



Time course of muscle fatty acid composition of cultured meagre (*Argyrosomus regius*) during the first sixteen months of a cage culture

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Submitted: 30 April 2013; Accepted: 23 September 2013; Published on line: 13/02/2014

SUMMARY: Juvenile meagre were sampled at intervals during their first 500 days in a cage-based fish farm. Fish muscle showed a relatively low fat content (0.65–2.0%) when compared with other species of farmed fish, corroborating the consideration of meagre as a lean fish. At the beginning of the assay, saturated and mono-unsaturated fatty acids were in a similar proportion (approximately 30%), while polyunsaturated were close to 39% of total FAs, the *n-3:n-6* ratio being of 3.8. Throughout the experiment a notable reduction in the sum of PUFA *n-3* and an increase of linoleic (18:2*n-6*) and oleic acid (18:1*n-9*) was found. These changes led to a fall of *n-3:n-6* ratio to values close to or lower than 1.0. Most probably, these changes reflect variations in the feeding regime applied during farming. In any case, fish offered good indices of lipid quality for human consumption.

KEYWORDS: *Aquaculture; Argyrosomus regius; Fatty acids profile; Muscle; n-3:n-6 ratio*

RESUMEN: *Evolución del contenido en lípidos y ácidos grasos del músculo de la corvina (Argyrosomus regius) durante los primeros dieciséis meses de cultivo en jaulas flotantes.* Se han muestreado corvinas juveniles durante los 500 primeros días de cultivo en jaulas flotantes. El contenido en grasa muscular fue relativamente bajo (0,65–2 %) en relación con otras especies de peces cultivados, lo que corrobora su consideración de pez magro. Al principio del ensayo, los ácidos grasos saturados y monoinsaturados mostraron una proporción del 30%, mientras que los poliinsaturados de un 39% del total de FAs, siendo la relación *n-3/n-6* de 3,8. A lo largo del periodo experimental hubo una reducción notable de PUFAs *n-3* y un incremento de ácido linoleico (18:2*n-6*) y, en menor proporción, de ácido oleico (18:1*n-9*), lo que llevó a una disminución de la relación *n-3/n-6* hasta valores cercanos o menores de 1,0. Estos cambios reflejan variaciones en la composición del alimento durante el cultivo. En cualquier caso, la corvina ofrece buenos índices de calidad lipídica para la salud del consumidor.

PALABRAS CLAVE: *Acuicultura; Argyrosomus regius; Músculo; Perfil de ácidos grasos; Relación n-3:n-6*

Citation/Cómo citar este artículo: García Mesa S, Suárez MD, Rincón Cervera MA, Guil Guerrero JL, González G, Cárdenas S, García Gallego M. 2014. Time course of muscle fatty acid composition of cultured meagre (*Argyrosomus regius*) during the first sixteen months of a cage culture. *Grasas Aceites* 65 (1): e006. doi: <http://dx.doi.org/10.3989/gya.049813>

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1. INTRODUCTION

There is growing evidence that future fish needs for human consumption must be covered, in progressively higher proportion, by aquaculture and thus a sustainable development of this activity is needed.

One of the main quality indexes in farmed fish for human consumption is the lipid amount and fatty acid (FA) profile stored in the main edible fraction, the muscle. Some *n*-3 PUFAs have been regarded as essential for human health (WHO, 1977) for their role in preventing and treating a wide variety of disorders. Experts in human nutrition and health agree that fish included in the daily diet helps prevent certain diseases such as cardiovascular ones (Pirini *et al.*, 2010). In this case, the beneficial effect seems to lie in the lipid component of fish and more specifically in the presence of certain inhibitors of platelet aggregation (Nasopoulou *et al.*, 2007) and certain MUFAs and PUFAs, mainly those of the *n*-3 series.

Numerous studies have demonstrated the influence of the dietary FAs on their profile in fish tissues (Lie, 2001; Yildiz *et al.*, 2008), but other factors could be also involved. The fish FA profile is certainly influenced by season (Leger *et al.*, 1977, Shirai *et al.*, 2002; Hassan *et al.*, 2010) since the biochemical composition of marine organisms can undergo seasonal changes in this respect (Ackman, 1995). Also, the maturation degree significantly alters the FA profile of different fish tissues (Hussain *et al.*, 2011).

Meagre (*Argyrosomus regius*) is receiving increasing attention for its reported good growth rate and easy adaptation to fish-farming practices. This species has been described as a particularly slender fish, even when it is cultured intensively, and even on a high-fat diet it provides high-quality commercial products. This fish has a high percentage of muscle, low fat, and healthy lipid content, so that it has a low index of atherogenicity and thrombogenicity (Poli *et al.*, 2003). The organoleptic properties show that farmed meagre has no accumulation of perivisceral and muscle fat compared with other cultivated species. Also, it can be cool stored for long periods of time without sensory characteristics being affected (Hernández *et al.*, 2009). Studies on the same species show that it provides low-fat meat even under intensive farming conditions (Piccolo *et al.*, 2008). The low range of muscle lipids observed among dietary treatments implies that meagre, like other species, has a restricted ability for lipid deposition in muscle.

Recently, Chatzifotis *et al.*, (2010) have observed that a 17% lipid diet was the most suitable for meagre in terms of growth, compared to 13% and 21%. Proximate analysis indicated that the lipid content of whole body and muscle was affected by the diets, whereas of the liver remained unaffected.

The aim of the present work was to evaluate the influence of the growth stage on the time course of muscle fatty acid composition of meagre, over a 500-day period of their production cycle in a fish farm based in off-shore cages. This experimental period was designed to complete the evaluation of the possible effects of different culture factors on total muscle lipids and fatty acid content.

2. MATERIAL AND METHODS

2.1. Animals, experimental conditions, and sampling

Juvenile meagre (*A. regius*) 80 days old were enclosed in a cage-based fish farm (Ceutamar S.L., Algeciras Bay, southern Spain). Throughout the experimental period (504 days long after a prior 15-day adaptation period), fish were subjected to the usual production conditions operating in the fish-farm facilities. The recorded average temperature over a full year was $17.9 \pm 0.1^\circ\text{C}$ (range 14.6 – 22.5°C , Figure 1). Throughout the experimental period the salinity was around 37 ppt. The dissolved oxygen reached the minimum values during summer but levels never dropped below 7.2 ppm.

Fish were fed three times a day with a total daily ration of 2% body weight throughout the experiment. Two commercial diets specially designed for meagre intensive culture (Dibaq-Diproteg S.A.), were used. The first diet (Microbaq) was provided during the adaptation period and the first month of the experimental one, and then was gradually replaced by the second diet (Alphamar). According to the manufacturers, the diets were based on fish meal and oil, soya protein and oil, wheat meal and gluten, pea meal, vitamins, and minerals. Diets were analysed for crude protein, crude lipid, and ash according to AOAC methods (2000) and FA composition. The composition is listed in Table 1, reflecting that the Microbaq diet had higher protein/lower fat levels and a higher protein:energy ratio than did Alphamar although the total energy content was similar in both diets. On the other hand, the diets varied in their FA profile, in that the Microbaq diet contained higher levels of PUFA *n*-3 but was lower of linoleic (LA) and oleic (OA) acids than Alphamar, resulting in a clearly higher *n*3:*n*6 ratio than in the starter diet. These situations most presumably reflect different amounts of fish and vegetable oils in the two diets.

Fish were sampled every 1–2 months except for sampling #9, which was delayed five months.

Each sample was based in 50–100 fish (the highest figure for the seven first samples, the lowest for later ones; according to the predefined practices at the fish farm) that were weighed and measured to follow the time course for growth; five of these fish, also taken at random, were independently analysed to determine the fillet composition. Samples were

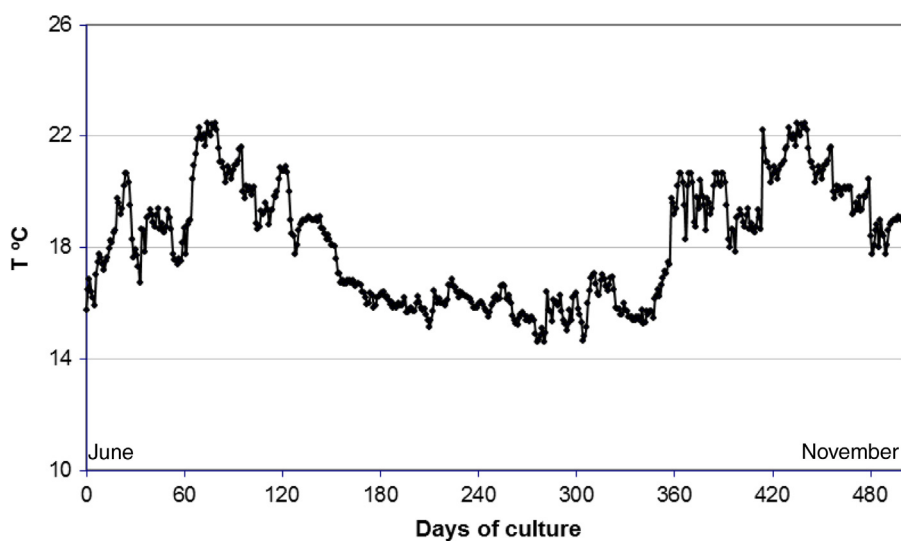


FIGURE 1. Time course of the water temperature.

taken after a 24-h food-deprivation period and the fish were rapidly killed following the Directive 2010/63/EU (overdose of metacaine, approved killing protocol).

2.2. Fish analyses

After skin removal, whole fillets of sampled fish were dissected. Muscles were ground, freeze-dried, and analysed for total lipids and fatty acid (FA) composition.

Total lipids were extracted gravimetrically after homogenizing twice in chloroform/methanol (2:1, v/v) for 3 min in a Waring blender (Folch *et al.*, 1957).

For FA extraction and transesterification, samples of diet and muscle (20 mg) were mixed with 20 mL of a solution of methanol:acetyl chloride (20:1, v/v) and 20 mL hexane, as previously described (Rodríguez-Ruiz *et al.*, 1998). The mixture was heated to 100 °C for 30 min under stirring, and, after cooling at room temperature, 20 mL of water were added and the FA methyl esters (FAMES) were extracted in the hexane layer. Three more extractions with hexane were made to ensure complete removal of methyl esters.

For the FAMES analyses an HP 5890 series II gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with an autoinjector (model HP 6890) and FID detector was used. The separation was made in a Supelco Omega wax 250 (30 m×0.25 mm) fused silica capillary column (0.25 µm). The oven-temperature profile was: 205 °C (10 min), 6 °C/min to 240 °C (9 min), for a total heating time of 25 min. The identity of each individual FAME was determined by comparing retention times with a Sigma

standard FAME mixtures. Replicates were made and mean values are shown in tables. The values of FAs are presented as percentages of the total FA mass. Heptadecanoic acid (17:0) methyl ester was used as the internal standard for quantitative analyses.

From the data on the FA composition, the index of atherogenicity (IA) and the index of thrombogenicity (IT) were calculated according to Ulbricht and Southgate (1991). The IA indicates the relationship between the sum of the main saturated and that corresponding to the main unsaturated FAs, the former being considered pro-atherogenic and the latter anti-atherogenic. The IT shows the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FA (MUFA, *n*-6 PUFA, *n*-3 PUFA). The value of flesh-lipid quality (FLQ) indicates the percentage relationship in which the main *n*-3 HUFA (EPA and DHA) appear in muscle with respect to total FAs. The higher value of this index, the greater the quality of the dietary FA source (Abrami *et al.*, 1992).

2.3. Statistics

Data were analysed using a one-way ANOVA followed by a comparison of means (Tukey's LSD procedure). A significance level of 95% was considered to indicate statistical differences ($p < 0.05$). When results were expressed as a percentage (e.g. FA), the data were normalized using the arcsine transformation of their square root prior to statistical analysis, following Sokal and Rohlf (1981). All statistics were conducted using specific software (Statgraphics Plus 4.0, Statistical Graphics Corp., Rockville, Maryland, U.S.A.).

TABLE 1. Proximate composition (g·Kg⁻¹ on dry weight) and fatty acid profile of the lipids in the diets (data are % of total fatty acids)

Proximate composition	Microbaq	Alphamar
Crude protein	547.4	432.5
Crude lipid	210.7	257.2
Ash	101.1	59.3
Nitrogen-free extract*	140.8	251.1
Energy ** (MJ·Kg ⁻¹)	22.00	22.32
Protein:energy ratio (g·MJ ⁻¹)	23.23	17.94
Fatty acid profile		
SFAs¹		
14:0 (MA)	4.87	2.47
16:0 (PA)	19.80	17.37
18:0 (SA)	4.47	4.70
MUFAs²		
16:1 <i>n</i> -7 (POA)	6.00	3.23
18:1 <i>n</i> -9 (OA)	14.90	22.20
18:1 <i>n</i> -7 (VA)	2.93	2.27
20:1 <i>n</i> -9 (EEA)	3.03	2.53
22:1 <i>n</i> -11 (GA)	3.10	2.43
PUFAs³		
18:2 <i>n</i> -6 (LA)	8.57	26.17
18:3 <i>n</i> -3 (ALA)	1.60	3.37
18:4 <i>n</i> -3 (SA)	1.53	0.70
20:4 <i>n</i> -6 (AA)	1.07	0.53
20:4 <i>n</i> -3 (ETA)	0.47	0.13
20:5 <i>n</i> -3 (EPA)	10.77	3.80
22:5 <i>n</i> -3 (DPA)	2.00	1.00
22:6 <i>n</i> -3 (DHA)	13.03	6.77
ΣSFAs	29.13	24.53
ΣMUFAs	29.97	27.70
ΣPUFAs	39.03	42.47
ΣPUFAs <i>n</i> -3 ⁴	29.40	15.77
ΣPUFAs <i>n</i> -6 ⁵	9.63	26.70
<i>n</i> -3: <i>n</i> -6	3.05	0.59

* calculated as 100 – (crude protein (g·kg⁻¹) + crude lipid (g·kg⁻¹) + ash (g·kg⁻¹))

** calculated on the basis of 24.3, 39.7 and 17.2 kJ·g⁻¹ of protein, lipid and NFE, respectively

¹Saturated fatty acids, ²Mono-unsaturated fatty acids,

³Polyunsaturated fatty acids, ⁴*n*-3 Polyunsaturated fatty acids,

⁵*n*-6 Polyunsaturated fatty acids.

3. RESULTS AND DISCUSSION

Regarding the lipid content of the main edible portion of fish (muscle), Table 2A shows that, over the period monitored, lipids steadily increased during the first year of caging (samples 1 to 8), after which the proportion of lipids in muscle stabilized or slightly fell.

The FA profiles of muscle lipids are shown in Tables 2A and 2B. In first samplings, the most abundant FAs were palmitic (PA, 16:0) among saturated (SFA), OA, 18:1*n*-9 among monounsaturated (MUFA), and docosahexaenoic (DHA, 22:6*n*-3) among polyunsaturated (PUFA). This situation persisted throughout the trial. For all groups, SFA and MUFA had a similar proportion of approximately 30%, while PUFA were in the top of the range, with 39%. In terms of the *n* series, *n*-3 clearly predominated, accounting for almost four times the content of *n*-6 at the beginning of the experiment.

Throughout the trial, there were some statistically significant changes in this profile, although many of them can be considered physiologically minor or irrelevant. The most relevant changes included the significant increase in LA, 18:2*n*-6 among PUFAs and OA, as well as, to a lesser extent, MUFAs. Among the SFA the changes observed were minor, with no clear trend detected.

Since the OA increase was offset by the decline in other figures of this group (e.g., palmitoleic acid, PA, 16:1*n*-7) the overall MUFAs presented a slight oscillation over time, i.e. around a total of 30% with a peak in the sampling 4 that coincided with that of OA.

The total content of PUFAs varied markedly in the first samplings, reaching over 45% in the sampling 6 and showing a similar behaviour until the end of the experiment. Within this group, the increase in *n*-6 series, due largely to LA, was accompanied by a decrease in *n*-3 that affected mainly eicosapentaenoic acid (EPA, 20:5*n*-3). These simultaneous changes in *n*-3 and *n*-6 led to a fall in the *n*-3:*n*-6 ratio from initial values of 3.8 to values similar to and in some cases lower than 1.0. In relative terms, the greatest reduction in this ratio occurred between the samplings 3 and 4, after which a slow but incomplete recovery was detected.

The muscle-lipid content increased as the fish grew in the farm from 0.65% to 1.7–2% in wet matter. Total FAs varied over the experiment between 60–70% of lipids, in agreement with previous results on fish muscle and liver (Kandemir and Polat, 2007; Guil-Guerrero *et al.*, 2011). Overall, lipid-content values proved clearly lower than those reported for other farmed fish (Grigorakis *et al.*, 2011) and fully justify the common name (meagre, from *maigre* in French, meaning “lean”) used for this species. The initial fattening of younger fish can be explained by the transition from a feeding based on high-protein/low-fat diets (hatchery diet and Microbaq) to a higher-fat diet. Both, Piccolo *et al.*, (2008) and Chatzifotis *et al.*, (2010) detected a higher proportion of lipids in meagre muscle when the fish was fed on high-fat diets (up to 21%), but the highest value recorded (20.7% lipids in diet, fish of 820 g) was only 3.6%. Poli *et al.* (2003) had previously reported meagre muscle fat-content values from 2.06% (fish

TABLE 2A. Total body length and weight, muscle-lipid content and FA profile at different sampling times in meagre production cycle. (Data are mean±SEM)

Sampling #	1	2	3	4	5	6	7	8	9	P
Days of experiment	0	33	62	119	167	258	307	340	504	
Total length (cm)	7.46 ^a ±0.05	10.15 ^b ±0.10	11.60 ^b ±0.11	17.86 ^b ±0.19	19.63 ^b ±0.17	20.27 ^c ±0.23	21.42 ^c ±0.24	22.73 ^c ±0.36	27.42 ^d ±0.40	<0.0001
Total weight (g)	4.10 ^a ±0.07	12.04 ^b ±0.43	18.85 ^b ±0.57	62.25 ^b ±2.00	84.56 ^b ±2.43	89.79 ^b ±3.44	107.50 ^b ±4.43	128.20 ^b ±6.49	211.38 ^c ±9.22	<0.0001
Total lipids (% wet matter)	0.79 ^a ±0.05	0.90 ^a ±0.04	0.91 ^a ±0.04	1.38 ^b ±0.10	1.18 ^{ab} ±0.14	1.53 ^c ±0.16	1.47 ^c ±0.14	2.04 ^d ±0.21	1.86 ^d ±0.10	<0.0001
FAs (% of total lipids)	66.23 ^b ±7.36	60.70 ^b ±5.52	65.31 ^{ab} ±2.37	67.60 ^b ±6.06	69.31 ^b ±6.59	66.32 ^b ±3.39	64.66 ^{ab} ±2.37	66.81 ^b ±4.49	67.43 ^b ±4.64	<0.0001
SFAs¹										
14:0 (PA)	3.96 ^a ±0.17	5.05 ^{ab} ±0.10	3.17 ^b ±0.40	3.91 ^c ±0.37	2.22 ^{ab} ±0.11	1.81 ^a ±0.10	2.10 ^a ±0.05	2.16 ^{ab} ±0.14	1.70 ^a ±0.13	<0.0001
16:0 (MA)	19.60 ^{abcd} ±0.37	22.73 ^d ±0.23	21.58 ^{cd} ±0.54	22.87 ^d ±1.79	20.45 ^{bcd} ±1.27	16.46 ^b ±0.48	18.16 ^{abc} ±0.37	17.13 ^b ±0.19	17.72 ^b ±0.26	<0.0001
18:0 (SA)	6.11 ^{abc} ±0.11	5.68 ^{ab} ±0.13	6.56 ^{bc} ±0.39	5.51 ^{ab} ±0.36	7.55 ^d ±0.50	6.62 ^{bc} ±0.22	5.76 ^{abc} ±0.33	6.16 ^{abc} ±0.44	3.27 ^a ±0.13	<0.0001
MUFAs²										
16:1n-7 (POA)	7.37 ^c ±0.21	7.65 ^c ±0.05	5.32 ^b ±0.43	5.92 ^b ±0.50	3.98 ^a ±0.10	3.08 ^a ±0.22	3.74 ^a ±0.13	3.92 ^a ±0.30	3.27 ^a ±0.13	<0.0001
18:1n-9 (OA)	18.48 ^a ±1.23	23.40 ^{abcd} ±0.01	20.52 ^{ab} ±0.86	27.73 ^d ±2.43	24.73 ^{bcd} ±1.40	21.00 ^{abc} ±0.98	23.22 ^{abcd} ±1.10	21.84 ^{abc} ±0.61	26.21 ^{cd} ±0.42	<0.0001
18:1n-7 (VA)	3.52±0.09	n.d.	3.49±0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7230
20:1n-9 (EEA)	2.57 ^{ab} ±0.10	3.40±0.20	3.18 ^a ±0.06	2.88 ^{ab} ±0.26	2.71 ^{ab} ±0.23	2.33±0.01	2.35±0.16	2.76 ^{ab} ±0.80	2.37±0.08	<0.0001
22:1n-11 (GA)	0.66 ^a ±0.06	2.30 ^{cd} ±0.10	2.41 ^a ±0.13	3.00 ^c ±0.25	1.65 ^b ±0.27	1.48 ^b ±0.03	1.68 ^b ±0.15	1.74 ^b ±0.15	1.60 ^b ±0.04	<0.0001
PUFAs³										
18:2n-6 (LA)	6.63 ^a ±0.13	8.13 ^a ±0.13	11.91 ^b ±0.50	17.03 ^c ±1.41	16.77 ^c ±1.09	19.24 ^c ±0.63	19.26 ^c ±0.83	17.02 ^c ±0.35	18.38 ^c ±0.30	<0.0001
18:3n-3 (ALA)	1.01 ^a ±0.01	1.20 ^{ab} ±0.00	1.29 ^{abc} ±0.11	1.52 ^{abcd} ±0.38	1.47 ^{abcd} ±0.12	1.62 ^{cd} ±0.05	1.95 ^d ±0.07	1.83 ^{cd} ±0.11	1.54 ^{abcd} ±0.06	<0.0001
18:4n-3 (SA)	1.39 ^a ±0.13	1.00 ^b ±0.00	0.61 ^a ±0.14	n.d.	n.d.	n.d.	n.d.	0.68 ^a ±0.09	n.d.	<0.0001
20:4n-6 (AA)	1.39 ^{cd} ±0.02	0.93 ^{bc} ±0.03	1.21 ^{cd} ±0.13	0.33 ^a ±0.22	0.78 ^{bc} ±0.05	1.41 ^{ab} ±0.11	1.17 ^{ab} ±0.03	1.37 ^{ab} ±0.11	1.52 ^{ab} ±0.07	<0.0001
20:4n-3 (ETA)	0.81 ^c ±0.03	0.53 ^b ±0.03	0.10 ^a ±0.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.0001
20:5n-3 (EPA)	10.70 ^a ±0.23	6.03 ^c ±0.08	4.85 ^{cd} ±0.33	2.41 ^a ±1.09	4.15 ^{ab} ±0.48	4.12 ^{ab} ±0.22	3.90 ^{ab} ±0.20	4.18 ^{ab} ±0.14	3.59 ^{ab} ±0.08	<0.0001
22:5n-3 (DPA)	2.93 ^a ±0.12	1.73 ^b ±0.03	1.37 ^b ±0.09	0.72 ^b ±0.40	1.58 ^b ±0.02	1.78 ^b ±0.14	1.54 ^b ±0.09	1.64 ^b ±0.04	1.46 ^b ±0.02	<0.0001
22:6n-3 (DHA)	13.76 ^{bcd} ±0.48	9.50 ^{ab} ±0.50	13.30 ^{bcd} ±0.99	5.08 ^a ±2.20	10.92 ^b ±0.98	17.13 ^d ±1.28	15.28 ^{cd} ±1.14	14.05 ^{cd} ±0.65	14.05 ^{bcd} ±0.65	<0.0001

Sample size for weight and length determinations: N=100 (samplings # 1-7) and N=50 (samplings # 8-9); for total lipids and fatty acid profile: N=5.

n.d.=no detected

¹ Saturated fatty acids, ² Mono-unsaturated fatty acids, ³ Polyunsaturated fatty acids

a, b, c... Values in the same row with different superscripts indicate significant differences (p<0.05) attributable to growing time.

TABLE 2B. Fatty acid profile of muscle lipids (continued)

Sampling #	1	2	3	4	5	6	7	8	9	P
Days of experiment	0	33	62	119	167	258	307	340	504	
ΣSFA ¹	29.22 ^b ±0.93	33.45 ^d ±0.77	31.06 ^c ±1.85	33.13 ^d ±2.75	30.22 ^{bc} ±3.64	24.89 ^a ±0.85	26.02 ^a ±0.97	25.45 ^a ±0.98	23.92 ^a ±1.54	<0.0001
ΣMUFA ²	29.36 ^{ab} ±1.87	31.05 ^c ±0.32	28.77 ^d ±2.62	35.01 ^e ±3.76	28.71 ^{cd} ±3.07	24.08 ^b ±2.39	26.96 ^b ±2.64	25.76 ^{ab} ±2.17	29.48 ^{de} ±1.80	<0.0001
ΣPUFA ³	38.91±1.04	29.03±0.51	34.67±1.72	27.99±2.51	35.67±4.00	45.30±2.41	41.86±2.97	46.24±1.58	40.54 ^{bc} ±2.12	<0.0001
ΣPUFA ³ n-3 ⁴	30.80 [±] 1.01	19.98 [±] 0.47	21.70 [±] 1.09	12.43 [±] 1.42	18.12 [±] 2.93	24.65 [±] 3.17	21.43 [±] 2.54	22.91 [±] 1.41	20.64 [±] 1.88	<0.0001
ΣPUFA ³ n-6 ⁵	8.11 ^a ±0.30	9.05 ^a ±0.18	12.97 ^b ±1.11	15.56 ^b ±1.69	17.55 ^b ±2.46	20.65 ^b ±1.17	20.43 ^c ±1.79	18.39 ^b ±0.56	19.90 ^c ±0.69	<0.0001
n-3/n-6	3.80 [±] 0.19	2.21 [±] 0.07	1.68 [±] 0.15	0.81 [±] 0.11	1.05 ^b ±0.21	1.20 ^b ±0.21	1.06 ^b ±0.16	1.25 ^c ±0.10	1.04 ^b ±0.10	<0.0001
IA ⁶	0.48 [±] 0.07	0.65 [±] 0.09	0.49 ^d ±0.13	0.56 [±] 0.47	0.43 ^c ±0.43	0.32 [±] 0.18	0.36 ^{ab} ±0.20	0.36 ^{ab} ±0.08	0.33 [±] 0.15	<0.0001
IT ⁷	0.25 ^a ±0.01	0.39 ^c ±0.01	0.35 ^b ±0.02	0.50 ^d ±0.05	0.37 ^{bc} ±0.06	0.25 ^b ±0.02	0.29 ^b ±0.02	0.27 ^b ±0.01	0.27 ^b ±0.20	<0.0001
FLQ ⁸	24.64 [±] 1.12	15.53 ^b ±1.02	18.13 [±] 0.96	10.33 [±] 0.85	15.07 ^b ±1.23	21.25 [±] 1.00	17.94 ^c ±0.98	18.76 [±] 0.85	17.64 [±] 0.95	<0.0001

¹Saturated fatty acids, ²Mono-unsaturated fatty acids, ³Polyunsaturated fatty acids, ⁴n-3 Polyunsaturated fatty acids, ⁵n-6 Polyunsaturated fatty acids,

⁶Index of atherogenicity=(12:0 + 4*14:0 + 16:0) / [(n-6 + n-3) PUFA + 18:1 + the sum of other MUFA];

⁷Index of thrombogenicity=(14:0+16:0+18:0) / (0.5*18:1 + 0.5*ΣMUFA³ + 0.5*PUFA³ n-3 + (n-3:n-6));

⁸Fish lipid quality=sum of 20:5n-3 and 22:6n-3.

a, b, c... Values in the same row with different superscripts indicate significant differences (p<0.05) attributable to growing time.

sampled in November) to 2.93% (fish sampled in July). Grigorakis *et al.* (2011) found 1.06% content in 1280-g meagre sampled in December and fed with a commercial feed of 10% fat. In all cases, these low values confirm the high capacity of this fish to maintain reduced fat accumulation in muscle, despite being fed fat-rich diets, apparently reflecting a peculiar metabolism which bears further study.

Not only the total fat content of a fillet but also its FA profile concern human consumers. At the beginning of the experimental period (sampling 0) the juveniles exhibited muscle with a FA pattern having the following main characteristics: a) PA, OA, and DHA as the figures most abundant in their respective groups of SFA, MUFA and PUFA; b) each of these groups represented approximately one third of total FA; and c) among the HUFA, a clear prevalence of the n-3 series with respect to n-6 series (ratio n-3:n-6 >> 1).

This situation could be considered representative of marine fish species in general (Lanari *et al.*, 1999; Grigorakis *et al.*, 2002; Yildiz *et al.*, 2006), including meagre (Poli *et al.*, 2003; Piccolo *et al.*, 2008; Grigorakis *et al.*, 2011). The high proportion of HUFAs n-3 is commonly attributed to the prevalence of these FA in the marine food web (Sargent and Henderson, 1995).

Frequently, factors such as water salinity, temperature, and physiological and dietary composition are considered the main determinants of possible changes in the fish FA profile. In this case the environmental salinity did not change appreciably throughout the experiment while temperature fluctuated only slightly (Figure 1). An increase in the proportion of HUFAs in fish lipids has been considered to be part of the process of adaptation to lower water temperatures, although data vary among species and type of lipids (neutral vs. polar lipids) (Greene and Selivonchick, 1987; Henderson and Tocher, 1987). In this case (Tables 2A, 2B), samples taken in the warmer season (samplings 2–3) were compared with those from colder one (samplings 5 and 9) showed no consistent differences in total HUFA levels or in EPA, DHA or AA. Data from other Mediterranean fish species (Yildiz *et al.*, 2006; Senso *et al.*, 2007) agree in that seasonal differences in the FA profile are minor or inexistent at least for the individual FA or FA groups cited above. Poli *et al.*, (2003) detected some differences in the FA content of cultured meagre sampled in November and May-July (although the authors reported that water temperature varied from 17.2 to 28.6 °C throughout the experiment, no data on water temperature in sampling days were provided) and thus n-3 PUFA, but also total SFA, were higher in fish sampled in November while total MUFA reduced in that season. The relatively high temperatures, even in winter, in the Mediterranean and adjacent Atlantic areas could underlie this lack of marked seasonal effects.

On the other hand, it is difficult to define with precision the possible effects of water temperature upon the fish FA profile, when the corresponding studies are based on successive samplings from wild and/or farmed fish, since several biological variables such as fish age, reproductive status, changes in dietary composition (natural prey or artificial feed) could mask strictly abiotic environmental ones such as temperature.

In our assay, the most significant change over the whole period was perhaps the marked reduction in the $n-3:n-6$ ratio from an initial value close to 4.0 to a ratio of only about 1.0. This resulted from both a reduction in $n-3$ PUFA, mainly EPA, and a rise in LA content, the latter increasing by a factor of more than 3. Other marked changes affected the MUFA group, with a reduction in POA, 16:1 $n-7$ and an increase in OA content. As a general trend, these changes were noticeable during the first part of the experiment, with a certain trend to stabilize thereafter.

Once again, the transition from the Microbaq diet, especially rich in $n-3$ HUFA (based on fish oil, as in commonly used hatchery diets), to the second diet used here (Alphamar), which incorporated higher levels of vegetable oils (as deduced from its high OA and LA levels, and the extremely low $n3:n6$ ratio, Table 1) was undoubtedly the main cause of the observed changes.

Figures 2 to 4, which display the time course of the fish-to-diet ratio (FtoD) for several representative individual or grouped FA, clearly show the convergence between FA composition of fish and diet for LA, 18:2 $n6$ (the main figure in a lot of vegetable oils) and $n-3$ HUFA (the most abundant in fish oil). Both circumstances have reduced

the FtoD $n-3:n-6$ ratio by a factor of close to four (Figure 4); a clear reduction in this ratio has also been detected in muscle of fish fed on diets with high proportions of plant oils in sea bass and sea bream (Grigorakis, 2007). Concerning individual $n-3$ HUFA, it is evident that fish have preserved the muscle levels of DHA at about two-fold higher than the dietary ones, while EPA FtoD showed a notable reduction to values close to one. This situation could be indicative of a specific greater need for DHA that could be accompanied by a conversion from EPA to DHA when the dietary supply of DHA was insufficient to meet the fish metabolic requirements. The practical disappearance of the initially reduced levels of other $\Delta 6$ desaturated $n-3$ precursors, such as stearidonic acid (SA, 18:4 $n-3$) and eicosatrienoic acid (ETA, 20:3 $n-3$), was also noticeable. The possible metabolic stress associated with this situation should be considered when formulating diets of low fish/high vegetal oil for fish culture, since not only muscle composition but fish growth and health could be negatively affected.

When the time course of the indices for fish-flesh lipid quality was considered (Table 2B), the situation proved unclear. In fact, FLQ became worse mainly due to EPA reduction, but when other HUFAs were included in the evaluation, such as in IA and IT, the negative effects of $n-3$ HUFA and $n-3:n-6$ reductions were partially counteracted by the beneficial effects of both lower SFA and higher MUFA and $n-6$. Previous studies with this species (Poli *et al.*, 2003; Piccolo *et al.*, 2008; Grigorakis *et al.*, 2011) reported values of IA from 0.38 to 0.69 and IT values from 0.09 to 0.39 in the range of those found in this experiment and found in other cultured marine fish (Grigorakis, 2007).

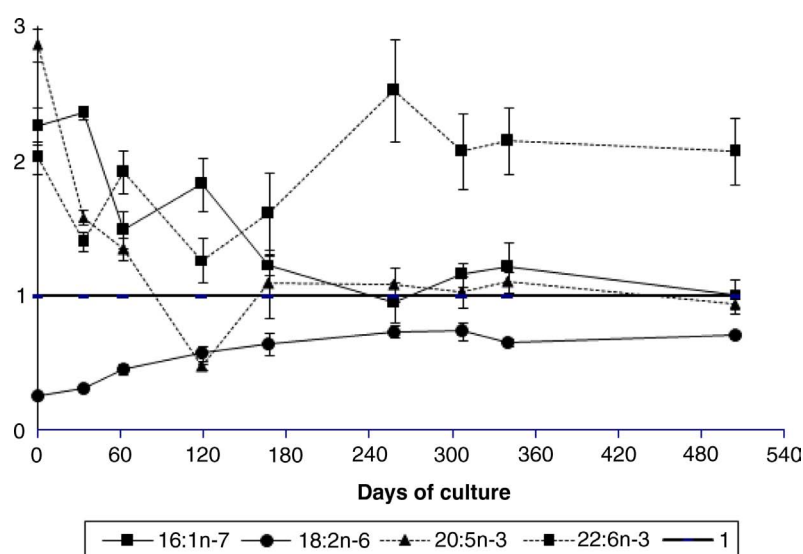


FIGURE 2. Time course of the ratio fish flesh to diet, (FtoD) of the relative content of several individual fatty acids of fish.

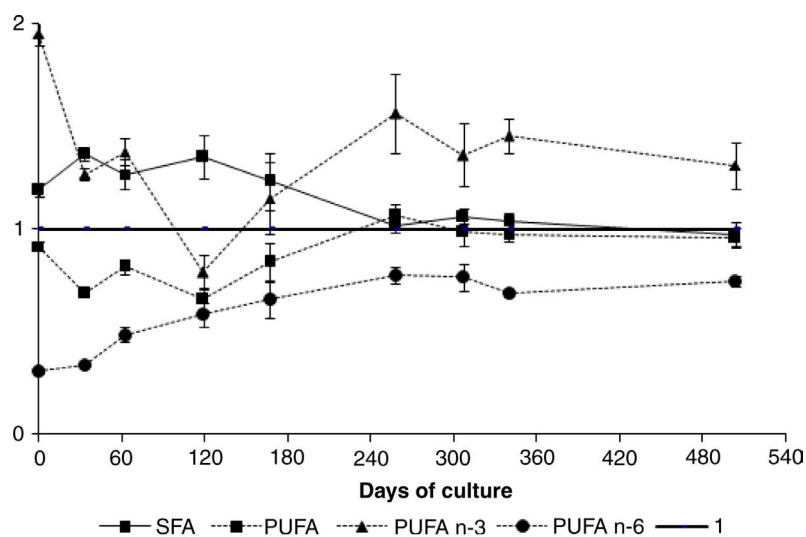


FIGURE 3. Time course of the ratio fish flesh to diet, (FtoD) of the relative content of several grouped fatty acids of fish. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA $n-3$: polyunsaturated fatty acids of the $n-3$ series; PUFA $n-6$: polyunsaturated fatty acids of the $n-6$ series.

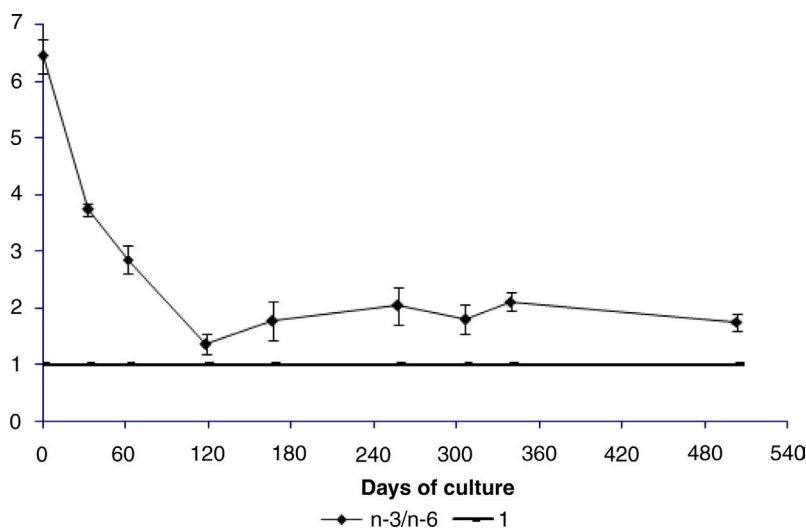


FIGURE 4. Time course of the diet fish flesh to diet ratio (FtoD) of the $n-3:n-6$ fatty acid ratio of fish.

4. CONCLUSIONS

The data compiled confirm the low fat content in meagre muscle in relation to other marine species, although values increased during culture. On the other hand, the muscle-fat FA content can be considered healthy for human consumption, values being similar to those found for cultured sea bass and sea bream, although when the culture plans include the use of plant-based lipid diets, the mixture of vegetal replacers of fish oil must be carefully designed. The use of high-HUFA $n3$ ending-diets could also be taken in consideration.

ACKNOWLEDGEMENTS

Fish were reared in Ceutamar S.L. (Algeiras, Cádiz, Spain) facilities, and the further experimental work was carried out in the CEIA $_3$ -UAL.

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