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Replacement of a large portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.)

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Abstract

In order to investigate the impact of dietary lipid sources on mechanisms involved in lipid deposition, three groups of European seabass fingerlings with average initial body weight of 5.2±1.0 g were fed three diets differing only by lipid source. These diets were: 100% anchovy oil (diet A), 40% anchovy oil–60% mix of vegetable oils (35% linseed, 15% palm, 10% rapeseed) (diet B) and 40% anchovy oil–60% mix of vegetable oils (24% linseed, 12% palm, 24% rapeseed) (diet C). After 64 weeks of rearing, when seabass reached the size of 160 g, the activity of lipogenic enzymes (fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme) in liver and of lipoprotein lipase (LPL) in perivisceral adipose tissue, liver and white muscle were measured. Transport of lipid by lipoproteins was examined by determining plasma lipid composition and lipoprotein classes. Dietary oil source did not modify growth performance or lipid content of flesh and liver of seabass. Replacement of 60% of fish oil by the two mixtures of vegetable oils had no significant effect on hepatic lipogenesis and activity of LPL in liver and adipose tissue. Activity of LPL in white muscle was decreased in fish fed diet C compared to those fed diets A and B. Diets containing the mixture of vegetable oils led to lowered plasma, VLDL and LDL cholesterol levels compared to diet A.

It is concluded that replacing 60% of fish oil by the two mixtures of vegetable oils in the feeds of European seabass fingerlings until they reach the size of 160 g has no marked effect on growth performance, lipogenesis and tissue lipid uptake but has a hypocholesterolemic effect.

Keywords: Fish oil; Lipogenesis; Lipoprotein lipase; Lipoproteins; European seabass; Vegetable oils

Abbreviations: AA, arachidonic acid (20:4n-6); CPO, crude palm oil; DHA, docosahexaenoic acid (22:6n-3); EPA, eicosapentaenoic acid (20:5n-3); FAS, fatty acid synthetase; FO, fish oil; G6PD, glucose-6-phosphate dehydrogenase; HDL, high density lipoprotein; LA, linoleic acid (18:2n-6); LDL, low density lipoprotein; LNA, α-linolenic acid (18:3n-3); LO, linseed oil; LPL, lipoprotein lipase; ME, malic enzyme; OA, oleic acid (18:1n-9); OO, olive oil; PUFA, polyunsaturated fatty acid; RO, rapeseed oil; SO, soybean oil; TAG, triacylglycerol; VLDL, very low density lipoproteins; VO, vegetable oil.

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1. Introduction

Endogenous lipids, synthesised via lipogenesis, and dietary lipids, not used for supply of energy, leads to lipid depots in tissues. Formation of tissue lipid depots involves transport of both absorbed and *de novo* synthesized lipids in peripheral tissues as lipoproteins, and release of fatty acids from triacylglycerol-rich core of circulating lipoproteins by lipoprotein lipase (LPL) for uptake by tissues.

All these pathways (lipogenesis, transport of lipids by lipoproteins and uptake of fatty acids by tissues *via* LPL action) involved in lipid deposition in fish tissues are similar to that of mammals (Sheridan, 1988; Babin and Vernier, 1989; Tocher, 2003). They are affected by dietary fatty acid composition in mammals (Blake and Clarke, 1990; Clarke et al., 1990; Grundy and Denke, 1990; Montalto and Bensadoun, 1993; Raclot et al., 1997; Salati and Amir-Ahmady, 2001; Fernandez and West, 2005).

In rainbow trout, n-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) and a high level of α-linolenic acid (LNA) inhibit the activities of fatty acid synthetase (FAS) and of glucose-6-phosphate dehydrogenase (G6PD), purveyor of NADPH, measured on hepatocytes (Alvarez et al., 2000). A n-3 PUFA rich diet also decreases the activity of malic enzyme (ME), another provider of NADPH, and G6PD in carp and Atlantic salmon (Shikata and Shimeno, 1994; Menoyo et al., 2003). But other reports show that hepatic lipogenic enzyme activities are not modified by partial or total replacement of fish oil (FO) by vegetable oils (VOs) in turbot (Regost et al., 2003), gilthead seabream (Menoyo et al., 2004) or Atlantic salmon (Torstensen et al., 2004).

The fatty acid composition of dietary lipid affects lipid composition of plasma and plasma lipoproteins in mammals (Jeffery et al., 1996; Temme et al., 1997; Salter et al., 1998; Chang et al., 2004). Plasma total cholesterol and LDL-cholesterol levels are increased by saturated fatty acids and decreased by PUFA such as linoleic acid (LA), EPA and DHA (Grundy and Denke, 1990; Spady, 1993). Similar results were observed in seabass with a decrease in plasma cholesterol and triacylglycerol with n-3 PUFA rich diet (Lemaire et al., 1991). In Atlantic salmon, a relationship between the fatty acid composition of dietary lipids and that of lipoprotein fractions has been shown (Lie et al., 1993; Torstensen et al., 2000, 2004).

Lipoproteins deliver endogenous and dietary lipid to peripheral tissues where LPL hydrolyses triacylglycerols (TAG) of TAG-rich lipoproteins (chylomicrons and VLDL). Fatty acids delivered by LPL are then taken up by tissues for oxidation or storage. In rat, a diet rich in n-3 PUFA increases LPL activity in the muscle (Baltzell et al., 1991) and its mRNA levels in epididymal adipose tissue (Murphy et al., 1993) and lowers the level of mRNA in retroperitoneal but not in subcutaneous adipose tissue (Raclot et al., 1997). n-3 PUFA also induces an increase in human plasma post-heparin LPL activity (Zampelas et al., 1994) whereas in earlier studies, no effect was observed on post-heparin LPL activity (Harris et al., 1988; Nozaki et al., 1991). In vitro studies have shown that LA and EPA but not oleic acid (OA) lowers the mRNA level, synthesis and secretion of LPL in chick adipocytes (Montalto and Bensadoun, 1993). On the other hand, in the rat, OA decreases LPL activity in adipocytes and epididymal cells (Kirkland et al., 1994) as well as the activity of post-heparin plasma LPL (Groot et al., 1988). From the above, there appears to exist inter-species differences in dietary fatty acid-induced lipid uptake. In fish, little information is available on the regulation of LPL by fatty acids. Available data in red seabream indicate that dietary fatty acids regulates, in a tissue-specific fashion, the LPL gene expression in liver and visceral adipose tissue (Liang et al., 2002a,b). In rainbow trout, total replacement of dietary fish oil by linseed oil did not modify the LPL activity in perivisceral adipose tissue, muscle or liver (Arantzamendi, 2002).

Since FO, one of the main components of fish feeds used in aquaculture is becoming increasingly scarce (FAO, 2004), research on the use of alternatives to FO is a major issue in aquaculture. Generally, marine fish are recognised as having a poor ability in converting LA to arachidonic acid (AA), and LNA to EPA and DHA (Sargent et al., 2002). Recent studies have shown that replacement of up to 60% dietary FO by VOs had no negative impact on growth, survival or health status of European seabass (Mourente and Dick, 2002; Izquierdo et al., 2003; Figueiredo-Silva et al., 2005; Mourente et al., 2005a,b) or gilthead seabream (Izquierdo et al., 2003, 2005). However, a higher level of substitution (80%) leads to decreased growth performances in both species (Montero et al., 2003, 2005; Menoyo et al., 2004; Izquierdo et al., 2005). This is in contrast to what has been shown in salmonids (Bell et al., 2001; Torstensen et al., 2004). In an earlier study, we also did not find any significant change in growth performances in rainbow trout fed diets in which all the FO was replaced by vegetable oils (Richard et al., 2006).

Our objective was to investigate the long term impact of dietary lipid source on the mechanisms involved in lipid deposition in European seabass fed diets in which FO was replaced by a vegetable oil mixture composed of rapeseed oil (RO), linseed oil (LO) and crude palm oil (CPO), which is becoming the most abundant VO in the

world (Gunstone, 2001). We studied the effects of this replacement on:

1) fat synthesis by measuring the activities of lipogenic enzymes in liver;
2) lipid transport by determining the composition of plasma and lipoprotein classes and the activities of LPL in different tissues.

2. Material and methods

2.1. Fish and diets

Fingerling European seabass (*Dicentrarchus labrax*) with average initial body weight of 5.2±1.0 g, were purchased from MARESA in Huelva (SW Spain) and reared in the wet laboratory facilities at the University of Cádiz in the Faculty of Marine and Environmental Sciences in Puerto Real (Cádiz). Fingerlings were randomly distributed in six 5000-l rectangular tanks at 0.6 kg/m^3, in seawater having a salinity of 39‰, saturated with oxygen, at a constant water temperature of 20±2 °C. After 2 weeks of acclimation, fish were fed to satiation with mechanical belt feeders with one of three isonitrogenous, isolipidic and isoenergetic diets (Table 1) differing only by lipid source, until they reached commercial size of 160 g (during 64 weeks). The composition of the blends of vegetable oils was designed in order to provide a similar balance of saturates, monoenes and PUFAs found in fish oil but without n-3 long chain PUFAs. Diet A contained 100% anchovy oil, diet B, 40% anchovy oil and 60% of a mixture of vegetable oils (35% LO, 15% CPO, 10% RO) and diet C contained 40% anchovy oil and 60% mixture of vegetable oils (24% LO, 12% CPO, 24% RO). Data on fatty acid composition of the diets are reported in Table 2. Diet A had the highest level of n-3 PUFA (EPA and DHA) and saturated fatty acids. Proportions of EPA and DHA were identical in diets B and C but the n-3/n-6 ratio was different between these diets (2.4, 1.9 respectively). The latter diets contained higher relative proportions of n-6 PUFA and monounsaturated fatty acids, mainly 18:1n-9 (OA) with the highest level in diet C. Diet B was the richest in 18:3n-3 (LNA) and diet C contained the highest percentage of 18:2n-6 (LA). The different diets were produced by Nutreco Aquaculture Research Center (Stavenger, Norway) in different pellet diameters (1, 3 and 5 mm) according to fish size.

Table 1
Ingredient composition (g/kg feed)* and proximate composition of experimental diets (size 5 mm)

	Diet A	Diet B	Diet C
Ingredient composition			
Fish meal[a]	400.0	400.0	400.0
Maize gluten[b]	262.7	262.7	262.7
Wheat[c]	152.3	152.3	152.3
Premixes[d]	25.0	25.0	25.0
Anchovy oil[e]	160.0	64.0	64.0
Rapeseed oil[f]	0	16.0	38.4
Linseed oil[g]	0	56.0	38.4
Palm oil[h]	0	24.0	19.2
Proximate composition			
Dry matter (DM), %	89.8	89.1	90.6
Crude protein (%DM)	53.2	51.8	52.8
Crude fat (%DM)	22.2	20.8	25.8
Carbohydrate (%DM)	17.1	20.3	14.6
Ash (%DM)	7.5	7.1	6.8

[a] Scandinavian LT-fish meal (Nordsildmel, Norway).
[b] Cargill, Staley, USA.
[c] Statkorn, Oslo, Norway.
[d] Following the requirements reported by NRC, 1993.
[e] Anchovy oil (Denofa, Fredrikstad, Norway) supplemented with 200 ppm BHT.
[f] Crude rapeseed oil (Oelmühle Hamburg, Germany) no antioxidant added.
[g] Crude E.C.C. linseed oil (N.V. Oliefabriek Lictervelde, Belgium) supplemented with 500 ppm Ronoxan A (Roche, Basel, Switzerland).
[h] Crude palm oil.
* All diets were produced at Nutreco Technology Centre, Norway, as extruded pellets.

2.2. Sampling procedure

At the end of the growth study (64 weeks), fish were killed by a blow on the head 16 h after the last meal. This interval was chosen to have empty digestive tracts and to avoid the presence of chylomicrons in plasma. Livers from six fish for each dietary treatment were sampled to analyse the activity of FAS, G6PD and ME. Muscle, liver and perivisceral adipose tissues from nine other fish were also sampled to measure activity of LPL. All the tissues sampled were frozen in liquid nitrogen and stored at −80 °C for analysis.

From 9 fish/diet, blood was withdrawn over 0.01% EDTA from the caudal vein. Plasma was obtained by centrifugation of blood (3000 g, 10 min). Sodium azide was added to the plasma (final concentration 0.04%). Plasma of three fish from the same tank was pooled. Three pools of plasma per dietary treatment were obtained. An aliquot of each plasma pool was stored at −20 °C for analysis of lipid class composition and the remainder was used for lipoprotein fractionation.

2.3. Gross composition analyses

Crude protein was determined using the Kjeldhal method (Association of Official Analytical Chemists,

Table 2
Fatty acid composition of the experimental diets (size 5 mm, g/100 g total fatty acid)

Fatty acid	Diet A	Diet B	Diet C
14:0	4.3	1.9	1.9
15:0	0.5	0.3	0.6
16:0	14.0	13.3	12.5
18:0	3.0	3.1	2.7
Saturates	22.9	19.5	18.6
16:1n-9	4.9	2.8	2.7
16:1n-7	8.7	8.6	7.5
18:1n-9	9.4	16.9	21.6
18:1n-7	2.2	/	2.0
20:1n-9	2.3	1.9	2.0
22:1n-11	2.0	1.7	1.7
MUFA	30.7	34.6	38.1
16:2	1.3	0.9	0.9
16:3	0.7	0.4	0.3
16:4	0.9	0.4	0.4
18:2 n-6	4.9	9.0	10.4
18:3n-3	1.5	12.3	10.3
18:4n-3	2.0	1.1	1.0
20:4n-6	0.6	0.3	0.3
20:4n-3	0.5	0.3	0.3
20:5n-3	9.8	5.3	5.2
22:5n-3	1.2	0.7	0.6
22:6 n-3	11.0	6.3	6.2
PUFA	34.4	37.0	35.9
Sat/PUFA	0.67	0.52	0.52
n-3/n-6	3.09	2.36	1.92

1990), crude fat by acid hydrolysis with a Soxtec System 1047 Hydrolysing Unit Tecator Application Note 92/87, followed by Soxhlet extraction using a Soxtec System HT6 Tecator Application Note 67/83 (Woyewoda et al., 1986).

2.4. Lipid and fatty acid analyses

Total lipid of diets, liver and muscle tissue was extracted according to Folch et al. (1957) and quantified gravimetrically. Fatty acid composition of diets was determined on the total lipid extract. Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids for 16 h at 50 °C, using tricosanoic acid (23:0) as an internal standard (Christie, 1989). Fatty acid methyl esters were extracted and purified (Tocher and Harvie, 1988) and then separated by a Hewlett-Packard 5890A Series II gas chromatograph equipped with a chemically bonded (PEG) Supercowax-10 fused silica wall coated capillary column (30 m×0.32 mm i.d., Supelco Inc., Bellefonte, USA), using an "on column" injection system and flame ionisation detection. Injector and detector temperature were respectively 240 and 260 °C. Hydrogen was used as the carrier gas and the oven thermal gradient was 50 to 180 °C at 25 °C/min, 180 to 235 °C at 3 °C/min and then a constant temperature of 235 °C for 10 min. Fatty acid methyl esters were identified by comparison with known standards and quantified by means of a direct-linked PC and Hewlett-Packard ChemStation software.

2.5. Lipogenic enzymes and lipoprotein lipase assays

For measurement of activities of lipogenic enzymes in liver, samples of liver were homogenised in three volumes of ice-cold buffer (0.02 M Tris–HCl, 0.25 M sucrose, 2 mM EDTA, 0.1 M NaF, 0.5 mM phenyl methyl sulphonyl fluoride, 0.01 M β-mercaptoethanol, pH 7.4). Homogenates were centrifugated at 15,000 g for 20 min at 4 °C and activities of lipogenic enzymes were measured on the supernatant. Activities of G6PD (EC 1.1.1.49) and ME (EC 1.1.1.40) were assayed by following NADPH production according to the methods of Bautista et al. (1988) and Ochoa (1955), respectively. FAS (EC 2.3.1.38) activity was determined by an isotopic method according to Hsu et al. (1969), measuring fatty acid synthetised from ^{14}C acetyl-CoA.

LPL activity was measured in liver, muscle and perivisceral adipose tissue. LPL assay was performed with 10% Intralipid (Fresenius Kabi, Sweden) which was labelled with tri(9,10-^{3}H)oleyl glycerol by sonication, and with rat serum as the source of apo C-II. Tissue samples (1 g) were homogenised in 9 volumes of ice-cold buffer (25 mM ammonia, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, 1250 IU heparin, 1 μl/ml pepstatin A, 10 μg/ml leupeptin, 4.25 IU aprotinin, pH 8.2). Homogenates were centrifuged at 20,000 g at 4 °C for 20 min. The clear supernatant was recovered and LPL activity determined according to Bengtsson-Olivecrona and Olivecrona (1992).

Enzyme activity units (IU), defined as μmoles of substrate converted to product per min at the assay temperature (25 °C for LPL and 30 °C for lipogenic enzymes), were expressed per mg of soluble protein (specific activity) and per g of tissue (wet weight). Protein concentration of tissue homogenates was determined by the method of Bradford (1976) using a protein assay kit (Bio Rad, München, Germany) with bovine serum albumin as a standard.

2.6. Separation of lipoproteins and analysis of lipid composition of plasma and lipoprotein classes

VLDL, LDL and HDL were obtained by sequential ultracentrifugal flotation of plasma as described by Torstensen et al. (2000) using a Kontron T-2060

Table 3
Morphometric parameters, feed utilisation, muscle lipid content and liver lipid content of European seabass (initial weight: 5.2±1.0 g) fed diets at 64 weeks

	Diet A	Diet B	Diet C
FBW[a] (g)	176±33	143±29	160±34
SGR[b] (%)	0.8±0.1	0.7±0.1	0.8±0.1
FE[c]	1.2±0.3	1.6±0.4	1.3±0.3
HSI[d]	2.0±0.3	1.9±0.2	2.1±0.3
Muscle lipids (%fresh)	2.6±1.6	1.7±0.3	2.5±1.0
Liver lipids (%fresh)	30.7±4.3	26.9±0.5	31.6±3.4

[a] FBW: final body weight.
[b] SGR: specific growth rate = 100 × ((ln(FBW) − ln(IBW))/days).
[c] FE: feed efficiency = wet weight gain (g)/feed intake (g).
[d] HSI: hepatosomatic index = (liver weight/body weight) × 100.

ultracentrifuge equipped with a TFT 65.13 rotor. The density intervals were obtained by addition of a KBr solution containing 0.04% NaN_3 and 0.01% EDTA. The density intervals were: VLDL, $d<1.015$ g/ml; LDL, $1.015<d<1.085$ g/ml and HDL, $1.085<d<1.21$ g/ml. The absence of chylomicrons and the purity of the different fractions were verified on agarose gel electrophoresis.

Concentrations of triacylglycerol (TAG), total cholesterol, phospholipid *and* free fatty acids were determined in plasma and lipoprotein fractions by colorimetric enzymatic methods using commercial kits (Triglycérides PAP 150, Cholestérol RTU, Phospholipides PAP 150, Biomérieux, France; NEFA C, Wako Chemicals Gmbh, Neuss, Germany).

2.7. Statistical analysis

Results are expressed as mean±SD. Data were analysed by one-way analysis of variance (ANOVA) to test the effect induced by dietary treatment on enzyme activities, lipid and protein composition of plasma and lipoprotein classes. Means were compared using Duncan's test. Differences were considered to be significant at a 0.05 probability level. All statistics were performed using software procedures contained in Statistical Analysis Systems (SAS, 1993).

3. Results

Growth data and morphometric parameters of seabass after 64 weeks of feeding are presented in Table 3. Growth performance of seabass was similar irrespective of dietary fat source and final body weights of fish were not significantly different among groups. There were no significant differences in muscle or liver lipid contents and hepatosomatic indices of seabass among dietary treatments.

G6PD activity was around 4 times higher than that of ME (Fig. 1). Activities of FAS, G6PD and ME were not

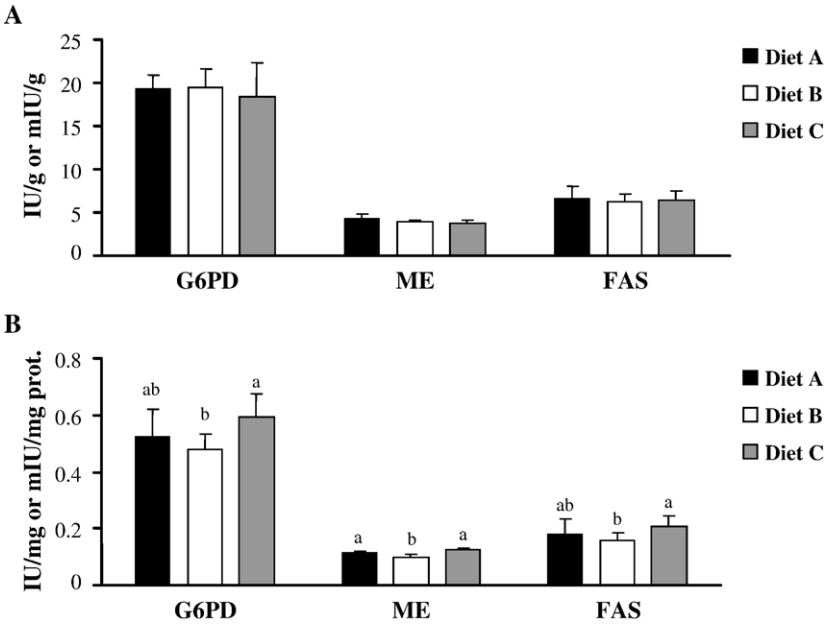

Fig. 1. Activities of G6PD, ME and FAS in liver of European seabass after 64 weeks of feeding expressed in (A) IU (G6PD and ME) or mIU/g tissue (FAS) and in (B) IU (G6PD and ME) or mIU/mg protein (FAS). Values are mean + SD ($n=6$). Mean values for a selected enzyme not sharing a common letter are significantly different ($P<0.05$).

Table 4
Triacylglycerol, total cholesterol, phospholipid, free fatty acid, and total protein in European seabass plasma (g/l) at 64 weeks

	Diet A	Diet B	Diet C
Triacylglycerol	19.9±1.1	21.8±1.0	20.8±1.5
Cholesterol	3.2±0.1[a]	2.6±0.1[b]	2.6±0.3[b]
Phospholipid	8.6±0.3[a]	8.4±0.3[a]	7.2±0.2[b]
Free fatty acid	0.25±0.04	0.20±0.05	0.17±0.05
Protein	33.9±1.4	36.0±3.8	36.7±5.3

Values are mean±SD (n=3). Means not sharing a common letter in a same line are significantly different (P<0.05).

modified by dietary treatment when expressed as IU/g of liver (Fig. 1.A). When expressed as IU/mg protein (Fig. 1.B) activities of the three enzymes were lower in fish fed diet B compared to those fed diet C.

Plasma total cholesterol levels decreased in fish fed diets containing VOs (Table 4). In seabass fed diet C, plasma phospholipid level was lower than in those fed diets A and B. Levels of TAG, protein and free fatty acid in plasma did not differ significantly among the three groups.

Data on the composition of plasma lipoprotein classes (VLDL, LDL and HDL), expressed as g/ml plasma are reported in Table 5. Fish fed diets containing VOs (diets B and C) had lower levels of cholesterol in VLDL and LDL fractions compared to fish fed diet A. Levels of phospholipid in VLDL were lower in fish fed diet C compared to that in fish fed diet A. There was no significant difference in lipid class composition of HDL among the three groups of fish. Amounts of VLDL, LDL and HDL in plasma were not significantly affected by dietary treatments. When components analysed in plasma lipoprotein classes are expressed as percentage of lipoprotein fraction (Table 6), composition of VLDL fraction was not modified among the three groups. LDL fraction had lower levels of cholesterol and phospholipids in fish fed diets containing VOs compared to fish fed diet A (FO) and the level of free fatty acids was significantly lower in fish of group C compared to those of group A. In HDL fraction, percentage of triacylglycerol was higher in group B than in groups A and C and percentage of all other components analysed in this lipoprotein fraction did not differ significantly among the three groups.

LPL activity, when expressed as mIU/g tissue, (Fig. 2.A) was slightly higher in liver than adipose tissue and muscle of seabass. LPL activity in muscle was lower in fish fed diet C compared to those fed diets A and B, but no differences were observed among fish fed the various diets when expressed as specific activity (mIU/

Table 5
Triacylglycerol, total cholesterol, phospholipid, free fatty acid and protein in VLDL, LDL, HDL of European seabass, expressed as concentration in lipoproteins of plasma (g/l plasma) and amount of lipoprotein classes (g/l plasma) after 64 feeding weeks

	Diet A	Diet B	Diet C
VLDL			
Amount VLDL	14.5±1.8	13.3±1.2	13.9±0.9
Triacylglycerol	10.3±1.5	9.4±1.3	10.2±0.8
Cholesterol	1.0±0.1[a]	0.8±0.0[b]	0.8±0.1[b]
Phospholipid	2.2±0.1[a]	2.0±0.1[ab]	1.9±0.1[b]
Free fatty acid	0.07±0.04	0.08±0.03	0.06±0.03
Protein	1.0±0.1	1.0±0.0	1.0±0.1
LDL			
Amount LDL	6.1±0.7	5.9±1.2	6.0±0.7
Triacylglycerol	3.6±0.5	3.6±0.9	3.7±0.7
Cholesterol	0.5±0.0[a]	00.4±0.1[b]	0.4±0.0[b]
Phospholipid	1.2±0.2	1.0±0.2	1.0±0.1
Free fatty acid	0.02±0.01	0.02±0.01	0.01±0.00
Protein	0.8±0.1	0.8±0.1	0.9±0.1
HDL			
Amount HDL	12.7±1.4	11.1±0.4	11.3±1.9
Triacylglycerol	1.9±0.2	1.9±0.1	1.8±0.4
Cholesterol	0.7±0.1	0.6±0.0	0.6±0.2
Phospholipid	4.0±0.5	3.4±0.3	3.4±0.8
Free fatty acid	0.12±0.03	0.10±0.02	0.09±0.04
Protein	5.8±0.6	5.1±0.1	5.5±0.6

Values are mean±SD (n=3). Means not sharing a common letter in a same line are significantly different (P<0.05).

Table 6
Triacylglycerol, total cholesterol, phospholipid, free fatty acid and protein in VLDL, LDL, HDL of European seabass, expressed as % of lipoprotein fraction after 64 feeding weeks

	Diet A	Diet B	Diet C
VLDL			
Triacylglycerol (%)	70.6±1.8	70.1±3.9	73.2±1.1
Cholesterol (%)	7.0±0.5	6.3±0.8	5.9±0.4
Phospholipid (%)	14.9±0.9	15.5±2.0	13.7±0.5
Free fatty acid (%)	0.5±0.2	0.6±0.3	0.4±0.2
Protein (%)	7.0±0.7	7.5±0.9	6.8±0.1
LDL			
Triacylglycerol (%)	57.3±2.2	60.3±3.2	61.6±4.4
Cholesterol (%)	10.2±0.9[a]	8.2±0.6[b]	8.1±1.2[b]
Phospholipid (%)	18.7±0.8[a]	16.8±0.5[b]	15.9±1.2[b]
Free fatty acid (%)	0.3±0.1[a]	0.2±0.0[ab]	0.2±0.0[b]
Protein (%)	13.4±0.9	14.4±2.3	14.1±2.0
HDL			
Triacylglycerol (%)	15.3±0.1[b]	17.4±0.1[a]	15.5±1.2[b]
Cholesterol (%)	5.9±0.2	5.3±0.4	5.0±0.6
Phospholipid (%)	31.8±0.7	30.5±1.5	29.7±3.1
Free fatty acid (%)	0.9±0.1	0.9±0.2	0.8±0.2
Protein (%)	46.1±0.5	45.8±1.3	49.0±4.9

Values are mean±SD (n=3). Means not sharing a common letter in a same line are significantly different (P<0.05).

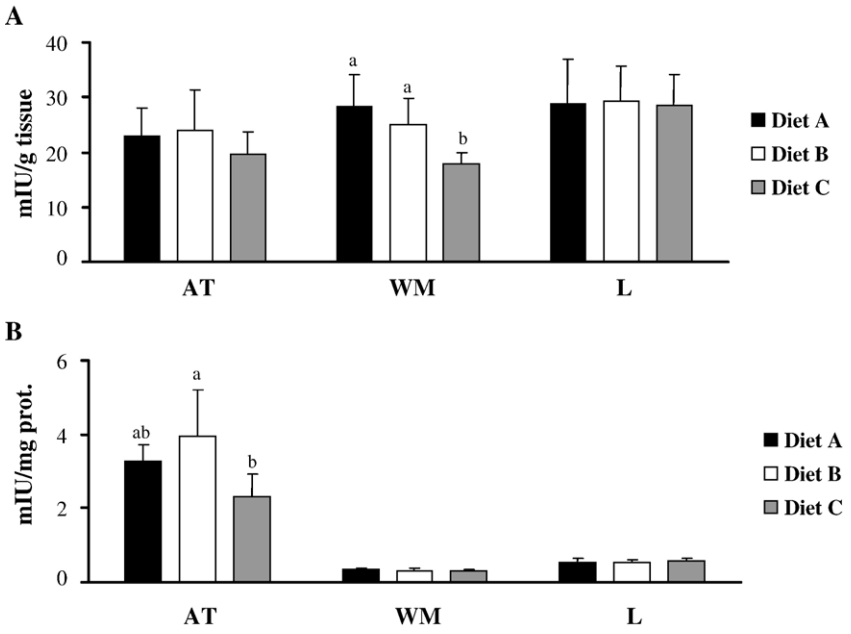

Fig. 2. LPL activity in perivisceral adipose tissue (AT), white muscle (WM) and liver (L) of European seabass after 64 weeks of feeding expressed in (A) mIU/g tissue and in (B) mIU/mg protein. Values are mean + SD ($n = 9$). Mean values in a same tissue not sharing a common letter are significantly different ($P < 0.05$).

mg protein). LPL activity did not differ significantly among fish fed the diets in liver, whatever the expression mode, and in adipose tissue when expressed per g/tissue. In this later, specific activity (Fig. 2.B), was lower in fish fed diet C compared to diet B, due to the tissue protein content. However, there were no significant differences between the two groups fed the VOs diets and the group fed the FO diet.

4. Discussion

We find here that European seabass fed diets containing 60% of VO blends (mixture of RO, LO and CPO in different proportions) for more than a year (64 weeks) had similar growth and feed utilization as those fed a FO diet. This is in agreement with data from earlier studies in European seabass and gilthead seabream fed diets with 60% VO for 15 weeks (Izquierdo et al., 2003; Figueiredo-Silva et al., 2005) or 30 weeks (Mourente and Dick, 2002; Izquierdo et al., 2005; Mourente et al., 2005a,b). When diets containing more than 60% of VO was fed, there was a reduction in growth of these species (Izquierdo et al., 2005; Menoyo et al., 2004; Montero et al., 2005), putatively related to their limited capacity in converting C18 fatty acids into long chain fatty acids such as AA, EPA and DHA.

Specific activities of FAS and ME in liver of seabass are in accordance with the data of Boujard et al. (2004) whereas G6PD specific activity was 2-fold higher in our study, probably due to the higher level of dietary carbohydrate in this study. G6PD activity in the liver of seabass was about 4 times higher than ME activity. This is in accordance with earlier data that pentose phosphate pathway is the principal purveyor of cytoplasmic reducing equivalents NADPH in European seabass (Dias et al., 1998; Boujard et al., 2004) as in gilthead sea bream (Gomez-Requeni et al., 2003), turbot (Regost et al., 2003) or rainbow trout (Gelineau et al., 2001). In Atlantic salmon however, G6PD activity is reported to be lower than that of ME (Menoyo et al., 2003; Torstensen et al., 2004). A high level of dietary n-3 PUFA (29% versus 15% of total fatty acid) decreases hepatic G6PD and ME activities in Atlantic salmon (Menoyo et al., 2003) and in vitro, there is also an inhibitory effect of EPA, DHA and LNA on G6PD and FAS activities in rainbow trout hepatocytes (Alvarez et al., 2000). Replacing FO partially by the two mixtures of VOs did not modify the activities of hepatic lipogenic enzymes in European seabass as also reported in gilthead seabream and turbot fed soybean oil (SO) (Regost et al., 2003; Menoyo et al., 2004) or in Atlantic salmon fed olive oil (OO) or RO (Torstensen et al., 2004). The differences in EPA, DHA and LNA content between FO and vegetable oils (SO, OO, RO) or the mixtures of VOs used in this study, did not seem to be enough to induce modifications in lipogenesis in seabass. Nevertheless, with LO, richer in LNA than the other vegetable oils

mentioned above, Menoyo et al. (2004) reported a decrease in hepatic lipogenesis in gilthead seabream, whereas in turbot this had no effect (Regost et al., 2003), indicating that the sensibility threshold of lipogenesis with fatty acids could be different among fish species.

Levels of plasma TAG and total cholesterol in plasma of seabass fed the different diets were similar to values reported by Dias et al. (2005). VLDL and HDL are the predominant lipoprotein classes in the plasma of seabass which is quite different from the contention that the highest lipoprotein class in teleost is HDL (Babin and Vernier, 1989). Differences in the postprandial status, 16 h fast in our study, could explain the high level of VLDL. As previously shown in rainbow trout and seabass, VLDL increase in post-absorptive phase, between 12 and 24 h (Sheridan, 1988; Santulli et al., 1988). Proportions of total cholesterol, phospholipid and TAG in each lipoprotein class are similar to earlier data in European seabass (Santulli et al., 1996). The major component of VLDL class was TAG (around 71% of fraction), reflecting its major role in the transport of TAG from liver to peripheral tissues. LDL, resulting from VLDL hydrolysis, contained less TAG (60%) than VLDL and was the richest class in cholesterol (9%). HDL class contained mainly protein (47%) and phospholipid (31%).

Both diets containing the VO blend induced a decrease in plasma total cholesterol by around 17% which appeared to be due to a decrease in proportion of cholesterol in LDL but the total amount of LDL was not modified. This is possibly due to the fact that diets B and C were richer than diet A in OA, LA and LNA which are known to reduce LDL-cholesterol (Dietschy, 1998; Fernandez and West, 2005). Plant oils also contain phytosterols (Phillips et al., 2002) which induce a decrease in total cholesterol and LDL-cholesterol in humans (Moghadasian and Frohlich, 1999; Matvienko et al., 2002) as well as in some teleosts (Gilman et al., 2003), by decreasing intestinal cholesterol absorption efficiency (Normen et al., 2000; Vanstone et al., 2002). Levels of phytosterol were not measured in the diets of the present study, but CPO contains around 0.5 g/kg of phytosterols and RO contains around 7.2 g/kg of phytosterols (Phillips et al., 2002). In rainbow trout, a diet based on RO, CPO and LO, with a high level of RO (55%), decreased plasma cholesterol, LDL-cholesterol as well as LDL (Richard et al., 2006). Earlier reports on the absence of any hypocholesterolemic effect of high levels of SO in rainbow trout and European seabass (Figueiredo-Silva et al., 2005), or OA-enriched sunflower oil or CPO in Atlantic salmon (Torstensen et al., 2000) may be due to the low phytosterol content of these oils (around 2.5 g/kg for soybean and sunflower oils (Phillips et al., 2002)).

The high level of LPL activity in the liver of seabass compared to rainbow trout is in relation with the fact that the liver is a high lipid deposition site in this species (Corraze and Kaushik, 1999). Tissue lipid uptake was not modified in perivisceral adipose tissue and liver of seabass by diets containing VOs compared to FO as there was no significant change in LPL activity in those tissues. Similar results have been obtained in rainbow trout, diets containing a blend of VOs had no impact on LPL activity in visceral adipose tissue, white muscle or liver (Richard et al., 2006).

In mammals several studies have reported an inverse relationship between LPL activity and plasma TAG levels (Groot et al., 1988; Baltzell et al., 1991; Zampelas et al., 1994; Harris et al., 1997). In our study, the similar level of plasma TAG among fish fed the diets is consistent with the absence of variation in LPL activity in adipose tissue and liver. Moreover, liver and muscle lipid contents of seabass were not affected by dietary oil source, as already seen in other studies in European seabass with partial replacement of FO by VOs (Figueiredo-Silva et al., 2005; Montero et al., 2005). The significant decrease in LPL activity in the muscle of sea bass fed diet C could be related to the high OA content in this diet. In red seabream, diets supplemented with OA decreased the LPL mRNA level in adipose tissue, but had no effect in liver (Liang et al., 2002a), indicating a tissue specific regulatory effect of LPL expression. In rat adipocytes exposed to OA, a decrease in LPL activity and mRNA level was observed (Kirkland et al., 1994), whereas this fatty acid had no effect on LPL mRNA level and synthesis of LPL in chick adipocytes (Montalto and Bensadoun, 1993) suggesting differences between species in the regulation of LPL by fatty acids.

5. Conclusions

Our data show that it is possible to replace 60% of dietary FO by a mixture of VOs (RO, LO and CPO) in the diets of European sea bass. Feeding sea bass with such diets for long periods (64 weeks) did not lead to differences in growth, tissue lipid content and mechanisms involved in lipid deposition (lipogenesis and tissue lipid uptake) although having a hypocholesterolemic effect.

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