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Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (Dicentrarchus labrax) juveniles: growth and fatty acid composition

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Abstract: European sea bass juveniles (14.4±0.1 g mean weight) were fed diets containing different levels of fish oil then of n-3 highly unsaturated fatty acids (n-3 HUFA) for 12 weeks. The fish performance as well as fatty acid (FA) composition of neutral and polar lipids from whole body after 7 and 12 weeks feeding were studied. The requirements of juvenile sea bass for n-3 highly unsaturated fatty acids (n-3 HUFA) were studied by feeding fish diets containing six different levels of n-3 HUFA ranging from 0.2% to 1.9% of the diet, with approximately the same DHA/EPA ratio (1.5:1).
The growth rate at the end of the trial showed significant differences. Fish fed low dietary n-3 HUFA (0.2% DM of the diet) showed significantly lower growth than the diet 3 (0.7%), then no further improvement (P>0.05) of growth performance was seen by elevating the n-3 HUFA level in the diet up to 1.9% (diet 6). No difference in feed efficiency, protein efficiency ratio or protein retention was observed among treatments, nor in protein and total lipid content. However, the n-3 HUFA levels in diets highly influenced fish fatty acid composition in neutral lipid, while polar lipid composition was less affected. Comparison of polar lipid content after 7 or 12 weeks indicated that DHA remained stable at the requirement level, while arachidonic acid decreased with time. Results of this experiment suggest that the requirement for growth of n-3 HUFA of juvenile sea bass of 14 g weight is at least 0.7% of the dry diet.

Keywords: European sea bass (Dicentrarchus labrax); Fatty acid composition; Growth; n-3 HUFA

1. Introduction

Lipids play an important role in fish nutrition for the provision of energy and essential fatty acids (EFA) (Sargent et al., 1989; Parpoura and Alexis, 2001). A considerable amount of research performed up to now demonstrates that marine species of finfish require n-3 highly unsaturated fatty acid (n-3 HUFA), specifically eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) for normal growth (New, 1986; Sargent et al., 1989, 1995; Bell et al., 1996).

The requirements of gilthead sea bream for n-3 HUFA have been investigated (Koven et al., 1992; Rodriguez et al., 1993, 1994; Ibeas et al., 1994). Kalogeropoulos et al. (1992) showed that the n-3 HUFA requirement in fingerlings sea bream (1 g weight) was 0.9% on a diet dry weight basis. Ibeas et al. (1994, 1996) have suggested that the requirement of juvenile gilthead sea bream for essential fatty acids were about 1% n-3 HUFA of the dry diet.

The fatty acid nutrition of European sea bass has been investigated for broodstock (Bell et al., 1997; Navas et al., 1997, 1998; Bruce et al., 1999) and larval stages (Navarro et al., 1995, 1997). Special consideration has been given to elucidate the effect that different dietary fatty acid compositions have on egg and larval composition and quality, nevertheless the lowest levels of n-3 fatty acids indispensable for adequate growth of sea bass has yet to be determined (Oliva-Teles, 2000; Parpoura and Alexis, 2001). The important increase in the global demand of fish oil and fish meal for animal consumption, together with the rather stable production of these products, is causing serious marketing problems and dependency. Thus, the quest for inclusion of alternative protein and lipid sources in marine fish diets is emerging as a priority in aquaculture research (Lodemel et

al., 2001; Ringo et al., 2002). In marine fish, partial substitution of fish oil by vegetable oils has been demonstrated in gilthead sea bream and European sea bass (Kalogeropoulos et al., 1992; Yildiz and Sener, 1997). Total replacement of fish oil by vegetable oil in a diet did not induce reduction in growth (Alexis, 1997) suggesting the minimum requirement for highly unsaturated fatty acids (HUFA) should be lower than the 0.9% DM provided by fish meal in this study. A growth reduction due to n-3HUFA deficiency may be expected when both vegetable oil and protein sources are simultaneously used in the diet.

The aim of the present study was to investigate the n-3 essential fatty acid requirement for growth of juvenile European sea bass and the effect of dietary levels on body composition. The minimal n-3 HUFA content, was obtain by using diets with the same protein source, low in fish meal and high in vegetable proteins, they differed by oil added in order to obtain a gradient of n-3 HUFA focused on expected minimal requirement for this species. This study use the conventional expression of n-3 requirement based on quantity of long chain (20-22 carbons) polyunsaturated fatty acid in % DM, however in order to take into account recent finding about essential fatty acid requirement (Sargent et al., 1999), diets were also designed in order to maintain similar DHA/EPA ratio.

2. Materials and methods

2.1. The fish and experimental conditions

The growth trial was conducted in the experimental facilities of IFREMER (Centre de Brest, France) for a period of 12 weeks. A total of 576 European sea bass (*Dicentrarchus labrax*) juveniles, of 14.4 g ± 0.1 mean initial body weight obtained from a commercial fish farm (Gravelines, France), were randomly distributed in 18 tanks of 75 l

(32 fish per tank). The water temperature during the experiment ranged between 21 and 22 °C and flow rate was 0.6 l min^{-1}. A 12/12 h light/dark cycle was adopted. The six experimental diets were randomly allotted in triplicate. Fish were fed by hand to visual satiety in four meals per day, feed intake was recorded. The fish were acclimated to the experimental conditions and fed a commercial fish feed for 2 weeks prior to starting the experiment. Each group was weighed every 2 weeks to follow growth and feed utilization. At day 52 (period 1), 16 fish per tank were removed (used for analysis). The remaining weighted fish were grown in the same tanks and same conditions until day 81 (period 2). Growth, expressed as the mean daily growth index (DGI) according to Kim and Kaushik (1992) was determined as:

$$DGI = \frac{(W_f^{1/3} - W_i^{1/3})}{Day}$$

Where W_f is the final mean weight and W_i the initial mean weight. Here two periods were pooled from day one to day 52 (period 1) and day 52 to day 81 (period 2), numerator of the previous formula was then :

$(W_{f1}^{1/3} - W_{i1}^{1/3}) + (W_{f2}^{1/3} - W_{i2}^{1/3})$

2.2. Experimental diets

Six experimental diets were formulated to be isoproteic and isolipidic containing 50% crude protein and 16% crude lipid. While the first diet (diet 1) contained rapeseed oil as the only added lipid source, in all the other diets rapeseed oil was partly replaced by fish oil. The fish oil was a blend of 85% cod liver oil and 15% EPAX 20/50 (20% EPA + 50% DHA) in order to have the DHA/EPA ratio near 1.5:1. Ingredient and chemical composition

of the diets are reported in Table 1 and fatty acid (FA) composition in Table 2. The major protein source was of vegetable origin (lupin meal, corn gluten) as a way to reduce n-3 HUFA content in the basal diet, and then to obtained a minimal level of HUFA n-3 near 0.2% DM in diet 1.

Table 1. Formulation (g /100 g diet) and chemical composition (% DM) of the experimental diets.

	Experimental diets					
	1	2	3	4	5	6
Ingredients[a] *(g 100 g^{-1})*						
Fish meal Norse LT 90	12.0	12.0	12.0	12.0	12.0	12.0
Fish hydrolysate GPSP-90	3.0	3.0	3.0	3.0	3.0	3.0
Lupin meal	52.0	52.0	52.0	52.0	52.0	52.0
Corn gluten	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin mixture[b]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mixture[c]	1.0	1.0	1.0	1.0	1.0	1.0
Dextrine	1.5	1.5	1.5	1.5	1.5	1.5
Betaine	0.5	0.5	0.5	0.5	0.5	0.5
D-L-Methionine	0.2	0.2	0.2	0.2	0.2	0.2
Marine fish oil[d]	0	1.0	1.6	2.1	2.7	6.0
Vegetable oil[e]	8.8	7.8	7.2	6.7	6.1	2.8
Chemical composition						
Dry matter (%)	92.1	91.0	91.8	91.5	91.5	93.2
Crude protein (% DM)	53.3	54.1	53.9	54.0	54.2	53.4
Crude fat (% DM)	18.1	18.1	18.2	18.2	17.9	17.0
Ash (% DM)	5.0	5.0	5.0	5.0	4.9	4.9
Nitrogen-Free Extract (%)	23.3	22.7	23.0	22.6	23.1	24.4
n-3 HUFA (% dry weight)	0.23	0.56	0.72	0.86	1.01	1.89

[a] Sources: fish meal and cod liver oil: La Lorientaise (Lorient, France); fish protein hydrolysates: Sopropêche (Boulogne sur mer, France); rapessed oil: Associated oil Packers, France.

[b] Vitamin mixture (g kg^{-1} vitamin mix): retinyl acetate, 1; cholecalciferol, 2.5; DL-α-tocopheryl acetate, 5; menadione, 1; thiamin–HCl, 0.1; riboflavin, 0.4; D-calcium panthothenate, 2; pyridoxine–HCl, 0.3; cyanocobalamin, 1; niacin, 1; choline, 200; ascorbic acid (ascorbyl polyphosphate), 5; folic acid, 0.1; D-biotin, 1; meso-inositol, 30. All ingredients were diluted with α-cellulose.

[c] Mineral mixture (g kg^{-1} mineral mix): KCl, 90; KI, 0.04; $CaHPO_4 \cdot 2H_2O$, 500; NaCl, 40; $CuSO_4 \cdot 5H_2O$, 3; $ZnSO_4 \cdot 7H_2O$, 4; $CoSO_4$, 0.02; $FeSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot H_2O$, 3; $CaCO_3$, 215; MgOH, 124; Na_2SeO_3, 0.03; NaF, 1.

[d] lipid mixture containing 80% of marine fish oil (cod liver oil) and 15% of EPAX (triacylglycerol mixture containing 70% of n-3HUFA).

[e] vegetable oil: rapeseed oil

Table 2

Composition of dietary fatty acids (in % total fatty acid methyl esters).

	Experimental diets					
FA %	1	2	3	4	5	6
Σ saturates	11.6	12.5	12.7	13.4	13.9	15.6
14:0	0.3	0.6	0.7	1.0	1.0	1.8
16:0	6.8	7.3	7.5	7.8	8.2	9.4
18:0	1.8	1.8	1.8	1.8	1.9	1.9
20:0	0.7	0.9	0.9	0.9	0.8	0.6
22:0	1.5	1.6	1.5	1.5	1.5	1.5
Σ monoenes	58.8	57.0	56.0	55.4	54.5	49.6
16:1n-7	0.5	0.8	1.0	1.1	1.3	2.3
18:1n-7	2.6	2.6	2.6	2.6	2.5	2.5
20:1n-7	0.1	0.1	0.1	0.1	0.1	0.2
18:1n-9	50.6	47.8	46.4	45.2	43.8	35.7
20:1n-9	3.0	3.3	3.4	3.5	3.7	4.5
22:1n-9	0.9	1.0	1.0	1.0	1.0	1.1
24:1n-9	0.2	0.2	0.2	0.3	0.3	0.3
20:1n-11	0.1	0.1	0.2	0.2	0.2	0.4
22:1n-11	0.7	0.9	1.1	1.3	1.5	2.5
Σ n-6	20.0	19.1	18.8	18.1	17.7	15.1
18:2n-6	19.6	18.7	18.3	17.6	17.2	14.4
18:3n-6	0.0	0.1	0.1	0.1	0.1	0.1
20:2n-6	0.2	0.2	0.2	0.2	0.3	0.3
20:3n-6	0.0	0.0	0.0	0.0	0.0	0.1
20:4n-6	0.1	0.1	0.1	0.1	0.2	0.3
Σ n-3	9.6	11.4	12.4	13.1	14.0	19.7
18:3n-3	7.9	7.4	7.3	7.1	6.8	5.6
18:4n-3	0.1	0.3	0.3	0.4	0.5	0.9
20:3n-3	0.0	0.0	0.1	0.1	0.1	0.1
20:4n-3	0.0	0.1	0.1	0.2	0.2	0.4
20:5n-3	0.5	1.4	1.7	2.1	2.4	4.7
22:5n-3	0.1	0.2	0.3	0.4	0.4	0.9
22:6n-3	0.9	1.9	2.5	3.0	3.6	7.0
DHA/EPA	1.7	1.4	1.5	1.4	1.5	1.5

2.3. Sampling

At the beginning of the growth trial, 15 fish from an initial pool of fish were sampled and frozen for analysis of whole body composition. At the end of the growth trial, the same protocol of slaughter was followed for each tank. Fish were fasted 24 h before slaughter. Five fish per tank were removed for comparative carcass analyses (moisture, protein and fat) and protein retention calculation.

2.4. Analytical methods

The fish for whole body composition were ground frozen and a representative portion was homogenised before analysis. Composition analyses of diets and chemical composition of whole body were made following standard methods (AOAC, 1984): dry matter after desiccation in an oven (105 °C for 12 h), crude protein (Dumas, Nitrogen Analyser, Fison instrument, N x 6.25). For lipid analyses of whole body, extraction was done according to Folch et al. (1957), with chloroform being replaced by dichloro-methane.

The separation of neutral lipids and phospholipids was performed according to the procedure described by Juaneda and Rocquelin (1985). The total lipid extracts were fractionated on silica cartridges (Sep-Pack, Waters); neutral lipids were eluted by chloroform and phospholipids by methanol. Fatty acids methyl esters (FAME) were prepared following the methods of Morrison and Smith (1964) and separated by gas chromatography (Auto-system Perkin-Elmer with a flame ionisation detector, BPX 70 capillary column: 25 m x 0.22 mm i.d. x 0.25 μm film thickness; split-splitless injector, with helium as carrier gas). The injector and detector temperatures were, respectively, 220 and 260 °C. Initial temperature of the oven was 50 °C, which increased to 180 °C by increments of 15 °C/min, maintained for 5 min, then increased to 220 °C by increments of 3 °C/min. Data acquisition and handling were carried out by connecting the GLC to a PE Nelson computer. The individual fatty acid methyl esters were identified by comparing the retention times of authentic standard mixtures. The results of individual FA composition were expressed as percent of total identified FA methyl esters.

2.5. Statistical analysis

All data were subjected to one-way analysis of variance to test the effects of experimental diets. Where significant ($P<0.05$) differences were detected, Duncan's multiple-range test was used to rank the groups. The data are presented as mean ± S.E. of the replicate groups. All statistical analyses were made using the SPSS 11.5 for Windows. Comparison of composition between the two periods was performed using a 2 way ANOVA.

3. Results

Survival was very high in all treatments, only one fish died during the first period (diet 4), another during the second period (diet 3), such mortality could not be related to dietary treatment. No difference in weight, feed efficiency protein efficiency ratio or protein retention was observed among treatments at the end of the first period (Table 3). During the second period significant differences occurred in weight. Considering the experimental design, split in two periods by an intermediate sampling, the best way to express growth is the DGI (calculated for both periods). The curve presenting these values in response to dietary n-3 HUFA (Fig. 1) was plotted in order to define requirement (Zeitoun et al., 1975). The broken line analysis seems here the best procedure and let to define the requirement at 0.70% DM n-3 HUFA. The sea bass fed diet 1 (0.2% n-3 HUFA) had the lowest growth, which did not significantly differ from those fed diet 2 but were significantly lower than those fed diets 3, 4, 5 and 6. External signs of abnormality were not observed among the groups during the feeding trial. The body composition of fish fed diets with different n-3 HUFA levels is shown in Table 4. No significant differences in the percentage of moisture,

protein and lipid were found among treatments. On the other hand, after 7 weeks of experiment, the fatty acid composition of fish showed pronounced change from the initial values.

Figure 1 : Daily growth index (DGI, %.d^{-1}) of fish fed the experimental treatments.
Symbols having different letters indicate that treatments are significantly different (P <0.05).

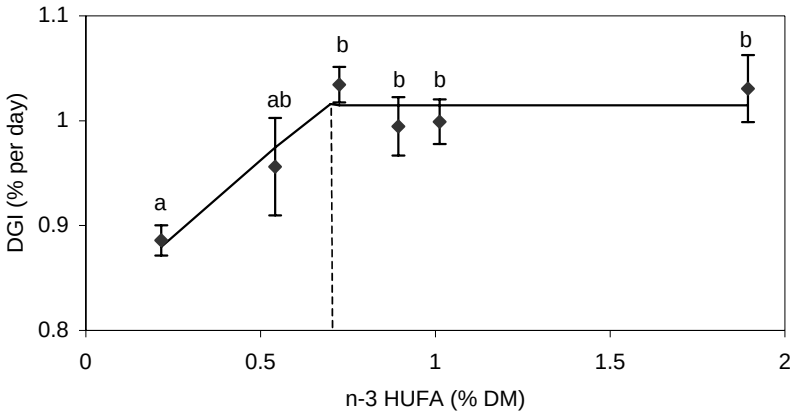

Table 3. Growth rate, feed efficiency and protein utilization of European sea bass fed diets with different levels of n-3 HUFA

	Diet no.					
	1	2	3	4	5	6
Period 1						
Survival (%)	100	100	100	98	100	100
Initial weight (g)	14.4±0.1	14.4±0.1	14.3±0.1	14.3±0.1	14.3±0.1	14.4±0.1
Final weight (g)	24.6±0.2	26.1±0.6	26.2±0.2	26.1±0.3	26.2±0.4	26.9±0.7
Weight increase (%)	70±3	81±6	84±2	82±1	83±4	87±4
Feed efficiency	0.61±0.02	0.69±0.06	0.68±0.01	0.65±0.01	0.67±0.04	0.70±0.04
Protein efficiency Ratio	1.14±0.05	1.27±0.12	1.25±0.03	1.21±0.02	1.25±0.08	1.31±0.08
Protein retention (%)	20.0±0.8	22.1±2.0	22.1±0.5	21.6±0.3	21.6±1.4	23.5±1.4
Period 2						
Survival (%)	100	94	98	100	100	100
Initial weight (g)	24.5±0.2^{a}	26.3±0.6^{b}	26.1±0.4^{b}	27.1±0.4^{b}	26.3±0.6^{b}	26.6±0.5^{b}
Final weight (g)	31.8±0.2^{a}	33.5±1.1^{ab}	34.9±0.6^{b}	35.5±0.6^{b}	34.6±0.7^{b}	35.1±1.0^{b}
Weight increase (%)	29.5±0.3	28.5±1.9	33.4±0.5	31.0±1.9	31.7±0.5	32.0±1.0

Values are means ± standard error (n=3), values in rows having different letters indicate that treatments are significantly different (P < 0.05).

Table 4. Whole body composition (% wet weight basis), viscerosomatic (VSI) index, nutrient and energy retention (% intake) of sea bass at beginning and the end of the two periods

		Experimental diets					
	initial	1	2	3	4	5	6
Period 1							
Moisture	70.3	66.4± 0.3	65.2 ± 0.4	65.4 ± 0.2	65.6 ± 0.2	65.8 ± 0.3	64.8 ± 0.1
Protein	17.9	17.8 ± 0.1	17.7 ± 0.4	17.8 ± 0.2	17.9 ± 0.1	17.6 ± 0.3	17.9 ± 0.3
Fat	8.1	13.1 ± 0.4	14.5 ± 0.5	13.9 ± 0.5	14.0 ± 0.3	13.6 ± 0.3	14.7 ± 0.2
VSI	--	10.4 ± 0.3	11.0 ± 0.5	10.5 ± 0.5	11.6 ± 0.1	10.6 ± 0.2	10.5 ± 0.3
Period 2							
Moisture		66.0± 0.4	65.2 ± 0.2	64.8 ± 0.3	65.3 ± 0.4	65.5 ± 0.8	65.8 ± 0.1
Protein		16.3 ± 0.3	17.0 ± 0.2	17.5 ± 0.2	17.4 ± 0.1	17.0 ± 0.2	17.1 ± 0.3
Fat		13.9 ± 0.4	14.3 ± 0.3	14.4 ± 0.2	13.6 ± 0.5	14.1 ± 0.5	13.8 ± 0.1

Values are means ± standard error (n=3 except for VSI where n= 18). All values were not significantly different (P>0.05).

The composition of fatty acids of the neutral lipids of carcass is shown in Table 5 for period 1. Compared to the initial sample, 18:1n-9 and 18:2n-6, the major dietary components gradually increased from diet 6 to diet 1 according to quantities of vegetable oil added and induced significant differences between treatments, and the same evolution was also observed concerning 18:3n-3. In contrast, fatty acids of marine origin (DHA, EPA and 22:1n-11) decreased compared to initial fish with a gradient from treatment 6 to treatment 1 according to the level of fish oil in the various diets. At day 81 (Table 6) the same tendencies were observed, with significant differences occurring according to dietary components. The impact of dietary fatty acid pattern was stronger than at the end of period 1, within each treatment a decrease of n-3 HUFA and an increase of 18:1n-9 and 18:2n-6 occurred. In fish fed diet 6 the level of DHA was close to the corresponding dietary level, and the level of EPA was lower than in the diet, while for other treatments DHA and EPA remained higher than their corresponding proportion in diets, but clearly lower than in initial fish. Arachidonic acid was very low in all treatments compare to initial diet, but a

little higher in treatment 6 than in others. The 18:3n-6 and 20:2n-6 are minor fatty acids, but encountered at relatively higher level than in the diets, and differences between treatments did not correspond to their dietary levels.

Table 5. Fatty acid composition of neutral lipids from carcass (area %). Results of 7-weeks feeding trial (period 1).

Fatty acids (FA)	Diet no.						
	1	2	3	4	5	6	initial
Σ saturates	17.1±0.7^{a}	18.6±0.1^{b}	18.7±0.2^{b}	18.5±0.1^{b}	18.8±0.2^{b}	20.6±0.1^{c}	24.9±0.1
14:0	1.76±0.04^{a}	1.80±0.04^{a}	1.78±0.04^{a}	1.83±0.01^{a}	1.97±0.08^{b}	2.31±0.04^{c}	4.47±0.01
16:0	11.5±0.4^{a}	12.7±0.1^{b}	12.7±0.2^{b}	12.6±0.1^{b}	12.8±0.2^{b}	14.2±0.1^{c}	16.8±0.1
18:0	2.9±0.2	3.1±0.0	3.1±0.0	2.9±0.0	2.9±0.0	3.1±0.0	3.3±0.0
20:0	0.5±0.0	0.5±0.0	0.4±0.0	0.5±0.0	0.4±0.0	0.4±0.0	0.2±0.0
22:0	0.5±0.0	0.5±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.1±0.0
Σ monoenes	54.6±0.2^{e}	54.0±0.1^{d}	53.7±0.2^{d}	53.1±0.2^{c}	52.6±0.2^{b}	49.6±0.1^{a}	41.6±0.3
16:1n-7	3.0±0.1^{a}	3.2±0.1^{ab}	3.2±0.1^{ab}	3.2±0.1^{ab}	3.4±0.1^{b}	4.1±0.1^{c}	6.1±0.0
18:1n-7	3.1±0.0	3.1±0.0	3.1±0.0	3.1±0.0	3.1±0.0	3.1±0.0	3.5±0.0
18:1n-9	41.3±0.3^{e}	40.2±0.1^{d}	39.9±0.3^{d}	38.9±0.3^{c}	38.1±0.3^{b}	33.3±0.1^{a}	19.0±0.1
20:1n-9	3.99±0.04^{a}	4.12±0.09^{ab}	4.23±0.07^{b}	4.19±0.06^{b}	4.31±0.06^{b}	4.57±0.04^{c}	5.08±0.02
24:1n-9	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.0	0.7±0.0
22:1n-11	1.52±0.06^{a}	1.64±0.02^{b}	1.68±0.03^{b}	1.79±0.04^{c}	1.88±0.02^{c}	2.36±0.02^{d}	4.52±0.06
Σ n-6	15.7±0.4^{c}	14.5±0.2^{b}	14.5±0.2^{b}	14.5±0.1^{b}	14.2±0.2^{b}	12.0±0.1^{a}	8.0±0.1
18:2n-6	14.2±0.4^{c}	13.2±0.1^{b}	13.1±0.2^{b}	13.1±0.1^{b}	12.9±0.2^{b}	10.6±0.1^{a}	6.0±0.1
18:3n-6	0.6±0.1	0.5±0.0	0.4±0.0	0.5±0.1	0.4±0.0	0.3±0.0	0.3±0.0
20:2n-6	0.50±0.00^{a}	0.50±0.01^{a}	0.52±0.01^{ab}	0.51±0.01^{ab}	0.54±0.00^{b}	0.53±0.01^{b}	0.52±0.01
20:3n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0
20:4n-6	0.19±0.01^{a}	0.19±0.01^{a}	0.19±0.01^{a}	0.19±0.01^{a}	0.22±0.01^{a}	0.28±0.00^{b}	0.63±0.03
Σ n-3	12.6±0.3^{a}	12.9±0.1^{ab}	13.2±0.2^{b}	13.9±0.2^{c}	14.4±0.2^{d}	17.9±0.1^{e}	25.5±0.3
18:3n-3	4.99±0.16^{b}	4.65±0.05^{b}	4.73±0.12^{b}	4.74±0.10^{b}	4.59±0.09^{b}	3.74±0.05^{a}	1.38±0.03
Σ n-3 HUFA	6.5±0.2^{a}	7.2±0.1^{b}	7.4±0.3^{b}	8.0±0.2^{c}	8.7±0.2^{d}	12.6±0.1^{e}	21.3±0.2
20:5n-3	2.37±0.07^{a}	2.56±0.04^{b}	2.65±0.08^{b}	2.84±0.04^{c}	3.07±0.06^{d}	4.38±0.05^{e}	7.64±0.03
22:5n-3	0.46±0.01^{a}	0.49±0.01^{ab}	0.54±0.01^{b}	0.59±0.02^{c}	0.66±0.02^{d}	0.98±0.02^{e}	1.51±0.02
22:6n-3	3.66±0.11^{a}	4.09±0.04^{b}	4.23±0.16^{b}	4.57±0.09^{c}	4.92±0.12^{d}	7.23±0.07^{e}	12.14±0.17
DHA/EPA	1.54±0.01^{a}	1.60±0.01^{b}	1.59±0.01^{b}	1.61±0.01^{bc}	1.60±0.02^{b}	1.65±0.01^{c}	1.59±0.02

Values are means ± standard error (*n=6*). Numbers in rows having different letters indicate that treatments are significantly different at $P < 0.05$.

Table 6. Fatty acid composition of neutral lipids from carcass (area %). Results of 13-week feeding trial (period 2), and comparison with results from the first period by a 2 way ANOVA.

Fatty acids (FA)	Diet no.						2 way ANOVA		
	1	2	3	4	5	6	Diet	Period	Interaction
Σ saturates	18.9±0.2^{a}	17.7±0.1^{b}	17.9±0.1^{bc}	17.9±0.1^{bc}	18.4±0.1^{c}	19.6±0.4^{d}	***	***	ns
14:0	1.49±0.04^{a}	1.62±0.03^{bc}	1.68±0.04^{c}	1.55±0.02^{ab}	1.70±0.03^{c}	2.16±0.05^{d}	***	***	ns
16:0	11.5±0.2^{a}	12.2±0.0^{b}	12.3±0.1^{b}	12.4±0.1^{b}	12.7±0.1^{b}	13.6±0.3^{c}	***	***	ns
18:0	2.86±0.03^{a}	2.88±0.04^{a}	2.96±0.03^{ab}	2.96±0.02^{ab}	3.04±0.02^{b}	2.96±0.07^{ab}	*	ns	ns
20:0	0.48±0.02^{c}	0.42±0.01^{bc}	0.41±0.01^{ab}	0.45±0.00^{bc}	0.49±0.03^{c}	0.36±0.01^{a}	***	ns	ns
22:0	0.54±0.02	0.53±0.01	0.56±0.02	0.6±0.0	0.6±0.0	0.5±0.0	ns	ns	ns
Σ monenes.	56.1±0.1^{e}	55.0±0.2^{d}	54.7±0.1^{cd}	54.0±0.1^{c}	53.4±0.1^{b}	50.1±0.1^{a}	***	***	**
16:1n-7	2.51±0.10^{a}	2.83±0.07^{b}	2.91±0.04^{bc}	2.83±0.04^{b}	3.06±0.03^{c}	3.85±0.06^{d}	***	***	ns
18:1n-7	3.06±0.02	3.02±0.02	3.07±0.02	3.02±0.02	3.09±0.02	3.04±0.01	ns	***	*
18:1n-9	44.1±0.3^{ab}	42.4±0.3^{ab}	41.6±0.1^{ab}	41.7±0.1^{a}	40.0±0.2^{ab}	34.7±0.2^{b}	***	***	**
20:1n-9	3.98±0.03^{a}	3.93±0.07^{a}	4.11±0.08^{ab}	3.37±0.68^{bc}	4.15±0.07^{ab}	4.47±0.04^{c}	*	ns	ns
22:1n-9	0.70±0.01^{a}	0.70±0.01^{a}	0.72±0.01^{ab}	0.75±0.01^{bc}	0.73±0.01^{b}	0.77±0.01^{c}	ns	***	ns
22:1n-11	1.16±0.04^{a}	1.31±0.04^{b}	1.40±0.02^{b}	1.36±0.03^{b}	1.52±0.03^{c}	2.07±0.04^{d}	***	***	ns
Σ n-6	16.2±0.3^{d}	15.5±0.1^{c}	15.1±0.1^{bc}	15.3±0.1^{c}	14.8±0.1^{b}	12.8±0.2^{a}	***	***	ns
18:2n-6	14.9±0.3^{d}	14.3±0.1^{c}	13.9±0.1^{c}	14.1±0.1^{c}	13.4±0.1^{b}	11.5±0.2^{a}	***	***	ns
18:3n-6	0.49±0.03^{c}	0.39±0.02^{abc}	0.41±0.04^{bc}	0.37±0.04^{ab}	0.48±0.04^{c}	0.31±0.02^{a}	**	ns	ns
20:2n-6	0.49±0.00^{a}	0.51±0.01^{a}	0.53±0.01^{b}	0.54±0.00^{b}	0.54±0.01^{b}	0.54±0.00^{b}	***	ns	ns
20:3n-6	0.07±0.02	0.08±0.01	0.07±0.01	0.05±0.01	0.06±0.00	0.09±0.01	ns	**	ns
20:4n-6	0.17±0.01^{ab}	0.18±0.01^{ab}	0.18±0.00^{ab}	0.16±0.01^{a}	0.19±0.01^{b}	0.27±0.01^{c}	***	***	ns
Σ n-3	10.8±0.2^{a}	11.9±0.2^{b}	12.3±0.1^{b}	12.3±0.1^{b}	13.4±0.1^{c}	17.4±0.2^{d}	***	***	**
18:3n-3	5.30±0.12^{d}	5.10±0.03^{d}	4.94±0.04^{bc}	5.08±0.05^{cd}	4.82±0.07^{b}	4.04±0.08^{a}	***	***	ns
Σ n-3 HUFA	4.6±0.3^{a}	5.8±0.2^{b}	6.4±0.1^{c}	6.3±0.1^{bc}	7.5±0.1^{d}	12.0±0.1^{e}	***	***	**
20:5n-3	1.74±0.09^{a}	2.13±0.07^{b}	2.29±0.02^{b}	2.27±0.03^{b}	2.66±0.03^{c}	4.13±0.06^{d}	***	***	*
22:5n-3	0.32±0.03^{a}	0.41±0.02^{b}	0.46±0.00^{c}	0.47±0.01^{c}	0.57±0.01^{d}	0.93±0.02^{e}	***	***	ns
22:6n-3	2.57±0.14^{a}	3.28±0.11^{b}	3.62±0.06^{c}	3.53±0.07^{bc}	4.27±0.10^{d}	6.93±0.09^{e}	***	***	**
DHA/EPA	1.48±0.01^{a}	1.54±0.02^{b}	1.57±0.02^{bc}	1.56±0.02^{bc}	1.60±0.02^{c}	1.68±0.01^{d}	***	**	*

Values are means ± standard error (*n=6*). Numbers in rows having different letters indicate that treatments are significantly. Different ($P < 0.05$). Influence of factors in 2 way ANOVA :* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

The fatty acid composition of the polar lipids is shown in Table 7 and 8 (period 1 and 2, respectively). Similar to the neutral lipids, in the initial sample, 16:0, 18:1n-9, 20:5n-3 and 22:6n-3 were the most abundant saturated, monounsaturated and polyunsaturated fatty acids, respectively. The 16:0 content was more abundant in the polar fraction than in the neutral fraction, while 18:1n-9 and 18:2n-6 were lower. The same tendencies were observed in fish under the various treatment, despite a marked influence of dietary fatty acid, less differences were observed than in neutral lipids. The n-3 HUFA content was higher in the polar fraction than in the neutral fraction. Compared to initial fish, DHA clearly decreased for animals fed on diet 6, and then more slightly for the other treatments according to dietary gradient. However DHA remained a major fatty acid of polar lipids even with treatment 1. The DHA/EPA ratio of the polar fraction (about 3:1) was generally higher than the neutral fraction (1.5:1). In contrast to neutral lipid, the highest DHA/EPA ratio in fish polar lipids was induced by diet 1 and the lowest by diet 6, as DHA decreased more slowly than EPA from fish fed diets 6 to 1. AA (20:4n-6) was represented in polar lipids about 10 times more than in neutral lipids, with significant differences according to the fish oil levels in diets. Significant differences occurred in minor n-6 fatty acids with higher levels of 18:3n-6 and 20:3n-6 (only after 7 weeks for this later) in treatment 1 than treatment 6, not related to their respective levels in diets. The fatty acids profiles of polar lipid of fish fed diets 3 to 5, were very similar, except 18:2n-6 and 20:5n-3 presenting a significant differences between diets 3 and 4, 5. Likewise, no other significant differences were found, with remarkable constancy of DHA and AA, particularly for analysis performed at day 81 (Table 8). The fatty acid composition of polar lipids further evolved during period 2 (Table 8 compared to Table 7), relatively less than in neutral lipids; only 55% of the identified fatty acids displayed significant change between the two periods in

the 2 way ANOVA for polar lipids compare to 70% in neutral lipids. As for neutral lipids observed differences in fish polar lipids corresponding to the gradients of fatty acids in respective diets, were increased at the end of the second period. DHA content decreased in polar lipid of fish fed diet 1 but seemed stabilized in other treatments, giving no significant change in the 2 way ANOVA between the two periods. However other essential fatty acids as AA and EPA decreased respectively around 16% and 5% (in mean for all treatments) during the second period.

Table 7. Fatty acid composition of polar lipids from carcass (area %). Results of 7-week feeding trial (period 1).

Fatty acids (FA)	Diet no.						
	1	2	3	4	5	6	initial
Σ saturates	26.1±0.9^{a}	28.5±0.3^{bc}	28.4±0.3^{bc}	27.1±0.6^{ab}	28.8±0.3^{bc}	29.5±0.7^{c}	30.7±0.3
14:0	0.7±0.1	1.0±0.2	1.0±0.3	0.7±0.3	1.1±0.4	0.8±0.2	2.0±0.3
16:0	16.7±0.5^{a}	17.8±0.3^{b}	17.7±0.5^{ab}	17.4±0.9^{ab}	18.1±0.7^{bc}	19.0±0.9^{c}	20.2±0.1
18:0	8.1±0.4^{a}	9.0±0.1^{bc}	9.0±0.5^{bc}	8.3±0.7^{ab}	9.0±0.3^{bc}	9.2±1.0^{c}	8.8±0.1
20:0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.1	0.4±0.0	0.3±0.1	0.2±0.0
22:0	0.2±0.1	0.3±0.0	0.3±0.0	0.3±0.1	0.3±0.0	0.3±0.1	0.1±0.0
Σ monenes.	30.0±0.2^{c}	29.2±0.3^{c}	28.3±0.7^{abc}	28.8±0.8^{bc}	26.9±0.4^{ab}	26.5±1.2^{a}	20.4±0.5
16:1n-7	1.3±0.1^{ab}	1.5±0.1^{bc}	1.4±0.3^{bc}	1.5±0.2^{bc}	1.1±0.1^{a}	1.7±0.4^{c}	2.0±0.1
18:1n-7	2.4±0.1	2.3±0.0	2.3±0.1	2.3±0.1	2.3±0.1	2.4±0.1	2.4±0.0
18:1n-9	23.4±0.3^{c}	22.3±0.2^{bc}	21.7±1.2^{b}	22.0±1.5^{bc}	20.2±0.6^{ab}	19.2±1.9^{a}	12.3±0.3
20:1n-9	1.8±0.0	1.8±0.1	1.8±0.2	1.9±0.2	1.8±0.1	1.9±0.3	1.1±0.4
24:1n-9	0.6±0.1^{ab}	0.7±0.1^{b}	0.5±0.1^{a}	0.6±0.1 ^{ab}	0.7±0.1^{b}	0.6±0.1^{ab}	0.6±0.0
22:1n-11	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.1^{a}	0.2±0.1^{a}	0.2±0.1^{a}	0.4±0.1^{b}	0.4±0.2
Σ n-6	15.7±0.2^{e}	13.4±0.0^{d}	12.7±0.2^{c}	12.2±0.2^{c}	11.5±0.1^{b}	9.3±0.3^{a}	6.3±0.0
18:2n-6	12.0±0.2^{e}	9.9±0.0^{d}	9.2±0.6^{c}	8.6±0.5^{c}	7.7±0.2^{b}	5.7±0.8^{a}	2.3±0.0
18:3n-6	0.59±0.03^{c}	0.42±0.02^{b}	0.38±0.02^{b}	0.37±0.03^{b}	0.33±0.02^{b}	0.21±0.05^{a}	0.20±0.07
20:2n-6	0.7±0.0	0.7±0.0	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.4±0.0
20:3n-6	0.27±0.01^{c}	0.23±0.03^{c}	0.19±0.01^{abc}	0.20±0.01^{abc}	0.19±0.01^{ab}	0.13±0.04^{a}	0.12±0.02
20:4n-6	1.6±0.0^{a}	1.6±0.0^{a}	1.7±0.1^{ab}	1.8±0.1^{b}	1.8±0.1^{b}	2.0±0.2^{c}	2.5±0.0
Σ n-3	28.2±0.6^{a}	29.0±0.5^{ab}	30.7±0.5^{bc}	31.9±0.6^{c}	32.9±0.5^{cd}	34.6±1.4^{d}	42.6±0.8
18:3n-3	2.9±0.1^{d}	2.4±0.1^{c}	2.2±0.2^{c}	2.3±0.2^{c}	1.8±0.1^{b}	1.5±0.3^{a}	0.4±0.0
Σ n-3 HUFA	24.7±0.5^{a}	25.9±0.5^{ab}	27.9±0.6^{bc}	29.2±0.7^{cd}	30.4±0.5^{de}	32.5±1.5^{e}	41.5±0.8
20:5n-3	5.1±0.1^{a}	5.9±0.1^{b}	6.5±0.4^{c}	6.8±0.3^{cd}	7.2±0.2^{d}	8.4±0.7^{e}	9.6±0.1
22:5n-3	0.85±0.04^{a}	0.93±0.04^{ab}	0.97±0.02^{b}	1.00±0.03^{bc}	1.01±0.05^{bc}	1.09±0.03^{c}	1.25±0.03
22:6n-3	18.7±0.4^{a}	19.1±0.4^{a}	20.4±1.1^{ab}	21.3±1.5^{bc}	22.4±1.1^{bc}	22.9±3.1^{c}	30.7±0.7
DHA/EPA	3.65±0.06^{c}	3.21±0.04^{b}	3.13±0.03^{b}	3.13±0.05^{b}	3.10±0.02^{b}	2.71±0.08^{a}	3.20±0.05

Values are means ± standard error (*n=7*). Numbers in rows having different letters indicate that treatments are significantly different at $P < 0.05$.

Table 8. Fatty acid composition of polar lipids from carcass (area %). Results of 13-week feeding trial (period 2), and comparison with results from the first period by a 2 way ANOVA.

Fatty acids (FA)	Diet no.						2 way ANOVA		
	1	2	3	4	5	6	Diet	Period	Interaction
Σ saturates	25.4±0.3^{a}	26.0±0.1^{a}	25.8±0.4^{a}	26.2±0.5^{a}	25.7±0.4^{a}	27.8±0.5^{b}	***	***	ns
14:0	1.0±0.3	0.7±0.1	0.6±0.1	0.7±0.1	0.7±0.1	1.2±0.2	ns	ns	*
16:0	15.7±0.2^{a}	16.3±0.2^{a}	16.1±0.1^{a}	16.3±0.4^{a}	16.2±0.2^{a}	17.2±0.2^{b}	***	***	ns
18:0	8.0±0.2	8.3±0.1	8.3±0.4	8.4±0.3	8.1±0.2	8.8±0.2	*	**	ns
20:0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.3±0.0	*	ns	ns
22:0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	ns	*	ns
Σ monenes.	30.9±0.6^{c}	29.7±0.2^{bc}	28.5±0.8^{b}	28.1±0.2^{b}	28.1±0.7^{b}	26.3±0.4^{a}	***	ns	ns
16:1n-7	1.1±0.1^{a}	1.5±0.0^{a}	1.4±0.1^{a}	1.5±0.2^{a}	1.5±0.1^{a}	1.9±0.1^{b}	***	ns	ns
18:1n-7	2.2±0.0	2.2±0.0	2.2±0.0	2.1±0.1	2.1±0.1	2.1±0.0	ns	***	ns
18:1n-9	24.5±0.5^{d}	22.9±0.2^{c}	21.9±0.7^{bc}	20.9±0.3^{b}	21.2±0.5^{bc}	18.5±0.4^{a}	***	ns	ns
20:1n-9	1.7±0.0	1.7±0.0	1.7±0.1	1.7±0.1	1.7±0.1	1.8±0.0	ns	**	ns
24:1n-9	0.8±0.1	0.8±0.1	0.7±0.0	0.8±0.1	0.7±0.0	0.9±0.1	ns	***	ns
22:1n-11	0.1±0.0	0.2±0.0	0.1±0.1	0.3±0.1	0.2±0.0	0.3±0.0	***	ns	ns
Σ n-6	17.1±0.1^{e}	14.5±0.1^{d}	13.5±0.2^{c}	12.6±0.2^{b}	12.3±0.2^{b}	9.6±0.1^{a}	***	***	*
18:2n-6	13.8±0.1^{e}	11.6±0.1^{d}	10.3±0.2^{c}	9.5±0.2^{b}	9.1±0.2^{b}	6.1±0.1^{a}	***	***	**
18:3n-6	0.6±0.0^{b}	0.4±0.0^{a}	0.4±0.0^{a}	0.4±0.0^{a}	0.4±0.0^{a}	0.3±0.0^{a}	***	ns	ns
20:2n-6	0.8±0.0	0.7±0.0	0.8±0.1	0.7±0.0	0.7±0.0	0.6±0.0	**	***	ns
20:3n-6	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	**	ns	ns
20:4n-6	1.3±0.0^{a}	1.3±0.0^{a}	1.4±0.0^{b}	1.5±0.0^{b}	1.5±0.0^{b}	1.8±0.0^{c}	***	***	ns
Σ n-3	26.5±0.5^{a}	29.7±0.3^{b}	32.2±0.6^{c}	32.9±0.6^{c}	33.7±0.8^{c}	36.3±0.6^{d}	***	ns	ns
18:3n-3	3.4±0.1^{d}	3.1±0.1^{c}	2.7±0.1^{b}	2.7±0.2^{bc}	2.5±0.1^{b}	1.8±0.1^{a}	***	***	ns
Σ n-3 HUFA	22.4±0.6^{a}	25.7±0.4^{b}	28.6±0.5^{c}	29.2±0.4^{c}	30.2±0.8^{c}	33.3±0.5^{d}	***	ns	ns
20:5n-3	4.4±0.1^{a}	5.7±0.1^{b}	6.2±0.1^{c}	6.7±0.2^{d}	6.8±0.2^{d}	8.2±0.2^{e}	***	***	ns
22:5n-3	0.74±0.03^{a}	0.93±0.01^{b}	1.04±0.02^{c}	1.07±0.05^{c}	1.11±0.03^{c}	1.21±0.03^{d}	***	ns	*
22:6n-3	17.3±0.4^{a}	19.1±0.3^{b}	21.4±0.4^{c}	21.5±0.2^{c}	22.3±0.6^{c}	24.0±0.4^{d}	***	ns	ns
DHA/EPA	3.96±0.07^{d}	3.37±0.05^{bc}	3.47±0.02^{c}	3.23±0.06^{b}	3.25±0.03^{b}	2.94±0.06^{a}	***	***	ns

Values are means ± standard error (*n=6*). Numbers in rows having different letters indicate that treatments are significantly different ($P < 0.05$). Influence of factors in 2 way ANOVA :* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant.

4. Discussion

Overall growth of sea bass during this experiment was slightly lower than the potential of this species. The high ratio of vegetable protein in the experimental diets could have reduced growth in the experimental treatments. Burel et al. (1998) in a set of experiments obtained either normal or reduced growth for rainbow trout fed on diets containing 70% lupin depending of the crop of lupin used. The other factor, which may be involved, is the low arachidonic acid (AA) content of the diets. AA is required for European sea bass broodstock in order to obtain good spawn quality (Bruce et al., 1999), but to our knowledge, there are no data on the AA requirement for European sea bass juveniles. In gilthead sea bream a requirement for AA has been demonstrated in larval stages (Bessonart et al., 1999), however in juveniles of the same species Fountoulaki et al. (2003) did not observe any effect of AA on growth (minimal level 0.2% of dietary fatty acid) whereas an effect of AA on growth was reported in turbot (Castell et al., 1994). Thus, AA may potentially interfere during n-3 HUFA requirement evaluation (Robin, 1995). In the diets used in the present study, AA was proportional to the quantity of fish oil blend added and 20:4n-6/20:5n-3/22:6n-3 ratios were quite similar.

With present data, maximal growth response was encountered with diet 3, close to the requirement found at 0.70% DM n-3 HUFA. However, the precision of this evaluation was affected by variability in growth mainly observed in treatment 2, suggesting an heterogeneous individual response to slightly deficient HUFA level. In any way, the n-3 HUFA as criterion for evaluation of requirement is open to criticism. The n-3 HUFA is evaluated as sum of n-3 PUFA with 20 and 22 carbons, while DHA is more effective than EPA (Watanabe et al., 1989), inducing influence of EFA balance (Sargent et al., 1999). The requirement also depends of dietary lipid level (Takeushi et al., 1992). In a study on prawn,

Glencross et al. (2002) suggested that EFA should be defined as a percentage of total fatty acids in conjunction with the amount of lipid in the diet. Then in the present study the requirement obtained at 0.70% DM n-3 HUFA should be consider with experimental conditions, specially a DHA:EPA ratio (1.5:1), and a diet containing 18% lipids.

With diet 1 (0.2% n-3 HUFA) the growth was 85% of the maximum observed. No mortality was encountered during this experiment with this diet, suggesting this species maybe relatively tolerant to dietary HUFA restriction. Gilthead sea bream juveniles have reduced growth at 0.6% (DHA+EPA DM) compared to 0.9% (Kalogelopoulos et al., 1992). Such tolerance of European sea bass may explain the lack of data on HUFA requirement in this species (Oliva-Teles, 2000), which is well studied in other respects, despite its early development in marine aquaculture production.

The importance of n-3 HUFA, in particular, EPA (20:5n-3) and DHA (22:6n-3) in marine fish for optimum performance is well demonstrated (Sargent et al., 1989; Rodriguez et al., 1994; Sargent et al., 1995; Ibeas et al., 1996; Oliva-Teles, 2000; Parpoura and Alexis, 2001). Fatty acids are the primary constituents of polar and neutral lipids, specifically phospholipids and triacylglycerols, respectively. Triacylglerols are depot fats while phospholipids are integral components of biomembranes. The degree of unsaturation of the fatty acids maintains the structural and functional integrity of cell membranes (Sargent et al., 1999). Phospholipids contain higher quantities of PUFA, and lower levels of monounsaturated compared to the neutral fraction (Henderson and Tocher, 1987). This tendency, confirmed in the present study, was also observed in gilthead sea bream (Kalogeropoulos et al., 1993; Ibeas et al., 1997).

The relative ratio of fish versus vegetable oil, in diets 1 to 6, induce a gradual decrease of the typical fatty acids of vegetable oils (18:1n-9, 18:2n-6 and 18:3n-3) and an

increase in marine type fatty acids (22:6n-3, 22:5n-3 and 22:1n-11). The dietary effect on fish neutral lipids corresponds well to these tendencies, according to the general rule that fish fatty acid profile tends to reflect diet composition. The analyses performed at two periods show this influence is increased with duration of the experiment and as fish weight (and total lipid quantity) increased, in accordance with a dilution of initial fish fatty acids by incorporation of dietary fatty acids (Robin et al., 2003). According to these authors polar lipids components evolved more slowly than neutral lipids. Polar lipid fatty acids were also influenced by dietary components, but fish under the various treatments tended to conserve a more similar fatty acid profile in their polar lipids than in their neutral lipids. Almost no differences were observed between polar lipids of fish fed diets 3, 4 and 5. DHA levels were quite similar within each treatment at the two periods, and a decrease was only observed in fish fed diet 1, severely depleted in n-3 HUFA. These results suggest that DHA content in polar lipids was more highly regulated. The relative high levels of AA and DHA in polar lipids versus neutral lipids is in accordance to the preferential incorporation of these fatty acids in phospholipids, described by Linares and Henderson (1991), contributing to maintenance of phospholipid quality.

Both polar and neutral lipids contained high levels of 16:0 and 18:1n-9. These fatty acids are involved in energy storage. They are also associated (mainly 16:0) with HUFAs in diacyl glycerophopholipids of fish (Bell and Dick, 1991), as well as stearic acid (18:0) in phosphatidyl inositol (Bell and Dick, 1990). Oleic acid (18:1n-9) was provided at high levels in the diets and increased in fish neutral lipids between the two periods analysed, reaching almost the same proportion than in the diets. Palmitic acid (16:0) was the primary saturated fatty acid, contributing more than 60% of these fatty acids in both polar and neutral fractions. Similar values for sea bass (Parpoura and Alexis, 2001; Alasalvar et al.,

2002) and sea bream (Ibeas et al., 1996; Grigorakis et al., 2002) have been reported. In contrast to oleic acid, palmitic acid was lower in diets (6.7 to 9.2%) compared to the levels observed in fish neutral lipids (11.5 to 13.6%) or polar lipids (15.7 to 17.2%) at the end of experiment. The same tendency was observed for 18:0 in lower proportions (1.8-2.0% in diets; 2.9-3.0% in neutrals, and 8.0-8.8% in polar lipids). These high levels of tissue saturates relatively to diets may reflect production by lipogenic activity (Dias et al., 1998).

The influence of dietary n-6 fatty acids on n-6 content in fish was in accordance with previous studies for a different marine species (Argyropoulou et al., 1992; Kalogeropoulos et al., 1993). The main representative of n-6 fatty acids in dietary lipids is linoleic acid, 18:2n-6. It is accumulated largely unchanged in the lipids of marine fish due to their reduced capacity for chain elongation and desaturation (Bell et al., 1986, 1994). However n-6 intermediates in desaturation elongation pathway as 18:3n-6, 20:2n-6 and 20:3n-6 were found in neutral and polar lipids at higher values than in diets. That was more clear in fish fed low HUFA diets containing also more 18:2n-6 (the precursor of n-6 fatty acids). Even if their absolute level were low, it suggest some desaturation and elongation might occur. If so, no clear influence on AA (the final product of desaturation elongation pathway in n-6 FA) can be observed: this fatty acid decreasing in fish neutral and polar lipids with the proportion in the diets and with the duration of experiment.

In the present experiment the low proportion of fish meal and replacement of fish oil by vegetable oil both contributed to lower n-3 long chain fatty acids. The minimal level for growth found at 0.7% DM of n-3 HUFA indicate that only a part of marine product either in protein or lipid sources can be used to reach this level. At the requirement level, the study of fish fatty acid in polar lipids, after the two periods, indicated a stability of DHA, but a decrease in AA content during time. That suggests quality of membranes in DHA can

be preserved during a longer feeding period, while a further decrease of AA could occurred. Dietary arachidonic acid is supplied at low level by marine products, but is lacking in available vegetable sources. Possible adverse effect of long term feeding of fish on diets containing low marine type products may involve both n-3 and n-6 EFA restriction, and should be further studied.

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