Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): effects on flesh fatty acid composition, plasma prostaglandins E_2 and $F_{2\alpha}$, immune function and effectiveness of a fish oil finishing diet

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Abstract

Triplicate groups of European sea bass (*Dicentrarchus labrax* L.), of initial weight 90 g, were fed four practical-type diets in which the added oil was 1000 g kg^{-1} fish oil (FO) (control diet), 600 g kg^{-1} rapeseed oil (RO) and 400 g kg^{-1} FO, 600 g kg^{-1} linseed oil (LO) and 400 g kg^{-1} FO, and 600 g kg^{-1} olive oil (OO) and 400 g kg^{-1} FO for 34 weeks. After sampling, the remaining fish were switched to the 1000 g kg^{-1} FO diet for a further 14 weeks. Fatty acid composition of flesh total lipid was influenced by dietary fatty acid input but specific fatty acids were selectively retained or utilized. There was selective deposition and retention of docosahexaenoic acid (DHA; 22:6n-3). Eicosapentaenoic acid (EPA; 20:5n-3) and DHA were significantly reduced and linolenic (LNA; 18:3n-3), linoleic (LA; 18:2n-6) and oleic (OA; 18:1n-9) acids significantly increased in flesh lipids following the inclusion of 600 g kg^{-1} RO, LO and OO in the diets. No significant differences were found among different treatments on plasma concentrations of prostaglandin E_2 and prostaglandin $F_{2\alpha}$. Evaluation of non-specific immune function, showed that the number of circulating leucocytes was significantly affected ($P < 0.001$), as was macrophage respiratory burst activity ($P < 0.006$) in fish fed vegetable oil diets. Accumulation of large amounts of lipid droplets were observed within the hepatocytes in relation to decreased levels of dietary n-3 HUFA, although no signs of cellular necrosis was evident. After feeding a FO finishing diet for 14 weeks, DHA and total n-3 HUFA levels were restored to values in control fish although EPA remained 18% higher in control than in the other treatments. This study suggests that vegetable oils such as RO, LO and OO can potentially be used as partial substitutes for dietary FO in European sea bass culture, during the grow out phase, without compromising growth rates but may alter some immune parameters.

KEY WORDS: rapeseed oil, linseed oil, olive oil, fish oil, polyunsaturated fatty acids, prostaglandins, immune function, European sea bass, finishing diet

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Introduction

The nutritional and health benefits from the consumption of seafood is a major reason for the continuing demand for fish and shellfish by consumers. The presence of long chain n-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), can provide significant health benefits (Sargent *et al.* 2001). As world food-grade fisheries have reached sustainable limits, the demands upon the aquaculture industry are increasing and the development of diets that are cost effective, have no detrimental effects on growth and health status in fish as well as maintaining a healthy product for consumers, are a current focus of research in aquaculture (Hunter & Roberts 2000). However, there is a concern that the feed grade fisheries, that provide fish oil and fish meal, have reached their limit of sustainability (Barlow & Pike 2001). In consequence, research on the substitution of dietary fish oil have indicated that it may be possible to replace fish oil by plant seed oils

(soybean oil, linseed oil, rapeseed oil, olive oil, palm oil and corn oil). In general, replacement of fish oil with vegetable oils has resulted in lower levels of n-3 highly unsaturated fatty acids (HUFA; $\geqslant$20:3), EPA and DHA and higher levels of C_{18} fatty acids (oleic acid, OA; linoleic acid, LA; and linolenic acid, LNA) in tissues of several salmonid species such as rainbow trout (Greene & Selivonchick 1990), Atlantic salmon (Bell *et al*. 1993), brown trout (Arzel *et al*. 1994) and brook charr (Guillou *et al*. 1995). However, some authors have described histological changes, mainly large lipid vacuoles in livers of turbot and Arctic charr when using dietary vegetable oils (Bell *et al*. 1995; Olsen *et al*. 1999, 2000). A number of recent studies on Atlantic salmon have suggested that dietary vegetable oil inclusion does not result in reduced growth performance, feed conversion or development of histopathology in the fish (Torstensen *et al*. 2000; Bell *et al*. 2001, 2003). However, at levels of vegetable oil inclusion above 50%, there was a significant accumulation of 18:2n-6 and reduction of 20:5n-3 and 22:6n-3 in the flesh (Torstensen *et al*. 2000; Bell *et al*. 2001, 2003).

The dependence of marine fish farming on fish oil has a solid scientific basis because marine fish have a high dietary requirement for n-3 polyunsaturated fatty acids (PUFA), reflecting the natural abundance of these nutrients in their cellular and depot lipids and in their natural prey in the marine environment (Sargent & Tacon 1999). Marine fish are traditionally fed high lipid diets with ingredients of marine origin containing high levels of n-3 fatty acids, particularly n-3 HUFA such as EPA and DHA. However, the recent decreasing worldwide supplies of marine oils and fish meal (Barlow 2000) have forced the industry to investigate alternative lipid sources for use in marine fish diets. The only sustainable alternative to fish oils are plant (vegetable) seed oils which are rich in C_{18} PUFA but devoid of the n-3HUFA abundant in fish oils. Few studies have been undertaken to study substitution of dietary fish oil with vegetable oils in marine fish. These type of studies have mostly involved salmonids (Tocher *et al*. 2000; Torstensen *et al*. 2000; Bell *et al*. 2002; Caballero *et al*. 2002) and freshwater fish (Ng *et al*. 2003) and only a few have investigated marine fish species such as gilthead sea bream (*Sparus aurata)* (Kalogeropoulos *et al*. 1992; Caballero *et al*. 2003; Montero *et al*. 2003), European sea bass (*Dicentrarchus labrax*) (Yildiz & Sener 1997; Parpoura & Alexis 2001) and turbot (*Psetta maxima*) (Bell *et al*. 1995; Regost *et al*. 2003). However, marine fish have a high requirement for n-3 HUFA, paricularly EPA and DHA, as they are not capable of bioconverting C_{18} PUFAs to C_{20} and C_{22} HUFAs (Sargent *et at*. 2002) and this must be taken into consideration when vegetable oils are used in their diets. Variations in dietary fatty acid profiles because of the inclusion of vegetable oils may alter fish metabolism, which may affect fish health and stress resistance. The inclusion of vegetable oils can produce inadequate ratios of n-3/n-6 fatty acids, which could affect fish health by altering the synthesis of eicosanoids (Sargent *et al*. 2002). However, the role of n-3 and n-6 fatty acids in fish immune response is unclear, and contradictory and further study of fish immune function is required (Montero *et al*. 2003).

The European sea bass, a strict carnivorous marine fish species, is among the most important marine finfish species cultured in Europe, particularly in the Mediterranean region, and production is still increasing. Nevertheless, production of this species is well-controlled but knowledge of its nutritional requirements is still incomplete compared with other fish species, such as salmonids and carps (Oliva-Teles 2000). In the present study, triplicate groups of juvenile European sea bass were fed diets containing a 600 g kg^{-1} replacement of fish oil (FO) with rapeseed oil (RO), linseed oil (LO) and olive oil (OO) versus a control diet containing 1000 g kg^{-1} FO, for 34 weeks. Following sampling, fish in all treatment groups were fed the control FO diet, for a further 14 weeks. Growth and feed utilization were recorded, along with flesh fatty acid composition, to assess restoration of flesh fatty acid compositions, when fish were returned to a FO diet. As variations in the dietary fatty acid profiles and the imbalance of n-3 to n-6 ratios, caused by the inclusion of vegetable oils, may alter fish metabolism, eicosanoid production and health status and stress resistance, we have assessed the immune response in European sea bass fed these diets by determining various haematological parameters, serum lysozyme activity, macrophage respiratory burst activity and histological examination of fish tissues. Furthermore, as eicosanoid production is associated very broadly with stressful situations and eicosanoids are modulators of immune function (Sargent *et al*. 2002), we also measured prostaglandins E_2 and $F_{2\alpha}$ in plasma of sea bass after 34 weeks of feeding the experimental diets.

Materials and methods

Animals, diets and experimental design

European sea bass (*D. labrax* L.) of 90 g average weight were purchased from MARESA in Huelva SW Spain and stocked in 5000 L cylindro-conical tanks at 100 fish per tank (approximately 2 kg m^{-3}). The salinity was 39 gL^{-1}, the temperature 20 ± 2 °C and dissolved oxygen above 5 mg mL^{-1}. The experimental diets were prepared by the

Nutreco Aquaculture Research Centre, Stavanger, Norway and were fed *ad libitum* to satiation with mechanical belt automatic feeders. Sea bass were fed diets containing various blends of FO and vegetable oils including RO, LO and OO over the grow out period until fish reached market size of approximately 420 g 34 weeks later. The experimental design consisted of 12 tanks with a triplicate for the control and the three experimental dietary treatments. The diets tested for sea bass contained approximately 220 g kg^{-1} oil. The control diet, (FO), contained 1000 g kg^{-1} anchovy oil and the added oil combinations for the experimental diets were as follows: diet RO, 400 g kg^{-1} FO and 600 g kg^{-1} RO; diet LO, 400 g kg^{-1} FO and 600 g kg^{-1} LO and diet OO, 400 g kg^{-1} FO and 600 g kg^{-1} OO. The majority of the dietary protein, of all diets, was provided by fish meal. However, as fish meal itself contains some fish oil, the replacement of fish oil with vegetable oils was maximized by reducing the level of fish meal in the diets by inclusion of maize gluten and wheat. Diet formulations, total oil and protein compositions of diets and total lipid fatty acid compositions are shown in Tables 1 and 2, respectively. Crude protein was determined by combustion using the Kjeldhal method (Association of Official Analytical Chemists 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). After sampling, 20 fish per treatment were switched to a FO finishing diet containing, for a further 14 weeks.

Sample collection and biometric determinations

Ten fish per tank were sampled at the beginning, 34 weeks and 48 weeks of commencing the experiment for biometry [total length, live mass, liver live and dry mass, hepatosomatic index (HSI) and specific growth rates (SGR)]. Flesh samples for lipid and fatty acid analyses and blood samples for red blood cell (RBC) lipid and fatty acid analysis and plasma eicosanoid measurement (see below) were also collected. Live masses were determined by blotting the biological material on filter paper before weighing, and dry mass was determined after heating in an oven at 60 °C for 24 h and cooling in vacuum before weighing. HSI was calculated and growth assessed by measuring the SGR as percentage weight gain per day (Wootten 1990). Mortality was measured at the end of the experiment and expressed as percentage of fish surviving. Samples of flesh representative of the edible portion (Norwegian Quality Cut) were dissected from four fish per replicate and dietary treatment, at each sampling point, and immediately frozen in liquid nitrogen and then stored at −80 °C until analysis.

Lipid analysis

Total lipid in samples was extracted after homogenization, using an Ultra turrax tissue disrupter, in 10 volumes of chloroform/methanol (2:1, v/v) containing 0.1 g kg^{-1} butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch *et al.* (1957) and essentially as described

Table 1 Composition of experimental diets (g kg^{-1} feed)

Diets	FO*	RO	LO	OO
Components				
Fish meal[1]	381.3	381.3	381.3	381.3
Maize gluten[2]	259.8	259.8	259.8	259.8
Wheat[3]	157.2	157.2	157.2	157.2
Premixes[4]	25.0	25.0	25.0	25.0
Fish oil (FO)[5]	176.7	70.7	70.7	70.7
Rapeseed oil (RO)[6]	0	106	0	0
Linseed oil (LO)[7]	0	0	106	0
Olive oil (OO)[8]	0	0	0	106
Gross composition (g kg^{-1} dry mass)				
Crude protein	475	485	501	487
Crude lipids	237	232	237	255

[1] Scandinavian LT-fish meal (Nordsildmel, Norway).
[2] Cargill, Staley, NC, USA.
[3] Statkorn, Oslo, Norway.
[4] Vitamin and mineral premix added minimum to NRC recommendations.
[5] Anchovy oil (Denofa, Fredrikstad, Norway) supplemented with 200 mg kg^{-1} BHT.
[6] Crude rapeseed oil (Oelmühle Hamburg, Germany) no antioxidant added.
[7] Crude E.C.C. linseed oil (N.V. Oliefabriek Lictervelde, Belgium) supplemented with 500 mg kg^{-1} Ronoxan A (Roche, Basel, Switzerland).
[8] Crude olive oil.
* Control and finishing diet.

Table 2 Total lipid content (% of dry mass) and fatty acid composition (mass percentage of total fatty acids) of the experimental diets fed to European sea bass (*Dicentrarchus labrax*) during 34 weeks

Dietary treatments	FO*	RO	LO	OO
Total lipid (%)	23.7 ± 3.4	23.2 ± 2.7	23.7 ± 3.5	25.5 ± 2.9
Fatty acid (%)				
14:0	6.6 ± 0.3^{a}	3.8 ± 0.6^{b}	3.0 ± 0.5^{b}	4.5 ± 1.1^{ab}
15:0 ISO	0.7 ± 0.2	nd	0.7 ± 0.1	1.1 ± 0.2
15:0	0.9 ± 0.1^{b}	1.7 ± 0.3^{a}	0.7 ± 0.1^{b}	0.9 ± 0.2^{b}
16:0	16.2 ± 0.3^{a}	10.3 ± 0.1^{c}	10.5 ± 0.1^{c}	13.1 ± 0.2^{b}
18:0	3.2 ± 0.1^{a}	2.4 ± 0.1^{c}	3.1 ± 0.0^{a}	2.7 ± 0.0^{b}
Total saturated	27.3 ± 0.2^{a}	18.7 ± 0.9^{c}	17.6 ± 0.7^{c}	21.6 ± 1.1^{b}
16:1n-9	5.8 ± 0.0^{a}	2.8 ± 0.1^{c}	2.7 ± 0.0^{c}	3.1 ± 0.0^{b}
16:1n-7	8.2 ± 0.2^{a}	6.9 ± 0.2^{b}	7.2 ± 0.1^{b}	7.4 ± 0.2^{b}
18:1n-9	13.4 ± 0.2^{d}	34.4 ± 1.3^{b}	15.6 ± 0.1^{c}	37.2 ± 0.6^{a}
20:1n-9	3.2 ± 0.1^{a}	2.7 ± 0.1^{ab}	2.1 ± 0.0^{b}	1.9 ± 0.5^{b}
22:1n-11	3.1 ± 0.3^{a}	2.1 ± 0.1^{bc}	1.8 ± 0.1^{c}	2.4 ± 0.1^{b}
Total monoenes	33.7 ± 0.3^{c}	48.9 ± 0.9^{b}	29.4 ± 0.3^{d}	52.0 ± 0.9^{a}
16:2	1.0 ± 0.2^{a}	0.5 ± 0.2^{ab}	0.4 ± 0.1^{b}	0.5 ± 0.0^{b}
16:3	1.0 ± 0.0^{a}	0.3 ± 0.2^{b}	0.5 ± 0.0^{ab}	0.4 ± 0.0^{ab}
16:4	1.2 ± 0.0^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}
18:2n-6	5.7 ± 0.2^{d}	12.3 ± 0.3^{a}	10.3 ± 0.1^{b}	9.0 ± 0.2^{c}
18:3n-3	0.8 ± 0.5^{c}	4.4 ± 0.2^{b}	25.9 ± 0.4^{a}	1.2 ± 0.0^{c}
18:4n-3	1.6 ± 0.4^{a}	0.9 ± 0.1^{b}	1.0 ± 0.0^{b}	0.9 ± 0.0^{b}
20:4n-6	0.8 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}
20:4n-3	0.4 ± 0.0^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{ab}	0.1 ± 0.0^{b}
20:5n-3	10.9 ± 0.2^{a}	5.0 ± 0.3^{b}	5.2 ± 0.1^{b}	4.7 ± 0.2^{b}
22:5n-3	1.3 ± 0.1^{a}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}
22:6n-3	9.2 ± 0.0^{a}	5.0 ± 1.5^{b}	4.4 ± 0.1^{b}	4.3 ± 0.2^{b}
Total polyenes	35.1 ± 0.8^{b}	30.3 ± 1.4^{c}	50.1 ± 0.6^{a}	23.1 ± 0.6^{d}
Unknown	4.0 ± 0.9^{a}	2.1 ± 0.4^{b}	2.9 ± 0.3^{ab}	3.3 ± 0.4^{ab}
Total n-9	22.4 ± 0.2^{b}	39.9 ± 1.2^{a}	20.4 ± 0.2^{b}	42.2 ± 1.0^{a}
Total n-7	8.2 ± 0.2^{a}	6.9 ± 0.2^{b}	7.2 ± 0.1^{b}	7.4 ± 0.2^{b}
Total n-6	8.4 ± 0.4^{d}	13.4 ± 0.2^{a}	11.4 ± 0.0^{b}	10.2 ± 0.2^{c}
Total n-3	26.7 ± 1.2^{b}	16.9 ± 1.5^{c}	38.5 ± 0.5^{a}	12.8 ± 0.4^{d}
HUFA n-6	1.5 ± 0.0^{a}	0.6 ± 0.0^{b}	0.7 ± 0.0^{b}	0.6 ± 0.0^{b}
HUFA n-3	22.0 ± 0.2^{a}	10.8 ± 1.4^{b}	10.6 ± 0.1^{b}	9.8 ± 0.3^{b}

Results are mean ± SD ($n = 3$). An SD of 0.0 implies an SD of <0.05. Significant effects because of dietary oil supplementation were determined by one-way ANOVA and Tukey multiple range test. Totals include some minor components not shown.
Values bearing different superscript letter are significantly different ($P < 0.05$).
HUFA, highly unsaturated fatty acid; nd, not detected; FO, fish oil; RO, rapeseed oil; LO, linseed oil; OO, olive oil.
* Control and finishing diet.

by Christie (1982). The lipid extract was desiccated overnight under vacuum following the removal of solvent by evaporation under a stream of nitrogen. After being weighed, the lipid extract was redissolved at a known concentration in chloroform/methanol (2:1, v/v) containing 0.1 g kg^{-1} BHT and stored at −20 °C prior to analysis.

Fatty acid methyl esters (FAME) were prepared from aliquots of total lipids by acid-catalysed transmethylation for 16 h at 50 °C, using tricosanoic acid (23:0) as internal standard (Christie 1989). FAME were extracted and purified as described previously (Mourente & Tocher 1994) and were separated in a Hewlett-Packard 5890A Series II gas chromatograph equipped with a chemically bonded (PEG) Supelcowax-10 fused silica wall coated capillary column (30 m × 0.32 mm i.d., Supelco Inc., Bellefonte, PA, USA), using an 'on column' injection system and flame ionization detection. Hydrogen was used as the carrier gas with an oven thermal gradient from an initial 50 to 180 °C at 25 °C min^{-1} and then to a final temperature of 235 °C at 3 °C min^{-1}, with the final temperature maintained for 10 min. Individual FAME were identified by comparison with known standards and quantified by means of a direct-linked PC and Hewlett-Packard ChemStation software.

Blood sampling

Two millilitre of blood were collected in heparinized syringes from 18 fish per dietary treatment, and centrifuged immedi-

ately (12 000 × g for 2 min), the plasma removed, acidified by addition of 50 μL mL^{-1} 2 M formic acid and immediately frozen in liquid nitrogen for further eicosanoid analysis. The remaining pellet was also immediately frozen in liquid nitrogen and kept at −80 °C for fatty acid analysis of red blood cells.

Extraction and measurement of prostaglandins E_2 and $F_{2\alpha}$ concentrations in plasma

The frozen acidified plasma samples were thawed and centrifuged at 12 000 × g for 2 min to remove any precipitate. The supernatants were extracted using octadecyl silyl (ODS, C18) 'Sep-Pak' mini-columns (Waters, Milford, MA, USA) by the method of Powell (1982) and as described in detail by Bell *et al*. (1994). C18 'Sep-Pak' mini columns were prewashed with 5 mL methanol and 10 mL of distilled water, plasma samples were charged on the mini-column, washed with a further 10 mL of distilled water and the eicosanoids eluted in 5 mL of ethyl acetate. Samples were dried under nitrogen and redissolved in immunoassay buffer. Quantitation of prostaglandins E and F were performed using enzyme immunoassay (EIA) kits, according to the manufacturers protocol (SPI-Bio, Massy, France).

Measurement of cellular immune parameters

Eight fish per dietary treatment were sampled after 34 weeks feeding with the experimental diets. Fish were anaesthetized with a lethal dose of tricaine methanesulphonate (MS-222; Sigma, Poole, UK). Blood samples were collected in heparinized vacuum tubes (Vacutainer® Becton Dickinson Vacutainer System, Oxford, UK) from the caudal vein.

Haematology

Blood was used immediately for haematological studies. Haematocrit values were obtained using heparinized microhaematocrit tubes and centrifuging at 10 000 × g for 4 min in a microcentrifuge (Microcentrifuge MH2; Sarstedt Ltd, Leicester, UK). Total erythrocyte and total leucocyte counts were made using phosphate buffered saline (PBS) for dilution and an improved Neubauer haemocytometer for counting (Hawksley, Lancing, UK).

Serum lysozyme activity

An aliquot of blood was allowed to clot at 4 °C overnight. Serum was then separated by centrifugation at 1500 × ***g*** for 15 min and stored at −20 °C until analysis. Serum lysozyme activity was assayed by a turbidimetric assay which measures the lytic activity of the sea bass serum against *Micrococcus lysodeikticus* according to Anderson & Siwicki (1994) and described by Rungruangsak-Torrisen *et al*. (1999). A suspension of 190 μL of bacteria (*M. lysodeikticus*; Sigma) and 10 μL of serum was measured spectrophotometrically at 540 nm in five replicate wells per serum sample after 1 and 5 min at 25 °C, using a Dynatech MRX 1.2 ELISA reader (Dynatech Laboratories Limited, West Sussex, UK). The bacterial suspension (0.2 mg mL^{-1}) was prepared in sodium phosphate buffer (0.04 M, pH 5.8). The results are given as units (U) mL^{-1} min^{-1} (1U = the amount of sample causing a decrease in absorbance of 0.001 min^{-1}).

Macrophage respiratory burst activity

The reduction of nitroblue tetrazolium (NBT) salt to formazan caused by oxygen radicals was measured spectrophotometrically. The method used for the isolation and culture of macrophages from head kidneys was a modification of the method described by Secombes (1990). Fish were anaesthetized by immersing in water containing MS-222, and killed by a sharp blow to the cranium. They were exsanguinated via the caudal vein to help reduce erythrocyte contamination. The head kidneys were removed aseptically into sterile bijoux bottles containing 5 mL Leibowitz L-15 medium supplemented with 1 g kg^{-1} foetal bovine serum (FBS). Samples were then placed in sterile Petri dishes and cell suspensions prepared by gently rubbing the tissue through a sieve fitted with a 100 μm sterile nylon mesh. The resulting suspensions were left in sterile siliconized bijou bottles for 2–3 min to allow debris and aggregates to settle. Two hundred microlitre of each kidney suspension was added to four replicate wells of a 96 well microtitre plate. Plates were sealed and incubated for 3 h before being washed gently three times to remove non-adherent cells. Two hundred microlitre volumes of L-15 with 10% FBS was added to all wells and cultures were incubated at 18 °C for 2–3 days, after which the respiratory burst activity of the macrophages was determined by the reduction of the redox dye NBT as described by Chung & Secombes (1988). The cells were washed to remove any unattached cells and 100 μL of the NBT (1 mg mL^{-1})/phorbol myristate acetate (PMA, 1 μg mL^{-1}) was added to three of the four wells and incubated at 18–20 °C for 40 min. Following incubation the contents of the well were removed using a multichannel pipette and 100 μL of methanol added to each well to wash the cells. A further 100 μL of methanol was added for 5–10 min to fix the cells. The formazan, which

results, was dissolved by adding 120 μL of potassium hydroxide (2 M) and 140 μL of dimethyl sulphoxide to each well and mixed by careful pipetting. Colour reactions were read using the Dynex MRX 1.2 microplate reader (Dynatech Laboratories Limited, West Sussex, UK) at a wavelength of 620 nm. The remaining fourth well was used to determine the numbers of macrophages attached to the plate for individual kidney samples (Secombes 1990). The results were expressed as 'macrophage activity' by calculating the mean optical density for each of the triplicate cultures and dividing the mean OD by the number of cells per well to obtain the OD per 10^5 cells and multiplying by 100 to obtain whole numbers.

Histological examination of fish tissues

Samples were collected at week 34 to identify any effects of oil type on the histology of the heart, liver or intestine. Samples of proximal, mid and distal intestine were collected from six fish from each dietary treatment, in addition to the heart and liver for histopathological examination. Sections were fixed in 10% buffered formalin at the time of dissection, embedded in paraffin wax and 5 μm sections were cut and stained with haematoxylin and eosin. Processed sections were examined 'blind' to eliminate bias in interpretation.

Stained sections of heart were assessed for signs of endocarditis and pericarditis. Liver sections were assessed on fat content, any indication of inflammation in the tissue, the degree of peri-vascular cuffing and finally the presence of single cell necrosis. Intestinal sections were examined on the integrity of the intestinal mucosa, the number, size and variability of the absorptive vacuoles in the mucosal enterocytes, the appearance of the submucosa and lamina propria and the presence of any inflammatory response. All parameters mentioned were scored from 0 to 5, with 0 being the lowest score or the least amount of pathology seen and 5, the highest score, or greatest amount of pathological change.

Statistical analysis

Results are reported as means ± SD ($n = 3$) unless otherwise stated. All statistical analyses were performed using a statistical computer package (Prism 4.0, GraphPad Software, Inc., San Diego, CA, USA). The significance of treatment effects on biometry and growth rates, flesh and red blood cell fatty acid compositions, haematology, serum lysozyme activity and macrophage respiratory burst activity were determined by one-way ANOVA followed where appropriate by Tukey's multiple comparison test. Percentage data and data which were identified as non-homogeneous (Bartlett's test) were subjected to either arcsine, square root or log transformation before analysis. Differences were reported as significant if $P < 0.05$ (Zar 1984). Immune parameter results are reported as means ± SD ($n = 8$) and for histology scoring ($n = 6$).

Results

There were no significant differences, between dietary treatments, in total length, live mass or SGR of the fish at the end of the 34 weeks of feeding the experimental diets, and also after 14 weeks of feeding the 1000 g kg^{-1} FO finishing diet (Table 3). Feed intake ranged between 2.2 and 2.7 g $fish^{-1}$ day^{-1} from beginning to the end of the experiment and no significant differences were observed among treatments. Flesh dry mass and flesh total lipid content did not show significant differences between different dietary treatments, either before or after the 'wash out' period (Table 3). Flesh total polar lipids accounted for approximately 350 g kg^{-1} of total lipids (largely phosphatidylcholine, ~180 g kg^{-1}) and flesh total neutral lipids were about 650 g kg^{-1} (largely triacylglycerol, ~570 g kg^{-1}), and no significant differences were found between the different dietary treatments (data not shown).

The fatty acid compositions of flesh total lipid of sea bass, following 34 weeks of feeding the experimental diets, as well as after 14 weeks of feeding the FO finishing diet, are shown in Table 4. After 34 weeks of feeding the experimental diets, total saturated fatty acids (primarily 16:0) were significantly higher in fish fed FO and OO diets than in fish fed RO and LO diets. Total monounsaturated fatty acids were significantly higher in RO and OO fish due largely to elevated levels of OA (18:1n-9) in rapeseed and olive oils. Flesh percentages of LA (18:2n-6) were highest in RO fish (because of the highest level of LA in rapeseed oil), secondly and identical in LO and OO fish and lowest in FO (control) fish. This same pattern was followed by total n-6 PUFA as LA is the major component. In contrast, arachidonic acid (AA; 20:4n-6) was highest in FO (control) fish and lowest, and identical, in fish from all other treatments. This very same pattern was followed by total n-6 HUFA, primarily AA. The percentage of LNA (18:3n-3) in flesh total lipids was highest in LO fish (because of the highest content of this fatty acid in linseed oil), followed by RO fish and thirdly, and identically, by FO and OO fish, respectively. Percentages of EPA, DHA and total n-3 PUFA were highest in FO (control) fish because of the highest content of these fatty acids in fish oil.

Table 3 Effect of partial replacement (600 g kg^{-1}) of dietary fish oil (FO) with vegetable oils (rapeseed oil, RO; linseed oil, LO and olive oil, OO) on growth and performance of European sea bass (*Dicentrarchus labrax*, L.) fed experimental diets for 34 weeks and finishing (FO) diet for 14 weeks, having previously been fed the experimental diets

Dietary treatment	Initial	FO*	RO	LO	OO
Final sampling point: after feeding the experimental diets (FO, RO, LO and OO) to fish during 34 weeks					
Fish length (cm)	20.8 ± 1.1	33.1 ± 1.9	33.1 ± 1.8	32.9 ± 1.8	32.8 ± 1.9
Fish live mass (g)	93.8 ± 16.6	442.0 ± 75.1	430.4 ± 81.8	434.3 ± 74.3	404.9 ± 90.7
Flesh dry mass (g kg^{-1})	244 ± 6	266 ± 55	264 ± 24	247 ± 11	243 ± 6
Flesh total lipid content (g kg^{-1} dry mass)	62 ± 8	69 ± 18	77 ± 29	71 ± 13	73 ± 22
SGR (% body mass day^{-1})[2]		0.65 ± 0.05	0.63 ± 0.07	0.64 ± 0.05	0.61 ± 0.06
Final sampling point: after feeding the finishing diet (FO) for 14 weeks, after feeding the experimental diets					
Fish length (cm)		36.5 ± 2.0	37.9 ± 1.9	37.8 ± 2.2	37.2 ± 1.1
Fish live mass (g)		559.5 ± 77.2	640.9 ± 101.8	654.8 ± 130.5	633.4 ± 85.5
Flesh dry mass (g kg^{-1})		256 ± 14	253 ± 13	255 ± 11	262 ± 17
Flesh total lipid content (dry mass g kg^{-1})		68 ± 21	69 ± 14	76 ± 35	59 ± 12
SGR[2]		0.47 ± 0.02	0.51 ± 0.02	0.51 ± 0.03	0.56 ± 0.03

Data are mean ± SD ($n = 30$). Values corresponding to different dietary treatments bearing different superscript letter are significantly different ($P < 0.05$).

[1] Hepato Somatic Index.

[2] Specific growth rate.

* Control diet.

Table 4 Effect of partial replacement (600 g kg^{-1}) of dietary fish oil (FO) with vegetable oils (rapeseed oil, RO; linseed oil, LO and olive oil, OO) on total lipid fatty acid composition (mass percentage of total fatty acids) of flesh from European sea bass (*Dicentrarchus labrax*, L.) fed experimental diets for 34 weeks and subsequent finishing (FO) diet for 14 weeks

	Experimental diets for 34 weeks				Finishing diet for 14 weeks			
Dietary treatment	FO*	RO	LO	OO	FO*	RO	LO	OO
14:0	1.9 ± 0.3^{a}	2.0 ± 0.2^{a}	1.1 ± 0.2^{b}	2.0 ± 0.3^{a}	2.9 ± 0.2^{a}	2.1 ± 0.1^{ab}	1.7 ± 0.5^{b}	1.8 ± 0.2^{b}
15:0	1.2 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	1.0 ± 0.5	1.3 ± 0.2	1.4 ± 0.1	1.3 ± 0.2	1.1 ± 0.2
16:0	17.0 ± 0.2^{a}	12.9 ± 0.2^{b}	14.2 ± 1.1^{b}	16.2 ± 0.2^{a}	16.0 ± 0.5^{a}	14.6 ± 0.1^{b}	14.7 ± 0.7^{b}	15.4 ± 0.3^{ab}
18:0	4.9 ± 0.2^{a}	3.5 ± 0.2^{b}	4.8 ± 0.5^{a}	4.4 ± 0.3^{a}	3.4 ± 0.1	3.2 ± 0.0	3.6 ± 0.5	3.6 ± 0.0
Total Saturated	25.3 ± 0.2^{a}	19.8 ± 0.4^{b}	21.5 ± 1.7^{b}	24.1 ± 0.7^{a}	23.3 ± 0.7^{a}	20.9 ± 0.0^{b}	20.9 ± 1.1^{b}	21.8 ± 0.2^{ab}
16:1n-9	3.8 ± 0.4^{a}	3.0 ± 0.2^{b}	2.2 ± 0.2^{c}	3.1 ± 0.2^{b}	4.6 ± 0.2^{a}	3.5 ± 0.0^{b}	3.3 ± 0.7^{b}	3.3 ± 0.2^{b}
16:1n-7	2.0 ± 0.1^{b}	7.3 ± 0.4^{a}	6.5 ± 3.9^{a}	1.5 ± 0.2^{b}	7.0 ± 0.5	8.2 ± 0.9	7.8 ± 0.6	7.0 ± 0.1
18:1n-9	15.5 ± 0.3^{b}	26.5 ± 1.6^{a}	18.8 ± 2.1^{b}	28.4 ± 2.6^{a}	15.4 ± 1.3^{b}	19.4 ± 0.9^{a}	14.7 ± 2.3^{b}	19.6 ± 0.9^{a}
18:1n-7	2.7 ± 0.1^{a}	2.4 ± 0.0^{b}	1.9 ± 0.1^{c}	2.2 ± 0.1^{b}	2.3 ± 0.1^{a}	2.3 ± 0.1^{a}	1.9 ± 0.1^{b}	2.1 ± 0.0^{ab}
20:1n-9	1.5 ± 0.1^{b}	2.5 ± 0.1^{a}	1.6 ± 0.1^{b}	2.1 ± 0.2^{a}	2.2 ± 0.2	2.1 ± 0.1	1.8 ± 0.4	2.0 ± 0.0
22:1n-11	0.6 ± 0.1^{b}	1.1 ± 0.1^{a}	0.7 ± 0.1^{b}	1.0 ± 0.1^{a}	1.2 ± 0.2	0.8 ± 0.0	0.8 ± 0.2	0.7 ± 0.1
Total Monoenes	26.5 ± 0.9^{b}	43.0 ± 1.7^{a}	31.9 ± 3.5^{b}	38.7 ± 3.0^{a}	33.0 ± 1.9^{ab}	37.6 ± 0.1^{a}	30.6 ± 3.1^{b}	36.6 ± 2.6^{a}
18:2n-6	3.0 ± 0.1^{c}	8.5 ± 0.3^{a}	5.7 ± 0.6^{b}	6.4 ± 0.3^{b}	3.9 ± 0.4^{b}	5.7 ± 0.2^{a}	5.2 ± 0.9^{ab}	4.5 ± 0.2^{ab}
20:4n-6	1.5 ± 0.1^{a}	0.6 ± 0.1^{b}	0.9 ± 0.2^{b}	0.9 ± 0.1^{b}	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.3	1.2 ± 0.0
Total n-6 PUFA	6.0 ± 0.3^{c}	10.3 ± 0.3^{a}	7.8 ± 0.8^{b}	8.2 ± 0.2^{b}	6.4 ± 0.1^{c}	8.0 ± 0.1^{a}	7.5 ± 0.4^{ab}	7.0 ± 0.1^{b}
18:3n-3	1.0 ± 0.3^{c}	2.7 ± 0.1^{b}	8.4 ± 0.3^{a}	0.9 ± 0.1^{c}	0.8 ± 0.1^{b}	1.7 ± 0.1^{b}	7.1 ± 2.4^{a}	0.9 ± 0.1^{b}
18:4n-3	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	1.0 ± 0.1^{a}	0.8 ± 0.0^{ab}	0.7 ± 0.2^{ab}	0.6 ± 0.1^{b}
20:5n-3	9.6 ± 0.6^{a}	5.0 ± 0.2^{b}	5.7 ± 1.0^{b}	6.3 ± 0.3^{b}	8.9 ± 0.4^{a}	7.0 ± 0.3^{b}	7.4 ± 0.8^{b}	7.6 ± 0.3^{b}
22:5n-3	1.1 ± 0.8	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.4 ± 0.1^{a}	1.1 ± 0.1^{b}	1.1 ± 0.1^{b}	1.1 ± 0.1^{b}
22:6n-3	20.2 ± 1.3^{a}	9.4 ± 1.1^{c}	14.4 ± 2.1^{b}	12.9 ± 1.4^{bc}	15.4 ± 1.8	15.1 ± 0.1	15.3 ± 4.2	16.2 ± 0.3
Total n-3 PUFA	35.0 ± 0.9^{a}	20.0 ± 1.2^{b}	31.9 ± 2.9^{a}	23.7 ± 2.0^{b}	28.7 ± 2.1^{ab}	26.8 ± 0.5^{b}	33.0 ± 2.7^{a}	28.1 ± 0.4^{b}
Total polyenes	41.2 ± 1.0^{a}	30.6 ± 0.9^{b}	39.9 ± 3.7^{a}	32.1 ± 1.8^{b}	35.1 ± 1.9^{b}	34.8 ± 0.3^{b}	40.5 ± 2.3^{a}	35.1 ± 0.4^{b}
Total Unknown	7.0 ± 0.9	6.5 ± 1.0	6.6 ± 2.3	5.0 ± 0.6	8.6 ± 0.7	7.7 ± 0.2	7.9 ± 0.5	6.6 ± 2.3
Total n-9	21.4 ± 0.9^{b}	32.6 ± 1.8^{a}	23.1 ± 2.4^{b}	34.2 ± 3.1^{a}	22.5 ± 1.7	25.4 ± 0.9	20.1 ± 3.4	26.7 ± 2.6
Total n-7	4.7 ± 0.1^{b}	9.7 ± 0.4^{a}	8.4 ± 1.5^{a}	3.7 ± 0.1^{b}	9.4 ± 0.5	10.5 ± 0.8	9.7 ± 0.5	9.1 ± 0.1
HUFA n-6	2.3 ± 0.2^{a}	0.9 ± 0.1^{b}	1.3 ± 0.2^{b}	1.3 ± 0.1^{b}	1.7 ± 0.2	1.4 ± 0.1	1.5 ± 0.3	1.7 ± 0.0
HUFA n-3	31.4 ± 0.8^{a}	15.7 ± 1.3^{c}	21.8 ± 3.1^{b}	20.6 ± 1.8^{bc}	26.3 ± 2.3	23.6 ± 0.5	24.4 ± 5.0	25.6 ± 0.4

Data are mean ± SD ($n = 3$). Values bearing different superscript letter are significantly different ($P < 0.05$). Total includes some minor components not shown.

PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid.

* Control diet and finishing diet.

The fatty acid composition of flesh total lipid, following 14 weeks of feeding the FO finishing diet, are also shown in Table 4. The percentages of 16:0 and total saturated fatty acids were significantly higher in fish previously fed FO diet. However, and although statistically significant, the differences can be considered as minimal and from the practical point of view the values for these saturated fatty acids were restored. In the monounsaturated fatty acids, OA was significantly higher, and identical, in RO and OO fish than in FO and LO fish that were lower and identical. The differences among total monoenes were less pronounced and only LO fish showed a significantly lower value. Again the differences can be considered as minimal and very close to total restoration. Percentages of LA were significantly higher in fish previously fed RO, LO and OO diets compared with fish previously fed FO diet. However, LA values for fish fed RO and OO diets were significantly reduced compared with 14 weeks previously (Fig. 1). Values for AA were restored and identical among all treatments. The percentage of flesh LNA was still highest in fish previously fed LO and only the value for RO fish decreased significantly during the 14 weeks of feeding the finishing diet (Fig. 2). The percentage of EPA was significantly highest in flesh of FO fish followed by 180 g kg^{-1} lower EPA values in flesh of fish from the other three treatments. However, the values of flesh EPA from RO, LO and OO fish increased significantly during the 14 weeks of feeding the finishing diet (Fig. 3). The percentages of DHA were not significantly different among any of the treatments previously fed the experimental diets. DHA values were apparently restored by a significant decrease of this fatty acid in flesh of FO fish and significant increases in flesh

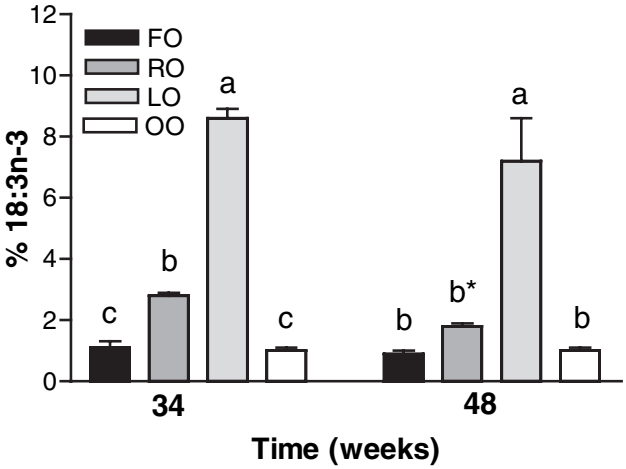

Figure 2 Linolenic acid (LNA; 18:3n-3) content (weight percentage of total) of total lipid from European sea bass (*Dicentrarchus labrax*) flesh after feeding experimental diets for 34 weeks and after 14 weeks of feeding the FO finishing diet up to 48 weeks. Columns assigned with different letters, within each time point, are significantly different ($P < 0.05$). An asterisk denotes significant difference ($P < 0.05$) between values belonging to the same treatment but at different time points.

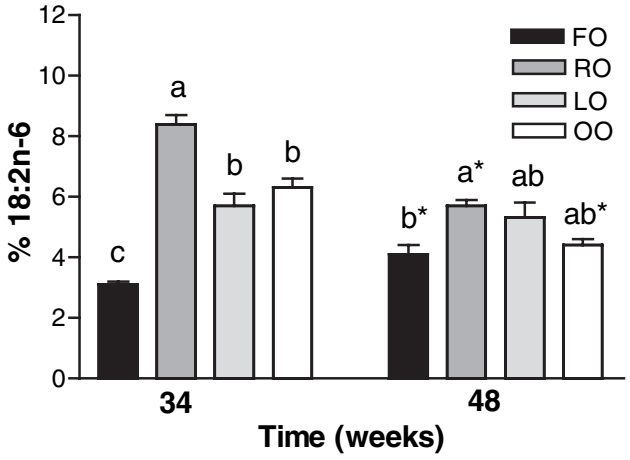

Figure 1 Linoleic acid (LA; 18:2n-6) content (weight percentage of total) of total lipid from European sea bass (*Dicentrarchus labrax*) flesh after feeding experimental diets for 34 weeks and after 14 weeks of feeding the FO finishing diet up to 48 weeks. Columns assigned with different letters, within each time point, are significantly different ($P < 0.05$). An asterisk denotes significant difference ($P < 0.05$) between values belonging to the same treatment but at different time points.

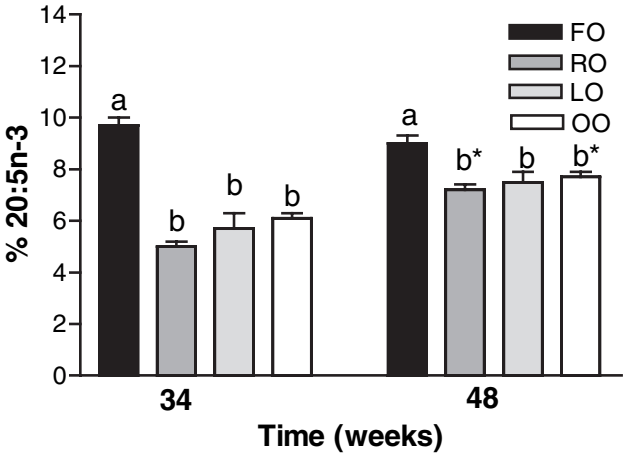

Figure 3 Eicosapentaenoic acid (EPA; 20:5n-3) content (weight percentage of total) of total lipid from European sea bass (*Dicentrarchus labrax*) flesh after feeding experimental diets for 34 weeks and after 14 weeks of feeding the FO finishing diet up to 48 weeks. Columns assigned with different letters, within each time point, are significantly different ($P < 0.05$). An asterisk denotes significant difference ($P < 0.05$) between values belonging to the same treatment but at different time points.

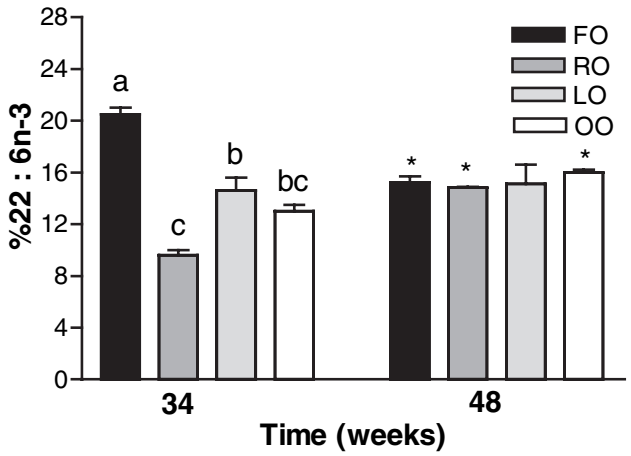

Figure 4 Docosahexaenoic acid (DHA; 22:6n-3) content (weight percentage of total) of total lipid from European sea bass (*Dicentrarchus labrax*) flesh after feeding experimental diets for 34 weeks and after 14 weeks of feeding the FO finishing diet up to 48 weeks. Columns assigned with different letters, within each time point, are significantly different ($P < 0.05$). An asterisk denotes significant difference ($P < 0.05$) between values belonging to the same treatment but at different time points.

of fish from RO, LO and OO diets (Fig. 4). The values of flesh total n-3 HUFA (mostly EPA and DHA) were identical in all treatments after feeding the finishing diet for 14 weeks (Table 4).

The effect of 600 g kg^{-1} replacement of dietary FO with vegetable oils (RO, LO and OO) on total lipid fatty acid composition of RBC from European sea bass after 34 weeks is shown in Table 5. No significant differences were found among total saturated fatty acids, mostly 16:0, although percentages of this fatty acid were significantly higher in the FO treatment. Total monoenes were significantly higher in the RO treatment, mainly because of the increased level of 18:1n-9 in this group. This was also reflected in total n-9 fatty acids that followed the same pattern. There were no statistical differences among total PUFA from the different dietary treatments but some differences were found among individual fatty acids of the n-6 and n-3 series. The highest values for LA were found in total lipids of RBC from RO and LO treatments, followed by OO with lowest levels in FO RBC. Total n-6 fatty acids followed the same pattern. In contrast, the highest values for AA were found in the FO group, with lower values in RO and LO fish. RBC LNA was highest in the LO treatment followed by the other three treatments with values reduced by ~900 g kg^{-1}. EPA was highest in the FO treatment followed by the other three treatments with values ~300 g kg^{-1} less. DHA was highest in FO and OO treatments followed by RO and LO RBC, which had values reduced by ~170 g kg^{-1}.

The effect of partial replacement (600 g kg^{-1}) of dietary FO with vegetable oils (RO, LO and OO) on PGE_2 and $PGF_{2\alpha}$ concentrations in plasma of European sea bass, during 34 weeks, are shown in Table 6. No statistical differences were found among different treatments on plasma concentrations of PGE_2 and $PGF_{2\alpha}$.

Of the blood parameters measured, there were no significant differences in the haematocrit or the number of circulating erythrocytes between fish fed the different experimental diets. Similarly, there were no significant differences in the serum lysozyme activity between the different groups. However, the number of circulating leucocytes was significantly affected ($P < 0.001$), with values in fish fed the vegetable oil diets being significantly lower than values in fish fed the FO diet (Fig. 5). Macrophage respiratory burst activity was significantly reduced in fish fed LO, RO and OO diets as measured by NBT reduction ($P < 0.006$) (Fig. 6).

Fish fed the control diet (FO) showed no significant histological abnormalities in the heart sections examined. Livers from fish fed the FO diet showed regular-shaped hepatocytes with centrally located nuclei and the intestinal sections showed no histopathological changes. Compared with the control fish, sea bass fed the RO diet showed livers with a relatively high fat content indicative of some marine species, however the vacuole size was very variable with some hepatocytes with large individual vacuoles and some with often small multiple vacuoles. All liver sections assessed showed very small and occasional foci of inflammatory cells but no necrosis or peri-vascular cuffing was observed. The intestines of the RO fed fish were generally normal, however the distal intestine appeared to have a relatively low absorptive capacity indicated by low levels of absorptive vacuoles in several of the sections examined. Livers from fish fed the LO diet had high fat levels similar to that found in the livers of RO fed fish. However, in this case the fat vacuoles appeared to be evenly distributed with less variability in size giving a more regular and even appearance to the architecture of the tissue. Some cellular infiltration was present in the proximal and distal sections, although no sign of cellular infiltration was observed in the mid sections. Overall the intestines of fish fed the LO diet were normal with a higher absorptive capacity than fish fed the RO diet. The majority of livers sampled from the OO dietary group showed a high fat content within the hepatocytes but no other associated pathology. Only one fish showed a variable vacuole appearance with some cellular breakdown. The intestines gave a similar appearance to those fed the FO diet with some increased cellular infiltration in the

Table 5 Effect of dietary 600 g kg^{-1} replacement of fish oil (FO) with vegetable oils (rapeseed oil, RO; linseed oil, LO and olive oil, OO) on total lipid fatty acid composition (mass percentage of total fatty acids) of red blood cells from European sea bass (*Dicentrarchus labrax*) fed on these diets for 34 weeks

Dietary treatments	FO*	RO	LO	OO
Fatty acid (%)				
14:0	1.2 ± 0.1	1.8 ± 1.1	1.4 ± 0.5	0.8 ± 0.1
15:0	2.0 ± 0.0	1.7 ± 0.4	1.7 ± 0.2	1.8 ± 0.2
16:0	13.1 ± 0.6^{a}	11.9 ± 0.1^{ac}	11.3 ± 0.7^{bc}	11.3 ± 0.3^{bc}
18:0	8.0 ± 0.5	9.0 ± 0.4	9.3 ± 1.2	8.8 ± 0.4
Total saturated	25.0 ± 1.2	24.9 ± 0.8	24.3 ± 0.6	23.4 ± 0.4
16:1n-9	1.6 ± 0.1	0.9 ± 0.2	1.1 ± 0.6	nd
16:1n-7	2.6 ± 0.2	2.6 ± 0.5	2.8 ± 0.4	2.5 ± 0.1
18:1n-9	6.2 ± 0.4^{c}	11.9 ± 1.0^{a}	9.3 ± 1.7^{ab}	9.0 ± 0.2^{b}
20:1n-9	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.2	1.3 ± 0.1
22:1n-11	0.6 ± 0.2	0.5 ± 0.2	0.8 ± 0.3	0.3 ± 0.3
24:1n-9	0.7 ± 0.1^{a}	0.5 ± 0.1^{b}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}
Total monoenes	14.4 ± 0.5^{b}	19.8 ± 1.3^{a}	17.2 ± 2.5^{ab}	15.3 ± 0.3^{b}
16:4n-3	2.5 ± 0.3	2.4 ± 0.1	2.0 ± 0.2	2.2 ± 0.2
18:2n-6	1.4 ± 0.1^{c}	3.8 ± 0.1^{a}	3.4 ± 0.7^{a}	2.4 ± 0.0^{b}
18:3n-3	0.2 ± 0.0^{b}	0.9 ± 0.1^{b}	4.9 ± 1.6^{a}	0.3 ± 0.0^{b}
20:2n-6	0.3 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	0.4 ± 0.1
20:4n-6	2.2 ± 0.1^{a}	1.8 ± 0.1^{b}	1.7 ± 0.2^{b}	1.9 ± 0.1^{ab}
20:5n-3	12.0 ± 0.7^{a}	8.4 ± 0.3^{b}	8.9 ± 0.9^{b}	8.9 ± 0.3^{b}
22:5n-6	0.8 ± 0.2^{a}	0.5 ± 0.1^{ab}	0.5 ± 0.1^{ab}	0.3 ± 0.1^{b}
22:5n-3	1.4 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.2
22:6n-3	30.0 ± 0.7^{a}	25.8 ± 1.2^{b}	25.7 ± 2.6^{b}	32.5 ± 0.7^{a}
Total polyenes	52.6 ± 0.3	47.6 ± 0.2	51.1 ± 1.6	52.6 ± 0.7
Unknown	8.0 ± 1.1	7.7 ± 0.9	7.5 ± 1.1	8.7 ± 0.0
Total n-9	9.7 ± 0.4^{b}	15.6 ± 1.5^{a}	12.5 ± 2.4^{ab}	11.7 ± 0.7^{b}
Total n-7	4.1 ± 0.1	4.3 ± 0.5	4.1 ± 0.4	3.9 ± 0.0
Total n-6	5.4 ± 0.2^{b}	7.3 ± 0.2^{a}	6.8 ± 0.4^{a}	5.4 ± 0.3^{b}
Total n-3	47.2 ± 0.2^{a}	39.6 ± 1.4^{b}	44.0 ± 1.9^{a}	46.6 ± 0.9^{a}
HUFA n-6	3.6 ± 0.2^{a}	2.8 ± 0.3^{b}	2.6 ± 0.2^{b}	2.4 ± 0.1^{b}
HUFA n-3	43.9 ± 0.4^{a}	35.5 ± 1.3^{b}	36.4 ± 3.6^{b}	43.5 ± 0.7^{a}

Results are mean ± SD ($n = 3$). An SD of 0.0 implies an SD of <0.05. Significant effects because of dietary oil supplementation were determined by one-way ANOVA and Tukey multiple range test. Totals include some minor components not shown.
Values bearing different superscript letter are significantly different ($P < 0.05$).
HUFA, highly unsaturated fatty acid; nd, not detected; FO, fish oil; RO, rapeseed oil; LO, linseed oil; OO, olive oil.
* Control diet.

distal segment only. Table 7. shows the mean histology scores ($n = 6 \pm$ SD) for the histological parameters assessed.

Discussion

This study showed that feeding European sea bass diets containing RO, LO or OO, at 600 g kg^{-1} of added oil, did not affect growth or survival when compared with fish fed 1000 g kg^{-1} marine fish oil (Table 3). This result is similar to a number of earlier studies with salmonids where vegetable and marine oil were used in feed formulations with levels of dietary lipids between 140 and 190 g kg^{-1} (Dosanjh *et al.* 1988; Polvi & Ackman 1992; Guillou *et al.* 1995; Tocher *et al.* 2000), or more recent studies using high energy/lipid formulations (Torstensen *et al.* 2000; Bell *et al.* 2001, 2002, 2003).

The fatty acid compositions of flesh lipids were closely related to dietary fatty acid input. However, while dietary fatty acids influence flesh fatty acids, specific fatty acids are selectively retained or utilized. DHA was selectively deposited and retained, as flesh DHA concentrations were always higher than diet concentrations. This has also been observed in Atlantic salmon (Bell *et al.* 2001, 2002, 2003), rainbow trout (Caballero *et al.* 2002), African catfish (Ng *et al.* 2003a) and turbot (Bell *et al.* 1994, 1995; Regost *et al.* 2003). The mechanism of selective deposition may include the high specificity of fatty acyl transferases for DHA and the relative resistance of DHA to β-oxidation because of the complex catabolic pathway required for this fatty acid (Bell *et al.* 2001). Furthermore, in European sea bass, the levels of DHA cannot be attributed to hepatic desaturation and elongation of LNA as sea bass hepatocytes have a very low level of fatty acid bioconversion and no nutritional stimulation of these

Table 6 Effect of partial replacement (600 g kg^{-1}) of dietary fish oil (FO) with vegetable oils (rapeseed oil, RO; linseed oil, LO and olive oil, OO) on PGE_2 and $PGF_{2\alpha}$ concentrations in plasma of European sea bass (*Dicentrarchus labrax* L.)

Eicosanoid	FO diet	RO diet	LO diet	OO diet
PGE_2 (pg mL^{-1})	236.0 ± 109.6	203.0 ± 96.3	241.0 ± 125.7	196.5 ± 103.4
$PGF_{2\alpha}$ (pg mL^{-1})	24.1 ± 16.5	27.9 ± 7.5	32.8 ± 13.2	37.8 ± 8.2

Values are mean ± SD ($n = 8$ fish per treatment).

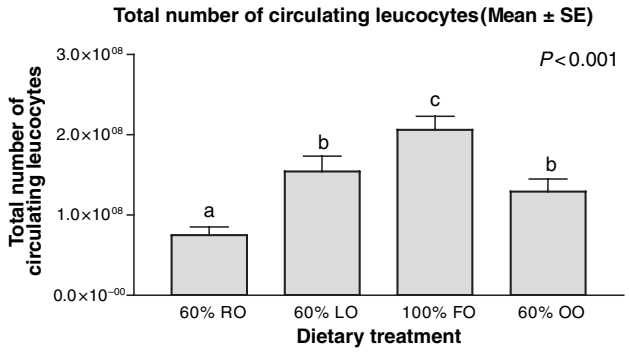

Figure 5 Total number of circulating leucocytes (mean ± SEM) ($n = 8$) in fish fed the experimental diets for 34 weeks. Significant differences are denoted by columns assigned different superscript letters.

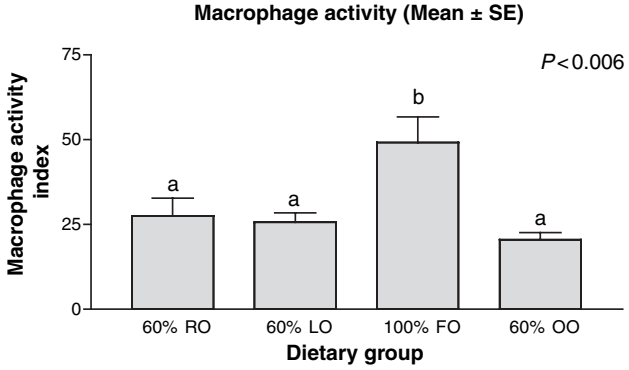

Figure 6 Macrophage activity (mean ± SEM) in the head kidney of fish ($n = 8$) fed the experimental diets for 34 weeks. Significant differences are denoted by columns assigned different superscript letters.

pathways was observed by the addition of VO to diets (Mourente & Dick 2002). In the present study, levels of LA and LNA in flesh were significantly lower in fish fed the RO, LO and OO diets than in diets suggesting these fatty acids were readily utilized for oxidation in sea bass. The selective oxidation of monoenoic and C_{18} PUFA supports similar data seen in salmonids and turbot (Bell *et al.* 1994, 1995, 2001, 2002, 2003; Caballero *et al.* 2002; Ng *et al.* 2003b; Regost *et al.* 2003).

Dietary FO replacement with VO affected the total lipid fatty acid composition of RBC, but to a lesser degree than flesh fatty acid compositions. Total n-3 HUFA were significantly higher in RBC of fish fed FO and OO than in RO and LO, respectively. This was mainly because of the significantly higher levels of DHA in RBC from fish fed FO and OO. This contrasts with results in gilthead sea bream fed similar diets with partial substitution of FO with VO where no differences in RBC DHA levels were seen in the different dietary treatments. Similar results were found for Atlantic salmon (Waagbø *et al.* 1993) and Atlantic cod (*Gadus morhua*) (Waagbø *et al.* 1995) fed vegetable oils. However, Klinger *et al.* (1996) found less susceptibility to lysis in the erythrocytes of channel catfish (*Ictalurus punctatus*) fed a menhaden oil-based diet when compared with fish fed a soybean oil diet. It may be that variations in RBC fatty acid profile may alter membrane properties such as osmostic resistance (Hagave *et al.* 1991).

Eicosanoid production is associated, very broadly, with stressful situations and is a normal physiological process, with excess eicosanoid production often occurring in pathological conditions (Sargent *et al.* 2002). Eicosanoids are produced from C_{20} PUFA by the action of cyclooxygenase and lipoxygenase resulting in metabolites that include prostaglandins, leukotrienes and lipoxins that are known to influence a wide range of immune functions (Uhing *et al.* 1990; Stankova & Rola-Pleszczynski 1993). Prostaglandins, especially AA-derived PGE_2, are produced by monocytes and macrophages, and are associated with modulation of immune cell function (Kinsella & Lokesh 1990), whereas $PGF_{2\alpha}$ is more related to environmental stress adaptation, such as temperature and salinity change (Mustafa & Srivastava 1989). There is good evidence that low concentrations of circulating PGE_2 ($<10^{-9}$ M) are required for normal immune function and T-cell differentiation, but that concentrations of PGE_2 $>10^{-8}$ are immunosuppresive in mammals (Kinsella & Lokesh 1990). In the present study, values for PGE_2 ranged between 5.6×10^{-10} and 9.7×10^{-10} M and no significant differences were observed

Table 7. Histological scores of European sea bass fed four experimental diets differing in oil source (mean ± SD)

Histology parameter	FO	RO	LO	OO
Heart				
Endocarditis	0.00 ± 0.00	0.13 ± 0.12	0.38 ± 0.37	0.13 ± 0.12
Pericarditis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pathology	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Liver				
Fat levels	3.38 ± 0.26^{a}	3.88 ± 0.12^{ab}	4.25 ± 0.16^{b}	3.88 ± 0.12^{ab}
Inflammation	0.13 ± 0.12^{a}	0.88 ± 0.12^{b}	0.38 ± 0.18^{a}	0.00 ± 0.00^{a}
Peri vascular cuffing	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Single cell necrosis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.12
Proximal				
Mucus activity	1.88 ± 0.12	2.25 ± 0.16	2.00 ± 0.00	2.00 ± 0.00
Vacuolation	1.13 ± 0.12	1.25 ± 0.25	1.25 ± 0.16	1.00 ± 0.00
Cellular infiltration	0.13 ± 0.12^{ab}	0.88 ± 0.30^{a}	0.75 ± 0.31^{ab}	0.00 ± 0.00^{b}
Sloughing	0.38 ± 0.18^{ab}	0.75 ± 0.31^{ab}	1.13 ± 0.23^{a}	0.25 ± 0.16^{b}
Mid				
Mucus activity	2.00 ± 0.00^{a}	2.38 ± 0.18^{b}	2.00 ± 0.00^{a}	2.00 ± 0.00^{a}
Vacuolation	1.88 ± 0.23	1.25 ± 0.16	1.50 ± 0.27	1.75 ± 0.16
Cellular infiltration	0.38 ± 0.18^{ab}	0.63 ± 0.18^{a}	0.00 ± 0.00^{b}	0.25 ± 0.16^{ab}
Sloughing	0.88 ± 0.30	0.63 ± 0.32	0.75 ± 0.37	0.38 ± 0.18
Distal				
Mucus activity	2.00 ± 0.00	2.25 ± 0.16	2.13 ± 0.12	2.00 ± 0.00
Vacuolation	2.50 ± 0.27	1.88 ± 0.35	2.13 ± 0.23	2.63 ± 0.26
Cellular infiltration	0.75 ± 0.16	1.13 ± 0.35	1.25 ± 0.25	1.13 ± 0.23
Sloughing	1.13 ± 0.30	0.75 ± 0.37	0.75 ± 0.41	0.88 ± 0.30

Values in the same row having different superscript letters are significantly different ($P < 0.05$).

among treatments. However, these data should be viewed with caution as important differences in the eicosanoid production pattern have been described among different fish species (Rowley 1991). With regard to nutritional regulation of immune function, Atlantic salmon fed diets containing linseed oil showed reduced production of leukotriene B_4 (LTB_4) and PGE_2 by stimulated kidney macrophages compared with those fed northern hemisphere fish oil or sunflower oil (Bell *et al.* 1996). This suggests that feeding vegetable oil could cause significant reductions in non-specific immune parameters such as haematocrit, total white blood cell and red blood cell counts, and macrophage respiratory burst (Good *et al.* 2001; Bell & Sargent 2003).

Several studies have shown that moderate amounts of vegetable oils in fish diets do not have a negative impact on fish growth, however, these oils have a fatty acid profile markedly different from that of marine oils and also can contain substances unnatural to fish. These differences may have several implications to the fish in long-term immune studies. In the present study, sea bass fed RO, LO and OO showed a significant reduction in the total number of circulating leucocytes and a reduction in macrophage respiratory burst activity. In a similar trial, gilthead sea bream (*S. aurata*) were fed diets containing soybean oil, RO and LO or a blend of these oils, compared with a FO control diet, for 29 weeks (Montero *et al.* 2003). While no differences were found in the haematocrit or haemoglobin contents the number of circulating RBC was significantly higher in fish fed the FO diet compared with fish fed the VO diets. Fish fed the soybean oil diet also had significantly lower complement activity than fish fed FO. In contrast to the present study, there was no effect of feeding VO on the macrophage respiratory burst activity, however, the phagocytic activity of the macrophages was significantly affected in fish fed either RO or soybean oil.

It is notable that Bell *et al.* (1996) observed that feeding Atlantic salmon diets containing either LO or sunflower oil had no significant effect on lysozyme activity, complement activity or haematocrit which agrees with the data from the present study with sea bass. However, in contrast to the present study, Bell *et al.* (1996) found no effect of feeding LO or sunflower oil on head kidney macrophage respiratory burst activity. In general, there are a few mechanisms by which dietary fatty acids may affect fish immune function and disease resistance. Firstly, by influencing the cell membrane lipid composition and its physical properties, which, in turn, can have profound effects on disease resistance because many immune responses are based on leucocyte cell membrane interactions (e.g. activation of cytokine production). Secondly, dietary fatty acids may affect immune function

through the production of eicosanoids from AA and EPA. Finally, dietary fatty acids may affect the immune system by alterations in signal transductions, possibly because of effects on protein kinase C (Balfry & Higgs 2001).

Literature evidence suggests that changing the concentration of dietary n-3 PUFA in fish feeds can have both beneficial and, in some instances, detrimental effects on disease resistance (Erdal *et al*. 1991; Sheldon & Blazer 1991; Kiron *et al*. 1995; Montero *et al*. 1999, 2003). Respiratory burst activity of phagocytes of European sea bass decreased with increasing dietary lipid level from 90 to 170 g kg^{-1} (Sitja-Bobadilla & Perez-Sanchez 1999). Mortality of channel catfish (*I. punctatus*) exposed to *Edwardsiella ictaluri* was significantly higher in fish fed a menhaden oil diet than those fed a catfish offal oil diet (Li *et al*. 1994). Fracalossi & Lovell (1994) found that channel catfish fed 70 g kg^{-1} menhaden oil or LO had a higher mortality after challenge with *E. ictaluri* than fish fed diets containing corn oil, or beef tallow or a mixture of the three lipid sources, at 28 °C. Conversely, Erdal *et al*. (1991) found that increasing the amount of dietary n-3 PUFA from 130 to 240 g kg^{-1} of the total fatty acids had an immunosuppressive effect on Atlantic salmon, and resulted in higher rates of mortality against *Yersinia ruckeri*. Bransden *et al*. (2003) found significantly increased cumulative mortalities in Atlantic salmon fed diets containing sunflower oil and challenged with *Vibrio anguillarum*. Increased activity of head kidney macrophages has been associated with increased dietary n-3 fatty acids in channel catfish (Blazer 1991; Sheldon & Blazer 1991) and rainbow trout (Ashton *et al*. 1994). Moreover, Atlantic salmon fed diets with a high ratio of n-3/n-6 PUFA had increased B lymphocyte responses following experimental challenges with *Aeromonas salmonicida* and *V. anguillarum* (Thompson *et al*. 1996). One possible mechanism of dietary fat effects on macrophages is altered signal transduction, which, in turn, could alter gene regulation and macrophage function. The role of lipids in fish immunity may involve modulation of cellular responses via changes in the membrane properties, such as permeability and fluidity, and hence the membrane-associated enzymes and receptors (Montero *et al*. 2003). Future studies may benefit by measuring additional immune parameters (humoral and cellular responses) to assess effects on fish health.

The major histological difference between fish fed the experimental diets was the occurrence of variable sized vacuoles and large amounts of lipid droplets within hepatocytes of fish fed RO and LO diets compared with fish fed FO. In addition, the distal intestine of the VO fed fish showed a higher degree of cellular infiltration in the mucosal folds compared with fish fed FO. These changes suggest an effect of dietary lipid on the transport and metabolism of fat, but further studies are required to clarify this. Accumulation of lipid droplets in livers of fish fed RO and LO diets may be due to the preference for specific fatty acids as energy sources resulting in storage of surplus fatty acids [e.g. 18:3 (n-3) and 18:1 (n-9)] in lipid droplets. Caballero *et al*. (2003) found that feeding gilthead sea bream (*S. aurata*) either LO, RO or soybean oil resulted in accumulation of supranuclear lipid droplets within intestinal enterocytes. The tendency for lipid accumulation may be related to decreased dietary n-3 HUFA, which would result in impaired lipoprotein synthesis (Watanabe 1982; Sargent *et al*. 1989; Olsen *et al*. 1999, 2000). However, accumulation of lipid droplets did not cause pathological damage to the intestinal epithelium and no signs of cellular necrosis were evident. In contrast, Olsen *et al*. (1999, 2000) found a significant impact on the gastrointestinal tract function and integrity when feeding large amounts of LO to Arctic char (*Salvelinus alpinus* L.), which may be explained by impairment of lipoprotein synthesis and lipid transport. Turbot (*Psetta maximus*) fed with diets containing coconut oil showed excessive lipid degeneration of the hepatic parenchyma and significant lesions in the lateral muscle wedge (Cowey *et al*. 1976).

Results from the present study are in broad agreement with earlier findings in other fish species (Caballero *et al*. 2002, 2003). In addition, hepatocytes with large lipid vacuoles and nuclei located at the periphery of the cell were observed in livers of red drum (*Sciaenops ocellatus*) fed diets containing soybean oil (Tucker *et al*. 1997) although no major histological changes in heart sections were observed in this study. However, studies by Bell *et al*. (1991, 1993) revealed that feeding Atlantic salmon diets containing sunflower oil resulted in development of severe heart lesions that caused thinning of the ventricular wall and muscle necrosis.

After 34 weeks, all fish were fed a FO finishing diet, for 14 weeks, and all groups showed identical growth rates (Table 3). The present study has shown that inclusion of VO, up to 600 g kg^{-1} of dietary oil, significantly reduces EPA and DHA and increases LA and LNA in sea bass flesh. The time required to restore individual fatty acids to values similar to those in fish fed FO were different for each fatty acid. After feeding the FO finishing diet for 14 weeks, 18:2n-6 remained 320 g kg^{-1} higher in RO fish, than in control fish, but was restored in LO and OO fish (Fig. 1). LNA remained 890 g kg^{-1} higher in LO fish than in control fish but was restored in RO and OO fish (Fig. 2) and total n-3 HUFA (mostly EPA and DHA) were restored to that in control fish (Figs 3 and 4).

Fish with oil-rich flesh provide a virtually unique source of n-3 HUFA in human diets. The benefits, in terms of human health, of increasing dietary intake of n-3 HUFA, and simultaneously reducing n-6 PUFA, largely as LA, are now well known (De Deckere *et al.* 1998; Horrocks & Yeo 1999; Simopoulos 1999; Hunter & Roberts 2000) and consumers have become more aware of the health benefits associated with consuming more fish. As capture fisheries decline, the demands upon the aquaculture industry to supply high quality seafood are increasing. The development of diets that are cost effective, produce good growth in fish, utilize sustainable raw materials and also preserve the n-3 HUFA content of the final product is an area of vital importance to aquafeed producers (Sargent & Tacon 1999; Hunter & Roberts 2000).

In summary, the results of this study suggest that RO, LO and OO can be used as 600 g kg^{-1} replacement of dietary FO for European sea bass, during the grow out period, without significantly compromising growth rates. However, reductions in the levels of EPA and DHA, and increases in the levels of C_{18} fatty acids arise when feeding VO. In consequence, the flesh fatty acid composition of sea bass fed RO, LO and OO is characterized by increased levels of LNA, LA and OA and reduced levels of EPA and DHA. However, after 14 weeks of feeding a FO finishing diet, flesh total lipid fatty acid profiles were almost completely restored, in all treatments, to similar values to those fed FO throughout. With regard to fatty acid restoration and LA retention after the wash out period the rank order of the oils tested would be FO > OO > LO > RO.

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References

Anderson, D.P. & Siwicki, A.K. (1994) Simplified assays for measuring non-specific defense mechanisms in fish. *Am. Fish. Soc. Fish Health section. Spec. Publ.*, **12,** 26–35. (Seattle, WA).

Arzel, J., Martínez-López, F.X., Métailler, R., Stéphan, G., Viau, M., Gandemer, G. & Guillaume, J. (1994) Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta*) reared in sea-water. *Aquaculture*, **123,** 361–375.

Ashton, I., Clements, K., Barrow, S.E., Secombes, C.J. & Rowley, A.F. (1994) Effects of dietary fatty acids on eicosanoid-generating capacity, fatty acid composition and chemotactic activity of rainbow trout (*Oncorhynchus mykiss*) leucocytes. *Biochim. Biophys. Acta*, **1214,** 253–262.

Association of Official Analytical Chemists (1990) *Official Methods of Analysis*, vol. 1, 15th edn. Association of Official Analytical Chemists, Arlington, VA, USA, pp. 71–72.

Balfry, S.K. & Higgs, D.A. (2001) Influence of dietary lipid composition on the immune system and disease resistance of Finfish. In: *Nutrition and Fish Health* (Lim, C. & Webster, C.D. eds), pp. 213–234. Food Products Press, Binghampton NY, USA.

Barlow, S. (2000) Fishmeal and oil: sustainable feed ingredients for aquafeeds. *Global Aquacult. Advocate*, **4,** 85–88.

Barlow, S.M. & Pike, I.H. (2001) Aquaculture feed ingredients in year 2010: fish meal and fish oil. In: *Aquavision* '98. 2nd Nutreco Aquaculture Business Conference. (Nash, C.E. & Julien, V. eds), pp. 71–74, Stavanger, Norway, 13–15 May 1998 Nutreco Aquaculture, Stavanger, Norway.

Bell, J.G. & Sargent, J.R. (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture*, **218,** 491–499.

Bell, J.G., McVicar, A.H., Park, M.T. & Sargent, J.R. (1991) High dietary linoleic-acid affects the fatty-acid compositions of individual phospholipids from tissues of Atlantic salmon (*Salmo salar*) – association with stress susceptibility and cardiac lesion. *J. Nutr.*, **121,** 1163–1172.

Bell, J.G., Dick, J.R., McVicar, A.H., Sargent, J.R. & Thompson, K.D. (1993) Dietary sunflower, linseed and fish oils affect phospholipid fatty-acid composition, development of cardiac lesions, phospholipase-activity and eicosanoid production in Atlantic salmon (*Salmo salar*). *Prostaglandins. Leukot. Essent. Fatty. Acids.*, **49,** 665–673.

Bell, J.G., Tocher, D.R., MacDonald, F.M. & Sargent, J.R. (1994) Effects of diets rich in linoleic (18:2n-6) and α-linolenic (18:3n-3) acids on the growth, lipid class and fatty acid compositions and eicosanoid production in juvenile turbot (*Scophthalmus maximus* L.). *Fish Physiol. Biochem.*, **13,** 105–118.

Bell, J.G., Tocher, D.R., MacDonald, F.M. & Sargent, J.R. (1995) Effects of dietary borage oil [enriched in γ-linoleic acid, 18:3(n-6)] or marine fish oil [enriched in eicosapentaenoic acid, 20:5(n-3)] on growth, mortalities, liver histopatology and lipid composition of juvenile turbot (*Scophthalmus maximus*). *Fish Physiol. Biochem.*, **14,** 373–383.

Bell, J.G., Ashton, I., Secombes, C.J., Weitzel, B.R., Dick, J.R. & Sargent, J.R. (1996) Dietary lipid affects phospholipid fatty acid compositions, eicosanoid production and immune function in Atlantic salmon (*Salmo salar*). *Prostaglandin, Leukot. Essent. Fat. Acids*, **54,** 173–182.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J. & Sargent, J.R. (2001) Replacement of fish oil with rape seed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid composition and hepatocyte fatty acid metabolism. *J. Nutr.*, **131,** 1535–1543.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R.P. & Sargent, J.R. (2002) Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. *J. Nutr.*, **132,** 222–230.

Bell, J.G., McGhee, F., Campbell, P.J. & Sargent, J.R. (2003) Rapeseed oil as an alternative to marine fish oil in diets of post-molt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid compo-

sition and effectiveness of subsequent fish oil 'wash out'. *Aquaculture*, **218,** 515–528.

Blazer, V.S. (1991) Piscine macrophage function and nutritional influences: a review. *J. Aquat. Anim. Health*, **3,** 77–86.

Bransden, M.P., Carter, C.G. & Nichols, P.D. (2003) Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. *Comp. Biochem. Physiol. B*, **135,** 611–625.

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. & Izquierdo, M.S. (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **214,** 253–271.

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Montero, D., Socorro, J., Fernández, A.J. & Rosenlund, G. (2003) Morphological aspects of intestinal cells from gilthead sea bream (*Sparus aurata*) fed diets containing different lipid sources. *Aquaculture*, **225,** 325–340.

Christie, W.W. (1982) *Lipid Analysis*, 2nd edn. Pergamon Press, Oxford.

Christie, W.W. (1989) *Gas Chromatography and Lipids: A Practical Guide*, 1st edn. The Oily Press, Ayr, Scotland.

Chung, S. & Secombes, C.J. (1988) Analysis of events occurring within teleost macrophages during respiratory burst. *Comp. Biochem. Physiol.*, **89,** 539–544.

Cowey, C.B., Adron, J.W., Owen, J.M. & Roberts, R.J. (1976) The effect of different dietary oils on tissue fatty acid and tissue pathology in turbot *Scophthalmus maximus*. *Comp. Biochem. Physiol.*, **53,** 399–403.

De Deckere, E.A.M., Korver, O., Verschuren, P.M. & Katan, M.B. (1998) Health aspects of fish and n-3 polyunsaturated fatty acids from plant and marine origin. *Eur. J. Clin. Nutr.*, **52,** 749–753.

Dosanjh, B.S., Higgs, D.A., Plotnikoff, M.D., Markert, J.R. & Buckley, J.T. (1988) Preliminary evaluation of canola oil, pork lard and marine lipid singly and in combination as supplemental dietary lipid sources for juvenile fall chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*, **68,** 325–343.

Erdal, J.I., Evensen, O.E., Kaurstad, O.K., Lillehaug, A., Solbakken, R. & Thorud, K. (1991) Relationship between diet and immune response in Atlantic salmon (*Salmo salar* L.) after feeding various levels of ascorbic acid and omega-3 fatty acids. *Aquaculture*, **98,** 363–379.

Folch, J., Lees, M. & Sloane-Stanley, G.H.S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226,** 497–509.

Fracalossi, D.M. & Lovell, R.T. (1994) Dietary lipid sources influence responses of channel catfish (*Ictalurus punctatus*) to challenge with the pathogen *Edwardsiella ictaluri*. *Aquaculture*, **119,** 287–298.

Good, J.E., Bell, J.G., Thompson, K.D. & Williams, P.W. (2001) Assessment of immune response in Atlantic Salmon (*Salmo salar*) receiving alternative oil diets. In: *An abstract and poster presentation at the 5th Nordic Fish Immunology Symposium*. pp. 42, June 2001, Institute of Pharmacy, University of Oslo, Oslo, Norway.

Greene, D.H.S. & Selivonchick, D.P. (1990) Effects of dietary vegetable, animal and marine lipids on muscle lipid and haematology of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **89,** 165–182.

Guillou, A., Soucy, P., Khalil, M. & Adambounou, L. (1995) Effects of vegetable and marine lipid on growth, muscle fatty acid composition and organoleptic quality of flesh of brook charr (*Salvelinus fontinalis*). *Aquaculture*, **136,** 351–362.

Hagave, T.A., Johansen, Y. & Christophersen, B. (1991) The effect of n-3 fatty acids on osmotic fragility of rat erythrocytes. *Biochim. Biophys. Acta*, **1084,** 251–254.

Horrocks, L.A. & Yeo, Y.K. (1999) Health benefits of docosahexaenoic acid (DHA). *Pharmacol. Res.*, **40,** 211–225.

Hunter, B.J. & Roberts, D.C.K. (2000) Potential impact of the fat composition of farmed fish on human health. *Nutr. Res.*, **20,** 1047–1058.

Kalogeropoulos, N., Alexis, M.N. & Henderson, R.J. (1992) Effects of dietary soybean and cod-liver oil levels on growth and body composition of gilthead sea bream (*Sparus aurata*). *Aquaculture*, **104,** 293–308.

Kinsella, J.E. & Lokesh, B. (1990) Dietary lipids, eicosanoids and the immune system. *Care Med.*, **18,** S94–S113.

Kiron, V., Fukuda, H., Takeuchi, T. & Watanabe, T. (1995) Essential fatty acid nutrition and defence mechanisms in rainbow trout *Oncorhynchus mykiss*. *Comp. Biochem. Physiol.*, **111A,** 361–367.

Klinger, R.C., Blazer, V.S. & Echevarria, C. (1996) Effects of dietary lipid on the haematology of channel catfish, *Ictalurus punctatus*. *Aquaculture*, **147,** 225–233.

Li, M.H., Wise, D.J., Johnson, M.R. & Robinson, E.H. (1994) Dietary menhaden oil reduced resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri*. *Aquaculture*, **128,** 335–344.

Montero, D., Blazer, V.S., Socorro, J., Izquierdo, M.S. & Tort, L. (1999) Dietary and culture influences on macrophage aggregate parameters in gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture*, **179,** 523–534.

Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M.J. & Izquierdo, M.S. (2003) Vegetable lipid sources for gilthead sea bream (*Sparus aurata*): effects on fish health. *Aquaculture*, **225,** 353–370.

Mourente, G. & Dick, J.R. (2002) Influence of partial substitution of dietary fish oil by vegetable oils on the metabolism (desaturation and β-oxidation) of [1-^{14}C]18:3n-3 in isolated hepatocytes of European sea bass (*Dicentrarchus labrax* L.). *Fish Physiol. Biochem.*, **26,** 297–308.

Mourente, G. & Tocher, D.R. (1994) In vivo metabolism of [1-^{14}C] linolenic acid [18:3(n-3)] and [1-^{14}C] eicosapentaenoic acid [20:5(n-3)] in a marine fish: time course of the desaturation/elongation pathway. *Biochim. Biophys. Acta*, **1212,** 109–118.

Mustafa, T. & Srivastava, K.C. (1989) Prostaglandins (eicosanoids) and their role in ectothermic organisms. *Adv. Comp. Environ. Physiol.*, **5,** 157–207.

Ng, W-K., Lim, P-K. & Boey, P-L. (2003a) Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, *Clarias gariepinus*. *Aquaculture*, **215,** 229–243.

Ng, W.K., Campbell, P.J., Dick, J.R. & Bell, J.G. (2003b) Interactive effects of dietary palm oil concentration and water temperature on lipid digestibility in rainbow trout, *Oncorhynchus mykiss*. *Lipids*, **38,** 1031–1038.

Oliva-Teles, A. (2000) Recent advances in European sea bass and gilthead sea bream nutrition. *Aquacult. Int.*, **8,** 477–492.

Olsen, R.E., Myklebust, R., Kaino, T. & Ringoe, E. (1999) Lipid digestibility and ultrastructural changes in the enterocytes of Arctic char (*Salvelinus alpinus* L.) fed linseed oil and soybean lecithin. *Fish Physiol. Biochem.*, **21,** 35–44.

Olsen, R.E., Myklebust, R., Ringoe, E. & Mayhew, T.M. (2000) The influences of dietary linseed oil and saturated fatty acids on caecal enterocytes in Arctic char (*Salvelinus alpinus* L.): a quantitative ultrastructural study. *Fish Physiol. Biochem.*, **22,** 207–216.

Parpoura, A.C.R. & Alexis, M.N. (2001) Effects of different dietary oils in sea bass (*Dicentrarchus labrax*) nutrition. *Aquacult. Int*., **9,** 463–476.

Polvi, S.M. & Ackman, R.G. (1992) Atlantic salmon (*Salmo salar*) muscle lipids and their response to alternative fatty acid sources. *J. Agric. Food Chem*., **40,** 1001–1007.

Powell, W.S. (1982) Rapid extraction of arachidonic acid metabolites from biological samples using octadecyl silica. In: *Methods in Enzymology*, Vol 86 (Packer, L. ed.), pp. 467–477. Academic Press, New York.

Regost, C., Arzel, J., Robin, J., Rosenlund, G. & Kaushik, S.J. (2003) Total replacement of fish oil by soybean or oil with return to fish oil in turbot (*Psetta maxima*) 1. Growth performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture*, **217,** 465–482.

Rowley, A.F. (1991) Lipoxin formation in fish leukocytes. *Biochim. Biophys. Acta*, **1084,** 303–306.

Rungruangsak-Torrisen, K., Wergeland, H.I., Glette, J. & Waagbø, R. (1999) Disease resistance and immune parameters in Atlantic salmon (*Salmo salar*) with genetically different trypsin isoenzymes. *Fish Shellfish Immunol*., **9,** 557–568.

Sargent, J.R., Henderson, R.J. & Tocher, D.R. (1989) The lipids. In: *Fish Nutrition*, (Halver, J.E. ed.), pp. 154–218. Academic Press, New York, USA.

Sargent, J.R. & Tacon, G.J. (1999) Development of farmed fish: a nutritional necessary alternative to meat. *Proc. Nutr. Soc*., **58,** 377–383.

Sargent, J.R., Bell, J.G., McGhee, J., McEvoy, J. & Webster, J.L. (2001) The nutritional value of fish. In: *Farmed Fish Quality* (Kestin, S.C. & Warriss, P.D. eds), pp. 3–12. Fishing News Books, Blackwell Science Ltd, Oxford.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002) The lipids. In: *Fish Nutrition*, 3rd edn. (Halver, J.E., Hardy, R.W. eds), pp. 181–257. Elsevier, USA.

Secombes, C.J. (1990) Isolation of salmonid macrophages and analysis of their killing activity. In: *Techniques in Fish Immunology* (Stolen, J.S. & Fletcher, T.C., Anderson, D.P., Robertson, B.S. & van Muiswinkel, W.B. eds), pp. 139–154. SOS Publications, Fair Haven, USA.

Sheldon, W.M. Jr & Blazer, V.S. (1991) Influence of dietary lipid and temperature on bactericidal activity of channel catfish macrophages. *J. Aquat. Anim. Health*, **3,** 87–93.

Simopoulos, A.P. (1999) Essential fatty acids in health and chronic disease. *Am. J. Nutr*., **70,** 560S–569S.

Sitja-Bobadilla, A. & Perez-Sanchez, J. (1999) Diet related changes in non-specific immune response of European sea bass (*Dicentrarchus labrax* L.). *Fish Shellfish Immunol*., **9,** 637–640.

Stankova, J. & Rola-Pleszczynski, M. (1993) Eicosanoids in defence. In: *Humoral Factors* (Sim, E. ed.), pp. 319–335. IRL Press, Oxford.

Thompson, K.D., Tatner, M.F. & Henderson, R.J. (1996) Effects of dietary (n-3) and (n-6) polyunsaturated fatty acid ratio on the immune response of Atlantic salmon, *Salmo salar* L. *Aquaculture Nutrition*, **2,** 21–31.

Tocher, D.R., Bell, J.G., Dick, J.R., Henderson, R.J., McGhee, F., Mitchell, D.F. & Morris, P.C. (2000) Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils. *Fish Physiol. Biochem*., **23,** 59–73.

Torstensen, B.E., Lie, O. & Froyland, L. (2000) Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.) effects of capelin oil, palm oil and oleic acid-enriched sunflower oil as dietary lipid sources. *Lipids*., **35,** 653–664.

Tucker, J.W., Ilellis, W.A., Vermeer, G.K., Roberts, D.E. & Woodward, P.N. (1997) The effects of experimental started diets with different levels of soybean or menhaden oil on red drum (*Sciaenops ocellatus*). *Aquaculture*, **149,** 323–339.

Uhing, R.J., Cowlen, M.S. & Adams, D.O. (1990) Mechanisms regulating the production of arachidonate metabolites in mononuclear phagocytes. *Curr. Top. Membr. Transp*., **35,** 349–374.

Waagbø, R., Sandnes, K., Lie, O. & Nilsen, E.R. (1993) Health aspects of dietary lipid sources and vitamin E in Atlantic salmon (*Salmo salar*): 1. Erythrocyte total lipid fatty acid composition, haematology and humoral immune response. *Fisk. Dir. Skr. Ser. Ernaering*., **6,** 47–62.

Waagbø, R., Hemre, G.I., Holm, J.C. & Lie, O. (1995) Tissue fatty acid composition, haematology and immunity in adult cod, *Gadus morhua* L., fed three dietary lipid sources. *J. Fish Dis*., **18,** 615–622.

Watanabe, T. (1982) Lipid nutrition in fish. *Comp. Biochem. Physiol*., **73,** 3–15.

Wootton, R.J. (1990) *Ecology of Teleost Fishes*. Fish and Fisheries Series 1, Chapman and Hall, Ashburton, UK, 404 pp.

Woyewoda, A.D., Shaw, S.J., Ke, P.J. & Burns, B.G. (1986) *Recommended Laboratory Methods for Assessment of Fish Quality*. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1448.

Yildiz, M. & Sener, E. (1997) Effect of dietary supplementation with soybean oil, sunflower oil or fish oil on the growth of sea bass (*Dicentrarchus labrax* L.). In: *Workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean*. 9pp. 24–26 June, Zaragoza, Spain.

Zar, J.H. (1984) *Biostatistical Analysis*, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ.