

Vegetable oils affect the composition of lipoproteins in sea bream (*Sparus aurata*)

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The aim of the present study was to determine the influence of the dietary fatty acid profile on the lipoprotein composition in sea bream fed different vegetable oils. Six experimental diets were formulated combining fish oil with three vegetable oils (soybean, rapeseed, linseed) in order to obtain 60–80% (w/w) fish-oil replacement. VLDL, LDL and HDL in plasma samples were obtained by sequential centrifugal flotation. The lipid class, protein content and fatty acid composition of each lipoprotein fraction were analysed. HDL was the predominant lipoprotein in sea bream plasma containing the highest proportion of protein (34%) and phosphatidylcholine. LDL presented a high content of cholesterol, whereas triacylglycerol comprised a larger proportion of VLDL. The lipid class of the lipoprotein fractions was affected by the dietary vegetable oils. Thus, a high dietary inclusion of soyabean and linseed oil (80%) increased the cholesterol in HDL and LDL in comparison to fish oil. Similarly, the triacylglycerol concentration of VLDL was increased in fish fed 80% soyabean and linseed oils owing to the low *n-3* highly unsaturated fatty acid content of these diets. Lipoprotein fatty acid composition easily responded to dietary fatty acid composition. VLDL was the fraction more affected by dietary fatty acid, followed by LDL and HDL. The *n-3* highly unsaturated fatty acid content increased in the order VLDL less than LDL and less than HDL, regardless of dietary vegetable oils.

Sea bream: Lipoproteins: VLDL: LDL: HDL: Vegetable oils: Cholesterol: Triacylglycerols: Phosphatidylcholine: Sterol esters: *n-3* highly unsaturated fatty acids

As in mammals, the transport of fatty acids and other lipid-soluble components to the peripheral tissues in fish is mediated mainly by lipoproteins. Exogenous lipids absorbed by the intestine are predominantly integrated into chylomicrons and VLDL (Sire *et al.* 1981; Caballero *et al.* 2003), whereas endogenous lipids are transported in VLDL, LDL and HDL (Sheridan, 1988; Babin & Vernier, 1989; Iijima *et al.* 1995). Proteins and lipids integrated into the different lipoproteins interact with enzymes or cellular receptors, resulting in lipoprotein degradation or transformation, with the subsequent transfer of products from tissues to lipoproteins or vice versa. Initially, chylomicrons and VLDL-triacylglycerols (TAG) are hydrolysed by the enzyme lipoprotein lipase, releasing fatty acids that are up taken by adipose tissue for storage or by other organs, such as muscle, for oxidation (Auwerx *et al.* 1992). The withdrawal of the LDL particles is mainly mediated by specific extrahepatic LDL receptors (Brown & Goldstein, 1997). One of the principal roles of HDL is to carry cholesterol from the peripheral cells to the liver. A key enzyme in this process is lecithin:cholesterol acyltransferase, responsible for transferring cholesterol and fatty acids from phosphatidylcholine to cholesterol esters on the surface of the HDL. Lecithin:cholesterol acyltransferase

activity has been observed in the plasma of teleost fish (Black *et al.* 1985).

The lipid proportion and composition of each lipoprotein are affected by, among other factors, dietary composition (Sheridan *et al.* 1985). For instance, the fatty acid composition of lipoproteins is reported to be affected by dietary fatty acid composition in Atlantic salmon (*Salmo salar*; Lie *et al.* 1993; Torstensen *et al.* 2000, 2001). The increasing global demand on fish oil and the unpredictability of its production imposes limitations on the utilization of vegetable oils in commercial aqua feeds (Food & Agriculture Organization, 1997). A partial replacement of fish oil by certain vegetable oils has, in several species, been proved to be feasible without affecting growth (Caballero *et al.* 2002; Bell *et al.* 2003; Regost *et al.* 2003; Izquierdo *et al.* 2003, 2005; Montero *et al.* 2005). Little information is, however, available on how these dietary fatty acids influence lipid transport in sea bream. Recent studies (Caballero *et al.* 2003, 2006) have reported that vegetable lipid sources affect intestinal TAG and phospholipid biosynthesis, interfering with the types of lipoprotein produced by the intestine of sea bream. In order to complete this information, an experiment was conducted to determine the influence of the dietary fatty

Abbreviations: HUFA, highly unsaturated fatty acids; TAG, triacylglycerol.

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acid profile on the lipoprotein composition in sea bream fed different vegetable oils.

Material and methods

Fish

The studies were carried out at the Instituto Canario de Ciencias Marinas (Canary Islands, Spain), and all fish were obtained from a local fish farm (ADSA, Canary Islands, Spain). Three hundred gilthead sea bream (*Sparus aurata*) with an average initial body weight of 10.06 g were randomly distributed in 100-litre polyethylene circular tanks (20 fish/tank). Tanks were supplied with continuous seawater (36‰) flow and aeration. The fish were fed under natural photoperiod conditions of approximately 12 h/12 h dark light. The water temperature and dissolved O₂ concentration during the experimental period ranged between 20.0 and 24.2 °C and 5.04 and 8.32 ppm, respectively. Fish were acclimatised to the experimental tanks and fed the control diet for 2 weeks before beginning the experiment. They were fed the experimental diets until apparent satiation three times a day, at 09.00, 12.00 and 15.00 hours, 6 days a week for 101 days. Each diet was assayed in triplicate tanks.

Experimental diets

Six isoenergetic and isonitrogenous experimental diets were formulated to contain 22 % crude lipid and 45 % crude protein. Whereas the control (100FO) diet contained anchovy oil as the only added lipid source, anchovy oil in all the other diets was partly replaced by vegetable oils (Table 1). Sixty per cent of the anchovy oil was replaced by either soyabean, rapeseed or linseed oil in diets 60SO, 60RO and 60LO, whereas 80 % was replaced by either soyabean or linseed oil in diets 80SO and 80LO. Anchovy oil was included in all the diets at a level high enough to keep the content of *n*-3 highly unsaturated fatty acid (HUFA), with twenty or more carbon atoms and three or more double bonds, over 3 % in order to meet the essential fatty acid requirements of this species (Montero *et al.* 1996).

An analysis of the fatty acid composition of the diets (Table 2) showed the highest saturated fatty acid and *n*-3

HUFA content in diet 100FO, the highest linoleic acid (18:2*n*-6) level in diets 60SO and 80SO, the highest linolenic acid (18:3*n*-3) in diets 60LO and 80LO, and the highest oleic acid (18:1*n*-9) concentration in diet 60RO. The dietary ratios of *n*-3 to *n*-6 differed between the experimental diets, mainly due to the different 18:2*n*-6 content of the plant oils used, ranging from 1.4 in diet 100FO to 0.5 in diet 60SO and 0.3 in diet 80SO. Diets 60LO and 80LO had an *n*-3:*n*-6 ratio higher than 2, mainly due to the higher 18:3*n*-3 content. Diets 80SO and 80LO had the lowest *n*-3 HUFA content, particularly in terms of arachidonic acid (20:4*n*-6), EPA (20:5*n*-3) and DHA (22:6*n*-3).

Sampling procedure

The fish were sampled at the end of the feeding trial. The fish were fasted for 24 h prior to sampling. Twenty randomly sampled fish per tank were anaesthetised with chlorobutanol (200 mg/l). Blood was collected from the caudal veins using EDTA-containing Vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was separated from the blood samples by centrifugation at 3000 rpm for 10 min. The twenty plasma samples from each tank were pooled into three samples (nine samples for each diet tested) and stored on ice (4 °C) until lipoprotein fractionation.

Separation of lipoproteins

VLDL, LDL and HDL in pooled plasma samples were obtained by sequential centrifugal flotation, as described by Lie *et al.* (1994), using a Pegasus 65 ultracentrifuge equipped with a 70-Ti fixed-angle rotor (Beckman Coulter Inc., Fullerton, CA, USA). Centrifugation was carried out at 37 500 rpm and 4 °C. The density intervals were obtained by adding solid KBr (Warnick *et al.* 1979). The run times for lipoprotein separation were: $d < 1.015$ g/ml over 20 h for VLDL; $1.015 < d < 1.085$ g/ml over 20 h for LDL; $1.085 < d < 1.21$ g/ml over 44 h for HDL. The different lipoprotein fractions obtained were weighed on an analytical balance (Mettler AJ100; Mettler Toledo S. A. E., L'Hospitalet de Llobregat, Barcelona) and stored at -80 °C until further analysis.

Table 1. Composition of experimental diets containing different lipid sources

	Diet					
	100FO (g/kg)	60SO (g/kg)	60RO (g/kg)	60LO (g/kg)	80SO (g/kg)	80LO (g/kg)
Fish meal (LT)	381.3	381.3	381.3	381.3	381.3	381.3
Corn gluten	259.8	259.8	259.8	259.8	259.8	259.8
Wheat	150.7	150.7	150.7	150.7	150.7	150.7
Lysine (99%)	7.2	7.2	7.2	7.2	7.2	7.2
Premix*	25	25	25	25	25	25
Anchovy oil	176	70.4	70.4	70.4	35.2	35.2
Soyabean oil†		105.6			140.8	
Rapeseed oil†			105.6			
Linseed oil†				105.6		140.8
Composition						
Crude lipids	23.74	24.15	22.94	23.19	22.59	25.48
Crude protein	47.52	48.84	48.51	48.52	49.43	48.71

LT, low temperature.

*Premix of vitamins and minerals according to National Research Council (1993) recommendations for fish.

†Crude vegetable oils.

Table 2. Main fatty acid composition of diets containing fish oil in combination with different vegetable lipid sources

	Diet					
	100FO (g/100 g fatty acids)	60SO (g/100 g fatty acids)	60RO (g/100 g fatty acids)	60LO (g/100 g fatty acids)	80SO (g/100 g fatty acids)	80LO (g/100 g fatty acids)
14:0	6.21	3.18	3.11	3.09	1.10	1.94
16:0	18.51	15.45	12.43	12.21	14.24	10.89
18:0	3.18	3.21	2.35	3.37	3.30	3.45
Sum saturated	27.90	21.84	17.89	18.67	18.64	16.28
16:1 <i>n</i> -7	6.46	3.41	3.39	3.31	2.20	2.10
17:1	1.28	0.63	0.63	0.62	0.37	0.35
18:1 <i>n</i> -9	16.26	18.48	36.84	17.03	21.07	18.71
18:1 <i>n</i> -7	2.47	1.84	ND	1.54	ND	ND
20:1 <i>n</i> -9	1.89	1.63	2.08	1.58	1.49	1.61
22:1 <i>n</i> -11	1.95	1.49	2.13	2.13	2.01	1.78
22:1 <i>n</i> -9	0.14	0.22	0.03	0.03	0.03	0.08
Sum monoenes	30.45	27.70	45.10	26.24	27.17	24.63
16:2 <i>n</i> -6	1.15	0.60	0.57	0.58	0.37	0.36
18:2 <i>n</i> -6	13.07	31.28	17.04	12.29	38.54	17.66
18:3 <i>n</i> -6	0.21	0.12	0.10	0.11	0.07	0.07
20:4 <i>n</i> -6	0.54	0.34	0.33	0.36	0.25	0.23
22:3 <i>n</i> -6	0.53	0.33	0.29	0.29	0.22	0.22
22:4 <i>n</i> -6	0.25	0.21	0.17	0.18	0.16	0.15
Sum <i>n</i> -6	15.75	32.88	18.50	13.81	39.61	18.69
16:4 <i>n</i> -3	1.85	0.88	0.85	0.88	0.44	0.44
18:3 <i>n</i> -3	1.41	3.24	4.64	26.72	4.03	30.63
18:4 <i>n</i> -3	1.43	0.79	0.76	0.78	0.48	0.54
20:4 <i>n</i> -3	0.43	0.25	0.26	0.25	0.22	0.19
20:5 <i>n</i> -3	11.19	5.88	5.84	6.19	3.73	3.79
22:4 <i>n</i> -3	0.19	0.15	0.12	0.14	0.10	0.08
22:5 <i>n</i> -3	1.16	0.75	0.63	0.79	0.50	0.45
22:6 <i>n</i> -3	4.99	3.20	3.26	3.58	2.36	2.92
Sum <i>n</i> -3	22.65	15.14	16.36	39.33	11.86	39.04
Sum <i>n</i> -3 highly unsaturated fatty acids	17.96	10.23	10.11	10.95	6.91	7.43
<i>n</i> -3: <i>n</i> -6	1.44	0.46	0.88	2.85	0.30	2.09
Lipids (% DW)	21.14	20.70	21.83	21.83	20.93	22.58

ND, not detected.

Analytical procedures

Lipids from pooled samples were extracted with a chloroform–methanol (2:1, v:v) mixture as described by Folch *et al.* (1957). Fatty acid methyl esters were obtained by transesterification with 1% H₂SO₄ in CH₃OH (Christie, 1982) using heneicosanoic acid (10% of total lipids) as an internal standard. Fatty acid methyl esters were purified by adsorption chromatography on NH₂ sep-pack cartridges (Waters SA, Milford, MA, USA) as described by Fox (1990), and separated and quantified by GLC following the conditions described by Izquierdo *et al.* (1990).

The lipid classes of each lipoprotein fraction were separated by high-precision TLC into sterol esters, TAG, cholesterol and phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine, and quantified by a flame ionisation detector system (Iatroscan MK-5, Iatron Laboratories Inc., Tokyo, Japan) according to the method described by Rønnestad *et al.* (1995). The total protein content of each lipoprotein fraction was determined by Lowry assay (Lowry *et al.* 1951).

Statistics

Results were expressed as means and standard deviations. Data were compared by ANOVA, and Tukey's test for comparison of means was applied ($P < 0.05$). When variances

were not normally distributed, the Kruskal–Wallis non-parametric test was applied to the data, and for comparison of medians, the Box and Whisker-notched test was applied (Sokal & Rolf, 1995).

Results

The amount of each lipoprotein fraction per gram of sea bream plasma was 264 (SD 37.6) mg/g for HDL, 194.2 (SD 43.7) mg/g for VLDL and only 131.6 (SD 41.5) mg/g for LDL.

Total protein and lipid content

Table 3 shows the total protein and lipid content of the lipoprotein fractions expressed as milligrams of protein or lipid per gram of lipoprotein fraction. No significant differences were found in the total protein (average 5.4 mg protein/g HDL) or lipid (average 11.2 mg lipid/g HDL) contents of the HDL fraction among fish fed the experimental diets. In the LDL fraction, the protein content was similar in all the experimental groups (average 0.4 mg protein/g LDL), whereas a higher content of total lipids was present in fish fed the 80SO and 80LO (1.2 mg lipid/g LDL) diets, fish fed 100FO showing the lowest lipid content (0.4 mg lipid/g LDL). No significant differences were found in the total protein (average 0.4 mg protein/g VLDL) for the VLDL fraction among fish

Table 3. Total protein and lipid content of the lipoprotein fractions of sea bream fed the experimental diets (Means and standard deviations; three samples from each tank, nine for each experimental diet)

Diets	Protein content						Lipid content					
	mg protein/g HDL		mg protein/g LDL		mg protein/g VLDL		mg lipid/g HDL		mg lipid/g LDL		mg lipid/g VLDL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
100FO	5.0	1.2	0.3	0.2	0.2	0.1	9.3	2.7	0.4	0.1 ^a	0.8	0.2 ^a
60SO	5.3	1.2	0.3	0.1	0.3	0.2	11.0	1.1	0.9	0.2 ^{bc}	1.0	0.2 ^{ab}
60RO	5.6	0.8	0.4	0.1	0.4	0.3	12.3	1.4	0.9	0.3 ^{bc}	1.2	0.2 ^{ab}
60LO	5.2	0.1	0.4	0.1	0.4	0.1	11.3	0.4	0.7	0.1 ^b	1.3	0.2 ^{ab}
80SO	5.6	0.6	0.5	0.2	0.4	0.2	12.3	1.8	1.2	0.2 ^{cd}	1.3	0.1 ^{ab}
80LO	5.3	0.8	0.3	0.1	0.5	0.3	10.7	0.9	1.2	0.3 ^d	1.8	0.1 ^b

Superscript letters in the same row denote significantly different ($P \leq 0.05$).

fed the experimental diets, but the lowest content of total lipid for this fraction was again found in fish fed diet 100FO (0.8 mg lipid/g VLDL).

Lipid class composition

The lipid class composition and percentage of protein in each lipoprotein fraction are shown in Fig. 1 and expressed as g lipid/g protein. An increase in the neutral lipid (sterol esters, TAG, cholesterol) content of the lipoproteins occurred concomitantly with a decreased protein content and density of lipoproteins (VLDL < LDL < HDL) for all the experimental diets tested. Phosphatidylcholine was the major phospholipids in all lipoprotein fractions, whereas only trace levels of the other glycerophospholipids (phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine) were found. In VLDL, TAG comprised a larger proportion than the other fractions. The proportion of sterol esters was higher in the lipid of LDL than in HDL. HDL consisted mainly of protein (34%) and phosphatidylcholine.

The lipid class composition of the different lipoprotein fractions expressed in mg lipid class/g plasma was affected by the experimental diets. In HDL (Fig. 2), fish fed diet 100FO had the lowest TAG content, being significantly different ($P < 0.05$) from that of fish fed diet 80SO.

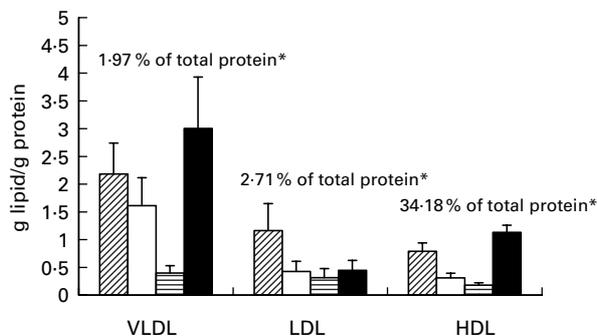


Fig. 1. Lipid class composition (g lipid/g protein) of VLDL, LDL and HDL from sea bream fed the experimental diets for 101 d (means and standard deviations). *Amount of protein in the lipoprotein fraction, calculated as (amount of protein in the lipoprotein fraction) / (amount of protein in VLDL + LDL + HDL + rest) \times 100%. ▨, Sterol esters; □, Triacylglycerol; ▤, Cholesterol; ■, Phosphatidylcholine.

In addition, these latter fish presented the highest cholesterol content ($P < 0.05$). No differences were found in the sterol esters and phosphatidylcholine content among fish fed the different experimental diets. In LDL (Fig. 3), fish fed the 100FO diet showed the lowest level of TAG, cholesterol and phosphatidylcholine in comparison to the other experimental groups. High levels of cholesterol and phosphatidylcholine were found in fish fed diets 80SO and 80LO. In VLDL (Fig. 4), lipid classes showed a large variation between the different experimental groups. Thus, fish fed the 60RO diet presented markedly high sterol esters content in comparison to other fish. In addition, fish fed the 100FO diet showed the lowest level of cholesterol, this being significantly ($P < 0.05$) different from those of the other fish, except for those fed 60RO. Finally, the highest values of TAG were found in fish fed diets 80SO and 80LO, values being significantly different from those of fish fed diets 100FO and 60RO.

Fatty acid composition

Table 4 shows the fatty acid composition of the VLDL fraction. The dietary influence of the composition of this fraction was demonstrated by the high level of 18:1n-9 (30%) in the 60RO fish, 18:2n-6 (21% and 26%) in the 60SO and 80SO fish, and 18:3n-3 (12% and 18%) in the 60LO and 80LO fish. The highest levels of n-3 HUFA were found in fish fed diet 100FO ($P < 0.05$), particularly due to the high content of DHA.

The LDL fraction (Table 5) was characterised by high levels of saturated fatty acid, particularly 16:0, in comparison with VLDL. Dietary fatty acid composition was also reflected in the contents of oleic, linoleic and linolenic acids, although to a lesser extent than those found in VLDL fraction. The DHA content of the LDL fraction was also elevated in all experimental groups, irrespective of dietary content. The HDL fatty acid composition (Table 6) in particular was characterised by a markedly high n-3 HUFA content in all experimental groups, with values ranging from 24.6% (80SO fish) to 38.8% (100FO fish), mainly due to the high level of DHA. In addition, this fraction also had a higher content of arachidonic acid (20:4n-6) in comparison with the other lipoprotein fractions. The HDL content of 18:1n-9; 18:2n-6 and 18:3n-3 reflected the dietary composition, although there was a considerable reduction in fatty acids of 18 carbons in

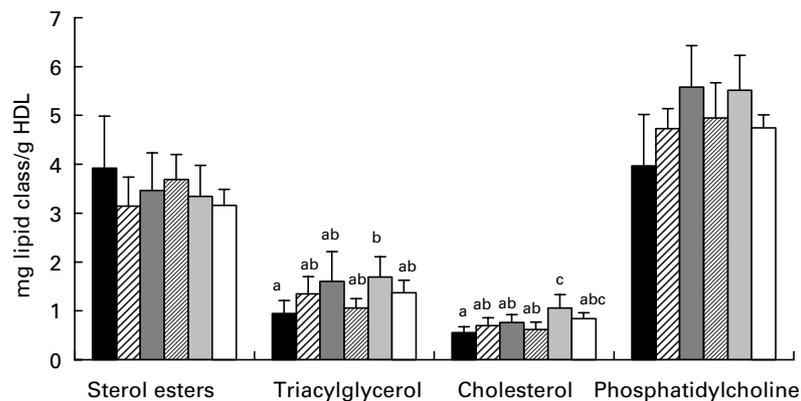


Fig. 2. HDL lipid class composition from sea bream fed the experimental diets (means and standard deviations; three samples from each tank, nine for each experimental diet). For the diet groups, mean values with unlike superscript letters were significantly different ($P \leq 0.05$). Diets: ■, 100FO; ▨, 60SO; ▩, 60RO; ▪, 60LO; ▧, 80SO; □, 80LO.

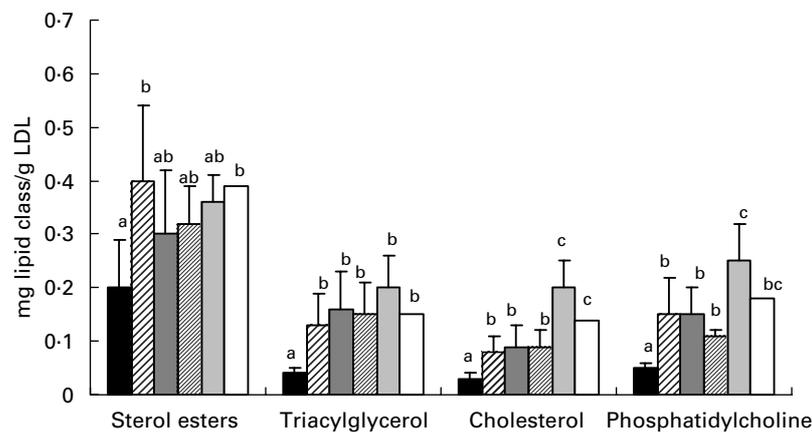


Fig. 3. LDL lipid class composition from sea bream fed the experimental diets (means and standard deviations; three samples from each tank, nine for each experimental diet). For the diet groups, mean values with unlike superscript letters were significantly different ($P \leq 0.05$). Diets: ■, 100FO; ▨, 60SO; ▩, 60RO; ▪, 60LO; ▧, 80SO; □, 80LO.

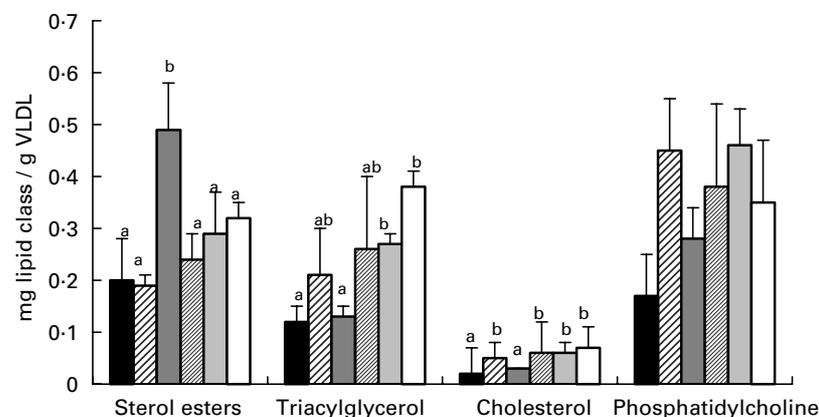


Fig. 4. VLDL lipid class composition from sea bream fed the experimental diets (means and standard deviations; three samples from each tank, nine for each experimental diet). For the diet groups, mean values with unlike superscript letters were significantly different ($P \leq 0.05$). Diets: ■, 100FO; ▨, 60SO; ▩, 60RO; ▪, 60LO; ▧, 80SO; □, 80LO.

Table 4. Fatty acid composition of VLDL from sea bream fed the experimental diets for 12 weeks (Means and standard deviations; three samples from each tank, nine for each experimental diet)

	Diet											
	100FO (g/100 g fatty acids)		60SO (g/100 g fatty acids)		60RO (g/100 g fatty acids)		60LO (g/100 g fatty acids)		80SO (g/100 g fatty acids)		80LO (g/100 g fatty acids)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	4.3	0.3	2.4	0.2	2.2	0.4	2.2	0.1	2.2	0.8	2.2	0.8
16:0	18.0	2.5	24.0	5.4	15.1	1.4	16.4	2.2	14.6	4.0	17.3	1.3
18:0	8.0	2.0	11.5	4.2	7.8	0.8	8.2	3.2	10.0	5.0	8.8	0.1
Sum saturated	30.3	4.3 ^a	37.9	9.4 ^a	25.1	1.8 ^a	26.8	5.3 ^a	26.7	8.9 ^a	28.3	0.7 ^a
16:1 <i>n</i> -7	6.0	1.1	2.7	0.3	3.1	1.0	3.0	0.3	2.4	0.4	2.4	0.5
18:1 <i>n</i> -9	19.0	1.2 ^a	17.9	2.8 ^a	30.0	3.1 ^b	20.2	1.0 ^a	19.8	3.9 ^a	19.7	1.2 ^a
18:1 <i>n</i> -7	3.4	0.3	2.2	0.1	3.0	0.6	2.4	0.6	2.0	0.4	2.1	0.4
22:1 <i>n</i> -11	1.3	0.1	0.8	0.1	1.6	0.4	0.5	0.0	0.8	0.4	1.2	0.1
22:1 <i>n</i> -9	0.4	0.0	0.3	0.1	ND		ND		0.7	0.4	0.4	0.0
Sum monoenes	30.1	2.3 ^{ab}	23.9	2.7 ^a	37.7	5.1 ^b	26.0	2.3 ^{ab}	25.7	4.2 ^a	26.0	1.4 ^{ab}
18:2 <i>n</i> -6	8.0	0.9 ^a	21.0	6.1 ^{bc}	19.3	1.5 ^b	18.3	1.4 ^b	26.2	6.5 ^c	12.4	0.7 ^{ab}
20:4 <i>n</i> -6	0.7	0.0 ^a	0.4	0.1 ^a	0.5	0.2 ^a	0.7	0.3 ^a	0.4	0.2 ^a	0.6	0.1 ^a
Sum <i>n</i> -6	8.8	0.9 ^a	21.5	6.1 ^{ab}	19.8	1.5 ^{ab}	19.0	1.9 ^{ab}	26.6	4.4 ^b	13.0	1.3 ^a
18:3 <i>n</i> -3	0.9	0.3 ^a	1.8	0.5 ^a	3.1	0.3 ^{ab}	12.3	0.6 ^c	4.0	2.5 ^{ab}	17.9	0.7 ^d
20:5 <i>n</i> -3	11.3	0.2 ^a	4.1	0.1 ^b	4.3	0.1 ^b	4.6	0.6 ^b	3.3	0.3 ^b	3.4	0.2 ^b
22:5 <i>n</i> -3	4.4	0.1	2.0	0.0	2.4	0.0	2.3	0.5	2.1	0.3	1.7	0.4
22:6 <i>n</i> -3	14.5	2.5 ^a	8.3	0.3 ^a	10.2	1.0 ^a	9.6	0.5 ^a	8.5	2.5 ^a	9.5	0.7 ^a
Sum <i>n</i> -3	31.1	1.9 ^a	16.3	0.7 ^b	20.0	1.8 ^b	28.8	1.1 ^c	18.0	0.4 ^b	32.5	0.7 ^d
Sum <i>n</i> -3 highly unsaturated fatty acids	30.2	1.7 ^a	14.8	0.2 ^b	16.9	2.0 ^b	16.5	0.6 ^b	14.0	2.7 ^b	15.0	1.1 ^b

Superscript letters in the same row denote significantly different ($P \leq 0.05$).
ND, not detected.

Table 5. Fatty acid composition of LDL from sea bream fed the experimental diets for 12 weeks (Means and standard deviations; three samples from each tank, nine for each experimental diet)

	Diet											
	100FO (g /100 g fatty acids)		60SO (g /100 g fatty acids)		60RO (g /100 g fatty acids)		60LO (g /100 g fatty acids)		80SO (g /100 g fatty acids)		80LO (g /100 g fatty acids)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	3.9	1.1	1.8	0.4	1.8	0.6	1.9	0.5	1.7	0.2	1.6	0.3
16:0	26.0	4.1	26.7	5.8	20.5	5.4	13.5	2.7	25.7	2.1	21.5 ^v	3.3
18:0	8.9	1.0	9.9	0.7	8.3	0.6	9.5	1.0	13.0	2.2	10.5	1.2
Sum saturated	38.8	2.9 ^a	38.4	3.8 ^a	30.6	5.5 ^{ab}	24.9	3.1 ^b	38.6	4.1 ^a	33.6	4.7 ^{ab}
16:1 <i>n</i> -7	4.4	0.1	2.2	0.0	3.9	0.1	3.0	0.2	1.6	0.2	1.9	0.1
18:1 <i>n</i> -9	14.9	0.9 ^a	15.0	2.6 ^a	26.0	0.9 ^b	20.5	1.5 ^{ab}	12.8	2.8 ^a	16.2	1.0 ^a
18:1 <i>n</i> -7	2.7	0.4	2.0	0.2	9.4	2.0	2.4	0.2	1.6	0.3	1.7	0.1
20:1 <i>n</i> -9	1.4	0.4	1.0	0.1	1.5	0.4	1.3	0.1	0.9	0.2	1.2	0.2
22:1 <i>n</i> -11	2.7	2.5	0.6	0.0	0.8	0.5	0.9	0.0	0.6	0.1	0.9	0.2
22:1 <i>n</i> -9	0.5	0.1	0.5	0.1	0.7	0.0	ND		0.4	0.1	0.5	0.0
Sum monoenes	26.6	1.4 ^{ab}	21.3	3.2 ^a	37.2	6.3 ^b	28.1	1.7 ^{ab}	17.9	3.6 ^a	22.3	1.2 ^a
18:2 <i>n</i> -6	3.6	0.2 ^a	14.8	1.4 ^b	10.8	2.6 ^{ab}	9.2	1.3 ^{ab}	16.9	4.5 ^b	10.7	2.1 ^{ab}
20:4 <i>n</i> -6	0.3	0.0 ^a	0.8	0.0 ^a	ND		ND		1.1 ^a	0.1	0.7	0.5 ^a
22:4 <i>n</i> -6	ND		0.3	0.0	ND		0.6	0.1	0.5	0.1	0.3	0.2
Sum <i>n</i> -6	3.9	1.2 ^a	15.9	2.2 ^b	10.8	2.6 ^{ab}	9.8	1.9 ^{ab}	18.5	4.1 ^{ab}	11.7	2.6 ^b
18:3 <i>n</i> -3	0.4	0.1 ^a	1.3	0.1 ^a	1.6	0.3 ^a	13.0	2.0 ^c	1.2	0.4 ^a	13.7	1.1 ^c
20:4 <i>n</i> -3	0.6	0.1	ND		ND		0.6	0.0	0.3	0.1	0.6	0.1
20:5 <i>n</i> -3	10.1	1.9 ^a	5.6	1.0 ^b	5.6	0.6 ^b	6.6	0.4 ^{ab}	3.9	0.1 ^b	4.6	0.6 ^b
22:5 <i>n</i> -3	3.7	0.3	2.5	0.4	1.9	0.0	3.1	0.2	1.8	0.3	2.0	0.1
22:6 <i>n</i> -3	14.5	1.8 ^a	13.3	3.0 ^a	12.3	3.0 ^a	13.7	2.3 ^a	12.4	0.1 ^a	14.5	1.6 ^a
Sum <i>n</i> -3	29.3	4.8 ^{ab}	22.7	5.1 ^{ab}	21.4	3.4 ^{ab}	37.0	2.3 ^b	19.6	0.5 ^a	35.4	4.6 ^b
Sum <i>n</i> -3 highly unsaturated fatty acids	28.5	4.5 ^a	21.4	4.2 ^a	19.8	3.6 ^a	24.0	2.2 ^a	18.4	0.1 ^a	21.7	2.5 ^a

Superscript letters in the same row denote significantly different ($P \leq 0.05$).
ND, not detected.

Table 6. Fatty acid composition of HDL from sea bream fed the experimental diets for 12 weeks (Means and standard deviations; three samples from each tank, nine for each experimental diet)

	Diet											
	100FO (g /100 g fatty acids)		60SO (g /100 g fatty acids)		60RO (g /100 g fatty acids)		60LO (g /100 g fatty acids)		80SO (g /100 g fatty acids)		80LO (g /100 g fatty acids)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.7	0.2	1.1	0.2	1.1	0.1	1.1	0.1	0.9	0.1	0.9	0.1
16:0	26.1	2.6	23.6	3.6	18.6	1.0	22.1	1.7	22.4	2.1	20.0	3.1
18:0	8.3	0.7	8.4	1.4	6.3	0.3	8.5	0.4	8.9	0.8	9.0	2.1
Sum saturated	36.1	3.4 ^a	33.1	5.1 ^{ab}	26.0	1.3 ^b	31.7	2.0 ^{ab}	32.1	2.8 ^{ab}	29.8	5.1 ^{ab}
16:1 n -7	2.8	0.2	2.0	0.3	1.5	0.8	1.9	0.1	1.5	0.1	1.5	0.1
18:1 n -9	12.2	0.9 ^a	15.1	1.6 ^b	21.8	0.7 ^c	14.9	0.7 ^b	14.6	0.5 ^{ab}	14.9	0.8 ^b
18:1 n -7	2.4	0.1	2.3	0.3	1.8	1.5	2.0	0.1	1.9	0.1	1.7	0.0
20:1 n -9	0.77	0.1	0.8	0.0	1.3	0.1	0.8	0.1	0.8	0.1	0.8	0.1
22:1 n -11	0.4	0.0	0.6	0.3	0.7	0.3	0.5	0.0	0.4	0.0	0.5	0.1
22:1 n -9	0.3	0.0	0.3	0.0	0.5	0.3	0.2	0.0	0.3	0.0	0.3	0.0
Sum monoenes	18.9	1.2 ^a	20.4	2.2 ^a	27.5	2.4 ^b	20.3	1.0 ^a	19.4	0.8 ^a	19.0	1.4 ^a
18:2 n -6	3.0	0.4 ^a	15.0	0.9 ^b	8.5	0.9 ^{cd}	6.8	0.2 ^c	20.5	1.2 ^e	9.1	0.8 ^d
20:2 n -6	0.2	0.1	0.8	0.1	0.6	0.1	0.2	0.2	1.0	0.1	0.4	0.0
20:4 n -6	1.7	0.1 ^a	1.0	0.4 ^{bc}	1.5	0.2 ^{ab}	1.3	0.0 ^b	0.8	0.4 ^{bc}	1.2	0.1 ^{bc}
22:4 n -6	1.3	0.9	0.9	0.0	0.6	0.5	0.9	0.4	0.9	0.0	0.9	0.4
Sum n -6	6.1	1.3 ^a	17.2	0.8 ^b	11.2	1.1 ^c	9.2	0.5 ^c	22.8	1.3 ^d	10.8	1.3 ^c
18:3 n -3	0.2	0.0 ^a	0.9	0.2 ^b	1.3	0.2 ^b	7.2	1.5 ^{cd}	1.2	0.1 ^b	10.8	2.1 ^d
20:4 n -3	0.8	0.3	0.5	0.1	0.9	0.4	0.8	0.1	0.4	0.0	0.8	0.1
20:5 n -3	13.1	1.2 ^a	8.0	2.0 ^{bc}	9.7	0.7 ^b	9.2	0.6 ^b	6.1	0.7 ^{bc}	7.0	1.0 ^c
22:5 n -3	3.6	0.4	2.7	0.7	3.3	0.6	2.7	0.3	2.1	0.4	2.0	0.1
22:6 n -3	21.4	1.8 ^a	17.3	4.0 ^a	20.0	1.2 ^a	19.0	0.8 ^a	16.1	2.4 ^a	18.3	2.2 ^a
Sum n -3	39.0	3.7 ^a	29.2	6.7 ^{ab}	35.3	2.5 ^{ab}	38.8	2.8 ^a	25.8	3.2 ^b	39.0	5.3 ^a
Sum n -3 highly unsaturated fatty acids	38.8	3.6 ^a	28.3	6.5 ^b	33.9	2.5 ^{ab}	31.6	1.4 ^{ab}	24.6	3.1 ^b	28.1	3.2 ^b

Superscript letters in the same row denote significantly different ($P \leq 0.05$).
ND, not detected.

comparison to the VLDL fraction. High levels of 16:0 fatty acids were also found in the HDL fraction.

Discussion

HDL was the mayor lipoprotein found in sea bream, confirming that this is the main lipoprotein involved in lipid transport as it also occurs in other species (Nelson & Shore, 1974; Chapman *et al.* 1980; Babin & Vernier, 1989; Santulli *et al.* 1989; Iijima *et al.* 1990; Lie *et al.* 1993). Such a predominance of HDL might be the consequence of a lower degradation of this fraction in comparison with other lipoproteins, a faster degradation of VLDL by lipoprotein lipase or an increased synthesis in liver and intestine (Léger, 1988; Iijima *et al.* 1990).

The lipid and protein composition of each lipoprotein fraction in sea bream was similar to that of other teleost fish. Thus, the chemical composition of HDL was characterised by a high content of protein and phospholipids, particularly phosphatidylcholine (Chapman, 1980; Lie *et al.* 1993). HDL and LDL were the two main lipoproteins involved in cholesterol transport in sea bream. Although the highest cholesterol contents were found in the LDL fraction, in agreement with other species (Farrell & Munt, 1983; Farrell *et al.* 1986; Lie *et al.* 1993; Torstensen *et al.* 2000), the main bulk of plasma cholesterol was transported by HDL, which was the more abundant lipoprotein in sea bream.

Despite this, VLDL was found to be present in minor amounts in the serum of other species (Santulli *et al.* 1997).

In sea bream plasma, the content of VLDL was found to be higher than that of LDL, reflecting the VLDL synthesis observed by electron microscopy in the enterocytes of this fish (Caballero *et al.* 2003). The VLDL fraction, rich in TAG as in other fish (McKay *et al.* 1985; Fainaru *et al.* 1988; Babin & Vernier, 1989; Torstensen *et al.* 2001), is implicated mainly in the transport of TAG to various tissues.

Interestingly, dietary fatty acids affected the lipid class composition of the lipoproteins. Thus, substitution of 80% fish oil by either soyabean or linseed oil raised the cholesterol content of the HDL and LDL. Although the fish-oil diets contained a higher proportion of saturated fatty acids (27.9% *v.* 18.6% and 16.3% in the vegetable-oil diets), which are reported to increase plasma cholesterol level (Hayes *et al.* 1997), this effect may be reduced in fish in view of their lower digestibility (about 85% in sea bream) in comparison with n -3 HUFA (95% in sea bream). The elevation in cholesterol caused by the reduction in dietary fish oil could instead be related to the high n -3 HUFA content of this oil (17.96% in fish-oil diets *v.* 6.9% and 7.4%, in vegetable-oil diets), in view of the high correlations found between the dietary n -3 HUFA and the HDL-cholesterol or LDL-cholesterol (Fig. 5(A), (B)). Despite the lack of studies in fish, considerable data are available on the effects of n -3 PUFA, with eighteen or more carbon atoms and two or more double bonds, on the plasma levels of cholesterol in mammals. Thus, in human subjects, the hypocholesterolaemic effect of fatty acids follows the pattern n -3 PUFA less than n -6 PUFA less than saturated fatty acids (Williams, 1998). As in

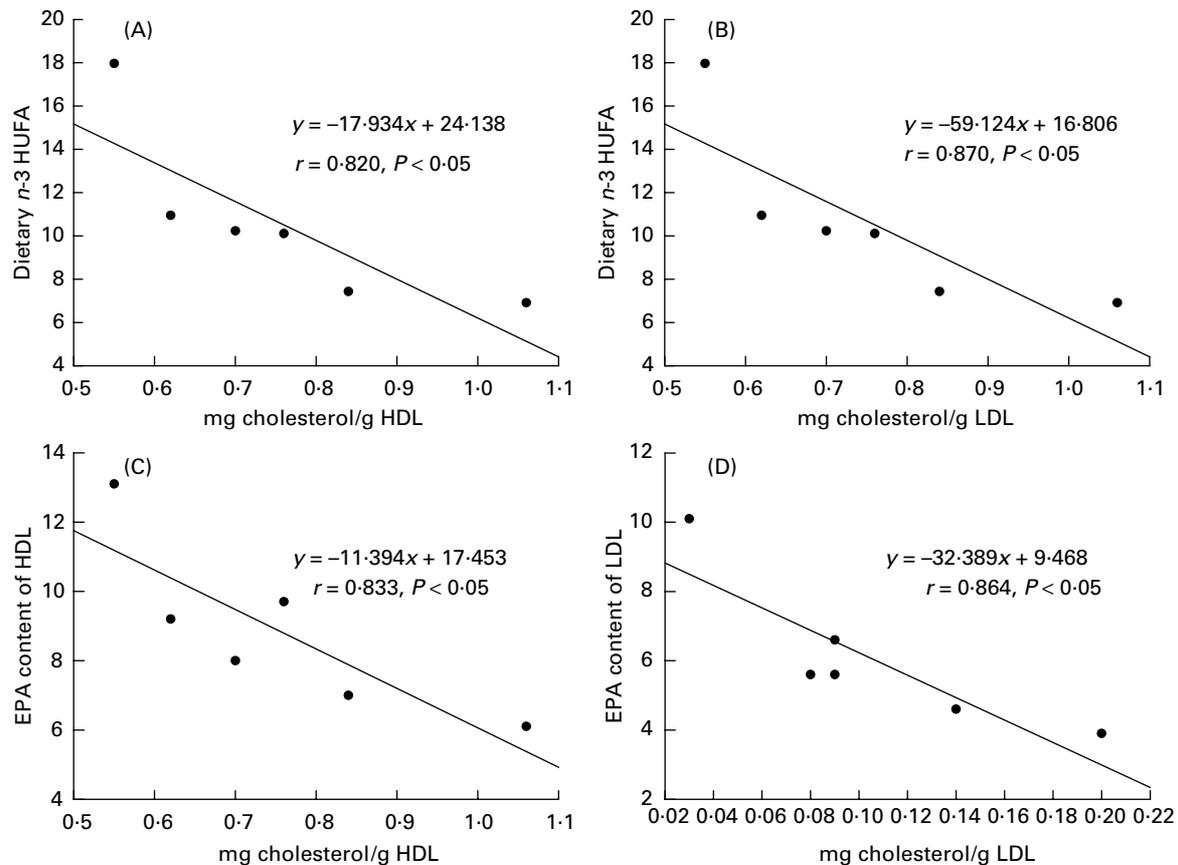


Fig. 5. Relationships between dietary *n*-3 highly unsaturated fatty acids (HUFA) and cholesterol level in HDL and LDL fractions ((A), (B)). Relationships between EPA content in HDL and LDL and the cholesterol level of those fractions ((C), (D)). Each point represents the dietary group.

mammals, dietary *n*-3 HUFA could, in the present experiment, enhance the activity of the enzyme lecithin:cholesterol acyltransferase located in the HDL (Thornburg *et al.* 1995; Parks *et al.* 2000), which catalyses the transfer of a fatty acid from phosphatidylcholine to cholesterol to form sterol esters, as the cholesterol:sterol esters ratios in HDL were doubled in fish fed 80% fish-oil substitution with vegetable oils than in those fed 100% fish oil. In addition, fish fed fish oil had a lower content of phosphatidylcholine, another substrate for lecithin:cholesterol acyltransferase.

In addition, significant inverse relationships were also found between EPA and HDL-cholesterol or LDL-cholesterol (Fig. 5(C), 5(D)). Spady (1993) described, in rats, how high levels of EPA and DHA decreased the LDL-cholesterol level by enhancing the activity of LDL-receptors in the liver. In turn, Tripodi *et al.* (1991) reported that the high affinity of the LDL fraction towards its liver receptors induced hypocholesterolaemia in rat fed those fatty acids. Similarly, in sea bream fed the fish-oil diet, with a higher content of these fatty acids, there was a reduction in LDL-cholesterol. As LDL receptors are proteins located in cell membranes, their function may be affected by the fatty acid composition of the membrane. Changes in the lipid composition of the membrane can induce structural and functional alterations in specific receptors (Gurr & Harwood, 1991).

The TAG content of all the lipoprotein fractions was also reduced by feeding fish oil. In human subjects, a high dietary content of *n*-3 PUFA reduces VLDL-TAG levels via a decrease in TAG synthesis in the liver (Harris *et al.* 1983; Berge *et al.* 1999).

The fatty acid composition of the VLDL was more readily affected by the diet than that of HDL, which could be related to the higher TAG content of the former lipoprotein as, in fish, neutral lipids are easily influenced by dietary fatty acids (Brodtkorb *et al.* 1997; Olsen & Henderson, 1997). In addition, the specific fatty acid composition of the lipoprotein would be related to the functional differences and the different metabolism and site of synthesis of the lipoproteins.

Finally, HDL was characterised by high levels of 22:6*n*-3, independent of dietary fatty acids. These results are in accordance with those of other studies (Lie *et al.* 1993; Torstensen *et al.* 2000) and suggest that HDL, the dominant lipoprotein in sea bream, is mainly implicated in the transport of DHA to the tissues (Brodtkorb *et al.* 1997).

In conclusion, dietary fish oil substitution by up to 60% of soyabean, linseed or rapeseed oil did not markedly affected lipoprotein composition, whereas 80% substitution by soyabean or linseed oil increased plasma cholesterol and TAG concentrations owing to the low *n*-3 HUFA content of these diets.

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