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Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: A time-course study on the effect of a re-feeding period with a 100% fish oil diet

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Abstract

In the present study 75 g European sea bass were fed for 8 months with different diets (22% dietary lipid content) containing vegetable oils at two inclusion levels. The control diet (Diet FO) contained anchovy oil as the only lipid source; in diets 60RO, 60LO, and 60SO of fish oil was substituted by rapeseed, linseed or soybean oils, respectively; finally, in diet 80LO, 80% of the fish oil was substituted by linseed oil. Fish were fed to apparent satiation three times a day. All fish were individually weighed once per month. Lipid and fatty acid composition of diets and fish fillets were determined at the beginning, middle and end of the experimental period. Once the commercial size was reached, all fish were fed a 100% FO containing diet during 150 days.

No significant differences were found in feed intake. Fish fed the diets containing 80% linseed oil or 60% rapeseed oil had significant ($P<0.05$) lower growth. Flesh fatty acid composition of total lipids reflected the fatty acids in the diets. Flesh contents of n-3 HUFA were reduced to about 45% in fish fed diets 60RO, 60LO or 60SO and to about 50% in diet 80LO. This reduction was markedly higher for EPA than for DHA. High levels of oleic, linoleic and linolenic acids were found in fish fed RO, SO and LO respectively.

After 150 days of re-feeding period with a 100% fish oil diet, DHA levels were restored in those fish previously fed diets containing vegetable oils, but EPA levels remained lower when compared with fish fed 100% FO diet. Flesh content of linoleic and linolenic acids remained higher in those fish previously fed soybean and linseed oil containing diets, respectively.

Keywords: Vegetable oil; EFA; DHA; EPA; Sea bass

Abbreviations: HUFA, Highly unsaturated fatty acid; ARA, Arachidonic acid (20:4n-6); EPA, Eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3); OA, Oleic acid (18:1n-9); LA, Linoleic acid: (18:2n-6); LNA, Linolenic acid: (18:3n-3).

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1. Introduction

The general trend to increase lipid content in aquafeeds for marine fish to improve growth, feed conversion and protein utilization has produced an increase in fish oil demand by aquaculture. The global production of fish oil based on fisheries landings is static, and it is predicted that by 2010 the fish feed industry will require at least 50% of the total world production of fish oil (Tacon, 1997; Barlow, 2000).

However, the capacity of the fisheries to cope with the increasing demand of fish oil has reached the limit of sustainability, due to different problems, including overfishing, climate alterations and increasing demand from other sectors (Sargent and Tacon, 1999). Thus, the use of alternative oils is necessary to supply lipids for aquafeeds, and those coming from plant seeds, such as linseed, rapeseed or soybean oils among others, are good candidates.

Some vegetable oils are considered as good alternative lipid sources in salmonids and freshwater fish feeds without affecting growth performance and feed conversion (Rosenlund et al., 2000; Bell et al., 2001; Caballero et al., 2002), However, marine fish have a very limited gene expression of delta 6 and delta 5 activity, and thus are not able to synthesize polyunsaturated fatty acids (PUFAs) (Mourente and Tocher, 1993) from linoleic and linolenic acids, abundant in many vegetable oils. PUFA of 20 or more carbons (highly unsaturated fatty acids; HUFAs) are essential for marine fish, with it being necessary to include arachidonic (20:4n-6) (ARA), ecicosapentaenoic (20:5n-3) (EPA) and docosahexaenoic (22:6n-3) (DHA) acids in marine fish diets. Partial replacement of fish oil by vegetable oils in marine fish diets will only be possible when the essential fatty acid requirements are met. The replacement of fish oil by vegetable oils in marine fish diets has been studied in turbot (Regost et al., 2003a,b) and gilthead sea bream (Kalogeropoulos et al., 1992; Caballero et al., 2002; Izquierdo et al., 2003, in press), but few focused on the European sea bass, one of the most important marine finfish species for Mediterranean aquaculture (Yildiz and Sener, 2002; Izquierdo et al., 2003).

Total replacement of fish oils by vegetable oils on low lipid content diet reduced sea bass growth (Yildiz and Sener, 2002). A high lipid content diet enables up to 60% of the fish oil to be replaced by several vegetable oils without compromising European sea bass growth and feed utilisation (Izquierdo et al., 2003), since the percentage of fish oil included is able to meet the EFA requirements of this species. Partial replacement of fish oil by vegetable oils for 3 months does not affect fillet taste and texture in gilthead sea bream and European sea bass (Izquierdo et al., 2003), but the use of some specific vegetable oils to partially replace fish oil can produce deleterious effects on fish health (Lodemel et al., 2001; Montero et al., 2003).

Not only fish health can be compromised by the inclusion of certain vegetable oils in marine fish diets, but also flesh nutritional quality can be affected, by the modification of muscle fatty acid profiles, including a reduction of n-3 HUFA, and particularly EPA (Izquierdo et al., 2003), and altering the relationship between n-3 and n-6 fatty acids (Izquierdo et al., in press). Thus, flesh quality of aquacultured fish in terms of n-3 HUFA source for human consumption can be affected by the use of vegetable oils. To avoid these problems, it is necessary to know how the different vegetable oils included in the diet can affect fish fatty acids and how feeding the last period of on-growing with 100% fish oil diets could enable recovery of the n-3 HUFA levels in fillets of fish previously fed vegetable oils (Izquierdo et al., in press).

Thus, the objective of this study was to determine the effect of long term feeding of diets with high levels of vegetable oil on growth performance and flesh quality of European sea bass, and the evolution of flesh fatty acid profiles when all fish were fed a fish oil diet during the final part of the on-growing period.

2. Materials and methods

2.1. Experimental diets

Five isoenergetic and isonitrogenous experimental diets with a lipid content of 22% were formulated (Table 1). Peruvian anchovy oil was the only added lipid source in Diet FO. All the other diets contained vegetable oils to substitute either 60% of the anchovy oil used in Diet FO, in diets 60SO (soybean oil), 60RO (rapeseed oil), 60LO (linseed oil), or 80% in diet 80LO (linseed oil). Fish oil was included in diets 60SO, 60RO, and 60LO at a level sufficient to meet

Table 1
Composition (g/kg) of experimental diets containing different lipid sources

Ingredients	FO	60SO	60RO	60LO	80LO
Fish meal (LT)	381	381	381	381	381
Corn gluten	260	260	260	260	260
Wheat	150.6	150.6	150.6	150.6	150.6
Lysine (99%)	7.23	7.23	7.23	7.23	7.23
Premix[a]	25	25	25	25	25
Anchovy oil	176	70.4	70.4	70.4	35.2
Soybean oil[b]		105.6			
Rapeseed oil[b]			105.6		
Linseed oil[b]				105.6	140.8
Crude lipids	22.50	22.11	22.37	19.52	24.06
Crude protein	49.27	49.03	49.25	48.37	47.92

[a] Premix of vitamins and minerals according to NCR (1993) recommendations for fish.
[b] Crude vegetable oils.

the reported EFA requirements of this species (Lanari et al., 1999) (Table 2).

2.2. Feeding trial

Six hundred and seventy five European sea bass (*Dicentrarchus labrax*) juveniles (75 g initial body weight) were distributed among 15 tanks of 500 l (45 fish/tank, each diet fed in triplicate). These tanks were supplied with seawater, temperature ranged between 17.8 and 22.8 °C, and continuous aeration. At the middle of the experiment, fish were transferred to 1000 l tanks. Fish were fed the experimental diets three times per day, 6 days per week, until apparent satiation, during 8 months when they reached the commercial size.

Feed intake was determined daily and all fish were individually weighed monthly. Conversion index (CI), and specific growth rate (SGR) were calculated using the following formulae: CI = Feed Intake/Weight Gain, SGR = ((Ln Final Weight − Ln Initial Weight)/t) × 100, t = experimental period (day).

2.3. Biochemical analysis

After 240 days of feeding, liver and muscle samples of 6 fish per tank (18 per diet) were collected and kept at −80 °C until they were analyzed. Lipids from diets and muscle were extracted with a chloroform methanol (2:1, v/v) mixture (Folch et al., 1957). The fatty acid methyl esters were obtained by trans-

Table 2
Main fatty acids of the experimental diets (g/100 g fatty acids)

	FO	60SO	60RO	60LO	80LO
14:0	7.91	3.40	2.35	3.18	2.19
14:1	0.20	0.09	0.02	0.08	0.06
15:0	0.48	0.32	0.22	0.21	0.15
16:0iso	n.d.	0.04	0.02	0.04	0.03
16:0	19.07	14.49	11.21	11.32	9.80
16:1n-7	7.11	3.16	3.39	3.12	2.06
16:1n-5	0.18	0.09	0.16	0.15	0.07
16:2	1.09	0.46	0.50	0.45	0.30
17:0	0.56	0.28	0.25	0.26	0.19
17:1	1.17	0.51	0.51	0.46	0.30
16:4n-1	0.09	0.04	0.04	n.d.	0.03
16:4n-3	1.53	0.63	0.64	0.59	0.35
18:0	3.21	2.98	2.24	2.90	3.01
18:1n-9	11.34	16.67	38.67	16.22	16.21
18:1n-7	2.57	2.04			1.48
18:2n-9	0.15	0.07	0.05	0.26	0.12
18:2n-6	3.92	30.54	13.67	12.01	13.18
18:2n-4	0.23	0.09	0.09	0.08	0.05
18:3n-6	0.29	0.12	0.12	0.12	0.07
18:3n-4	0.13	0.06	0.05	0.02	0.03
18:3n-3	0.88	4.66	5.03	27.11	37.50
18:4n-3	2.12	1.00	1.04	1.28	0.75
18:4n-1	0.17	0.08	0.09	0.24	0.11
20:0	0.26	0.27	0.37	0.24	0.20
20:1n-9	2.46	1.79	2.28	2.17	1.65
20:1n-7	0.26	0.14	0.16	0.17	0.09
20:2n-9	0.12	0.05	0.05	0.04	0.03
20:2n-6	0.14	0.09	0.10	0.10	0.07
20:3n-6	0.10	0.05	0.05	0.09	0.03
20:4n-6	0.88	0.41	0.40	0.38	0.24
20:3n-3	0.06	0.02	0.04	0.07	0.07
20:4n-3	0.57	0.26	0.26	0.27	0.16
20:5n-3	13.47	5.85	6.10	5.86	3.34
22:0	0.20	0.26	0.26	0.17	0.11
22:1n-11	2.47	2.03	1.75	2.49	1.83
22:4n-6	0.48	0.22	0.23	0.21	0.13
22:5n-6	0.14	0.08	0.07	0.08	0.02
22:4n-3	0.25	0.11	0.13	0.11	0.08
22:5n-3	0.07	0.02	0.04	0.04	0.03
24:0	1.51	0.66	0.70	0.62	0.33
22:6n-3	11.67	5.58	6.14	6.32	3.49
Saturated	31.73	20.10	17.00	18.55	15.69
Monoenoics	28.06	28.75	47.22	25.08	23.81
n-3	31.88	34.56	20.00	42.16	46.02
n-6	6.34	15.63	14.82	13.13	13.86
n-9	14.27	20.45	41.13	18.63	17.98
n-3 HUFA	27.36	12.63	13.28	13.18	7.41
n-3/n-6	5.03	2.21	1.35	3.21	3.32

HUFA: Highly unsaturated fatty acid.

esterification with 1% sulphuric acid in methanol (Christie, 1982) and separated and quantified by gas–liquid chromatography under the conditions previously reported (Izquierdo et al., 1990).

2.4. Organoleptic test and texture analysis

Nine fish per dietary treatment were sampled for organoleptic tests. Fillets from those fish were kept at 4 °C for 24 h. After this, fillets were cooked during 10 min in a steam oven at 150 °C. Immediately after cooking in aluminium boxes, fillets were offered to a panel of 12 selected trained judges. Tests were conducted in isolated and air conditioned rooms with standardized light. Judges were randomly offered closed food boxes labelled with codes containing the fillets (3×4 cm). Odour (marine, earthy, off-odour, oily), appearance (juiciness, compactness, shininess, whiteness), texture (adhesiveness, hardness, cohesiveness, juiciness), flavour (marine, earthy, off-odour, oily) and persistence of the flavour were tested for samples of fish fed the experimental diets and classified by the judge on a continuous scale from 0 to 100 for each parameter.

In addition, 6 fish per tank (18 per dietary treatment) were sampled for texture using a 4465 INSTRON UTM texture analyser. All tests were carried out at refrigeration temperature, keeping the fillet cooled with ice. Fish fillets were compressed and punctured following the methodology described by Borderias et al. (1983). For the puncture tests, cylindrical pieces of fillet of 5.3 cm in diameter and 1.2 cm in height were cut from the fish left upper flank. Flesh puncture was measured after the fillet had been completely penetrated by a 0.8 cm wide cylinder at a speed of 80 mm/min. Similarly, for the pressure tests, cylindrical pieces of fillet of 2.6 cm wide and 1.2 cm high were cut from the fish left flank. The flesh compression was measured as the force required to compress the thickness of the fillet by 30% using a 3.6 cm wide cylindrical piston at a speed of 50 mm/min. Instrumental colour analysis was performed with a MINOLTA CHROMAMETER CR200 (Minolta, Osaka, Japan) giving results in CIE (1976) values for lightness (L*), associated with the brightness intensity of the stimulus, chroma $((a^{*2}+b^{*2})^{1/2})$, in relation with the proportion of pure colour, and the angle of hue $(\tan^{-1}(b^*/a^*))$ pertaining to the predominant colour (Wyszecki and Stiles, 1982). Intramuscular pH of the fillets was also determined using a pH-meter.

2.5. Re-feeding trial with 100% fish oil diet

After sampling fish for growth performance, biochemical composition and flesh quality, the remaining fish from all dietary treatments were fed diet FO for 150 days. During this period, fish were sampled after 10, 30, 50 and 150 days. At each sampling point 9 fish per treatment were sampled and pooled in 3 samples of 3 fish each. Samples of flesh were analysed for lipid content and fatty acid composition as described above.

2.6. Statistical analysis

All the data was statistically treated using ANOVA, and Tukey's test at $P<0.05$ was applied as a multiple sample comparison analysis using a SPSS Statistical Software System 10.0 (SPSS Inc., Chicago, Illinois) (Sokal and Rolf, 1995).

3. Results

Fatty acid analysis of total lipids in diet reflected those characteristic from each added vegetable oil (Table 2). Total saturated fatty acids ranged from 15.69% in diet 80LO to 31.23% in diet FO. Oleic acid (OA) ranged from 11.34% in diet FO to 38.67% in diet 60RO. Linoleic acid (LA) was higher in the diet with soybean oil, ranging from 3.92% in diet FO to 30.98% in diet 60SO. Similarly, linolenic acid (LNA) increased, particularly with the increasing inclusion of linseed oil, from 0.88% in diet FO to 37.50% in diet 80LO. Percentages of EPA ranged from 13.47% in diet FO to 3.34% in diet 80LO, while DHA ranged from 11.67% in diet FO to 3.49% in diet 80LO, and ARA ranged from 0.88% in diet FO to 0.24% in diet 80LO.

All diets were well accepted by European sea bass and thus no significant differences were found in feed intake during the experimental period. The use of vegetable oils in European sea bass diets did not affect fish survival. However, the use of 80% of linseed oil in diet significantly ($P<0.05$) reduced growth after

142 days of feeding when compared with fish fed FO diet, in terms of body weight (Table 3). However, no significant differences were found between eviscerated body weight of fish fed FO or 80LO (Table 3). On the other hand, the use of 60% of rapeseed oil in diet (60RO) also significantly ($P<0.05$) reduced European sea bass growth in terms of total and eviscerated body weight after 240 days of feeding. No significant differences were found using 60% of either soybean oil or linseed oil in diet (Table 3). Specific growth rate (SGR) for all periods was similar in all experimental groups, and around 0.52%/day and feed conversion ratio from 1.44 (FO diet) to 1.54 (60SO diet). No significant differences were found in liver weight and hepatosomatic index (HSI) among experimental groups (Table 3). Lipid content of liver and muscle were similar in all the dietary treatments (Table 3).

In both muscle and liver, fatty acid composition of total lipids showed the effect of the diets. Saturated fatty acids in liver were higher, but not significantly, in fish fed FO diet (Table 4). Besides, fish fed either 60RO or 80LO showed significantly ($P<0.05$) lower content of saturated fatty acids in their muscles (Table 5). Monoenoic fatty acids and particularly OA significantly ($P<0.05$) increased in both liver and muscle of the fish fed the rapeseed oil containing diet (Tables 4 and 5). LA significantly ($P<0.05$) increased in fish fed the soybean oil containing diet, whereas LNA significantly ($P<0.05$) increased in fish fed the linseed oil containing diets in both tissues (Tables 4 and 5). N-3 HUFA content in muscle was reduced down to around 55% in flesh from fish fed linseed oil containing diets, to 63% in rapeseed oil containing diet and 66% in soybean oil containing diet (Table 5). A significant ($P<0.05$) reduction of n-3 HUFA could be also observed in livers of fish fed vegetable oil containing diets (Table 4). Furthermore, the relationship between n-3 and n-6 fatty acids in fish flesh was significantly ($P<0.05$) affected by the use of vegetable oils in diets. This ratio decreased from 4.18 in fish fed FO diet to around 3 in the linseed oil containing diets, to 1.76 in the rapeseed oil containing diet and 0.93 in the soybean oil containing diet (Table 5), this ratio being a good indicator of the nutritional value of fillets for human health.

Regarding flesh quality in terms of texture and appearance, analysis of the sensory profiling showed that the use of vegetable oils in diets for European sea bass did not significantly affect texture parameters despite a tendency to a less resistance to compression in fish fed linseed oil containing diets (Fig 1). Regarding the appearance of flesh, only fish fed the rapeseed oil containing diet showed a significantly ($P<0.05$) higher flesh yellowness when compared with fish fed the FO diet (Fig. 1). Vegetable oil addition to European sea bass diets did not significantly ($P>0.05$) alter organoleptic properties of fish fillets, particularly appearance and texture (Figs 2 and 3), and only fish fed the soybean oil containing diet showed any effect, but not significant ($P>0.05$), on odour and flavour from fish fed fish oil diet (Fig. 2).

In order to recover the levels of those fatty acids that are of interest for human consumption, a re-feeding experiment with 100% FO diet was conducted. The results showed that the DHA fillet content

Table 3
Growth of European sea bass fed different vegetable lipid sources (mean ± S.D.)

Ingredients	Diets				
	FO	60SO	60RO	60LO	80LO
Final body weight (g)*	378.31 ± 27.18a	371.73 ± 34.93a	356.31 ± 41.41b	358.38 ± 4.75a	365.96 ± 38.33b
Eviscerated weight (g)*	312.47 ± 50.68a	310.02 ± 64.11a	274.83 ± 29.93b	331.56 ± 44.48a	310.35 ± 45.89a
SGR	0.53 ± 0.01	0.52 ± 0.02	0.51 ± 0.02	0.52 ± 0.01	0.51 ± 0.01
CI	1.44 ± 0.05	1.55 ± 0.05	1.49 ± 0.05	1.47 ± 0.05	1.49 ± 0.04
Liver weight**	9.40 ± 2.10	9.28 ± 2.80	8.64 ± 2.23	9.67 ± 2.71	9.70 ± 2.30
HIS**	2.55 ± 0.42	2.55 ± 0.42	2.57 ± 0.36	2.55 ± 0.46	2.62 ± 0.39
Liver lipid content (% d.w.)	63.1 ± 8.85	65.63 ± 1.98	60.91 ± 7.25	61.74 ± 8.78	64.06 ± 2.84
Muscle lipid content (% d.w.)	13.41 ± 1.65	14.39 ± 1.86	13.52 ± 1.69	13.42 ± 0.59	14.86 ± 2.64

Different letters in the same line denote statistically significant difference ($P<0.05$).
$n=3$; * $n=35\times3$; ** $n=6\times3$.

Table 4
Main fatty acids of the liver of fish fed the experimental diets (g/100 g fatty acids) (Mean ± S.D.)

	Diets				
	FO	60SO	60RO	60LO	80LO
14:0	2.30 ± 0.1a	1.73 ± 0.0b	1.93 ± 0.1b	1.74 ± 0.0b	1.58 ± 0.0b
14:1	0.10	0.04	0.06	0.05	0.04
15:0	0.15	0.09	0.08	0.09	0.07
16:0iso	0.05	0.03	0.03	0.03	0.02
16:0	24.12	23.44	21.97	22.08	21.65
16:1n-7	7.53 ± 0.4a	4.83 ± 0.1ab	3.45 ± 2.9b	5.28 ± 0.2ab	4.73 ± 0.2ab
16:1n-5	0.24	0.14	0.11	0.14	0.12
16:2	0.45	0.25	0.27	0.25	0.19
17:0	0.32	0.23	0.23	0.22	0.18
17:1	0.55	0.28	0.32	0.29	0.22
16:4n-1	0.02	0.01	0.04	0.01	0.01
16:4n-3	0.13	0.05	0.06	0.05	0.03
18:0	4.11 ± 0.1ab	4.89 ± 0.3b	3.64 ± 0.1a	4.51 ± 0.3ab	4.36 ± 0.5ab
18:1n-9	38.72 ± 2.4a	37.85 ± 1.1a	46.66 ± 0.8b	37.77 ± 0.7a	37.86 ± 0.4a
18:1n-5	0.12	0.26	0.39	0.17	0.07
18:2n-9	0.46	0.51	0.66	0.51	0.43
18:2n-6	2.80 ± 0.7a	12.64 ± 0.3c	6.78 ± 0.2b	5.61 ± 0.4b	6.28 ± 0.7b
18:2n-4	0.19	0.09	0.12	0.07	0.04
18:3n-6	0.19	0.37	0.27	0.18	0.18
18:3n-4	0.15	0.08	0.41	0.06	0.03
18:3n-3	0.44 ± 0.1a	1.56 ± 0.2a	1.51 ± 0.7a	9.70 ± 0.9b	12.84 ± 1.4c
18:4n-3	0.67	0.40	0.46	0.76	0.78
18:4n-1	0.07	0.02	0.03	0.04	0.02
20:0	0.12	0.13	0.14	0.10	0.10
20:1n-9	1.97	1.72	2.16	1.85	1.74
20:1n-7	0.15	0.09	0.10	0.09	0.07
20:2n-9	0.04	0.03	0.05	0.03	0.03
20:2n-6	0.18 ± 0.0a	0.49 ± 0.0b	0.26 ± 0.0a	0.22 ± 0.2a	0.34 ± 0.1ab
20:3n-6	0.05	0.04	0.03	0.03	0.02
20:4n-6	0.36 ± 0.0a	0.19 ± 0.0c	0.21 ± 0.0b	0.19 ± 0.0b	0.13 ± 0.0c
20:3n-3	0.04 ± 0.0a	0.05 ± 0.0a	0.05 ± 0.0a	0.16 ± 0.0b	0.20 ± 0.0c
20:4n-3	0.33 ± 0.1a	0.17 ± 0.0b	0.19 ± 0.0b	0.20 ± 0.0b	0.15 ± 0.0b
20:5n-3	4.37 ± 0.5a	2.13 ± 0.2b	2.29 ± 0.1b	2.20 ± 0.1b	1.52 ± 0.2b
22:0	0.05	0.08	0.06	0.04	0.05
22:1n-11	0.97	0.98	0.88	1.00	0.94
22:1n-7	0.22	0.07	0.22	0.07	0.07
22:4n-6	0.20	0.07	0.10	0.10	0.06
22:5n-6	0.10	0.05	0.05	0.05	0.03
22:4n-3	0.04	0.02	0.02	0.02	0.01
22:5n-3	0.83 ± 0.1a	0.41 ± 0.1b	0.44 ± 0.0b	0.41 ± 0.0b	0.27 ± 0.0b
22:6n-3	5.92 ± 1.0a	3.37 ± 0.5b	3.60 ± 0.2b	3.51 ± 0.2b	2.53 ± 0.3b
Saturated	31.23	30.63	28.07	28.81	28.00
Monoenoics	50.28 ± 2.6ab	46.13 ± 1.2a	53.81 ± 1.9b	46.57 ± 0.4a	45.70 ± 0.3c
n-3	12.76 ± 1.6a	8.15 ± 0.9b	8.61 ± 0.9ab	17.01 ± 0.8c	18.34 ± 2.0c
n-6	4.25 ± 0.7a	14.02 ± 0.3b	8.24 ± 0.c	6.55 ± 0.4c	7.11 ± 0.8c
n-9	41.37 ± 2.4a	40.21 ± 1.1a	49.65 ± 1.0b	40.23 ± 0.7a	40.11 ± 0.3a
n-3 HUFA	11.52 ± 1.6a	6.14 ± 0.8b	6.58 ± 0.3b	6.50 ± 0.2b	4.68 ± 0.6b
n-3/n-6	3.02 ± 0.3a	0.58 ± 0.1d	1.05 ± 0.2c	2.60 ± 0.1ab	2.58 ± 0.6b

HUFA: Highly unsaturated fatty acid.
*Different letters in the same line denote statistically significant difference ($P<0.05$).
$n=3$.

Table 5
Main fatty acids of the muscle of fish fed the experimental diets (g/100 g fatty acids) (Mean ± S.D.)

	Diets				
	FO	60SO	60RO	60LO	80LO
14:0	5.0 ± 0.1a	2.8 ± 0.2b	3.1 ± 0.1b	3.0 ± 0.2b	2.3 ± 0.1c
14:1	0.10	0.07	0.06	n.d.	0.05
15:0	0.4 ± 0.0a	0.2 ± 0.0b	0.2 ± 0.0b	0.24 ± 0.1b	0.19 ± 0.1b
16:0iso	0.16	0.17	0.07	0.15	0.16
16:0	21.6 ± 0.3ab	19.9 ± 0.9ab	19.2 ± 1.7ab	23.0 ± 1.5ab	17.1 ± 0.6c
16:1n-7	4.42	3.82	3.82	3.55	3.33
16:1n-5	0.16	0.09	0.11	0.09	0.08
16:2	0.75	0.38	0.38	0.34	0.31
17:0	0.37	0.25	0.25	0.27	0.21
17:1	0.69	0.33	0.34	0.29	0.27
16:4n-1	0.13	0.19	0.10	0.13	0.14
16:4n-3	0.53	0.29	0.26	0.26	0.26
18:0	3.57	4.10	3.44	5.26	4.00
18:1n-9	18.5±0.8a	20.1 ± 0.4a	31.2 ± 1.3b	19.90 ± 0.7a	18.8 ± 3.2a
18:1n-5	0.20	0.17	0.23	0.17	0.17
18:2n-9	0.22	0.17	0.23	0.18	0.18
18:2n-6	4.6 ± 0.4a	18.9 ± 0.6d	9.1 ± 1.0bc	7.5 ± 0.3b	9.8 ± 0.4c
18:2n-4	0.18	0.08	0.09	0.07	0.06
18:3n-6	0.21	0.23	0.15	0.14	0.13
18:3n-4	0.11	0.06	0.07	0.05	0.04
18:3n-3	1.2 ± 0.2a	2.9 ± 0.0a	2.9 ± 0.4a	13.3 ± 1.1b	20.9 ± 0.7c
18:4n-3	1.3 ± 0.1a	0.7 ± 0.1b	0.7 ± 0.1b	0.7 ± 0.1b	0.8 ± 0.1b
18:4n-1	0.11	0.06	0.05	0.06	0.05
20:0	0.17	0.16	0.22	0.17	0.12
20:1n-9	2.93	2.41	3.25	2.64	2.40
20:1n-7	0.21	0.13	0.16	0.12	0.10
20:2n-9	0.07	0.04	0.04	0.03	0.03
20:2n-6	0.28	0.56	0.31	0.35	0.45
20:3n-6	0.08	0.06	0.05	0.04	0.04
20:4n-6	0.8 ± 0.0a	0.5 ± 0.0b	0.5 ± 0.0b	0.5 ± 0.0b	0.4 ± 0.0b
20:3n-3	0.09	0.07	0.07	0.15	0.21
20:4n-3	0.45	0.26	0.25	0.23	0.23
20:5n-3	9.2 ± 0.5a	4.9 ± 0.1b	4.8 ± 0.7b	4.2 ± 0.4b	4.0 ± 0.3b
22:0	0.08	0.10	0.08	0.07	0.06
22:1n-11	1.98	1.83	2.31	2.13	1.69
22:1n-7	0.29	0.22	0.05	0.04	0.13
22:4n-6	0.08	0.04	0.04	0.04	0.03
22:5n-6	0.23	0.13	0.13	0.12	0.13
22:4n-3	0.05	n.d.	0.04	0.03	0.03
22:5n-3	1.5 ± 0.1a	0.8 ± 0.1b	0.8 ± 0.1b	0.6 ± 0.1b	0.6 ± 0.1b
24:0	0.03	n.d.	n.d.	0.02	n.d.
22:6n-3	14.1 ± 2.1a	9.4 ± 0.4b	8.8 ± 1.1b	7.7 ± 0.9b	8.1 ± 1.2b
Saturated	31.3 ± 0.5a	27.8 ± 1.3ab	26.6 ± 2.0b	32.1 ± 1.9a	24.1 ± 1.6b
Monoenoics	31.9 ± 3.6a	31.2 ± 0.9a	43.3 ± 1.8b	30.9 ± 0.9a	28.9 ± 2.9a
n-3	28.4 ± 2.8a	19.4 ± 0.3b	18.6 ± 2.5b	27.2 ± 2.1a	35.1 ± 1.9c
n-6	6.8 ± 0.5a	20.7 ± 0.6d	10.6 ± 1.2bc	8.9±0.3b	11.1 ± 0.5c
n-9	21.9 ± 0.7a	22.8 ± 0.5a	34.8 ± 1.7b	22.8 ± 0.8a	21.5 ± 3.1a
n-3 HUFA	25.4 ± 2.6a	15.5 ± 0.4b	14.7 ± 1.9b	12.9 ± 1.3b	13.1 ± 1.5b
n-3/n-6	4.2 ± 0.2a	0.9 ± 0.0d	1.8 ± 0.0c	3.1 ± 0.1b	3.2 ± 0.1b

HUFA: Highly unsaturated fatty acid.
*Different letters in the same line denote statistically significant difference ($P<0.05$).
$n=3$.

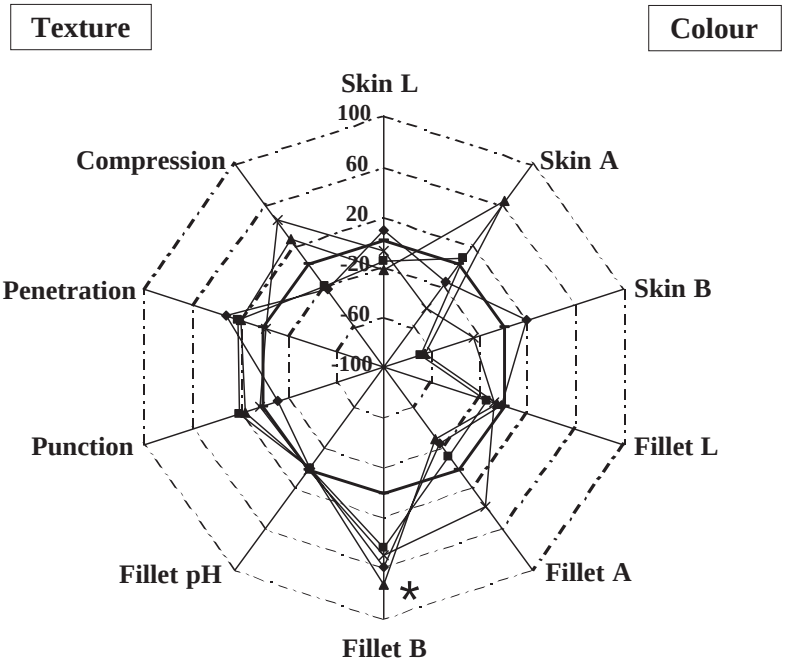

Fig. 1. Texture parameters and colour of both fillet and skin of fish fed the experimental diets during 8 months. Values are presented as percent of variation from fish fed FO diet values. * denotes significant differences ($P<0.05$) from fish fed FO diet. L: lightness; A: Redness; B: Yellowness. $N=6\times3$. +: FO; ×: 60SO; ▲: 60RO; ■: 60LO; ◆: 80LO.

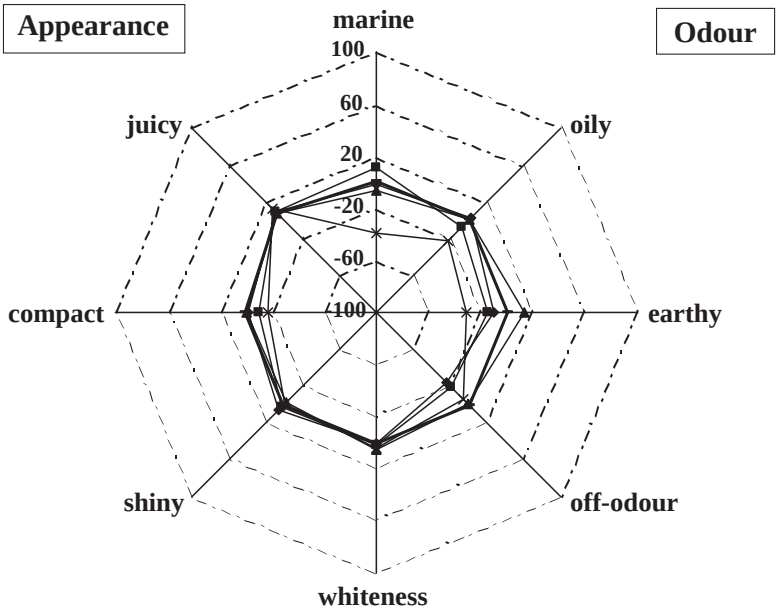

Fig. 2. Organoleptic parameters of fillets from fish fed experimental diets during 8 months and determined by a trained judge: fillet appearance and odour. Values are presented as percent of variation from fish fed FO. $N=18$. +: FO; ×: 60SO; ▲: 60RO; ■: 60LO; ◆: 80LO.

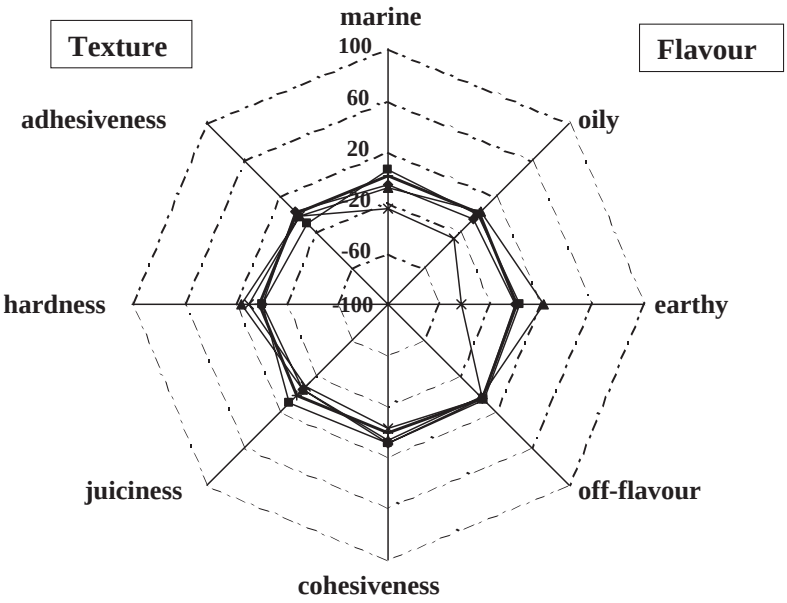

Fig. 3. Organleptic parameters of fillets from fish fed experimental diets during 8 months and determined by a trained judge: fillet texture and flavour. Values are presented as percent of variation from fish fed FO. $N=18$. +: FO; ×: 60SO; ▲: 60RO; ■: 60LO; ◆: 80LO.

approached those of fish fed fish oil after 150 days of re-feeding with 100% FO diet in all fish previously fed with vegetable oil containing diets (Fig. 4). However, the EPA fillet content did not return to the levels of fish oil fed fish even after 150 days of re-feeding period, with fish previously fed vegetable oils showing a reduction in EPA from 13% (60SO) to 35% (60LO) when compared with fish previously fed FO diet (Fig. 5).

LA and LNA contents in fish previously fed the vegetable oils progressively decreased after feeding with a FO diet. Nevertheless, even after 150 days of feeding the FO diet, LA was only reduced to 36% of its initial value in fish previously fed diet 60SO and remained significantly ($P<0.05$) higher in the other dietary treatments when compared with fish previously fed FO diet (Fig. 6), whereas LNA was reduced to 50% of its initial value in fish previously fed either 60LO or 80LO (Fig. 7).

4. Discussion

The present study has shown that it is possible to replace up to 60% of fish oil by soybean oil or linseed oil as described previously for this species (Izquierdo et al., 2003), even for long periods of substitution. However, the use of rapeseed oil at 60% substitution over long periods affected European sea bass growth, in contrast with the effect of inclusion of this oil over shorter periods for this species (Izquierdo et al., 2003), or the use of this oil in diets for Atlantic salmon (Bell et al., 2003). In addition, the use of 80% linseed oil for substitution of fish oil also seems to affect fish growth as described for other marine species such as gilthead sea bream (Izquierdo et al., in press), denoting the low availability of these fish species to elongate and desaturate fatty acids (Mourente and Tocher, 1993). However, although fish fed 80% of linseed oil in diet showed total body weight affected, they did not show significant differences in eviscerated body weight when compared with fish fed FO diet. Further studies on dietary linolenic acid metabolism would clarify if linseed oil in high substitution levels constitutes a good alternative oil for European sea bass.

The fatty acid composition of fillet lipids are closely related to dietary fatty acids, but DHA seems to be selectively retained in muscle, since flesh DHA concentrations are higher than dietary concentrations, as described for gilthead sea bream (Izquierdo et al., 2003), rainbow trout (Caballero et al., 2002) or turbot (Regost et al., 2003a). However, levels of EPA in liver

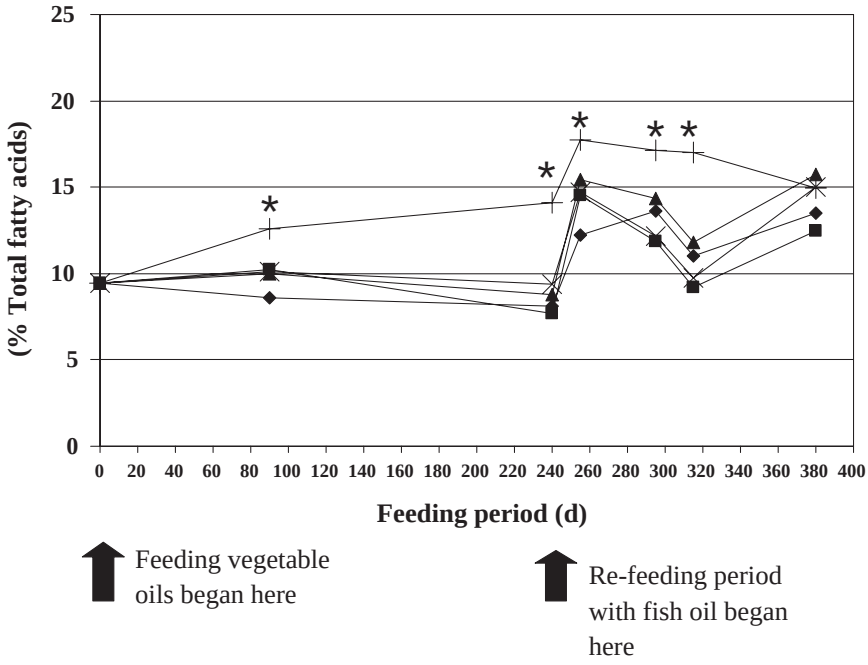

Fig. 4. Evolution of fillet DHA content (g/100 g F.A.) during the experimental period, including re-feeding period. * denotes significant differences ($P<0.05$) among fish fed FO diet and fish fed the rest of the diets (vegetable oil containing diets). +: FO; ×: 60SO; ▲: 60RO; ■: 60LO; ◆: 80LO.

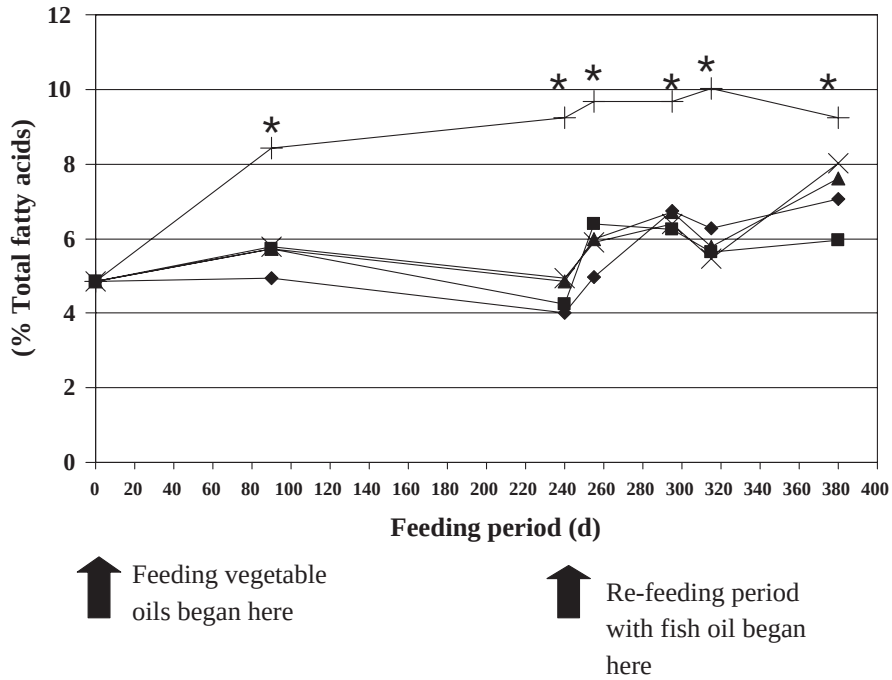

Fig. 5. Evolution of fillet EPA content (g/100 g F.A.) during experimental period, including re-feeding period. * denotes significant differences ($P<0.05$) among fish fed FO diet and fish fed the rest of the diets (vegetable oil containing diets). +: FO; ×: 60SO; ▲: 60RO; ■: 60LO; ◆: 80LO.

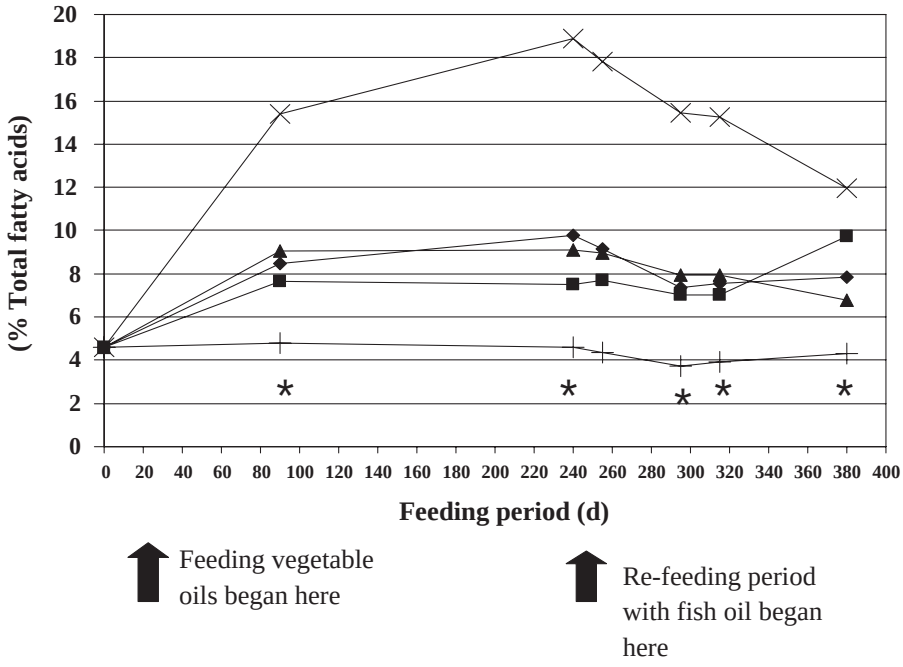

Fig. 6. Evolution of fillet linoleic acid (LA) content (g/100 g F.A.) during experimental period, including re-feeding period. * denotes significant differences ($P<0.05$) among fish fed FO diet and fish fed the rest of the diets (vegetable oil containing diets). +: FO; ×: 60SO; ▲: 60RO; ■: 60LO; ◆: 80LO.

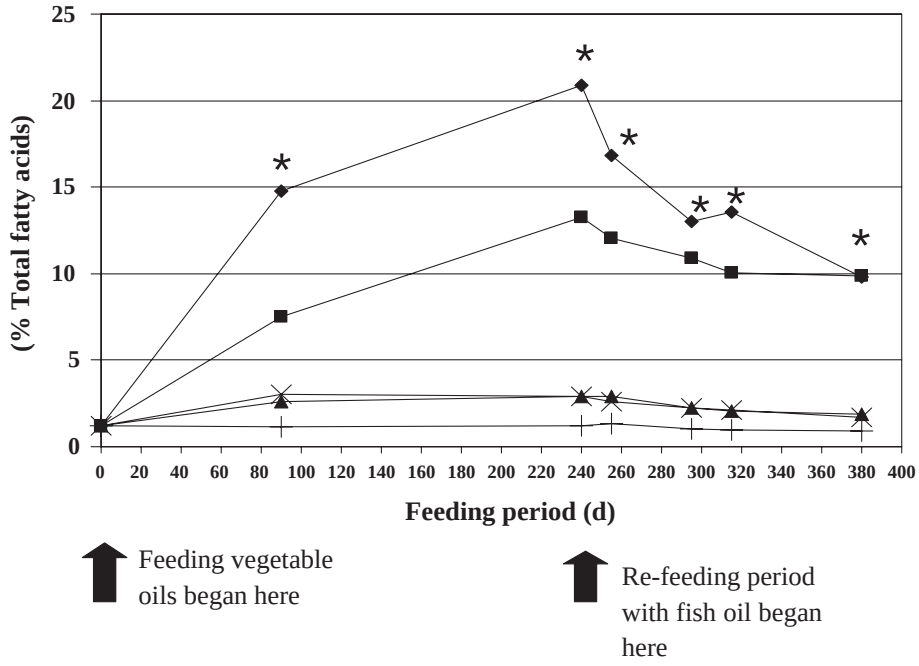

Fig. 7. Evolution of fillet linolenic acid (LNA) content (g/100 g F.A.) during the experimental period, including re-feeding period. * denotes significant differences ($P<0.05$) among fish fed FO diet and fish fed linseed oil containing diets. +: FO; ×: 60SO; ▲: 60RO; ■: 60LO; ◆: 80LO.

and muscle total lipids are lower than those presented in diets, denoting a preferred utilization of this fatty acid. The selective retention of DHA could be related to a higher peroxisomal beta-oxidation of EPA when compared with DHA (Madsen et al., 1998), due to the complex catabolism of this fatty acid (Bell et al., 2001). This selective retention is not reflected in liver, since levels are lower than those found in diets, in contrast to finds on Atlantic salmon, where liver seems to retain DHA more effectively than red or white muscle (Torstensen et al., 2004).

LA and LNA were accumulated in liver and muscle proportional to their levels in the diet, with levels in muscle higher than those found in liver. The higher content of these fatty acids in muscle could be related to the direct absorption and esterification of dietary fatty acids in muscle (Henderson, 1996) and with a mobilization from liver to muscle due to the high affinity of the acyltransferases synthesizinng phospholipids that contain these fatty acids. The accumulation of LA seems to be more important than that of LNA, since the ratio of level in muscle/level in diet is higher for LA, denoting that this fatty acid tends to be accumulated and retained in muscle of European sea bass more than LNA.

Oleic acid is also retained in both tissues studied, but mainly in liver where levels are higher than those found in the diets, even in fish fed the diet containing rapeseed oil, as described for other species such as coho salmo and rainbow trout (Skonberg et al., 1994). The ratio between oleic acid in tissue/oleic acid in diet is higher for the sea bass when compared with gilthead sea bream fed similar diets (Izquierdo et al., in press), denoting that this fatty acid is not well utilized. Oleic acid is a good substrate as the CoA-derivative for mitochondrial carnitine palmitoyltransferase I (CPT I) (Gavino and Gavino, 1991) and an increase of this fatty acid could indicate a low activity of mitochondrial fatty acid oxidation enzymes. Foxworthy and Eacho (1988) showed that increased peroxisomal beta-oxidation by using 2 hydroxy-3-propyl-4-(6(tretrazol-5-yl)hexyloxy)acetophenone (4-THA) inhibited the oxidation of 18:1 fatty acids and decreased mitochondrial redox state. In rat hepatocytes, EPA and DHA induced the gene expression of peroxisomal fatty acyl-CoA oxidase (Totland et al., 2000), DHA inhibiting the mitochondrial beta-oxidation of other fatty acids (Osmundsen and Bjornstand, 1985). Thus, an increase of selected fatty acids in diet, such as LA, LNA or OA could produce an accumulation of these fatty acids due to a reduced mitochondrial fatty acid oxidation in the presence of DHA and EPA. Mitochondrial beta-oxidation is of special importance in muscle (Froyland et al., 2000), thus the levels of C18 fatty acids were higher in liver than in muscle.

The use of vegetable oils did no affect flesh quality in terms of fillet texture and organoleptic qualities, as described for other species such as Atlantic salmon (Hardy et al., 1987; Bjerkeng et al., 1997), differing from those results found by Regost et al. (2003b) who described a more potato (earthy) odour and less flesh moisture of fish fed soybean oil containing diet when compared with fish fed 100% FO. However, it is difficult to compare these results among different species and different countries, since preferences for specific odour or flavour varies due to species and cultural reasons. Only fish fed the rapeseed oil containing diet showed a more yellowness flesh coloration when compared with fish fed the 100% fish oil diet. This difference was detected using a colorimeter, but no differences were found by the trained judges in terms of flesh colour, denoting that the differences in colour were not detected by human eye.

Although trained judges did not find significant differences in flesh quality, the nutritional quality of flesh changed when using vegetable oils in diets, since the relationship between n-6 and n-3 fatty acids changed. Increased dietary n-3 HUFA and decreased dietary n-6 produced some benefits in human health (Herold and Kinsella, 1986; Hwang, 1989), and the use of vegetable oils in fish diets alters the ratio of n-3/n-6. Fish fed soybean containing diets showed the lower n-3/n-6 ratio, and levels of n-3 HUFA were diminished in all vegetable oil treatments, decreasing the nutritional value of the fillets.

DHA levels recovered at the end of the re-feeding period with 100% FO, with all fish previously fed vegetable oils showing a similar DHA value than those fish continuously fed on FO diet. However, this recovery is slower than that shown by gilthead sea bream fed on similar diets (Izquierdo et al., in press), where DHA was recovered after a 60 day re-feeding period. Similar recovery has been described for turbot under similar feeding conditions (Regost et

al., 2003a). However, levels of EPA were not recovered in any of the experimental groups after 150 days, as described for gilthead sea bream (Izquierdo et al., in press). The percentage of EPA recovered at the end of the re-feeding period with fish oil was only 65–87% after 150 days, being these values quite similar to those values showed by gilthead sea bream after 104 days of re-feeding period (Izquierdo et al., in press). Regost et al. (2003a) also found incomplete recovery of EPA after 8 weeks of re-feeding period in turbot previously fed vegetable oil containing diets.

Besides, all fish previously fed vegetable oils showed higher values of LA in their fillets after the re-feeding period, with LA fillet contents of fish previously fed soybean oil more than 3 fold higher than those of fish fed fish oil even after 150 days. Gilthead sea bream in similar feeding conditions also showed 60% of LA remaining after a re-feeding period of 104 days (Izquierdo et al., in press). Similar results have been found for turbot fed a soybean oil containing diet after a re-feeding period of 8 weeks with a 100% FO diet, with values of LA higher in muscle phospholipids, denoting the poor utilization of this fatty acid even in a species able to elongate LA to 20:2n-6 (Regost et al., 2003a). Atlantic salmon fed on a rapeseed oil containing diet had higher LA levels after 12 weeks of re-feeding period with 100% FO diet (Bell et al., 2003).

Thus, the results of the re-feeding period showed that European sea bass has a lower capacity to use vegetable oils, as levels of n-3 HUFA recovered slower and LA remained higher when compared with gilthead sea bream (Izquierdo et al., in press). Special care must be taken in the use of vegetable oils in European sea bass feeds in order to keep the nutritional value of fillets similar to those from fish fed fish oil as unique lipid source.

In conclusion, soybean and linseed oils can be used at a 60% of fish oil substitution without affecting European sea bass growth and flesh quality, but rapeseed oil at this level of substitution decreased fish growth. However, the use of vegetable oils, and specially the use of soybean oil, alters the nutritional quality of European sea bass fillets, decreasing the levels of n-3 HUFA and increasing levels of C18 fatty acids. A re-feeding period of 150 days with 100% FO was enough to restore levels of DHA in fillets of fish fed vegetable oils previously, but levels of EPA were not fully recovered. Besides, after the re-feeding period, the amounts of C18 fatty acids remained higher in fillets from fish previously fed vegetable oil containing diets, and particularly LA in those fish fed previously soybean oil containing diet. Further experiments are required in the use of blends of vegetable oils to obtain the best dietary fatty acid profile to substitute 60% of fish oil.

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