on the amount of the polyunsaturated fatty acid diet content (Bjerkeng et al. 1999); therefore, the FA composition was determined on the fish feeds, flesh, and skin fish samples. The lipid extraction of the samples was performed according to Hara and Radin (1978) and the transmethylation of the FA according to Christie (1982), with the modifications described by Chouinard et al. (1999). The FA methyl esters in hexane were then injected into a gas chromatograph (GC 1000 DPC, Dani Instruments S.P.A., Cologno Monzese, Italy) equipped with a flame ionization detector. The separation of the FA methyl esters was performed using a Famewax™ fused silica capillary column (30 m × 0.25 mm (i.d.), 0.25 μm; Restek Corporation, Bellefonte, PA, USA). The peak area was measured using a Dani Data Station DDS 1000. Each peak was identified and quantified by pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

Table 5 Main fatty acids (mg/g of total lipids) of the skin at beginning and end of experiment

	Initial	GFP0	GFP30	GFP60
C14:0	17.9 ± 7.4	17.5 ± 7.7	17.9 ± 5.3	16.8 ± 2.2
C16:0	56.5 ± 15.9	67.8 ± 2.3	70.3 ± 14.9	112.5 ± 15.1
C18:0	13.6 ± 2.3	16.7 ± 2.8	21.1 ± 2.8	36.1 ± 15.5
C20:0	0.7 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	2.1 ± 1.4
C16:1 n-7	16.0 ± 3.2	19.3 ± 4.4	21.8 ± 6.4	23.8 ± 2.3
C18:1 n-9	42.1 ± 0.4	50.0 ± 4.2	62.6 ± 14.1	132.0 ± 20.2
C18:1 n-7	7.1 ± 2.0	9.5 ± 0.8	11.1 ± 3.0	18.7 ± 8.3
C20:1 n-11	4.4 ± 1.9	7.2 ± 1.6	5.4 ± 1.3	20.5 ± 4.7
C18:3 n-3	3.8 ± 0.7	4.9 ± 0.8	6.1 ± 11.6	10.4 ± 3.7
C18:4 n-3	1.8 ± 0.1	3.5 ± 1.1	2.9 ± 0.8	5.3 ± 1.9
C20:5 n-3	10.0 ± 1.0	18.7 ± 6.3	18.9 ± 4.2	29.5 ± 11.5
C22:5 n-3	3.2 ± 0.6	5.3 ± 1.9	5.2 ± 1.1	8.4 ± 3.9
C22:6 n-3	9.8 ± 0.5	17.9 ± 2.2	22.3 ± 3.8	40.3 ± 27.2
C18:2 n-6	31.0 ± 7.6	39.7 ± 3.5	56.0 ± 12.2	120.3 ± 25.7
C18:3 n-6	1.2 ± 0.3	1.4 ± 0.2	1.8 ± 0.3	2.9 ± 1.0
C20:4 n-6	1.0 ± 0.0	2.4 ± 1.4	5.4 ± 0.9	6.1 ± 4.0
C22:5 n-6	-	3.1 ± 2.2	1.1 ± 0.7	1.2 ± 0.4
SFA[a]	91.3 ± 26.6	99.4 ± 6.3	113.1 ± 22.9	164.7 ± 49.7
MUFA[b]	63.1 ± 34.7	95.9 ± 8.0	107.7 ± 24.8	190.0 ± 78.0
PUFA[c]	69.5 ± 4.5	118.4 ± 33.8	131.3 ± 28.6	289.5 ± 171.5
PUFA n-3	29.8 ± 0.1	63.2 ± 26.6	58.5 ± 10.8	96.5 ± 49.6
PUFA n-6	34.5 ± 5.6	48.8 ± 7.9	65.8 ± 18.0	114.8 ± 49.7
n-3/n-6	0.9 ± 0.1	1.3 ± 0.3	0.9 ± 0.2	1.3 ± 0.5

[a]Saturated fatty acids; [b]monounsaturated fatty acids; [c]polyunsaturated fatty acids.

Proximate analysis

Diets and fish muscle were analyzed to determine the proximate composition according to standard methods (AOAC 1995). The gross energy content was determined using an adiabatic calorimetric bomb (IKA C7000, IKA Werke GmbH Co. KG, Staufen, Germany). The total nitrogen content was determined using a nitrogen analyzer (Rapid N III, Elementar Analysensysteme GmbH, Hanau, Germany) according to the Dumas method, and the crude protein was calculated as total $N \times 6.25$. Total lipid content was gravimetrically determined following the extraction method of Hara and Radin (1978).

Table 6 Chemical composition (% wet weight) of fillets of red porgy at beginning and of experiment

	Initial	GFP0	GFP30	GFP60
Dry matter	26.59 ± 0.88	28.94 ± 1.50	28.61 ± 1.49	27.60 ± 0.16
Crude protein	20.43 ± 0.41	22.48 ± 1.09	23.42 ± 0.14	21.60 ± 1.45
Crude fat	3.75 ± 0.20 a	2.44 ± 1.24 b	2.20 ± 0.26 b	3.25 ± 0.44 a
Ash	1.72 ± 0.41	1.56 ± 0.04	1.58 ± 0.05	1.53 ± 0.07

In the rows, different letters indicate statistical differences $P \leq 0.05$.

Table 7 Skin fat content during the feeding experiment

	GFP0	GFP30	GFP60
Start of the trial	6.14 ± 0.46 a	6.20 ± 0.47 a	2.27 ± 0.39 b
End of the trial	13.36 ± 1.44 a	6.56 ± 0.61 b	6.39 ± 1.06 b

In the rows, different letters indicate statistical differences $P \leq 0.05$.

Digestive enzyme assays

At the end of the experiment, the fish were killed 6 h after the last meal; five fish per tank were sampled. The intestine was separated, and all visible fat were removed. Tissues with their digestive contents were immediately frozen in liquid nitrogen and stored at −80°C before analysis. Tissues were homogenized using a polytron (Kinematica AG, Lucerne, Switzerland) in a cold Tris–HCl 50-mM (pH 7.0) buffer to a final concentration of 50 mg/mL. The homogenate was then centrifuged at 4°C at 7,500 × *g* for 10 min. The supernatant containing the crude extracts was picked up and stored at −20°C before analysis as proposed by Santigosa et al. (2008).

Total alkaline protease activity

The effect of different pH incubations on the proteolytic activity of the crude enzyme extract was determined on the base of the casein hydrolysis assay as described in a previous work (Gai et al. 2012). Results are reported as enzyme units (U) released per gram of wet tissue (U/g tissue).

Lipase assay

Lipase (E.C. 3.1.1.3) activity was determined spectrophotometrically by hydrolysis of *ρ*-nitrophenyl myristate according to Iijima et al. (1998) with a modified method of Albro et al. (1985). One unit of enzyme activity was defined as 1 μg of *ρ*-nitrophenol released per minute. Results are reported as enzyme units (U) released per milliliter of homogenate (U/mL homogenate).

Histological assays

The histological analyses were performed in order to check any possible detrimental effect of the experimental diets on the intestine integrity. Intestinal sections were evaluated following the criteria based on soybean meal-induced enteritis in the Atlantic salmon (Baeverfjord and Krogdahl 1996). Fish were sampled at the same time and in the same condition as those utilized for digestive enzyme assays. Samples of the liver and the proximal and distal intestine were sampled and fixed in 4% buffered (pH 7.2) formalin. After 1 week, the fixed tissues were embedded in paraffin wax, following normal histological procedures, and stained with the normal Mayer hematoxylin-eosin and Sudan Black stains in the intestine and liver sections, respectively. The Sudan Black stain was performed to observe and confirm the possible presence of cereoid substances.

Table 8 TPA and lipase activities measured in intestinal digestive tract of red porgy fed with experimental diets

	GFP0	GFP30	GFP60
TPA (U/g of tissue)	0.17 ± 0.05 a	0.20 ± 0.05 a	0.13 ± 0.02 b
Lipase (U/mL of homogenate)	6.82 ± 0.92	6.53 ± 1.40	6.56 ± 0.43

In the rows, different letters indicate statistical differences $P \leq 0.05$.

Table 9 Red porgy histological observations of the intestinal tract

	Intestinal tract		
	NC	MC	SC
GFP0	9	1	-
GFP30	7	3	-
GFP60	4	2	4

NC, no changes; MC, medium changes; SC, severe changes.

Statistical analysis

As far as the statistical treatments are concerned, the percentages and ratios were transformed into the arcsin square roots; on the transformed and normalized data, variance analyses were performed; any significant differences were ranged according to the Duncan test using the significant level of $P < 0.05$. The homogeneity of the variance was assessed on statistically significant results using a goodness-of-fit KS test.

Results

At the end of the trial, the final weight of the fish groups were as follows: GFP0 142.9 ± 21.5 g, GFP30 120.5 ± 27.9 g, GFP60 139.9 ± 23.8 g. Table 3 shows the measured and calculated values of the parameters that describe the skin color of the farmed red porgy. Significant differences were observed for lightness (L*) which is higher in the skin of the control fish; redness (a^*) and chroma values increased in the fish fed with GFP30 and GFP60 diets. Figure 1 shows the trend of the carotenoid content in the skin showing a growing gap in time between the liver of the fish fed with GFP30 and GFP60 diets and those of the fish fed with GFP0 diet. The FA composition of the diets was reported in Table 2. The FA composition of the GFP30 and GFP60 diets showed higher percentages of C18:1n9 (oleic acid (OA)) and C18:2n-6 (linoleic acid (LA)) than that of the GFP0 diet. These FAs increased in the diets with an increased carotenoid inclusion, while the other FAs did not change. The monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) n-6 content was higher in the GFP30 and GFP60 diets than in the control diet (GFP0). Due to the high content of LA in the GFP30 and GFP60 diets, the PUFA n-6 content increased, and the n-3/n-6 ratio decreased in these diets. The FA composition of the fillets and skin of *Pagrus* are reported in Tables 4 and 5, respectively. FAs in the fillet and skin reflected those of the diets. The analysis of these tables show that in the fillets (Table 4), the FA content, expressed as milligrams per gram (mg/g) of total lipids, tends to increase when compared to the fillets at the beginning of the trial; moreover, the fish fed with diets supplemented with carotenoids

Table 10 Red porgy histological observations of the liver

	Cereoid substance		
	AB	MP	HP
GFP0	2	3	-
GFP30	3	2	-
GFP60	3	2	-

AB, cereoid substances absent; MP, medium presence of cereoid substance; HP, high presence of cereoid substances.

showed a higher MUFA and PUFA n-6 content. The skin FA profile is similar to the flesh FA composition but lower in terms of FA contents.

The chemical composition of the fish fillets is shown in Table 6. The fillet protein content was not influenced by the diets. The fillet samples analyzed at the beginning of the feeding trials and those analyzed at the end of the trials showed a protein content ranging between 20.43% and 23.42% (Table 6). The lipid deposition in the skin showed an increasing deposition, proportional with dietary pigment inclusion (Table 7).

The total alkaline protease activity (TPA) and lipase are reported in Table 8; statistical differences appeared only for TPA, in particular, between GFP0 and GFP30 vs. GFP60, with the first two diets higher than the second.

The histological analyses were performed on the intestine tract and liver, and the results are summarized in Tables 9 and 10, respectively. No histopathological changes were observed in the liver for any of the dietary treatments, while severe changes were recorded in the intestinal tract of four animals fed with diet GFP60.

Discussion

Many papers in the scientific literature on fish nutrition for aquaculture reported the use of microalgae, yeast, and other plant material which are useful to increase the skin or fillet pigmentation. Zatkova et al. (2010) used spray-dried biomass of green algae *Scenedesmus*, *Chlorella*, or *Haematococcus* enriched in carotenoids (12 to 60 mg/kg of feed) as feed supplement for a juvenile wels catfish. Chatzifotis et al. (2011) fed red porgies with diets supplemented with red (mainly astaxanthin esters) and yellow (mainly β-carotene, lutein, and zeaxanthin) carotenoids that affected significantly the carotenoid deposition in the skin as well as the skin hue and chroma. Among these papers, results are reported on the efficacy of the sources of astaxanthin esters or free astaxanthin (Tejera et al. 2007). In the present study, a feed pigment supplementation based on astaxanthin derived from yeast (*P. rhodozyma*) and plant pigments, alfalfa (*Medicago sativa*), oil and meal maize, red pepper extract, and algae was tested. The yeast *P. rhodozyma* is a pigment source with an easy market availability as well as the other raw materials of the pigmentation pool, such as the xanthophylls.

Torrissen (1985) and Nickell and Bromage (1998) reported that increasing the dietary lipid level resulted in a higher deposition rate of carotenoids in the muscle of the rainbow trout, while Regost et al. (2001) detected in turbot a significant subcutaneous fat accumulation. In our study, GFP0 and GFP60 diets showed high skin lipid values during the experimental period. However, Lee et al. (2010) did not observe any effects of different dietary lipid levels on the deposition of carotenoids in the pale chub. Skin and muscle lipid content was influenced by dietary lipids. As expected, astaxanthin deposition reduces the lightness, while the accumulation of dietary carotenoids led to a statistical significant increase in a^* and b^* values. Kalinowski et al. (2011) reported that the skin coloration of red porgy fed with a diet supplemented with unesterified astaxanthin (100 mg/kg) in a 120-day feeding trial presented adequate hue values throughout the 7 days post-mortem and skin chroma up to day 3 post-mortem, having values similar to those reported for wild red porgy.

The total carotenoids of the three diets (5 to 18 mg/kg) reported in Table 1 are similar to the values reported by Chatzifotis et al. (2011) (5 to 20 mg/g), and this means

that the pigment supplement was also similar. Grigorakis and Alexis (2005) reported that 4 weeks of carotenoid deprivation determined a discoloration of the fish skin in the dorsal area in red porgy. In rainbow trout, the pigmentation changes during its life cycle, and it increases during the development. Bjerkeng et al. (1992) showed that the fish deposited small amounts of carotenoids in the flesh (<3 mg/kg) up to 50 weeks. A minimum of 9 weeks of feeding with xanthophylls is necessary for proper pigmentation of the Arctic char. However, the maximum uptake of carotenoids occurs after 15 weeks of feeding with diets enriched in xanthophylls (Shahidi et al. 1994).

Our results indicate that the sustainable production of red porgy with a natural red hue is possible on the basis of proper adjustment of dietary astaxanthin as also reported by Tejera et al. (2010).

As far as red porgy fillet FAs are concerned, the fish fed with the GFP60 diet showed higher values of MUFA, PUFA n-3, and PUFA n-6 than the fish fed with GFP30 and GFP0 diets. These differences are determined mainly by OA, C20:5n-3 (EPA), and C22:6 n-3 (DHA) acids contained in the diets. The skin FA profile was not so different in comparison to those of the fillets. A similar trend was observed for the different FA fractions even if the skin values were lower than those of the fillets, in particular, for the following FAs: OA, LA, EPA, and DHA.

The total alkaline protease activity values obtained in this study are comparable to those reported by Sicuro et al. (2010).

Results of the skin color, carotenoid content, and increased lipid content of the skin showed a good correlation with the experimental diets, even if the skin of red porgy did not show a very strong staining as has been suggested. Likely, given the upward trend of carotenoids, an additional period of feeding the experimental diets could lead to the desired results but can also cause a darker coloration. Experimental diets did not affect fillet FA composition and quality.

Although the digestive process in fish is not studied as much as in mammals, the enzyme profile measured in this experiment is a good physiological indicator of feed utilization. In particular, when an anti-nutritional factor effect is suspected, digestive enzyme analysis is a useful method that gives clear information for the utilization of a new formulated fish feed by the fish (Refstie et al. 2006; Corrêa et al. 2007).

The parameters related to the intestinal tract integrity and liver lipidosis showed an increased number of subjects at the increasing level of carotenoid supplementation. This increase was probably due to the different lipid metabolisms of the fat soluble pigments contained in the diets supplemented with carotenoids. On the contrary, the presence of cereoid substances in the liver was negligible with no difference among the experimental diets.

Conclusions

The present study show encouraging results for developing new research, but they are not satisfying to apply this carotenoid supplement feed for this species in production farming.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions

AM,GBP, and MZ developed the initial idea and designed the study. GF, MS, and FD were responsible for the feed manufacturing, feeding trial, and sample collection. FG, FD, AR, and EL carried out the analytical work. GBP, FG, MS, and MZ were responsible for data analysis. FG, AM, and MS wrote the manuscript with assistance from all other authors. All authors read and approved the final manuscript.

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