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Efficacy of dietary methyl esters of $n-3$ HUFA vs. triacylglycerols of $n-3$ HUFA by gilthead seabream (*Sparus aurata* L.) juveniles

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Abstract

A feeding experiment was carried out on gilthead seabream juveniles to investigate the utilization of dietary $n-3$ highly unsaturated fatty acids ($n-3$ HUFA), when presented as methyl esters or as triacylglycerols. Three groups of gilthead seabream juveniles, of an initial mean weight of 62 g, were fed diets containing the same level of $n-3$ HUFA (about 2% dry weight basis, DWB) but where these essential fatty acids (EFA) were supplied in the form of methyl esters, triacylglycerols or as a mixture of these two chemical forms (diets 1, 2 and 3, respectively). A fourth group of 62-g individuals was fed a diet containing a particularly high level of triacylglycerols of $n-3$ HUFA (about 5% DWB). After 8 weeks of feeding, the results showed that fish growth, hepatosomatic index, total lipid content, and fatty acid composition of neutral and polar lipids of brain, liver, gills and muscle were not affected by the chemical form of the lipids given in the diet. However, individuals fed the very high level of EFA (diet 4) showed a lower growth rate than the other three groups of fish. In addition, eicosapentaenoic acid (EPA; $20{:}5n-3$) and docosahexaenoic acid (DHA; $22{:}6n-3$) levels in both neutral and polar lipids from liver, gills and muscle were higher in this group of fish, with the brain fatty acid composition being less affected by dietary regime.

Keywords: Methyl esters; Triacylglycerols; $n-3$ HUFA; Gilthead seabream; Juveniles

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1. Introduction

Dietary lipids play an important role in fish nutrition as a source provision of body energy and essential fatty acids (EFA) (Sargent et al., 1989). The nutritional aspects of EFA in fish have been extensively studied (Watanabe, 1982; Greene and Selivonchick, 1987; Sargent et al., 1989). In particular, considerable attention has been focused on the $n-3$ highly unsaturated fatty acids ($n-3$ HUFA) requirements of marine fish, chiefly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The requirements of gilthead seabream for $n-3$ HUFA have been investigated in larvae (Koven et al., 1989, 1990, 1992; Mourente et al., 1993; Rodríguez et al., 1993, 1994, 1997, 1998; Salhi, 1997), fingerlings (Kalogeropoulus et al., 1992), juveniles (Ibeas et al., 1994, 1996, 1997) and broodstock (Fernández-Palacios et al., 1995). The minimum level of dietary $n-3$ HUFA, including EPA and DHA, required by gilthead seabream for optimum growth and development has been reported to be about 1.5% dry weight basis (DWB)

Table 1
Ingredients and proximate composition (wt.%) of experimental diets

	Diet no.			
	1	2	3	4
Ingredients				
Defatted sardine meal[a]	52.5	52.5	52.5	52.5
Casein[b]	15.0	15.0	15.0	15.0
Corn starch	10.0	10.0	10.0	10.0
α-Cellulose	5.0	5.0	5.0	5.0
Vitamin mix[c]	5.0	5.0	5.0	5.0
Mineral mix[d]	2.0	2.0	2.0	2.0
Choline chloride	0.9	0.9	0.9	0.9
Vitamin E	0.1	0.1	0.1	0.1
Carboxymethyl cellulose	0.5	0.5	0.5	0.5
Beef tallow	6.5	4.8	5.6	0.0
EPA 28[e]	0.0	4.2	2.2	9.0
ME 75[f]	2.5	0.0	1.2	0.0
Proximate composition				
Crude protein	50.1	49.9	50.3	49.9
Crude lipid	11.9	12.3	11.7	12.5
Ash	15.6	15.2	15.7	15.1
Moisture	19.4	19.8	20.1	19.1
$n-3$ HUFA (% dry weight)	2.2	2.1	2.1	4.9

[a] Sardine meal was defatted by using chloroform.

[b] From bovine milk.

[c] Contained (mg/kg of dry diet): retinol (IU) 50000; cholecalciferol (IU) 2000; α-tocopherol (IU) 300; thiamin 37; riboflavin 48; pyridoxine 20; cyanocobalamin 0.1; folic acid 10; calcium pantothenate 74; menadione 11; ascorbic acid 240; myo-inositol 337; biotin 0.5; nicotinic acid 300.

[d] Contained (mg/kg of dry diet): Mn 9.3; Cu 0.9; Co 2.2; I 2; Zn 44; ethoxyquin 130.

[e] Triacylglycerol mixture containing 40% $n-3$ HUFA.

[f] Methyl ester mixture containing 75% $n-3$ HUFA.

Table 2
Performance factors of gilthead seabream juveniles fed experimental diets for 8 weeks

	Diet no.			
	1	2	3	4
Initial weight (g)	62.3±1.2	62.5±1.3	61.5±1.1	61.8±1.1
Final weight (g)	100.3±1.6[b]	101.5±1.7[b]	102.2±1.5[b]	95.5±1.4[a]
Weight gain (%)	61.0	62.4	66.2	54.5
Condition factor[a]	2.2±0.0	2.2±0.0	2.2±0.0	2.1±0.0
Hepatosomatic index[b]	1.6±0.1	1.6±0.1	1.7±0.1	1.6±0.1

Data are mean±SEM ($n=45$ fish). Values within a given row followed by different superscript letters are significantly different ($P<0.05$).

[a]The condition factor was determined: (final body weight (g)/[final total body length (cm)]^3)×100.

[b]The hepatosomatic index was determined: (liver weight (g)/final body weight (g))×100.

for both larvae (Salhi, 1997; Rodríguez et al., 1998) and broodstock (Fernández-Palacios et al., 1995) and about 1% for both fingerlings (1 g fish) and juveniles (11.5 g fish) (Kalogeropoulus et al., 1992; Ibeas et al., 1996).

Table 3
Main fatty acid composition (area %) of experimental diets

	Diet no.			
	1	2	3	4
ΣSaturates	36.3	36.0	36.6	23.4
14:0	5.9	5.1	5.7	5.2
16:0	22.3	23.4	22.9	16.3
18:0	7.3	6.7	7.1	1.3
Σ$n-9$	18.5	18.3	18.9	13.8
16:1$n-9$	0.6	1.0	0.9	1.3
18:1$n-9$	17.8	16.7	17.6	11.9
18:2$n-9$	0.1	0.2	0.2	0.4
Σ$n-6$ PUFA	4.8	5.1	5.1	4.5
16:3$n-6$	0.3	0.6	0.8	0.4
18:2$n-6$	3.1	3.0	2.6	1.9
20:4$n-6$	0.9	1.1	1.0	1.0
Σ$n-3$ PUFA	21.5	20.4	21.3	45.4
18:3$n-3$	0.5	0.6	0.6	1.1
18:4$n-3$	1.6	2.3	1.9	4.6
Σ$n-3$ HUFA	18.6	17.1	18.1	39.0
20:5$n-3$	11.7	10.5	11.4	25.0
22:5$n-3$	0.6	0.7	0.7	2.6
22:6$n-3$	5.7	5.4	5.5	11.0
18:1$n-9$/$n-3$ HUFA	1.0	1.0	1.0	0.3
Saturated/unsaturated	0.6	0.6	0.6	0.3

Table 4
Main fatty acid composition (area %) of brain and liver neutral lipids

	Initial	Diet no.			
		1	2	3	4
Brain					
ΣSaturates	36.0 ± 0.3$^{(ab)}$	36.5 ± 0.3$^{(b)}$	38.2 ± 0.4$^{(c)}$	37.1 ± 0.5$^{(bc)}$	35.1 ± 0.5$^{(a)}$
14:0	5.9 ± 0.2$^{(a)}$	6.5 ± 0.3$^{(ab)}$	7.1 ± 0.2$^{(b)}$	6.3 ± 0.2$^{(ab)}$	5.9 ± 0.2$^{(a)}$
16:0	23.9 ± 0.3$^{(ab)}$	24.7 ± 0.3$^{(b)}$	24.9 ± 0.4$^{(b)}$	25.2 ± 0.5$^{(b)}$	23.2 ± 0.4$^{(a)}$
18:0	5.0 ± 0.2	4.5 ± 0.2	5.4 ± 0.3	4.8 ± 0.3	5.1 ± 0.2
Σ$n-9$	19.7 ± 0.4	21.5 ± 0.5	21.1 ± 0.3	21.0 ± 0.3	20.8 ± 0.3
18:1$n-9$	18.3 ± 0.4	20.2 ± 0.5	19.8 ± 0.2	19.5 ± 0.3	19.2 ± 0.3
18:2$n-9$	nd	tr	tr	tr	0.5 ± 0.1
Σ$n-6$ PUFA	5.4 ± 0.2	5.3 ± 0.3	4.8 ± 0.2	5.3 ± 0.2	5.0 ± 0.3
18:2$n-6$	3.1 ± 0.2$^{(b)}$	3.0 ± 0.3$^{(b)}$	2.4 ± 0.2$^{(ab)}$	2.9 ± 0.2$^{(ab)}$	1.8 ± 0.2$^{(a)}$
20:4$n-6$	1.7 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.9 ± 0.2
Σ$n-3$ PUFA	18.4 ± 0.3$^{(a)}$	22.1 ± 0.4$^{(b)}$	21.3 ± 0.4$^{(b)}$	22.0 ± 0.3$^{(b)}$	27.3 ± 0.4$^{(c)}$
18:3$n-3$	1.2 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	1.3 ± 0.2
18:4$n-3$	1.6 ± 0.1$^{(b)}$	1.1 ± 0.1$^{(a)}$	0.9 ± 0.1$^{(a)}$	1.3 ± 0.2$^{(ab)}$	1.6 ± 0.2$^{(b)}$
Σ$n-3$ HUFA	14.9 ± 0.3$^{(a)}$	19.5 ± 0.4$^{(b)}$	18.8 ± 0.4$^{(b)}$	19.4 ± 0.3$^{(b)}$	23.6 ± 0.4$^{(c)}$
20:5$n-3$	3.0 ± 0.2$^{(a)}$	4.5 ± 0.2$^{(b)}$	3.7 ± 0.3$^{(ab)}$	4.6 ± 0.2$^{(b)}$	6.8 ± 0.3$^{(c)}$
22:5$n-3$	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.7 ± 0.1
22:6$n-3$	10.6 ± 0.3$^{(a)}$	13.6 ± 0.4$^{(b)}$	14.2 ± 0.4$^{(bc)}$	13.5 ± 0.3$^{(b)}$	15.7 ± 0.4$^{(c)}$
Saturated/unsaturated	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
18:1$n-9$/$n-3$ HUFA	1.2 ± 0.0$^{(c)}$	1.0 ± 0.0$^{(b)}$	1.1 ± 0.0$^{(bc)}$	1.0 ± 0.0$^{(b)}$	0.8 ± 0.0$^{(a)}$
EPA/DHA	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
Liver					
ΣSaturates	32.2 ± 0.3$^{(bc)}$	30.5 ± 0.2$^{(a)}$	31.2 ± 0.4$^{(ab)}$	33.0 ± 0.3$^{(c)}$	29.9 ± 0.3$^{(a)}$
14:0	4.0 ± 0.1$^{(b)}$	3.9 ± 0.1$^{(b)}$	3.1 ± 0.1$^{(a)}$	4.6 ± 0.2$^{(bc)}$	4.7 ± 0.2$^{(c)}$
16:0	20.7 ± 0.3$^{(b)}$	19.1 ± 0.2$^{(a)}$	21.1 ± 0.4$^{(bc)}$	22.0 ± 0.3$^{(c)}$	18.9 ± 0.3$^{(a)}$
18:0	7.2 ± 0.3$^{(b)}$	7.0 ± 0.2$^{(b)}$	6.7 ± 0.2$^{(ab)}$	6.0 ± 0.2$^{(a)}$	5.9 ± 0.2$^{(a)}$
Σ$n-9$	39.7 ± 0.4$^{(c)}$	36.6 ± 0.3$^{(b)}$	36.0 ± 0.3$^{(b)}$	36.6 ± 0.4$^{(b)}$	31.0 ± 0.3$^{(a)}$
18:1$n-9$	35.5 ± 0.4$^{(c)}$	34.8 ± 0.3$^{(bc)}$	33.5 ± 0.3$^{(b)}$	33.4 ± 0.4$^{(b)}$	29.7 ± 0.3$^{(a)}$
18:2$n-9$	2.3 ± 0.2^{d}	0.5 ± 0.1$^{(ab)}$	0.7 ± 0.1$^{(b)}$	1.4 ± 0.1$^{(c)}$	tr$^{(a)}$
Σ$n-6$	3.2 ± 0.2$^{(a)}$	4.4 ± 0.2$^{(b)}$	4.4 ± 0.2$^{(b)}$	4.0 ± 0.2$^{(ab)}$	4.9 ± 0.2$^{(b)}$
18:2$n-6$	2.2 ± 0.2	2.5 ± 0.2	2.8 ± 0.2	2.4 ± 0.2	2.2 ± 0.2
18:4$n-6$	tr$^{(a)}$	0.8 ± 0.1$^{(bc)}$	0.5 ± 0.1$^{(ab)}$	0.7 ± 0.1$^{(b)}$	1.2 ± 0.1$^{(c)}$
Σ$n-3$	7.3 ± 0.2$^{(a)}$	12.4 ± 0.3$^{(b)}$	12.7 ± 0.3$^{(b)}$	11.8 ± 0.2$^{(b)}$	20.8 ± 0.3$^{(c)}$
18:3$n-3$	tr$^{(a)}$	0.5 ± 0.1$^{(ab)}$	tr$^{(a)}$	tr$^{(a)}$	0.8 ± 0.1$^{(b)}$
18:4$n-3$	0.8 ± 0.2$^{(a)}$	1.1 ± 0.2$^{(a)}$	0.9 ± 0.2$^{(a)}$	1.0 ± 0.2$^{(a)}$	2.5 ± 0.2$^{(b)}$
Σ$n-3$ HUFA	6.1 ± 0.2$^{(a)}$	10.6 ± 0.3$^{(b)}$	11.3 ± 0.3$^{(b)}$	10.2 ± 0.2$^{(b)}$	16.6 ± 0.3$^{(c)}$
20:5$n-3$	1.7 ± 0.1$^{(a)}$	4.7 ± 0.3$^{(b)}$	5.3 ± 0.3$^{(b)}$	4.2 ± 0.2$^{(b)}$	7.4 ± 0.3$^{(c)}$

Table 4 (*continued*)

	Initial	Diet no.			
		1	2	3	4
Liver					
22:5$n-3$	$0.8 \pm 0.1^{(a)}$	$0.9 \pm 0.1^{(a)}$	$1.5 \pm 0.1^{(b)}$	$1.0 \pm 0.1^{(ab)}$	$2.2 \pm 0.1^{(c)}$
22:6$n-3$	$3.2 \pm 0.1^{(a)}$	$4.2 \pm 0.2^{(b)}$	$4.0 \pm 0.1^{(b)}$	$4.4 \pm 0.2^{(b)}$	$5.4 \pm 0.2^{(c)}$
Saturated/unsaturated	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
18:1$n-9$/$n-3$ HUFA	$5.8 \pm 0.1^{(c)}$	$3.3 \pm 0.1^{(b)}$	$3.0 \pm 0.1^{(b)}$	$3.3 \pm 0.1^{(b)}$	$1.8 \pm 0.0^{(a)}$
EPA/DHA	$0.5 \pm 0.0^{(a)}$	$1.1 \pm 0.0^{(b)}$	$1.3 \pm 0.1^{(b)}$	$1.0 \pm 0.0^{(b)}$	$1.4 \pm 0.1^{(b)}$

Data are mean $\pm$ SEM ($n = 3$ fish); nd, not detected; tr, fatty acid values $\leq 0.4\%$. Values within a given row having different superscript letters are significantly different ($P < 0.05$).

The lipid sources used in experiments undertaken by our group on gilthead seabream juveniles were either fatty acid methyl esters (FAME) or triacylglycerols. However, the efficacy of dietary $n-3$ HUFA when these EFA are supplied in the form of methyl esters or as triacylglycerols has not yet been determined. Early studies by Yu and Sinnhuber (1975) raised the question of possible stress imposed on fish by esters of fatty acids. Red seabream larvae (Izquierdo et al., 1989) and gilthead seabream larvae (Rodríguez, 1994) fed rotifers enriched with methyl esters of $n-3$ HUFA showed high mortality and poor growth compared with larvae fed rotifers enriched with triacylglycerols of $n-3$ HUFA. On the other hand, suppression of weight gain by dietary $n-3$ HUFA (2% DWB) has been observed in juvenile red drum fed fatty acid ethyl esters (Lochman and Gatlin, 1993). Finally, a growth reduction was also seen in rainbow trout fed high levels of $n-3$ fatty acid ethyl esters (Castell et al, 1972; Yu and Sinnhuber, 1972), whereas, rainbow trout fed a diet containing up to 5% of triacylglycerols of $n-3$ fatty acids showed optimal growth (Yu and Sinnhuber, 1976). The aim of the present study was to compare the efficacy of methyl esters of $n-3$ HUFA vs. triacylglycerols of $n-3$ HUFA as lipid sources for gilthead seabream. To this purpose, three groups of juveniles were fed diets containing about 2% of $n-3$ HUFA (DWB), supplied as methyl esters, triacylglycerols or as a mixture of these two lipid sources. Previous studies performed in our laboratory on gilthead seabream larvae (Rodríguez et al., 1993, 1994), have shown a detrimental effect upon larval development when fed high levels of dietary $n-3$ HUFA, supplied either as methyl esters or triacylglycerols. For this reason, a possible excess of these EFA presented in the form of triacylglycerols, was also assessed in a fourth group of fish.

2. Materials and methods

Gilthead seabream (*Sparus aurata*) previously fed a commercial diet and of an average weight of 62 g, were randomly sorted into 12 groups of 23 fish each (three groups/diet). Each group of fish was stocked in a 1000-l tank with a seawater supply of

Table 5
Main fatty acid composition (area %) of gills and muscle neutral lipids

	Initial	Diet no.			
		1	2	3	4
Gills					
ΣSaturates	30.7±0.3$^{(b)}$	30.0±0.3$^{(b)}$	29.8±0.2$^{(b)}$	30.4±0.3$^{(b)}$	28.5±0.2$^{(a)}$
14:0	4.6±0.2$^{(b)}$	3.7±0.2$^{(ab)}$	3.9±0.2$^{(ab)}$	3.8±0.1$^{(ab)}$	3.5±0.1$^{(a)}$
16:0	22.5±0.3$^{(b)}$	22.2±0.3$^{(b)}$	21.8±0.2$^{(ab)}$	22.6±0.3$^{(b)}$	20.9±0.2$^{(a)}$
18:0	3.1±0.1	3.7±0.1	3.6±0.1	3.5±0.2	3.8±0.2
Σ$n-9$	24.6±0.3$^{(b)}$	25.4±0.4$^{(b)}$	25.9±0.5$^{(b)}$	24.9±0.4$^{(b)}$	19.2±0.3$^{(a)}$
18:1$n-9$	23.3±0.3$^{(b)}$	23.9±0.4$^{(b)}$	24.3±0.5$^{(b)}$	23.2±0.4$^{(b)}$	18.1±0.3$^{(a)}$
18:2$n-9$	tr	tr	tr	0.5±0.1	tr
Σ$n-6$ PUFA	3.9±0.2$^{(a)}$	4.7±0.2$^{(b)}$	4.5±0.2$^{(ab)}$	4.2±0.2$^{(ab)}$	4.2±0.2$^{(ab)}$
18:2$n-6$	2.3±0.2$^{(ab)}$	3.1±0.2$^{(b)}$	2.9±0.2$^{(b)}$	2.6±0.2$^{(ab)}$	1.9±0.2$^{(a)}$
20:4$n-6$	0.6±0.1	0.6±0.1	0.6±0.1	0.7±0.1	0.8±0.1
Σ$n-3$ PUFA	21.6±0.2$^{(a)}$	22.2±0.3$^{(ab)}$	22.3±0.3$^{(ab)}$	22.8±0.3$^{(b)}$	32.7±0.3$^{(c)}$
18:3$n-3$	1.1±0.2	0.9±0.1	0.9±0.1	0.8±0.1	0.9±0.1
18:4$n-3$	2.0±0.2$^{(ab)}$	1.4±0.1$^{(a)}$	1.8±0.2$^{(ab)}$	1.6±0.2$^{(ab)}$	2.7±0.2$^{(b)}$
Σ$n-3$ HUFA	18.1±0.2$^{(a)}$	19.6±0.3$^{(b)}$	19.3±0.2$^{(b)}$	20.1±0.3$^{(b)}$	28.2±0.3$^{(c)}$
20:5$n-3$	6.1±0.2$^{(a)}$	6.6±0.3$^{(a)}$	6.7±0.3$^{(a)}$	6.5±0.3$^{(a)}$	12.1±0.3$^{(b)}$
22:5$n-3$	1.8±0.1$^{(a)}$	2.0±0.1$^{(a)}$	2.1±0.2$^{(a)}$	2.1±0.2$^{(a)}$	3.3±0.2$^{(b)}$
22:6$n-3$	9.4±0.2$^{(a)}$	10.3±0.2$^{(a)}$	9.6±0.2$^{(a)}$	10.5±0.2$^{(a)}$	11.7±0.2$^{(c)}$
Saturated/unsaturated	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0
18:1$n-9$/$n-3$ HUFA	1.3±0.0$^{(b)}$	1.2±0.0$^{(b)}$	1.3±0.0$^{(b)}$	1.2±0.0$^{(b)}$	0.6±0.0$^{(a)}$
EPA/DHA	0.6±0.0$^{(a)}$	0.6±0.0$^{(a)}$	0.7±0.0$^{(a)}$	0.6±0.0$^{(a)}$	1.0±0.0$^{(b)}$
Muscle					
ΣSaturates	30.1±0.3$^{(ab)}$	31.1±0.3$^{(bc)}$	32.3±0.2$^{(c)}$	32.0±0.3$^{(c)}$	29.0±0.3$^{(a)}$
14:0	4.2±0.2	3.9±0.2	4.1±0.2	4.3±0.2	4.0±0.2
16:0	21.7±0.3$^{(a)}$	22.7±0.3$^{(b)}$	23.4±0.2$^{(b)}$	23.1±0.3$^{(b)}$	21.3±0.3$^{(a)}$
18:0	3.8±0.2$^{(ab)}$	4.0±0.2$^{(ab)}$	4.3±0.3$^{(b)}$	4.1±0.2$^{(b)}$	3.1±0.2$^{(a)}$
Σ$n-9$	27.7±0.3$^{(b)}$	28.6±0.4$^{(bc)}$	29.5±0.4$^{(c)}$	28.9±0.3$^{(bc)}$	22.6±0.3$^{(a)}$
18:1$n-9$	26.0±0.3$^{(b)}$	27.1±0.4$^{(bc)}$	28.0±0.4$^{(c)}$	27.0±0.3$^{(bc)}$	20.8±0.3$^{(a)}$
18:2$n-9$	tr	tr	tr	0.6±0.0	0.6±0.1
Σ$n-6$	3.7±0.2	4.3±0.2	3.8±0.2	4.1±0.3	3.7±0.2
18:2$n-6$	2.4±0.2$^{(ab)}$	3.0±0.2$^{(b)}$	2.5±0.2$^{(ab)}$	2.7±0.3$^{(b)}$	1.6±0.2$^{(a)}$
18:4$n-6$	0.5±0.1	0.6±0.1	0.5±0.1	0.5±0.1	0.7±0.1
Σ$n-3$	19.7±0.2$^{(a)}$	20.1±0.2$^{(a)}$	20.4±0.3$^{(a)}$	19.2±0.2$^{(a)}$	26.5±0.3$^{(b)}$
18:3$n-3$	0.6±0.1	0.5±0.1	tr	tr	0.6±0.1
18:4$n-3$	1.9±0.2$^{(a)}$	2.0±0.2$^{(a)}$	1.8±0.2$^{(a)}$	1.8±0.2$^{(a)}$	3.1±0.3$^{(b)}$
Σ$n-3$ HUFA	16.8±0.2$^{(a)}$	17.3±0.2$^{(a)}$	17.8±0.3$^{(a)}$	16.6±0.2$^{(a)}$	21.8±0.2$^{(b)}$
20:5$n-3$	7.2±0.2$^{(a)}$	7.2±0.2$^{(a)}$	7.4±0.2$^{(a)}$	6.9±0.2$^{(a)}$	9.1±0.3$^{(b)}$
22:5$n-3$	1.0±0.1$^{(a)}$	0.9±0.1$^{(a)}$	0.7±0.1$^{(a)}$	0.9±0.2$^{(a)}$	1.9±0.2$^{(b)}$
22:6$n-3$	8.1±0.2$^{(a)}$	8.7±0.2$^{(ab)}$	9.3±0.3$^{(bc)}$	8.4±0.2$^{(ab)}$	10.2±0.2$^{(c)}$

Table 5 (*continued*)

	Initial	Diet no.			
		1	2	3	4
Muscle					
Saturated/unsaturated	0.4±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.4±0.0
18:1 $n-9/n-3$ HUFA	1.5±0.0	1.6±0.0	1.6±0.0	1.6±0.0	1.0±0.0
EPA/DHA	0.9±0.0	0.8±0.0	0.8±0.0	0.8±0.0	0.9±0.0

Data are mean±SEM ($n=3$ fish); nd, not detected; tr, fatty acid values $\leq 0.4\%$. Values within a given row having different superscript letters are significantly different ($P<0.05$).

4.5 l/min which ensured suitable water conditions and an oxygen level close to saturation. During the experiment, the water temperature ranged from 21.0°C to 22.5°C. The experimental diets were formulated with the basal composition shown in Table 1. Sardine meal was purchased from Canagrosa (Lanzarote, Spain). Casein, corn starch, α-cellulose and vitamin E (added as an antioxidant for the lipids) were provided by Sigma (St. Louis, MO). The mineral and vitamin mixtures used were supplied by DIBAQ (Madrid, Spain) and the choline chloride by Fluka Chemie (Buchs, Switzerland). EPA 28 is a triacylglycerol mixture containing 40% $n-3$ HUFA and ME 75 consists of a mixture of FAME containing about 75% $n-3$ HUFA. Both lipid sources were provided by Nippai (Tokyo, Japan).

Total lipid and $n-3$ HUFA contents (% DWB) of the commercial diet used before the start of the experiment were 18.15% and 1.76%, respectively. The experimental diets 1, 2 and 3 were formulated to contain about 2% (DWB) of $n-3$ HUFA in the form of methyl esters, triacylglycerols or as mixture (2:1) of these two chemical forms (1:1.8, respectively). Diet 4 contained 4.9% (DWB) $n-3$ HUFA from triacylglycerols. The fish were fed for 8 weeks and the diets were fed to apparent satiation, five times a day. Every 2 weeks, the animals were individually weighed and checked for external signs of abnormality. At the end of the experiment, the fish were sacrificed and brain, liver, gill and muscle tissues were collected for biochemical analysis. The livers were also weighed for hepatosomatic index determination. Further samples of the initial fish fed the commercial diet were also taken at the begining of the experiment in order to establish the fish weight gain as well as changes in the corporal composition at the end of the trial.

The lipid content was gravimetrically measured after extraction with chloroform/methanol (2:1, v/v), according to the method of Folch et al. (1957). Crude lipid extracts were subsequently separated into polar and non-polar fractions by means of silica Sep-pak cartridges (Waters, Milford, MA) using chloroform, a mixture of chloroform/methanol (49:1, v/v) and methanol as solvent systems (Juaneda and Rockelin, 1985). Fatty acid mixtures were prepared from the crude lipids by saponification with KOH (50%) and FAME, subsequently prepared by esterification with borontrifluoride in methanol as described by Metcalfe and Schmitz (1961). The FAME were separated by gas–liquid chromatography on a Shimadzu GC-14A equipped with a flame ionization detector, and fitted with a fused silica capillary column (Supelcowax 10; 30 m × 0.32 mm i.d., Supelco, Bellefonte, PA). Helium was used as the carrier gas.

Table 6
Main fatty acid composition (area %) of brain and liver polar lipids

	Initial	Diet no.			
		1	2	3	4
Brain					
ΣSaturates	33.2±0.2$^{(c)}$	31.9±0.2$^{(b)}$	31.8±0.3$^{(b)}$	32.6±0.3$^{(bc)}$	29.4±0.2$^{(a)}$
14:0	1.1±0.2	1.5±0.2	1.6±0.2	1.9±0.2	1.9±0.2
16:0	20.7±0.2$^{(c)}$	19.7±0.2$^{(b)}$	19.2±0.2$^{(ab)}$	20.1±0.2$^{(bc)}$	18.6±0.2$^{(a)}$
18:0	10.9±0.2$^{(c)}$	10.5±0.2$^{(bc)}$	10.7±0.2$^{(bc)}$	9.9±0.2$^{(b)}$	8.1±0.2$^{(a)}$
Σ$n-9$	20.2±0.3$^{(b)}$	21.4±0.2$^{(c)}$	22.2±0.3$^{(c)}$	21.6±0.2$^{(c)}$	18.2±0.3$^{(a)}$
18:1$n-9$	17.2±0.3$^{(b)}$	18.6±0.2$^{(c)}$	19.2±0.3$^{(c)}$	18.1±0.1$^{(bc)}$	15.2±0.3$^{(a)}$
18:2$n-9$	0.5±0.1	tr	tr	tr	tr
Σ$n-6$ PUFA	2.9±0.1	2.8±0.1	2.9±0.2	2.7±0.1	3.1±0.1
18:2$n-6$	0.7±0.1	0.5±0.1	0.5±0.1	tr	tr
20:4$n-6$	1.8±0.1	1.9±0.1	1.8±0.2	1.8±0.1	2.0±0.2
Σ$n-3$ PUFA	31.9±0.3$^{(a)}$	37.9±0.3$^{(b)}$	37.4±0.4$^{(b)}$	37.0±0.3$^{(b)}$	39.6±0.4$^{(c)}$
18:3$n-3$	tr	tr	tr	tr	0.5±0.1
18:4$n-3$	0.6±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
Σ$n-3$ HUFA	29.7±0.3$^{(a)}$	35.5±0.3$^{(b)}$	35.0±0.4$^{(b)}$	34.9±0.3$^{(b)}$	37.1±0.4$^{(c)}$
20:5$n-3$	5.5±0.2$^{(a)}$	5.3±0.2$^{(ab)}$	4.6±0.3$^{(a)}$	5.2±0.2$^{(ab)}$	5.7±0.3$^{(b)}$
22:5$n-3$	0.9±0.2$^{(ab)}$	1.0±0.2$^{(ab)}$	1.2±0.1$^{(b)}$	0.7±0.1$^{(a)}$	1.2±0.2$^{(b)}$
22:6$n-3$	21.9±0.3$^{(a)}$	28.0±0.3$^{(b)}$	27.8±0.4$^{(b)}$	27.5±0.3$^{(b)}$	28.1±0.5$^{(b)}$
Saturated/unsaturated	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.4±0.0
18:1$n-9$/$n-3$ HUFA	0.6±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.4±0.0
EPA/DHA	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
Liver					
ΣSaturates	34.0±0.3$^{(b)}$	35.7±0.4$^{(bc)}$	36.8±0.4$^{(c)}$	35.9±0.5$^{(c)}$	31.7±0.4$^{(a)}$
14:0	1.7±0.1	2.2±0.2	1.9±0.1	1.8±0.1	1.8±0.1
16:0	22.4±0.3$^{(a)}$	25.7±0.4$^{(b)}$	26.5±0.4$^{(b)}$	26.1±0.5$^{(b)}$	23.0±0.4$^{(a)}$
18:0	9.2±0.2$^{(c)}$	7.0±0.2$^{(b)}$	7.5±0.2$^{(b)}$	7.1±0.2$^{(b)}$	5.9±0.2$^{(a)}$
Σ$n-9$	16.5±0.3$^{(c)}$	10.7±0.2$^{(b)}$	11.7±0.2$^{(b)}$	11.1±0.2$^{(b)}$	9.0±0.2$^{(a)}$
18:1$n-9$	14.7±0.3$^{(c)}$	9.0±0.2$^{(b)}$	9.5±0.2$^{(b)}$	8.7±0.2$^{(b)}$	7.2±0.2$^{(a)}$
18:2$n-9$	0.7±0.1$^{(a)}$	0.9±0.1$^{(ab)}$	1.4±0.2$^{(b)}$	1.3±0.1$^{(b)}$	1.0±0.1$^{(ab)}$
Σ$n-6$	8.4±0.2$^{(c)}$	6.9±0.2$^{(b)}$	6.8±0.1$^{(b)}$	7.2±0.2$^{(b)}$	5.1±0.2$^{(a)}$
18:2$n-6$	4.0±0.2$^{(c)}$	2.6±0.2$^{(b)}$	1.7±0.1$^{(b)}$	2.0±0.2$^{(b)}$	0.8±0.1$^{(a)}$
18:4$n-6$	2.8±0.2$^{(a)}$	3.9±0.2$^{(b)}$	4.2±0.3$^{(b)}$	4.3±0.3$^{(b)}$	4.1±0.3$^{(b)}$
Σ$n-3$	35.1±0.3$^{(a)}$	36.9±0.2$^{(b)}$	36.8±0.3$^{(b)}$	36.7±0.3$^{(b)}$	45.9±0.4$^{(c)}$
18:3$n-3$	tr$^{(ab)}$	tr$^{(a)}$	tr$^{(a)}$	tr$^{(a)}$	0.6±0.1$^{(b)}$
18:4$n-3$	0.6±0.1$^{(b)}$	tr$^{(a)}$	tr$^{(a)}$	tr$^{(a)}$	0.5±0.1$^{(ab)}$
Σ$n-3$ HUFA	33.8±0.3$^{(a)}$	35.9±0.2$^{(b)}$	36.0±0.3$^{(b)}$	35.8±0.3$^{(b)}$	44.6±0.4$^{(c)}$
20:5$n-3$	10.6±0.3$^{(a)}$	14.0±0.2$^{(b)}$	13.8±0.3$^{(b)}$	13.4±0.2$^{(b)}$	17.0±0.3$^{(c)}$
22:5$n-3$	1.3±0.2$^{(a)}$	1.2±0.2$^{(a)}$	1.4±0.2$^{(a)}$	1.4±0.2$^{(a)}$	2.5±0.2$^{(b)}$
22:6$n-3$	20.7±0.3$^{(a)}$	20.0±0.2$^{(a)}$	20.1±0.3$^{(a)}$	20.2±0.3$^{(a)}$	24.6±0.4$^{(b)}$

Table 6 (*continued*)

	Initial	Diet no.			
		1	2	3	4
Liver					
Saturated/unsaturated	0.5±0.0	0.6±0.0	0.6±0.0	0.6±0.0	0.5±0.0
18:1 $n-9/n-3$ HUFA	0.4±0.0	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.0
EPA/DHA	0.5±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0

Data are mean±SEM ($n=3$ fish); nd, not detected; tr, fatty acid values $\leq 0.4\%$. Values within a given row having different superscript letters are significantly different ($P<0.05$).

Injector and detector temperatures were 250°C. The column temperature was programmed to rise from 180°C (maintained for 10 min) to a final temperature of 215°C at a rate of 2.6°C/min. Individual FAME were identified by reference to known standards (Sigma) and to a well characterized fish oil (Nippai), and quantified by a Shimadzu C-R4A integrator.

Ash and moisture contents of the experimental diets were measured according to Official Methods of Analysis (A.O.A.C., 1980). The crude protein content was also determined according to A.O.A.C. (1980) by using the Kjeldahl method.

In order to facilitate the observation of general trends in this comparative study, data of fatty acids were restricted to the principal groups: saturates, $n-9$, $n-6$ PUFA, $n-3$ PUFA and $n-3$ HUFA.

Each value is presented as the mean ± SEM of three fish for the analytical results and of 45 fish for performance factors determination. Statistical testing to verify differences between treatments were carried out by using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test (Zar, 1984) where significance was accepted at $P<0.05$.

3. Results

Table 2 summarizes the performance factors of gilthead seabream juveniles fed the experimental diets for 8 weeks. During the feeding trial, mortality or external signs of abnormality were not observed in any group of fish. In addition, no differences in the hepatosomatic index were found among groups. However, final body weight was lowest when a high level of EFA was fed (diet 4), whereas, no differences in terms of growth were observed among the three groups of fish fed the diets containing 2% $n-3$ HUFA, whatever the chemical form these EFA were presented.

In general terms, total lipid content of fish tissues did not vary with respect to those of the initial sample or according to diet, with values of about 49%, 46% and 21% DWB, for brain, gills and muscle, respectively. However, the liver presented values of around 37% in both the initial sample and fish fed 5% $n-3$ HUFA, significantly higher than those present in livers from fish fed 2% $n-3$ HUFA (25.1%, 24.7% and 23.7% DWB for diets 1, 2 and 3, respectively).

The fatty acid composition of total neutral and polar lipids from all the tissues examined are shown in Tables 4–7. In both lipid fractions, and independent of dietary

Table 7
Main fatty acid composition (area %) of gills and muscle polar lipids

	Initial	Diet no.			
		1	2	3	4
Gills					
ΣSaturates	42.7 ± 0.5$^{(b)}$	42.8 ± 0.4$^{(b)}$	42.2 ± 0.4$^{(b)}$	41.0 ± 0.5$^{(ab)}$	39.4 ± 0.3$^{(a)}$
14:0	3.1 ± 0.2	3.5 ± 0.2	2.9 ± 0.1	3.0 ± 0.3	3.2 ± 0.2
16:0	30.2 ± 0.5	30.7 ± 0.4	30.9 ± 0.4	29.6 ± 0.5	29.3 ± 0.3
18:0	8.4 ± 0.3$^{(c)}$	6.8 ± 0.2$^{(b)}$	7.0 ± 0.2$^{(b)}$	6.4 ± 0.3$^{(ab)}$	5.6 ± 0.2$^{(a)}$
Σ*n*−9	18.2 ± 0.3$^{(c)}$	13.8 ± 0.4$^{(b)}$	14.9 ± 0.3$^{(b)}$	15.0 ± 0.3$^{(b)}$	11.9 ± 0.3$^{(a)}$
18:1*n*−9	16.2 ± 0.3$^{(c)}$	11.8 ± 0.4$^{(ab)}$	13.0 ± 0.3$^{(b)}$	12.6 ± 0.3$^{(b)}$	10.6 ± 0.3$^{(a)}$
18:2*n*−9	tr	nd	nd	tr	nd
Σ*n*−6 PUFA	6.9 ± 0.3$^{(b)}$	5.8 ± 0.2$^{(ab)}$	5.6 ± 0.2$^{(ab)}$	6.0 ± 0.2$^{(b)}$	4.9 ± 0.2$^{(a)}$
18:2*n*−6	3.8 ± 0.3$^{(c)}$	3.1 ± 0.2$^{(bc)}$	2.4 ± 0.3$^{(ab)}$	2.7 ± 0.2$^{(ab)}$	1.8 ± 0.2$^{(a)}$
20:4*n*−6	2.6 ± 0.2	2.9 ± 0.2	3.0 ± 0.3	2.9 ± 0.3	3.0 ± 0.2
Σ*n*−3 PUFA	21.3 ± 0.2$^{(a)}$	28.8 ± 0.3$^{(b)}$	27.2 ± 0.1$^{(b)}$	27.8 ± 0.3$^{(b)}$	36.5 ± 0.4$^{(c)}$
18:3*n*−3	nd$^{(a)}$	tr$^{(b)}$	tr$^{(b)}$	0.5 ± 0.1$^{(b)}$	tr$^{(b)}$
18:4*n*−3	0.6 ± 0.1$^{(b)}$	tr$^{(a)}$	tr$^{(a)}$	tr$^{(a)}$	0.5 ± 0.1$^{(b)}$
Σ*n*−3 HUFA	18.5 ± 0.2$^{(a)}$	25.4 ± 0.3$^{(b)}$	24.4 ± 0.2$^{(b)}$	24.7 ± 0.3$^{(b)}$	32.3 ± 0.4$^{(c)}$
20:5*n*−3	7.1 ± 0.2$^{(a)}$	12.0 ± 0.3$^{(b)}$	11.6 ± 0.3$^{(b)}$	11.1 ± 0.3$^{(b)}$	15.2 ± 0.4$^{(c)}$
22:5*n*−3	0.9 ± 0.1$^{(a)}$	0.5 ± 0.1$^{(a)}$	0.7 ± 0.1$^{(a)}$	0.5 ± 0.1$^{(a)}$	1.8 ± 0.1$^{(b)}$
22:6*n*−3	9.1 ± 0.2$^{(a)}$	12.4 ± 0.2$^{(b)}$	11.9 ± 0.3$^{(b)}$	12.6 ± 0.3$^{(b)}$	14.6 ± 0.3$^{(c)}$
Saturated/unsaturated	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0
18:1*n*−9/*n*−3 HUFA	0.9 ± 0.1$^{(c)}$	0.5 ± 0.0$^{(b)}$	0.5 ± 0.0$^{(b)}$	0.5 ± 0.0$^{(b)}$	0.3 ± 0.0$^{(a)}$
EPA/DHA	0.8 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0
Muscle					
ΣSaturates	28.7 ± 0.3$^{(b)}$	29.4 ± 0.4$^{(b)}$	28.8 ± 0.3$^{(b)}$	30.2 ± 0.4$^{(b)}$	26.2 ± 0.3$^{(a)}$
14:0	1.3 ± 0.2$^{(b)}$	1.1 ± 0.1$^{(b)}$	1.2 ± 0.2$^{(b)}$	1.5 ± 0.2$^{(b)}$	0.5 ± 0.1$^{(a)}$
16:0	21.8 ± 0.2$^{(b)}$	22.7 ± 0.4$^{(b)}$	21.2 ± 0.3$^{(ab)}$	21.8 ± 0.4$^{(b)}$	20.3 ± 0.3$^{(a)}$
18:0	5.2 ± 0.3$^{(ab)}$	5.0 ± 0.2$^{(ab)}$	5.4 ± 0.2$^{(ab)}$	6.0 ± 0.3$^{(b)}$	4.6 ± 0.2$^{(a)}$
Σ*n*−9	13.6 ± 0.3$^{(b)}$	14.8 ± 0.4$^{(bc)}$	14.0 ± 0.3$^{(b)}$	15.2 ± 0.4$^{(c)}$	11.9 ± 0.2$^{(a)}$
18:1*n*−9	13.0 ± 0.3$^{(b)}$	13.8 ± 0.4$^{(b)}$	13.5 ± 0.3$^{(b)}$	14.3 ± 0.4$^{(b)}$	11.3 ± 0.2$^{(a)}$
18:2*n*−9	nd	tr	tr	tr	tr
Σ*n*−6	6.4 ± 0.2	6.8 ± 0.1	6.9 ± 0.2	6.7 ± 0.2	5.9 ± 0.2
18:2*n*−6	4.1 ± 0.2$^{(b)}$	4.0 ± 0.1$^{(b)}$	4.2 ± 0.2$^{(b)}$	3.2 ± 0.2$^{(ab)}$	2.5 ± 0.2$^{(a)}$
18:4*n*−6	1.9 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
Σ*n*−3	38.2 ± 0.2$^{(b)}$	32.4 ± 0.3$^{(a)}$	33.7 ± 0.3$^{(a)}$	32.7 ± 0.2$^{(a)}$	41.9 ± 0.4$^{(c)}$
18:3*n*−3	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	tr	0.5 ± 0.1
18:4*n*−3	1.0 ± 0.1$^{(b)}$	tr$^{(a)}$	0.5 ± 0.1$^{(a)}$	tr$^{(a)}$	0.5 ± 0.1$^{(a)}$
Σ*n*−3 HUFA	35.9 ± 0.2$^{(b)}$	31.3 ± 0.2$^{(a)}$	32.6 ± 0.2$^{(a)}$	31.7 ± 0.2$^{(a)}$	40.5 ± 0.4$^{(c)}$
20:5*n*−3	14.0 ± 0.2$^{(b)}$	11.3 ± 0.3$^{(a)}$	12.3 ± 0.2$^{(a)}$	12.0 ± 0.2$^{(a)}$	16.2 ± 0.4$^{(c)}$
22:5*n*−3	1.6 ± 0.1	1.9 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	2.0 ± 0.2
22:6*n*−3	19.4 ± 0.2$^{(b)}$	17.3 ± 0.2$^{(a)}$	18.1 ± 0.3$^{(ab)}$	17.1 ± 0.2$^{(a)}$	21.3 ± 0.3$^{(c)}$

Table 7 (*continued*)

	Initial	Diet no.			
		1	2	3	4
Muscle					
Saturated/unsaturated	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0
18:1$n-9$/$n-3$ HUFA	0.4±0.0	0.4±0.0	0.4±0.0	0.5±0.1	0.3±0.0
EPA/DHA	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.8±0.0

Data are mean±SEM ($n=3$ fish); nd, not detected; tr, fatty acid values $\leq 0.4\%$. Values within a given row having different superscript letters are significantly different ($P<0.05$).

treatment, fatty acids such as 16:0 and 18:1$n-9$ were the most abundant within the saturates and $n-9$ series, respectively. Among the polyunsaturates, the $n-6$ fatty acids were always less abundant than the $n-3$ series, the latter being particularly rich in DHA and EPA. Both in neutral and polar lipid fractions, the highest levels of DHA were present in brain.

When comparing the fatty acid composition of neutral and polar lipids from the initial sample (Tables 4–7), 16:0 content in liver and gills was lower in the neutral fraction than in the polar fraction, whereas in the brain, the opposite pattern was observed. In muscles, this fatty acid was similarly represented. The 18:1$n-9$ content of the neutral fraction was higher than that of the polar fraction, the exception being for the brain, where this fatty acid was similarly represented in both fractions. The $n-3$ HUFA content in brain, liver and muscle was notably higher in the polar lipid fraction than in the neutral lipid, whereas in gills, these differences were not found.

When gilthead seabream juveniles were fed diets 1, 2 and 3, only minor differences were found among groups in terms of saturates, $n-9$, $n-6$ or $n-3$ fatty acids from either the neutral or polar lipid fraction. However, some differences were found between these three dietary groups of fish and the initial sample. For instance, the $n-3$ HUFA level significantly increased with respect to that of the initial sample in all tissues studied, except for the neutral and polar lipid fraction of muscle (Tables 4–7).

When fish were fed a lower level of saturates and a high level of EFA (diet 4, Table 3), a significant decrease in saturates was observed in the polar lipid fraction from brain, liver and muscle with respect to the the initial sample and the other three dietary groups (Tables 6 and 7). In addition, an accretion can be observed in the total $n-3$ HUFA in all the tissues examined with respect to the amount present in the other three treatments or in the initial sample (Tables 4–7). Only the levels of EPA and DHA present in the brain polar lipids did not vary in fish fed diet 4 with respect to those of treatments 1, 2 and 3, despite the different contribution of EFA in the diets (Table 6).

4. Discussion

From the fatty acid analysis of both the neutral and the polar lipid fraction of the initial sample, it is concluded that in most tissues studied, the most abundant fatty acids were 16:0, 18:1$n-9$, 22:6$n-3$ and 20:5$n-3$. These results are in agreement with

those previously reported for the same species (Ibeas et al., 1994, 1996, 1997), and indicate that these fatty acids are not only the main energy source in the tissues but also, the primary fatty acids selectively incorporated into membrane phospholipids.

When fish were fed diets equally contributing in saturates, $n-9$, $n-6$ and $n-3$ HUFA, presented either as methyl esters or triacylglycerols, only minor differences were found in fish tissue levels of any of these fatty acids. This may suggest that the methyl ester form of fatty acids did not have any obvious detrimental effect on lipid metabolism of gilthead seabream juveniles. In addition, no differences were observed in terms of growth, mortality or in gross external signs of abnormality, among groups 1, 2 and 3 over the 8-week feeding trial. Therefore, both the body fatty acid composition and fish growth support the conclusion that in gilthead seabream juveniles (62 g of initial body weight), the feed efficiency of dietary $n-3$ HUFA given as methyl esters is similar to that achieved when triacylglycerols are used. These results differ from those obtained in our laboratory for gilthead seabream larvae when fed rotifers enriched on methyl esters of $n-3$ fatty acids. In that experiment, the larvae displayed a much lower weight gain and a very high mortality rate compared with larvae fed rotifers enriched with triacylglycerols of $n-3$ HUFA (Rodríguez et al., 1993). Poor growth and a high mortality was also observed in red seabream larvae when they were fed rotifers (Izquierdo et al., 1989) or *Artemia* (Takeuchi et al., 1992) enriched on methyl esters of $n-3$ fatty acids. The reason for this negative effect of methyl esters of fatty acids on seabream larvae but not on seabream juveniles is not clear. It could be thought that larvae are more vulnerable than juveniles to toxic methanol produced during digestion of methyl esters.

Another explanation to the different results obtained in the present study compared with those obtained with larvae could be found in the fact that larvae were not directly fed with $n-3$ HUFA methyl esters. This means that there is an intermediary organism altering, through its metabolism, the chemical form of lipids that will finally reach the larvae. In fact, Izquierdo (1998) found that rotifers fed $n-3$ HUFA methyl esters accumulated the $n-3$ HUFA mainly as free fatty acids, whereas, when they were fed $n-3$ HUFA from triacylglycerols, these fatty acids were esterified into triacylglycerols and phospholipids. These phospholipids could have exerted a beneficial effect during the digestion of lipids in larvae, since dietary phospholipids heve been shown to increase the efficiency of the digestion proccesses in fish larvae (Kanazawa, 1993; Geurden et al., 1997; Fontagne et al., 1998; Sargent et al., 1999). Finally, the free fatty acids accumulated in rotifers when enriched with methyl esters could have also had a gastric irritant effect in larvae, as previously reported in humans (Ackman and Ratnayake, 1989).

On the other hand, the FAME utilized in the study carried out on gilthead seabream larvae, contained 85% $n-3$ HUFA. Therefore, it is possible that an excess of $n-3$ HUFA in the enriched rotifers could also have contributed to the poor larval growth, since HUFA requirements cannot be meaningfully considered without considering the overall balance of polyunsaturated, monounsaturated and saturated fatty acids and their relative proportions in dietary phospholipids and triacylglycerols (Sargent et al., 1999).

In this sense, Rodríguez et al. (1994) have shown that larvae fed rotifers containing 7.42% (DWB) of triacylglycerols of $n-3$ HUFA displayed poorer growth and survival

than fish given rotifers containing 5.5% (DWB) of $n-3$ HUFA also as triacylglycerols. In the present study, when gilthead seabream juveniles were fed a high level of triacylglycerols of $n-3$ HUFA (diet 4), some differences were found with respect to fish given the other three diets, some of these differences being poorer growth and high levels of both EPA and DHA in the neutral and polar lipid fractions from liver, gills and muscle. This marked increase in tissue $n-3$ HUFA content was acompained by a decrease of saturates and $n-9$, which was probably due to the fact that 16:0, 18:0 and 18:1$n-9$ levels were lower in diet 4 than in diets 1, 2 and 3. It is well established that the fatty acid pattern of fish tissue lipid reflects the fatty acid composition of dietary lipids (Bell et al., 1994; Sargent et al., 1995). Therefore, not only the possible excess of EPA and DHA present in tissues from fish fed diet 4, but also the changes observed in the levels of 16:0, 18:0 and 18:1$n-9$, might involve a disturbance in membrane phospholipids and to be responsible for the poorer growth achieved by this group of fish. In fact, Lochman and Gatlin (1993) suggested that the mechanism underlying a suppression of fish weight gain in juvenile red drum when fed an excess of $n-3$ PUFA ethyl esters might involve a disturbance in optimal levels of tissue lipid saturation induced by levels of $n-3$ PUFA not normally encountered in natural diets. There seems to be a tissue optimum level of unsaturation for an optimum growth rate. Therefore, an excess of dietary $n-3$ fatty acids may create an abnormal situation which obliges the animal to metabolically respond to these changes (Yu and Sinnhuber, 1976), thus reducing fish growth.

In contrast to liver, gills and muscle, the brain tissue from gilthead seabream juveniles did not undergo an accretion of EPA and DHA in its polar lipids as a result of the high intake of dietary $n-3$ HUFA. Although the brain may be vulnerable to dietary changes imposed during periods of growth and development, its chemical composition is relatively constant and is more resistant to the influence of external factors than are other organs. This being particularly true after maturity (Odutuga, 1977). Since the brain is the principal organ involved in the regulation of the physiological functions, there must be some mechanisms involved in the membrane structure metabolism aiming to preserve the structure and function of brain cells and membranes.

In conclusion, no differences were found in terms of $n-3$ HUFA efficiency when these fatty acids were fed to fish in the form of methyl esters or as triacylglycerols. A reduction of fish growth was noticeable when they were fed a high level of EFA, with tissue composition being markedly influenced by the fatty acid composition of the diet. The exception being for the brain which particularly preserved the $n-3$ HUFA levels in its polar lipids.

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