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Effect of different levels of synthetic astaxanthin on growth, skin color and lipid metabolism of commercial sized red porgy (*Pagrus pagrus*)

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ABSTRACT

A study was undertaken to evaluate the effects of supplementing commercial feeds with Carophyll® Pink, a gelatin-encapsuled astaxanthin, on growth parameters, lipid composition, pigment concentration and skin color of commercial size red porgy. Three isonitrogenous diets were formulated containing different levels of astaxantin: Ctrl diet (no pigment added), A_{50} diet (with 50 mg/kg of astaxanthin) and A_{80} diet (with 80 mg/ kg of astaxanthin). Four groups of fish (386.29 ± 29.50 g initial weight) were grown in triplicate tanks (10 fish per tank). Ctrl, A_{50} and A_{80} groups were fed the respective diets to apparent satiation for 180 days, while a fourth group was fed A_{50} diet for 90 days followed by A_{80} diet for another 90 days ($A_{50}A_{80}$). At the end of trial muscle samples were collected for composition analysis, liver and mesenteric fat for lipid composition determination and skin samples for carotenoids quantification. Dietary astaxanthin supplementation had no effect on growth performance and hepatosomatic index. However, $A_{50}A_{80}$ group displayed a lower muscle protein content and higher fat content when compared to the other groups. There was no clear effect of dietary astaxanthin supplementation on the liver's and mesenteric fatty acid profile. Although there was no significant effect on skin total carotenoids, the astaxanthin content tended to increase in fish fed astaxanthin supplemented diets, which was reflected on redness values. Accordingly, dietary astaxanthin supplementation (at 50 or 80 mg/kg for 6 months or at 50 mg/kg for 3 months followed by 80 mg/kg for 3 months) influenced positively skin hue and chroma of red porgy on both pectoral and caudal areas, improving the skin color in commercial sized red porgy and achieving hue and chroma values close to those previously reported for wild individuals. The results reported here provide evidence

Abbreviations: Ctrl, diet not supplemented with astaxanthin; A_{50}, diet supplemented with 50mg/kg of astaxanthin; A_{80}, diet supplemented with 80 mg/kg of astaxanthin; $A_{50}A_{80}$, diet supplemented with 50 mg/kg of astaxanthin for three months followed with 80 mg/kg of astaxanthin for the three next months; IBW, initial body weight; FBW, final body weight; SGR, specific growth rate; DGI, daily growth index; FCR, feeding conversion ratio; PER, protein efficiency ratio; HIS, hepatosomatic index; L*, lightness; a*, redness/greenness chromaticity; b*, yellowness/bluish chromaticity; TL, total lipids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; DHA, docosahexaenoic acid (C22:6ω3); EPA, eicosapentaenoic acid (C20:5ω3); ARA, arachidonic acid (C20:4ω6); DMSO, dimethyl sulphoxide.

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of the applicability of Carophyll® Pink to improve skin color of large size commercial red porgy, but further studies are needed to optimize this carotenoid supplementation in production farming.

1. Introduction

There is a great challenge of supplying fish to an increasing and demanding market of animal proteins for human consumption. The undertaking of diversification processes within aquaculture industry is essential to maintain high production growth rates. The red porgy (*Pagrus pagrus*) is a protogynous hermaphrodite sparid fish widely distributed in the temperate zone of both sides of the Atlantic Ocean and Mediterranean Sea (Manooch, 1975). In the past, several studies showed that this high price species presented a good adaptability and a high growth rate in captivity (Pavlidis and Mylonas, 2011). Hatchery techniques for this species evolved from other sparids culture and are well established (Aristzabal et al., 2009; Morris et al., 2008; Andrade et al., 2011, 2012, 2013). However, under culture conditions, the natural red-silver color of red porgy body changes into an overall dark grey, most prominently in the tail and fins, differentiating from wild counterparts (Stephanou et al., 1995; Cejas et al., 2003; Almansa et al., 2001; Pavlidis et al., 2008; Kalinowski et al., 2015) and hence remaining as the bottleneck for the commercialization of the species.

Fish flesh and skin color is due to the presence of oxygenated carotenoids or xanthophylls (da Costa and Miranda-Filho, 2020), an important class of pigments in animals, synthesized by all photosynthetic organisms (Carvalho and Caramujo, 2017). Nevertheless, teleost fish do not possess the ability to synthesize carotenoids endogenously (Goodwin, 1984), but they can modify dietary carotenoids and store them in the integument and other tissues. In the wild, fish obtain carotenoids from prey, but in intensive fish cultures, the carotenoids requirements should be met through dietary additives (Noori and Razi, 2017). Still, carotenoids deposition and pigmentation efficiency are source-dependent and species-specific (Ha et al., 1993**),** as not all fish species possess the same pathways for metabolizing carotenoids.

The carotenoid compounds have also been associated with other biological functions such as the synthesis of vitamin A (White et al., 2003), immune functions (Amar et al., 2012) and high antioxidant activity (Wang et al., 2006), preventing the harmful effects of lipid peroxidation (Liebler, 1993). Together with lipid protection, carotenoids may as well exert influence on lipogenesis and lipid composition (Mary et al., 2003; Trattner et al., 2007; Woo et al., 2010; Hu et al., 2012). Marine fish lipids, namely the docosahexaenoic acid (DHA, C22:6ω3), eicosapentaenoic acid (EPA, C20:5ω3) and arachidonic acid (ARA, C20:4ω6) play important physiological roles in fish as components of membranes phospholipids and as precursors of active eicosanoids (Bell and Sargent, 2003). As in other marine fish, red porgy tissues are naturally enriched with these fatty acids (Rueda et al., 1997; Cejas et al., 2003). Dietary supplementation with carotenoids has been shown to decrease total lipids in the whole fish and liver, as well as palmitic acid (C16:0) (Kalinowski et al., 2011), and to increase liver EPA and DHA content (Kalinowski et al., 2011). Conversely, there is a controversy regarding the effect of carotenoids on growth (Chatzifotis et al., 2005; Kalinowski et al., 2015). Although some studies reported growth enhancement with carotenoid supplementation (Torrissen et al., 1995; Christiansen and Torrissen, 1996; Amar et al., 2001), others have found no effect at all (Nakano et al., 1995; Nickell and Bromage, 1998; Rahman et al., 2016).

The keto-carotenoid astaxanthin (3,3′-dihydroxy-*β,β*-carotene-4,4′-dione) is the main carotenoid responsible for the reddish or pinkish color of most wild fish (Dhankhar et al., 2012; Carvalho and Caramujo, 2017). Therefore, many studies used it, from natural or synthetic sources, to induce pink or red skin coloration (Tejera et al., 2007; Chatzifotis et al., 2011; Kalinowski et al., 2011, 2015). The continued growth of the aquaculture industry has led to a massive demand for astaxanthin (Lim et al., 2018). Nowadays, the commercialization of the synthetic products is widespread not only because it lowers the pigments production costs (Lim et al., 2018), but because these are standardized products, chemically stable with high carotenoid concentration (Teimouri and Amirkolaie, 2015). Still, carotenoid supplementation in aquaculture feeds, further increases the already high management costs (Lim et al., 2018), decreasing profits in aquaculture operation (Pham et al., 2014). For that reason, the research on supplementation protocols to optimize the inclusion of these pigments in commercial sized fish is key to assure profitability. To our knowledge previous studies on red porgy tegument coloration dealt with alevins and juveniles up to 220 g at the beginning of the experiments. Surprisingly, larger sized fish (600 g) have received little attention though it is more valued and with a higher potential for product diversification in several Mediterranean countries (e.g. filleting) (Basurco et al., 2011). Therefore, the purpose of the current study was to investigate the effects of supplementing commercial feeds with Carophyll® Pink, a gelatin-encapsuled astaxanthin, on large commercial sized red porgy growth parameters and lipid composition. Red porgy juveniles were fed two astaxanthin supplementation levels and a third group was sought to ascertain whether an increase in astaxanthin concentration would lead to a change in retention efficiency, ultimately affecting feed production costs. To accomplish the objective of this study, the following parameters were evaluated: growth performance, skin pigmentation, tissues proximate composition (dry matter, ash, crude protein and total lipid), lipid classes and fatty acid composition of the liver and mesenteric fat.

2. Material and methods

2.1. Experimental diets and dietary treatments

A diet formulated to meet the nutritional requirements of Sparidae (Guillaume and Choubert, 2001; Koshio, 2002; Schuchardt et al., 2007; Kalinowski et al., 2011) was used as a control diet (Ctrl), not being supplemented with astaxanthin. Two other diets were

supplemented with 50 mg/kg (diet A_{50}) and 80 mg/kg (diet A_{80}) of synthetic astaxanthin (3, 3′-dihydroxy-*β, β*-carotene-4, 4′ -dione) (Carophyll® Pink 10 % CWS, DSM Nutritional Products Ltd). Astaxanthin was prediluted with a mineral carrier and added to the mixer prior to extrusion, to ensure homogeneous mixing occurred. The formulation included antioxidants to minimize losses of the pigment due to oxidative stress. All feeds were formulated and manufactured by Aquasoja (Soja de Portugal, Portugal). Ingredients, diet composition and the carotenoid content of the diets are presented in Table 1.

Four groups were assigned different dietary treatments. The Ctrl, A_{50} and A_{80} groups were fed the Ctrl, A_{50} and A_{80} diets, respectively, for six months. A fourth group ($A_{50}A_{80}$) was fed upon the A_{50} diet for three months followed by the A_{80} diet for the next three months.

2.2. Husbandry and experimental set-up

One hundred and twenty red porgy (*Pagrus pagrus*) juveniles hatched and reared at Mariculture Center of Calheta (CMC) facilities, with an average initial weight of 386.29 ± 29.50 g, were distributed by twelve white circular fiberglass 500 L white tanks in a flow-through system, at an initial density of 7.5 kg/m^3. Prior to the beginning of the trial, fish underwent a 2-weeks acclimation period and were fed the Ctrl non-carotenoid supplemented diet. Then, the dietary treatments (Ctrl, A_{50}, A_{80}, $A_{50}A_{80}$) were randomly assigned to the twelve tanks (three tanks per treatment). Throughout the trial, fish were exposed to natural photoperiod (10 h light/14 h dark), dissolved oxygen levels were kept near saturation and water temperature ranged between 20.0 and 23.6 °C. The trial lasted 180 days, during which, fish were hand fed until apparent satiation (fish were given as much feed as they would consume within a 15-minute period), twice per day, six days a week (Kalinowski et al., 2015). The feed consumption in each tank was daily recorded.

2.3. Fish performance and color measurements

At the beginning and at the end of the feeding trial, fish were starved for 24 h, anesthetized with tricaine methanesulfonate (MS222; Sigma, St. Louis, MO, USA) at a concentration of 100 mg/L and individually weighed. The final mean body weight of each

Table 1
Ingredients (%) and composition of the experimental diets (% dry matter).

	Diets		
	Ctrl	A_{50}	A_{80}
Ingredients (%)			
Fishmeal 67 [a]	35	35	35
Maize gluten [b]	15	15	15
Fish meal 62 [c]	14	14	14
Fish oil [d]	11	11	11
Soybean meal 48 [e]	8.5	8.5	8.5
Wheat meal [f]	8	8	8
Rapeseed meal [g]	5	5	5
SCP [h]	2.5	2.5	2.5
Premix [i]	1	1	1
Astaxanthin (mg/kg) [j]	–	50	80
Diet composition			
Moisture (%)	9.0	9.0	9.0
Ash (%)	11.2	11.2	11.2
Crude fibre (%)	2.0	2.0	2.0
Crude protein (%)	55.0	55.0	55.0
Fat (%)	17.6	17.6	17.6
Gross energy (kJ/g)	20.8	20.8	20.8
Calcium (%)	2.3	2.3	2.3
Phosphorus (%)	1.7	1.7	1.7
Sodium (%)	0.5	0.5	0.5

[a] Whole sardine based fishmeal from Sovapec SA, Morocco.
[b] Copam SA, Portugal.
[c] Tuna byproduct fishmeal from Aucosa SA, Spain.
[d] Industrias Afines SL, Spain.
[e] Non GM dehulled and defatted soybean meal, from Bunge Iberica Portugal SA, Portugal.
[f] Reagro, Importação e Exportação SA, Portugal.
[g] Oleocom SA, Portugal.
[h] Single Cell Protein from *Corynebacterium glutamicum* (Protorsan) - Ajinomoto Foods Europe SAS, France.
[i] Vitamin A 5.000 I.U./kg; Vitamin D3 1.000 I.U./kg; Vitamin E 200 I.U./kg; Vitamin C 100 mg/kg; Vitamin K3 5 mg/kg; VItamin B1 4 mg/kg; Vitamin B2 10 mg/kg; Vitamin B6 5 mg/kg; Vitamin B12 10 µg/kg; Biothin 0.35 mg/kg; Inositol 150 mg/kg; Folic acid 3 g/kg; Nicotinic acid 35 mg/kg; Panthotenic acid 15 mg/kg; $CuSO_4$ 3 mg/kg; $FeSO_4$ 20 mg/kg; KI 1 mg/kg; MnO_2 12 mg/kg; Na_2SeO_3 0.15 mg/kg; ZnO 15 mg/kg Valouro SA, Portugal; Betafin S1 0.4 g/kg; Choline chloride 0.5 g/kg.
[j] Synthetic astaxanthin, Carophyll® Pink, DSM.

experimental treatment was determined by dividing the total fish weight of the three replicates by the number of fish. Specific growth rate (SGR), daily growth index (DGI), feed conversion ratio (FCR), protein efficiency ratio (PER) and the hepatosomatic index (HSI) were calculated as:

$SGR = [\ln_{\text{average final weight (g)}} - \ln_{\text{average initial weight (g)}})/\text{days}] \times 100$

$DGI = [\text{final weight (g)}^{1/3} - \text{initial weight}^{1/3}\ \text{(g)/days}] \times 100$

FCR = feed intake during the whole experimental period (g)/gained weight during the whole experimental period (g)

PER = weight gain (g)/protein intake (g) (based on the diets protein content, Table S1)

HSI = liver weight (g)/fish weight (g) × 100)

In the current study, colorimetric analysis was done using a colorimeter (MiniScan EZ, HunterLab) calibrated on a white reference plate (with reflectance values of L*= + 95.91, a*= +0.09 and b*= +2.02). According to the concept of International Commission on Illumination (CIE, 1976), color is a three-dimensional characteristic of appearance consisting of a lightness attribute (L*) and two chromatic attributes, hue and chroma. L* ranges from 0 for black and 100 for white. Hue (H°) is determined by the dominant wavelength and is the name of a color as found in its pure state in the spectrum. Chroma (C*) refers to the saturation of a color and it is a measure of how much grey and white light is mixed in with the 'pure' focal color. The skin color parameters registered from the colorimeter were: L* (lightness), a* (redness/greenness chromaticity) and b* (yellowness/bluish chromaticity). From a* and b* values, hue and chroma were then calculated as $H^\circ = \tan^{-1}$ (b*/a*) and $C^* = ((a^{*2}+b^{*2})^{0.5})$, respectively (Boccard et al., 1981). Color measurements were taken in all fish, immediately after anesthetization. A total of three readings was taken in the caudal and pectoral regions, always on the left side of the fish.

2.4. Analytical methods

Twelve fish per treatment (four fish per tank) were killed in iced water, following European Directive nº. 2010/63/UE on the protection of animals used for scientific purposes. Skin samples were collected from the left-hand side of all fish, in the front lateral zone (Kalinowski et al., 2005) for carotenoid and astaxanthin determination. The livers from nine fish per treatment (three per replicate) were excised and weighed (W), for hepatosomatic index determination (HSI, see 2.3). Samples of dorsal muscle and mesenteric fat were taken from twelve fish for subsequent analysis of tissues proximate composition (dry matter, ash, crude protein and total lipid), lipid classes and fatty acids profile. Following the excision, all tissue samples were immediately snap-frozen in liquid nitrogen and kept at −80 °C until they were freeze dried and again stored at −80 °C until further analysis.

2.4.1. Tissues proximate composition

Samples of red porgy muscle and liver were analysed following AOAC (1975) procedures: dry matter (105 °C to constant weight), ash (550 °C to constant weight), crude protein (N × 6.25) by the Kjeldahl method after acid digestion and total lipids (TL) were extracted with a chloroform-methanol mixture (1:2, v/v), containing 0.01 % (w/v) BHT (butylated hydroxytoluene), according to Bligh and Dyer (1959). Prior to lipid and protein determinations, residual moisture was determined using a Gibertini-Eurotherm dry weight balance.

2.4.2. Lipid classes

Lipid classes were separated from total lipids using a silica column at atmospheric pressure. Before making the column, the silica (60 mesh, Sigma) was activated at 100 °C . The column was compacted by the dichloromethane. The elution sequence, of growing polarity, followed Guckert et al. (1985) and Smith et al. (1986) procedure: first dichloromethane, then acetone and finally methanol. The fractions were dried by low nitrogen flow. These elutions allow the separation of the different lipid fraction: neutral lipids (dichloromethane; NL), phospholipids (acetone; PhL) and glycolipids (methanol; GL). PhL and GL were the polar lipids.

2.4.3. Fatty acids profile

Fatty acids content was determined as fatty acid methyl esters (FAME) according to the procedure of Lepage and Roy (1986) modified by Cohen et al. (1988), by gas chromatography-mass spectrometry (GC–MS). In brief, analyses were performed in a gas chromatograph (GC, Agilent HP 6890) equipped with a flame ionisation detector and a mass selective detector (Agilent 5973). The separation was performed in a polyethylene glycol capillary column (Supercolwax) with 30 m of length, 0.25 mm i.d. and 0.25 μm film thickness from Supelco. The chromatographic conditions were as follows: oven initial temperature was 150 °C for 2 min; increasing 3 °C/min to 205 °C and kept for 2 min, 3 °C/min to 230 °C and 30 °C/min until reaching the final temperature of 300 °C for 5 min; transfer line temperature 260 °C; temperature detector, 270 °C; split ratio, 40:1. Helium was used as a carrier gas with a flow rate of 1 mL/min. The FAME identification was accomplished by comparing the retention times and mass spectra fragmentation to those of known standards (Supelco 37 component FAME and mix bacterial acid methyl esters CP mix from Supelco). Four replicates were performed for each GC analysis and FAME are expressed as percentage of dry mater. The internal standard used was the heneicosanoic acid (C21:0).

2.4.4. Carotenoids extraction and astaxanthin determination

Carotenoids were extracted from dried skin red porgy using dimethyl sulphoxide (DMSO) and acetone as solvents extractors. Briefly, DMSO was added to the samples and heated for 30 min in a water bath with a temperature between 45 and 50 °C. The solutions were centrifuged at 3720 *g* for 10 min and the supernatant was removed. Extraction was repeated with acetone until the organic phase was devoid of color. All the supernatants were combined in a volumetric flask and used for estimation of total carotenoid and to astaxanthin determination.

The total carotenoids content was calculated using the absorbance value measured in a spectrophotometer UV at 471−477 nm and an extinction coefficient of $\varepsilon = 2500$ L/mol cm (Davies, 1976).

Astaxanthin was separated and determined from the total carotenoids extract by high performance liquid chromatography (HPLC) according Cifuentes et al. (2003) with some modifications. The HPLC apparatus consisted of an Agilent 1110 Series (Palo Alto, CA, USA), equipped with an autosampler (Agilent G1313A), quaternary pump (Agilent G1311A) and UV–vis detector (Agilent 1100 G1315B DAD). The separation was carried out using a reversed phase C18 (Phenomenex, 250 × 4.6 mm) column, in an isocratic solvent system (80:20, acetonitrile:ethyl acetate, v/v), at a flow rate of 1.0 mL/min for 10 min. Astaxanthin was detected at 477 nm (Davies, 1976) and identified by its time retention and absorption spectra using standard substance.

2.5. Statistical analysis

Statistical analyses followed reported methods (Zar, 2010). IBM SPSS Statistics 25 was the software used for all the statistical analysis performed. All data were tested for normality, using a Kolmogorov–Smirnov (whenever n>30) or Shapiro–Wilk (whenever n<30) test, and for homogeneity of variance, using a Levene's test. All percentage data were arcsine transformed prior to analysis. The overall influence of dietary treatment on performance parameters (IBW, FBW, SGR, DGI, FCR and PER), HSI and tissues composition, lipid classes and fatty acid content, as well as skin carotenoid and astaxanthin content was tested by one-way ANOVA followed by a Tukey's post-hoc test. A non-parametric Kruskal-Wallis test followed by a Games-Howell post-hoc test was used instead, whenever data did not meet normality and homoscedasticity requirements. The results are presented as the mean value ± standard deviation (SD). Significance levels were set at $P < 0.05$.

3. Results

3.1. Fish performance

Dietary supplementation with astaxanthin did not affect red porgy growth, as elicited from the results on final body weight, specific growth rate and daily growth index (Table S1). Feed conversion ratio, protein efficiency utilization and hepatosomatic index were also not affected by dietary astaxanthin supplementation (Table S1). However, there was a significant effect on the lipid ($P < 0.01$) and protein ($P < 0.01$) content of the muscle, with the $A_{50}A_{80}$ group displaying a lower protein content and higher fat content when compared to the other groups (Table 2).

3.2. Lipid metabolism

Total lipids (TL) accounted for 40–50 % of liver and for 88–93 % of mesenteric fat, dry matter (Fig. 1). The neutral lipids (NL) were overrepresented in mesenteric fat (62–69 %) whereas in liver a more even distribution was found between phospholipids (PhL; 41–59 %) and neutral lipids (37–55 %) (Fig. 1). Fish grown solely upon the A_{50} diet were found to have a significant lower percentage of phospholipids ($P < 0.01$) and higher percentage of neutral lipids ($P < 0.01$) when compared to the other groups.

Overall, the major fatty acids found in the different fractions of the liver's and mesenteric fat were palmitic acid (C16:0); oleic acid (C18:1), followed by linoleic acid (LA, C18:2ω6), eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3).

Although there were some differences on the liver's fatty acid profile among dietary treatments, there was no clear trend related with dietary supplementation with astaxanthin. Within the neutral lipids, the A_{50} and A_{80} groups had a higher percentage of myristic acid (C14:0). Conversely, the $A_{50}A_{80}$ group had a higher amount of α-linolenic acid (C18:3ω3) than the Ctrl group and both the A_{50} and $A_{50}A_{80}$ groups had a higher amount of linoleic acid (C18:2ω6) than the Ctrl group, which reflected on the total ω6-fatty acids (Table 3). Fatty acid profile of liver's phospholipids showed that the group fed the A_{80} diet had a significantly higher percentage of myristic acid (C14:0) than the groups fed A_{50} and $A_{50}A_{80}$ and a lower percentage of stearidonic acid (C18:4ω3) (Table 4). The glycolipids fraction

Table 2
Muscle composition (%, dry matter) of the commercial size red porgy (*P. pagrus*) fed with different experimental diets.

	Diets				
	Ctrl	A_{50}	A_{80}	$A_{50}A_{80}$	*P-value*
Lipids	10.0 ± 0.94 [b]	9.48 ± 0.81 [b]	9.84 ± 2.20 [b]	14.8 ± 1.78 [a]	*<0.01*
Protein	86.2 ± 3.36 [a]	86.9 ± 1.80 [a]	89.1 ± 2.24 [a]	82.9 ± 3.20 [b]	*<0.01*
Water content	74.1 ± 0.95	73.6 ± 1.55	74.6 ± 1.18	73.2 ± 1.31	*0.45*
Ash	5.10 ± 0.54	6.21 ± 0.60	6.21 ± 0.80	5.14 ± 0.47	*0.10*

Data are expressed as mean ± SD; Different letters within the same row represent statistical differences.

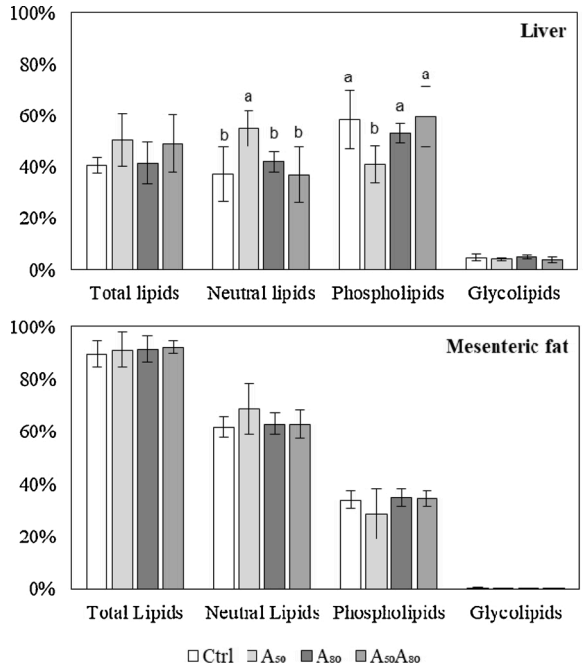

Fig. 1. Total lipids (%, dry matter) and lipid classes (% of total lipid) in the liver and mesenteric fat of the commercial size red porgy (*P. pagrus*) fed different experimental diets. Data are expressed as mean ± SD. Different letters represent statistical differences ($P < 0.05$).

accounted for less than 5% of liver total lipids (Fig. 1). Within this fraction, the $A_{50}A_{80}$ group had a higher amount of stearic acid (C18:0) than the A_{50} group, which in turn presented a higher amount of α-linolenic acid (C18:3ω3) than any other group. These variations did not reflect on the total ω3-fatty acids, in which EPA and DHA were the predominant fatty acids, accounting for

Table 3
Neutral lipids fatty acids profile (% detected) in the liver of commercial size red porgy (*P. pagrus*) fed with different experimental diets.

	Diets				
Fatty acids	Ctrl	A_{50}	A_{80}	$A_{50}A_{80}$	*P-value*
Saturated fatty acid					
C14:0	4.93 ± 0.29 [b]	5.64 ± 0.10 [a]	5.68 ± 0.41 [a]	4.63 ± 0.30 [b]	*<0.01*
C16:0	18.9 ± 0.55	19.5 ± 1.53	20.2 ± 0.88	18.6 ± 1.83	*0.33*
C18:0	7.99 ± 0.48	8.03 ± 1.84	8.07 ± 0.78	8.22 ± 1.13	*1.00*
Other	1.39 ± 0.04 [b]	1.60 ± 0.09 [a]	1.60 ± 0.07 [a]	1.42 ± 0.12 [b]	*<0.01*
Total	*33.2 ± 0.92*	*34.7 ± 3.53*	*35.6 ± 1.51*	*32.8 ± 3.14*	*0.30*
Monosaturated fatty acid					
C16:1	10.1 ± 0.34	10.7 ± 1.12	10.41 ± 1.40	10.2 ± 1.08	*0.81*
C18:1	19.3 ± 0.37	18.7 ± 0.72	19.6 ± 1.56	20.2 ± 1.07	*0.29*
C20:1	2.76 ± 0.12	3.37 ± 0.64	3.39 ± 0.13	2.96 ± 0.41	*0.15*
Other	2.17 ± 0.21	2.79 ± 0.70	2.83 ± 0.21	2.12 ± 0.55	*0.11*
Total	*34.4 ± 0.77*	*35.6 ± 0.93*	*36.2 ± 2.68*	*35.4 ± 1.00*	*0.31*
Polyunsaturated fatty acid					
C18:2ω6	6.48 ± 0.02 [b]	7.31 ± 0.38 [a]	6.85 ± 0.17 [ab]	7.16 ± 0.04 [a]	*0.01*
C18:3ω3	0.86 ± 0.05 [b]	0.90 ± 0.02 [ab]	0.88 ± 0.02 [ab]	0.94 ± 0.02 [a]	*0.02*
C18:4ω3	1.35 ± 0.10	1.25 ± 0.22	1.18 ± 0.14	1.25 ± 0.12	*0.55*
C20:3ω6	0.68 ± 0.02	0.64 ± 0.14	0.65 ± 0.12	0.69 ± 0.21	*0.97*
C20:4ω6	0.88 ± 0.04	0.79 ± 0.18	0.74 ± 0.04	0.96 ± 0.21	*0.20*
C20:5ω3	7.80 ± 0.43	6.38 ± 1.50	6.23 ± 1.14	7.36 ± 0.96	*0.18*
C22:5ω3	2.91 ± 0.16	2.36 ± 0.72	2.17 ± 0.22	2.78 ± 0.67	*0.28*
C22:6ω3	8.94 ± 0.52	7.44 ± 1.64	7.14 ± 1.96	8.19 ± 1.55	*0.20*
Other	2.54 ± 0.15	2.64 ± 0.45	2.42 ± 0.07	2.43 ± 0.28	*0.78*
Total	*32.4 ± 1.23*	*29.7 ± 4.44*	*28.2 ± 3.43*	*31.8 ± 3.82*	*0.25*
Total ω3	22.4 ± 1.06	18.8 ± 4.11	18.0 ± 3.44	21.0 ± 3.31	*0.19*
Total ω6	8.04 ± 0.19 [b]	8.74 ± 0.06 [a]	8.25 ± 0.05 [ab]	8.81 ± 0.31 [a]	*<0.01*
ω3/ ω6	2.78 ± 0 12	2.16 ± 0.49	2.19 ± 0.42	2.38 ± 0.29	*0.02*
DHA/EPA	1.15 ± 0.07	1.17 ± 0.02	1.13 ± 0.11	1.11 ± 0.07	*0.82*
EPA/ARA	8.91 ± 0.21	8.08 ± 0.18	8.34 ± 1.14	7.77 ± 0.75	*0.17*

Data are expressed as mean ± SD; Different letters within the same row represent statistical differences ($P < 0.05$).

approximately 7 and 16 %, respectively (Table 5).

Regarding the mesenteric fat, there was no significant effect of dietary astaxanthin supplementation on the lipid classes. Although minor differences in the content of some fatty acids were detected in all lipid classes, there was no obvious relation with astaxanthin supplementation (Tables 6–8). Within the neutral lipids the A_{80} group presented higher amounts of palmitic acid (16:0) than any other group, reflecting on total SFA (Table 6). As for the PUFA, results showed that the $A_{50}A_{80}$ group had a higher amount of linoleic acid (C18:2ω6) than the A_{80} and Ctrl groups, which reflected on the total ω6-fatty acids. DHA/EPA ratio and ω3/ω6 ratio differences were explained by the fact that the A_{80} group had higher EPA content than any other group, while the $A_{50}A_{80}$ had the lowest EPA levels (Table 6). In the phospholipids class, the A_{80} group had a higher amount of palmitic (16:0) and stearic acids (18:0) and a lower amount of oleic (C18:1) and eicosenoic (C20:1) acids when compared to the $A_{50}A_{80}$ group, reflecting on the total saturated fatty acids (SFA) and the mono-unsaturated fatty acids (MUFA) (Table 7). Total ω6-fatty acids was significantly higher in fish fed the $A_{50}A_{80}$ than in those fed the Ctrl or A_{80} diets, which reflected on ω3/ω6 ratio that was significantly higher in fish fed the Ctrl and A_{80} diets as compared to those grown upon the $A_{50}A_{80}$ diet (Table 7). Within the diminutive glycolipids fraction (less than 0.46 % of mesenteric fat total lipids) (Fig. 1), changes in fatty acids profile did not reveal a clear response to dietary astaxanthin supplementation (Table 8).

3.3. Skin color and carotenoid accumulation

Dietary astaxanthin supplementation had an effect on color of both the pectoral and caudal regions of red porgy, affecting the recorded and calculated values of the parameters used to describe skin color of red porgy (Table 9; Fig. 2). The supplementation with synthetic astaxanthin increased redness (a*) in both pectoral and caudal regions. Although no significant differences were observed in the yellowness (b*), hue values decreased in all the diet groups, making their skin look less yellowish than the fish fed the Ctrl diet (70.09 ± 4.71 in the pectoral region and 67.42 ± 3.22 in the caudal region, Fig. 2), but still yellowish (56–60 and 53–58 in the pectoral and caudal regions, respectively). Chroma results showed that the A_{80} and $A_{50}A_{80}$ groups displayed a brighter color in the pectoral region than the Ctrl group, while A_{50} displayed intermediate brightness. The A_{80} and $A_{50}A_{80}$ group displayed a significantly brighter color in the caudal region than the Ctrl group, while A_{50} groups displayed intermediate values (Fig. 2). Significant differences were also found in the astaxanthin content, which increased with increasing dietary astaxanthin (Fig. 3). However, these differences were not clearly reflected in the total carotenoids content measured in the skin of red porgy, which was highly variable and without significant differences among groups (Fig. 3).

Table 4
Phospholipids fatty acids profile (% detected) in the liver of commercial size red porgy (*P. pagrus*) fed with different experimental diets.

	Diets				
	Ctrl	A_{50}	A_{80}	$A_{50}A_{80}$	*P-value*
Saturated fatty acid					
C14:0	8.13 ± 0.76 ^{ab}	7.40 ± 0.21 ^{b}	9.60 ± 1.26 ^{a}	6.76 ± 1.30 ^{b}	***0.03***
C16:0	30.2 ± 0.66	24.8 ± 3.61	32.9 ± 2.53	25. 8 ± 7.78	*0.08*
C18:0	9.35 ± 1.25	8.08 ± 3.20	9.59 ± 0.66	7.61 ± 1.66	*0.31*
Other	1.82 ± 0.11	1.82 ± 0.20	2.05 ± 0.43	1.62 ± 0.21	*0.42*
Total	*49.6 ± 0.38*	*42.1 ± 6.97*	*54.1 ± 4.41*	*41.8 ± 10.9*	***0.07***
Monosaturated fatty acid					
C16:1	12.6 ± 1.25	13.0 ± 1.34	13.2 ± 1.02	12.1 ± 0.38	*0.49*
C18:1	26.0 ± 1.24	21.2 ± 2.22	23.9 ± 1.64	23.9 ± 5.14	*0.17*
C20:1	2.92 ± 0.37	2.94 ± 0.78	3.19 ± 0.11	2.70 ± 0.75	*0.79*
Other	2.54 ± 0.92	2.28 ± 0.66	2.56 ± 0.23	2.04 ± 0.70	*0.56*
Total	*44.0 ± 2.71*	*39.4 ± 2.44*	*42.8 ± 2.83*	*40.7 ± 6.20*	*0.36*
Polyunsaturated fatty acid					
C18:2ω6	1.84 ± 0.54	5.73 ± 0.92	1.14 ± 0.84	3.95 ± 3.68	*0.11*
C18:3ω3	0.41 ± 0.04	0.71 ± 0.20	0.33 ± 0.08	0.67 ± 0.44	*0.15*
C18:4ω3	0.65 ± 0.26 ^{ab}	0.99 ± 0.49 ^{ab}	0.44 ± 0.08 ^{b}	1.20 ± 0.42 ^{a}	***0.03***
C20:3ω6	0.21 ± 0.01	0.64 ± 0.51	0.00 ± 0.00	0.55 ± 0.64	*0.08*
C20:4ω6	0.15 ± 0.08	0.51 ± 0.29	0.08 ± 0.07	0.58 ± 0.59	*0.09*
C20:5ω3	1.11 ± 0.98	3.74 ± 2.63	0.25 ± 0.24	4.21 ± 4.80	*0.20*
C22:5ω3	0.32 ± 0.29	1.22 ± 0.85	0.06 ± 0.08	1.43 ± 1.50	*0.09*
C22:6ω3	0.86 ± 0.55	2.60 ± 1.86	0.19 ± 0.14	3.14 ± 3.47	*0.10*
Other	0.87 ± 0.22	2.34 ± 1.07	0.54 ± 0.14	1.84 ± 1.50	*0.07*
Total	*6.43 ± 2.94*	*18.5 ± 8.79*	*3.04 ± 1.77*	*17.6 ± 17.04*	*0.11*
Total ω3	3.48 ± 2.17	9.67 ± 6.14	1.36 ± 0.66	11.0 ± 10.93	*0.20*
Total ω6	2.21 ± 0.62	6.88 ± 1.71	1.22 ± 0.91	5.08 ± 4.91	*0.11*
ω3/ω6	1.46 ± 0.58	1.30 ± 0.57	1.40 ± 0.59	1.99 ± 0.24	*0.28*
DHA/EPA	1.08 ± 0.46	0.69 ± 0.02	1.24 ± 0.72	1.60 ± 1.11	*0.20*
EPA/ARA	6.03 ± 3.10	6.77 ± 1.36	2.59 ± 1.02	4.20 ± 3.98	*0.14*

Data are expressed as mean ± SD; Different letters within the same row represent statistical differences ($P < 0.05$).

Table 5
Glycolipids fatty acids profile (% detected) in the liver of commercial size red porgy (*P. pagrus*) fed with different experimental diets.

	Diets				
Fatty acids	Ctrl	A50	A80	A5080	*P-value*
Saturated fatty acid					
C14:0	2.80 ± 0.51	3.42 ± 1.19	3.43 ± 0.69	2.78 ± 0.90	*0.53*
C16:0	24.3 ± 2.49	26.6 ± 1.07	28.4 ± 2.68	25.0 ± 4.60	*0.27*
C18:0	6.45 ± 0.76 ^{ab}	6.37 ± 0.27 ^{b}	6.62 ± 0.78 ^{ab}	7.66 ± 0.41 ^{a}	***0.03***
Other	1.53 ± 0.03	1.75 ± 0.04	1.62 ± 0.22	1.69 ± 0.11	*0.24*
Total	*35.1 ± 2.49*	*38.1 ± 1.95*	*40.0 ± 3.82*	*37.1 ± 5.86*	*0.44*
Monosaturated fatty acid					
C16:1	5.62 ± 1.52	6.72 ± 1.53	7.24 ± 1.93	5.73 ± 1.19	*0.46*
C18:1	15.1 ± 2.66	17.1 ± 4.39	18.8 ± 3.76	15.7 ± 3.84	*0.36*
C20:1	1.31 ± 0.25	1.97 ± 0.80	1.94 ± 0.09	1.40 ± 0.18	*0.13*
Other	2.30 ± 0.55	3.41 ± 0.94	2.74 ± 0.17	2.48 ± 0.61	*0.48*
Total	*24.4 ± 4.90*	*29.2 ± 7.64*	*30.8 ± 5.47*	*25.3 ± 5.78*	*0.33*
Polyunsaturated fatty acid					
C18:2ω6	3.59 ± 0.50	4.65 ± 1.20	4.24 ± 0.25	3.98 ± 0.36	*0.30*
C18:3ω3	1.05 ± 0.14 ^{b}	1.42 ± 0.20 ^{a}	0.91 ± 0.07 ^{b}	1.13 ± 0.07 ^{b}	***<0.01***
C18:4ω3	1.32 ± 0.17	1.62 ± 0.32	1.58 ± 0.39	1.67 ± 0.27	*0.25*
C20:3ω6	1.55 ± 0.46	1.28 ± 0.44	1.01 ± 0.29	1.61 ± 0.44	*0.20*
C20:4ω6	0.58 ± 0.07	0.49 ± 0.06	0.41 ± 0.13	0.60 ± 0.14	*0.15*
C20:5ω3	7.77 ± 0.99	6.36 ± 2.74	5.48 ± 1.81	7.06 ± 2.11	*0.38*
C22:5ω3	2.48 ± 0.26	1.75 ± 0.68	1.73 ± 0.38	2.16 ± 0.68	*0.15*
C22:6ω3	20.8 ± 6.37	13.6 ± 7.35	12.4 ± 5.90	17.6 ± 8.78	*0.36*
Other	1.38 ± 0.19 ^{b}	1.54 ± 0.21 ^{ab}	1.40 ± 0.13 ^{b}	1.80 ± 0.14 ^{a}	***0.02***
Total	*40.6 ± 7.21*	*32.7 ± 9.59*	*29.2 ± 8.26*	*37.6 ± 11.52*	*0.36*
Total ω3	33.8 ± 7.40	25.1 ± 10.5	22.4 ± 7.78	30.0 ± 11.3	*0.31*
Total ω6	5.72 ± 0.44	6.42 ± 0.70	5.66 ± 0.53	6.19 ± 0.29	*0.26*
ω3/ω6	5.94 ± 1.43	4.07 ± 2.07	3.91 ± 1.08	4.81 ± 1.67	*0.30*
DHA/EPA	2.64 ± 0.52	2.05 ± 0.28	2.17 ± 0.36	2.38 ± 0.54	*0.21*
EPA/ARA	13.4 ± 0.45	12.6 ± 4.17	13.6 ± 1.75	11.7 ± 1.16	*0.56*

Data are expressed as mean ± SD; Different letters within the same row represent statistical differences ($P < 0.05$).

Table 6
Neutral lipids fatty acids profile (% detected) in the mesenteric fat of commercial size red porgy (*P. pagrus*) fed with different experimental diets.

	Diets				
Fatty acids	Ctrl	A50	A80	A5080	*P-value*
Saturated fatty acid					
C14:0	5.51 ± 0.14	5.42 ± 0.27	5.65 ± 0.14	5.38 ± 0.15	*0.41*
C16:0	16.8 ± 0.16 ^{b}	16.4 ± 0.06 ^{c}	17.8 ± 0.04 ^{a}	16.2 ± 0.36 ^{bc}	***0.01***
C18:0	6.01 ± 0.37 ^{a}	6.09 ± 0.52 ^{a}	6.71 ± 0.26 ^{a}	4.54 ± 0.52 ^{b}	***<0.01***
Other	1.15 ± 0.12 ^{b}	1.21 ± 0.14 ^{b}	1.25 ± 0.10 ^{b}	1.97 ± 0.22 ^{a}	***<0.01***
Total	*29.4 ± 0.29* ^{b}	*29.1 ± 0.92* ^{b}	*31.4 ± 0.07* ^{a}	*28.1 ± 0.77* ^{b}	***<0.01***
Monosaturated fatty acid					
C16:1	8.94 ± 0.16	8.86 ± 0.28	9.01 ± 0.11	8.62 ± 0.24	*0.17*
C18:1	18.2 ± 0.54	18.1 ± 1.17	17.7 ± 0.05	19.7 ± 0.69	***0.04***
C20:1	3.17 ± 0.17 ^{ab}	3.42 ± 0.17 ^{a}	2.86 ± 0.00 ^{b}	3.25 ± 0.02 ^{a}	***0.04***
Other	2.33 ± 0.16 ^{ab}	2.67 ± 0.19 ^{a}	2.01 ± 0.08 ^{b}	2.64 ± 0.20 ^{a}	***<0.01***
Total	*32.6 ± 0.89*	*33.1 ± 1.26*	*31.6 ± 0.25*	*34.2 ± 0.89*	*0.06*
Polyunsaturated fatty acid					
C18:2ω6	7.22 ± 0.05 ^{b}	7.32 ± 0.36 ^{ab}	6.84 ± 0.08 ^{b}	7.90 ± 0.16 ^{a}	***0.02***
C18:3ω3	1.09 ± 0.03	1.09 ± 0.06	1.02 ± 0.06	1.15 ± 0.09	*0.21*
C18:4ω3	1.85 ± 0.02	1.81 ± 0.07	1.91 ± 0.04	1.85 ± 0.05	*0.23*
C20:3ω6	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.00	0.20 ± 0.01	*0.31*
C20:4ω6	0.09 ± 0.00	0.12 ± 0.03	0.09 ± 0.00	0.09 ± 0.01	***0.06***
C20:5ω3	10.3 ± 0.20 ^{abc}	10.4 ± 0.03 ^{b}	10.7 ± 0.02 ^{a}	10.0 ± 0.09 ^{c}	***0.02***
C22:5ω3	2.77 ± 0.12	2.79 ± 0.05	2.50 ± 0.03	2.55 ± 0.18	*0.10*
C22:6ω3	10.0 ± 0.38	9.99 ± 0.22	9.67 ± 0.04	10.1 ± 0.35	*0.48*
Other	3.41 ± 0.04	3.14 ± 0.26	3.14 ± 0.24	2.94 ± 0.35	*0.13*
Total	*37.9 ± 0.69*	*37.8 ± 0.41*	*37.0 ± 0.32*	*37.8 ± 0.26*	*0.18*
Total ω3	27.3 ± 0.74	27.3 ± 0.24	27.0 ± 0.00	26.9 ± 0.30	*0.26*
Total ω6	7.50 ± 0.05 ^{b}	7.63 ± 0.38 ^{ab}	7.11 ± 0.08 ^{b}	8.19 ± 0.16 ^{a}	***0.02***
ω3/ω6	3.64 ± 0.12 ^{a}	3.59 ± 0.16 ^{ab}	3.80 ± 0.04 ^{a}	3.28 ± 0.09 ^{b}	***0.01***
DHA/EPA	0.97 ± 0.03 ^{ab}	0.97 ± 0.02 ^{ab}	0.90 ± 0.00 ^{b}	1.01 ± 0.04 ^{a}	***0.02***
EPA/ARA	121 ± 6.37	92.0 ± 20.38	120.0 ± 1.66	109 ± 9.97	*0.05*

Data are expressed as mean ± SD; Different letters within the same row represent statistical differences ($P < 0.05$).

Table 7
Phospholipids fatty acids profile (% detected) in the mesenteric fat of the commercial size red porgy (*P. pagrus*) fed with different experimental diets.

	Diets				
Fatty acids	Ctrl	A50	A80	A5080	*P-value*
Saturated fatty acid					
C14:0	6.64 ± 0.20	7.24 ± 0.59	6.76 ± 0.11	6.27 ± 0.50	*0.07*
C16:0	18.6 ± 0.14 ^{ab}	18.8 ± 0.58 ^{ab}	19.7 ± 0.48 ^{a}	18.1 ± 0.54 ^{b}	***0.02***
C18:0	5.57 ± 0.87 ^{ab}	5.02 ± 0.67 ^{ab}	6.70 ± 1.20 ^{a}	4.35 ± 0.39 ^{b}	***0.02***
Other	1.29 ± 0.14 ^{b}	1.39 ± 0.12 ^{b}	1.38 ± 0.04 ^{b}	2.00 ± 0.11 ^{a}	***<0.01***
Total	*32.1 ± 0.99* ^{b}	*32.4 ± 0.74* ^{ab}	*34.5 ± 1.55* ^{a}	*30.7 ± 0.69* ^{b}	***<0.01***
Monosaturated fatty acid					
C16:1	9.66 ± 0.15 ^{ab}	10.1 ± 0.25 ^{a}	9.64 ± 0.08 ^{ab}	9.14 ± 0.40 ^{b}	***<0.01***
C18:1	18.4 ± 0.77 ^{ab}	18.4 ± 0.56 ^{ab}	17.6 ± 0.66 ^{b}	20.4 ± 1.32 ^{a}	***0.02***
C20:1	2.87 ± 0.07 ^{ab}	2.88 ± 0.12 ^{ab}	2.59 ± 0.09 ^{b}	3.30 ± 0.43 ^{a}	*0.05*
Other	1.93 ± 0.05	1.88 ± 0.22	1.68 ± 0.10	2.23 ± 0.36	*0.09*
Total	*32.9 ± 0.86* ^{ab}	*33.2 ± 0.40* ^{ab}	*31.5 ± 0.54* ^{b}	*35.1 ± 1.76* ^{a}	***0.02***
Polyunsaturated fatty acid					
C18:2ω6	7.19 ± 0.07 ^{ab}	7.20 ± 0.44 ^{ab}	6.75 ± 0.10 ^{b}	7.79 ± 0.24 ^{a}	***0.04***
C18:3ω3	1.15 ± 0.04	1.16 ± 0.05	1.07 ± 0.04	1.17 ± 0.20	*0.75*
C18:4ω3	1.94 ± 0.08	2.03 ± 0.11	1.99 ± 0.11	1.91 ± 0.29	*0.76*
C20:3ω6	0.17 ± 0.00	0.18 ± 0.03	0.21 ± 0.06	0.17 ± 0.02	*0.76*
C20:4ω6	0.09 ± 0.00 ^{b}	0.12 ± 0.03 ^{ab}	0.10 ± 0.00 ^{a}	0.09 ± 0.00 ^{b}	***0.02***
C20:5ω3	10.1 ± 0.02	10.4 ± 0.36	10.2 ± 0.09	9.60 ± 0.52	*0.16*
C22:5ω3	2.47 ± 0.10	2.34 ± 0.13	2.25 ± 0.00	2.31 ± 0.22	*0.35*
C22:6ω3	7.12 ± 0.21	6.05 ± 0.99	6.91 ± 0.33	7.16 ± 0.47	*0.18*
Other	3.86 ± 0.14	3.93 ± 0.43	3.54 ± 0.25	3.12 ± 0.67	*0.14*
Total	*35.0 ± 0.22*	*34.3 ± 0.87*	*34.0 ± 1.01*	*34.2 ± 1.81*	*0.68*
Total ω3	24.0 ± 0.24	23.2 ± 0.84	23.7 ± 0.59	23.3 ± 1.08	*0.48*
Total ω6	7.45 ± 0.08 ^{b}	7.50 ± 0.49 ^{ab}	7.06 ± 0.16 ^{b}	8.05 ± 0.26 ^{a}	***0.05***
ω3/ω6	3.23 ± 0.06 ^{a}	3.10 ± 0.12 ^{ab}	3.36 ± 0.01 ^{a}	2.89 ± 0.05 ^{b}	***0.01***
DHA/EPA	0.71 ± 0.02	0.59 ± 0.11	0.68 ± 0.03	0.78 ± 0.08	*0.14*
EPA/ARA	118 ± 2.99	93.4 ± 25.7	102 ± 0.57	105 ± 6.80	*0.12*

Data are expressed as mean ± SD; Different letters within the same row represent statistical differences (P < 0.05).

Table 8
Glycolipids fatty acids profile (% detected) in the mesenteric fat of commercial size red porgy (*P. pagrus*) fed with different experimental diets.

	Diets				
Fatty acids (%)	Ctrl	A50	A80	A5080	*P-value*
Saturated fatty acid					
C14:0	5.09 ± 0.39	4.40 ± 0.15	4.96 ± 0 .40	5.37 ± 0.83	*0.09*
C16:0	19.0 ± 0.58 ^{a}	15.7 ± 1.92 ^{b}	17.9 ± 0.77 ^{ab}	18.6 ± 0.66 ^{a}	***0.01***
C18:0	5.96 ± 0.32 ^{a}	5.57 ± 0.34 ^{ab}	5.03 ± 0.10 ^{b}	6.11 ± 0.40 ^{a}	***0.02***
Other	2.19 ± 0.20 ^{a}	2.02 ± 0.12 ^{a}	1.44 ± 0.04 ^{b}	1.49 ± 0.03 ^{b}	***0.02***
Total	*32.2 ± 1.04* ^{a}	*27.7 ± 1.51* ^{c}	*29.2 ± 1.31* ^{bc}	*31.6 ± 0.85* ^{ab}	***<0.01***
Monosaturated fatty acid					
C16:1	7.09 ± 0.25	6.23 ± 0.84	7.37 ± 0.23	7.13 ± 0.39	*0.08*
C18:1	18.1 ± 1.10	16.8 ± 0.56	18.7 ± 0.66	18.3 ± 0.52	*0.06*
C20:1	1.99 ± 0.31	1.88 ± 0.10	1.87 ± 0.03	2.06 ± 0.22	*0.62*
Other	3.00 ± 0.38	3.77 ± 0.34	3.20 ± 0.09	3.05 ± 0.11	*0.10*
Total	*30.2 ± 1.87*	*28.7 ± 1.36*	*31.2 ± 0.55*	*30.6 ± 0.72*	*0.21*
Polyunsaturated fatty acid					
C18:2ω6	5.37 ± 0.14 ^{ab}	5.22 ± 0.36 ^{b}	5.26 ± 0.10 ^{b}	5.89 ± 0.22 ^{a}	*0.10*
C18:3ω3	2.19 ± 0.18 ^{b}	2.81 ± 0.23 ^{a}	1.69 ± 0.00 ^{c}	0.85 ± 0.09 ^{d}	***<0.01***
C18:4ω3	4.76 ± 0.38 ^{b}	6.63 ± 2.37 ^{abc}	3.46 ± 0.21 ^{c}	6.96 ± 0.67 ^{a}	***0.05***
C20:3ω6	0.40 ± 0.28	0.54 ± 0.29	0.77 ± 0.02	0.24 ± 0.02	*0.07*
C20:4ω6	0.76 ± 0.02 ^{b}	1.81 ± 0.96 ^{ab}	1.93 ± 0.04 ^{a}	0.77 ± 0.08 ^{b}	***0.02***
C20:5ω3	10.0 ± 0.93	9.70 ± 0.81	10.6 ± 0.31	9.59 ± 0.57	*0.50*
C22:5ω3	2.06 ± 0.31	2.09 ± 0.21	1.96 ± 0.09	1.89 ± 0.25	*0.65*
C22:6ω3	9.01 ± 1.07	10.4 ± 2.32	9.28 ± 0.02	8.79 ± 1.52	*0.79*
Other	3.01 ± 0.17 ^{b}	3.86 ± 0.05 ^{a}	3.90 ± 0.14 ^{a}	2.89 ± 0.52 ^{ab}	***0.02***
Total	*37.6 ± 2.26* ^{b}	*43.5 ± 2.61* ^{a}	*39.5 ± 0.77* ^{ab}	*37.9 ± 1.33* ^{b}	***<0.01***
Total ω3	*28.6 ± 2.24*	*32.8 ± 3.97*	*28.6 ± 0.77*	*28.6 ± 1.77*	*0.27*
Total ω6	*6.53 ± 0.37*	*7.57 ± 1.49*	*7.96 ± 0.16*	*6.90 ± 0.26*	*0.30*
ω3/ω6	*4.39 ± 0.52*	*4.54 ± 1.38*	*3.59 ± 0.02*	*4.14 ± 0.25*	*0.51*
DHA/EPA	*0.90 ± 0.05*	*1.08 ± 0.30*	*0.88 ± 0.02*	*0.91 ± 0.12*	*0.90*
EPA/ARA	*13.2 ± 1.33* ^{a}	*6.67 ± 3.36* ^{ab}	*5.46 ± 0.05* ^{b}	*12.5 ± 0.92* ^{a}	***0.02***

Data are expressed as mean ± SD; Different letters within the same row represent statistical differences (P < 0.05).

Table 9
Skin color parameters of commercial size red porgy (*P. pagrus*) fed with different experimental diets.

	Pectoral Region		Caudal Region	
	a*	b*	a*	b*
Ctrl	4.01 ± 0.92^{c}	11.19 ± 1.59	5.19 ± 1.76^{b}	12.42 ± 1.76
A_{50}	5.92 ± 2.02^{b}	10.93 ± 2.16	9.06 ± 1.92^{a}	13.44 ± 2.29
A_{80}	8.23 ± 2.09^{a}	11.80 ± 2.28	10.84 ± 2.82^{a}	13.03 ± 2.57
$A_{50}A_{80}$	8.58 ± 2.12^{a}	12.11 ± 2.21	10.77 ± 2.75^{a}	13.26 ± 1.84
P-value	<0.01	0.42	<0.01	0.60

a*: redness/greenness chromaticity; b*: yellowness/bluish chromaticity. Data are expressed as mean ± SD (n = 25/treatment); Different letters within the same column represent statistical differences (P < 0.05).

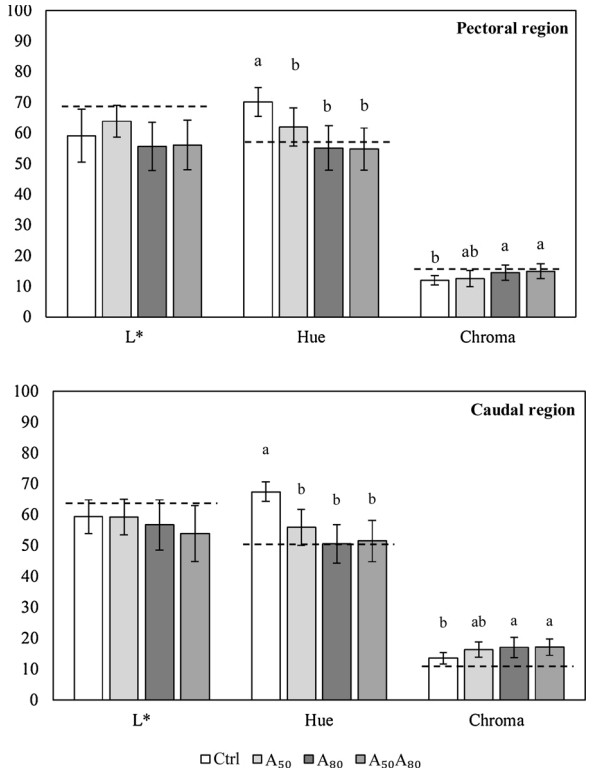

Fig. 2. Skin color in the pectoral and caudal regions of the commercial size red porgy (*P. pagrus*) fed different experimental diets. Data are expressed as mean ± SD. Different letters represent statistical differences (P < 0.05). Dotted lines represent mean values reported for wild red porgy in Kalinowski et al. (2007).

4. Discussion

4.1. Fish performance

Carotenoids are responsible for fish skin and flesh pigmentation, but increasing attention is being directed towards other biological functions of these compounds in aquatic animals, such as promoting growth and reproduction, antioxidant and as immunostimulant activities. Dietary supplementation with astaxanthin at the tested concentrations did not affect growth performance, hepatosomatic index and feed utilization of commercial sized red porgy, which agrees with earlier observations for the same species fed different sources and concentrations of dietary astaxanthin (Cejas et al., 2003; Chatzifotis et al., 2005; Kalinowski et al., 2005; Tejera et al., 2007; Manganaro et al., 2012). The present results are also in line with studies carried out with Australian snapper, *Pagrus auratus* (Doolan et al., 2009), olive flounder, *Paralichthys olivaceus* (Pham et al., 2014), gilthead seabream *Sparus aurata* (Gomes et al., 2002; Wassef et al., 2010), Atlantic Salmon, *Salmo salar* (Baker et al., 2002) and rainbow trout, *Oncorhyncus mykiss* (Rahman et al., 2016; Noori and Razi, 2017). Conversely, an increasing number of research work revealed significant positive influence between dietary astaxanthin supplementation and somatic growth of several fish species, like rainbow trout (*Oncorhynchus mykiss*; Bazyar et al., 2010), Atlantic cod (*Gadus morhua*; Hansen et al., 2016), large Yellow Croaker (*Pseudosciaena crocea;* Li et al., 2014). Kalinowski et al. (2011)

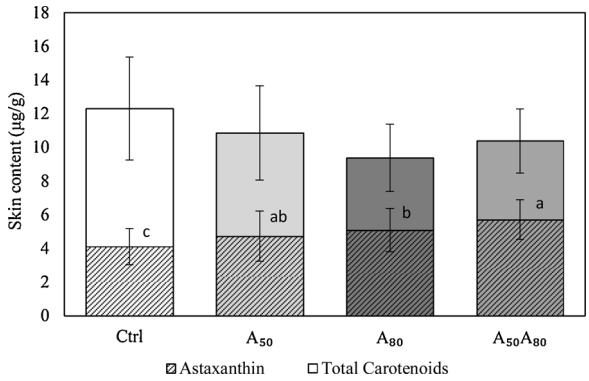

Fig. 3. Total carotenoids and astaxanthin content (µg/g) in the skin of commercial sized red porgy (*P. pagrus*) fed different experimental diets. Data are expressed as mean ± SD. Different letters represent statistical differences ($P < 0.05$).

reported increased final body weight, weight gain and eviscerated weight after feeding red porgy juveniles (226.9 g) for 90 days with a diet supplemented with 100 mg/kg of Carophyll® Pink. In the current study, fish initial weight averaged 386 g, which is within the range size (325–425 g) that P. *pagrus*, a protogynous hermaphrodite species, has sexual reversal (Manooch, 1975) thus requiring different priorities of energy allocation. In fact, data inferred from red porgy growth trials in intensive culture conditions shows that exponential growth phase from hatching (day 0) onwards (weight(g) = $11.601e^{0.677(days)}$; $r^2 = 0.912$; n = 66,873) is followed by an inflexion point at 370 g of slower growth (Kentouri et al., 1995), which could explain the lower SGRs achieved in the present study, when compared to those reported by Kalinowski et al. (2011). That may also contribute to the fact that there was no noticeable effect of dietary astaxanthin supplementation on somatic growth in the present study, though there was no sign of sexual maturation yet. These various results highlight the fact that the mechanisms by which carotenoids may influence fish growth are not well elucidated (Kalinowski et al., 2015). Being considered as micronutrients in fish nutrition, carotenoids are expected to affect other physiological responses not necessarily reflecting into somatic growth (Noori and Razi, 2017).

4.2. Lipid metabolism

Depending on the species, nutritional state, the life-stage or the physiological state, fish tend to accumulate lipids in different anatomical sites (muscle, liver, mesenteric fat), (Flynn et al., 2009; Weil et al., 2013; He et al., 2015). Also, cultured fish fed commercial diets generally exhibit a greater body fat content than wild specimens (Ackman, 1989), reflecting lipid composition of the diets. Regardless the astaxanthin supplementation, the present results on white muscle composition allow considering red porgy as a "lean fish" (below 2% lipid content in wet weight) according to the classification of Ackman (1989), which agrees to what was previously observed by Rueda et al. (1997) and García-Romero et al. (2014).

Not surprisingly, preferential lipid storage occurred in mesenteric fat, followed by the liver. Astaxanthin supplementation did not affect the lipid content of mesenteric fat or the liver, for what it can be considered that the astaxanthin dietary supplementation levels used in this experiment were not high enough to produce significant metabolic changes in red porgy. Furthermore, the results on fatty acid composition, particularly, SFA, MUFA and PUFA of the individual lipid classes (phospholipids, glycolipids and neutral lipids) of the liver of red porgy were similar across all dietary treatments. It could be expected that astaxanthin supplementation would enhance lipid utilization in tissues such as the liver and mesenteric fat that are rich in readily oxidized PUFA (Halliwel and Chirico 1993). Kalinowski et al. (2011) found that feeding red porgy juveniles with astaxanthin supplemented diets for 120 days (100 mg of Carophyll® Pink per kg diet) decreased total lipids and palmitic acid (SFA; C16:0) content in the liver, while increasing EPA (PUFA; C20:5ώ 3) and DHA (PUFA, 22:6ώ3) levels. These authors suggested that a more efficient lipid utilization, reflected from a lower total lipid content in the whole body and liver, would have translated into improved growth performance. Conversely, Tejera et al. (2007, 2010) did not find any effects of shrimp meal or Carophyll Pink supplementation on the lipid composition of the skin of red porgy fry. Likewise, Pham et al. (2014) did not find clear differences in the liver fatty acids composition of olive flounder juveniles fed diets supplemented with 100 and 200 mg/kg Carophyll® Pink, except for a slight decrease in C18:2ω-6 and increase of C18:3ω-3. In agreement with the previous authors (Tejera et al., 2007, 2010; Pham et al., 2014), in the present study there was no effect on the unsaturated fatty acids content in any of the three lipid classes. Further studies on the astaxanthin supplementation on primary lipid peroxidation, measured by thiobarbituric acid reactive substances of the liver should be performed. Alternately, antioxidant properties of astaxanthin could be evaluated by the activity of antioxidant enzymes. Antioxidant activity involves a wide range of enzymes, including superoxide dismutase; plasma catalase that are known to be inversely related to the dietary carotenoid content (Rahman et al., 2016), as previously shown in juvenile olive flounder by Pham et al. (2014). Also, mesenteric fat, which is the main lipid depot and well suited for long-term lipid storage (Rueda et al., 1997), was composed by more than 85 % of lipids, without significant differences across different diets ($P > 0.05$). Neutral lipids were overrepresented in this tissue, whereas relative higher amounts of phospholipids and glycolipids were found in the liver. Analysis of the fatty acid patterns of the lipid fractionation showed that the mesenteric fat fatty acids composition followed the general liver composition and percentages of several fatty acids differed between

fractions, regardless of the dietary treatment. As reported for other fish, in all analyzed tissues, ω3-fatty acids levels made up a larger fraction than ω6-fatty acids (Greene and Selivonchick, 1987; Ackman, 1992; Tornaritis et al., 1994). According to Ackman (1967), MUFA and SFA constitute the main groups of fatty acids in neutral lipids of most fish and phospholipids usually contain very high levels of PUFA, particularly of DHA (Brockerhoff et al., 1963; Delgado et al., 1994). However, the present results showed that liver phospholipid fraction had the lowest PUFA (3–18 %) and DHA (0.6–3 %) content, mostly due to higher content in SFA (42–54 %). Similar to Rueda (1997) findings in wild red porgy, in the present study liver PUFA contents were higher in both neutral (triglycerides fraction-28–32 %) and glycolipids fraction (29–40 %) than in the phospholipids fraction, the glycolipids showing the highest DHA levels (12–21 %). Nonetheless, comparison of fatty acids composition of different lipid fractions between liver and mesenteric fat, revealed consistently higher PUFA (34–44 %) contents in all three fractions of mesenteric fat, while PUFA content of the liver varied between 3–18 % in phospholipids, between 29–41 % in glycolipids and 28–32 % in the neutral fraction. In the present study, PUFA and DHA contents in liver and mesenteric fat were higher those reported by Kalinowski et al. (2011, 2015), who found 18–21 % PUFA and 7.71–9.34 % DHA in the liver and 19.5–21.4 % PUFA and 8.1–9.3 % DHA in mesenteric fat of red porgy.

4.3. Skin color and carotenoid accumulation

Carotenoid pigments, primarily astaxanthin diester and tunaxanthin, are responsible for the bright reds and yellows seen in several species of seawater red skinned fish (Matsuno et al., 1985; Miki et al., 1985), including red porgy (Tejera et al., 2007). Achieving natural pigmentation is of outmost importance to commercial operations, since it affects consumers perception of product quality (e.g. health, freshness, nutritive value and taste) and ultimately, product price (Carvalho and Caramujo, 2017; Lim et al., 2018). The measurement of color variables such as redness (a*), yellowness (b*), hue and chroma give information on the deposition of the referred skin carotenoids (Kalinowski et al., 2007) specially in the front lateral zone, as this area seems to accumulate more astaxanthin with less variability (Kalinowski et al., 2005). In the current study, there was a high variation in the carotenoid content between groups and no tendency was observed with dietary astaxanthin supplementation. However, according to what was expected, astaxanthin content tended to increase in fish fed the astaxanthin supplemented diets, which was also reflected in redness values. Like what was reported by Chatzifotis et al. (2005) and Kalinowski et al. (2011), the dietary supplementation with astaxanthin (at 50, 80 or 50 followed by 80 mg/kg) also influenced positively skin hue and chroma of red porgy. Hue values of both pectoral and caudal regions were similar amongst the groups fed the astaxanthin supplemented diets, but lower than those of the control group and closer to those referenced for wild individuals (Kalinowski et al., 2007; García-Romero et al., 2010). Chroma also increased in fish fed astaxanthin supplemented diets, the present values being closer to those reported for wild fish, though slightly lower in the pectoral region. Lower chroma values were mostly linked with lower b* values (yellowness), reflecting possible low yellow carotenoids content (tunaxanthin). Although synthesis of this yellow xanthophyll from dietary astaxanthin has been reported for red porgy by Tejera et al. (2007) and for Japanese red sea bream (Allahpichay et al., 1984), Kalinowski et al. (2007) suggested that red porgy may have a limited synthesis capacity, similarly to what has been suggested by Yi et al. (2014) for the yellow croaker, *Larimichthys croceus*.

Red porgy skin lightness (L*) was not affected by astaxanthin supplementation in the diets, though cultured red porgy presented lower L* values, when compared to wild red porgies (Kalinowski et al., 2007; García-Romero et al., 2010). Likewise, several other authors (Chatzifotis et al., 2005; Kalinowski et al., 2005; García-Romero et al., 2010, 2014) concluded that this color parameter was not influenced by different pigment supplementation concentrations and feeding trial duration in red porgy. It has been suggested that several factors may influence skin lightness, including overproduction of melanin as a response to culture conditions, such as background color or light intensity (Booth et al., 2004; Van der Salm et al., 2004, 2006; Pavlidis et al., 2008; Tejera et al., 2010; Papandroulakis et al., 2013). This effect has also been reported in red sea bream (Lin et al., 1998) and Australian snapper (Booth et al., 2004).

5. Conclusions

Red porgy has great potential for large-scale commercial production. Aquaculture operators recognize the opportunity for red porgy production in several international markets once discoloration problems are bypassed. In the present study the dietary astaxanthin supplementation (at 50 or 80 mg/kg for 6 months or at 50 mg/kg for 3 months followed by 80 mg/kg for 3 months) improved the skin color in large size commercial red porgy, as it increased redness/greenness chromaticity and the achieved hue and chroma values were close to those previously reported for wild individuals. The present study results are encouraging but call for further research on carotenoid supplementation in finishing diets for red porgy. As the commercial grow out of red porgy may demand the use of offshore cages or net pens, future works should consider performing the trials in offshore cages, as this may influence final coloration of red porgy (the lightness in particular). Finally, studies on the combination of astaxanthin with tunaxanthin should be considered, as both xanthophylls seem to contribute for an adequate skin pigmentation of this sparid species.

Author statement

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anifeedsci.2021.114916.

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