

Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils

D. Menoyo^{1,3}, M. S. Izquierdo², L. Robaina², R. Ginés², C. J. Lopez-Bote³ and J. M. Bautista^{1*}

¹Departamento de Bioquímica y Biología Molecular IV, Facultad de Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain

²Grupo de Investigación en Acuicultura (ULPGC & ICCM), P. O. Box 56, 35200 Telde, Islas Canarias, Spain

³Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain

(Received 5 May 2003 – Revised 1 March 2004 – Accepted 3 March 2004)

Linseed (LO) and soyabean (SO) oils were evaluated as fish-oil (FO) substitutes in the diets of marketable-sized gilthead sea bream (*Sparus aurata*). Practical diets were designed factorially with the lipid added as follows (%): FO 100, LO 60+FO 40, LO 80+FO 20, SO 60+FO 40, SO 80+FO 20. The effects of experimental diets on growth, fatty acids patterns in liver and muscle, flesh quality variables and activities of selected enzymes involved in lipid synthesis and catabolism were determined at the end of a 7-month trial. Fatty acid composition of liver and muscle generally reflected the fatty acid composition of the diets. The *n*-3 PUFA levels were significantly reduced by the inclusion of vegetable oils. This tendency was more pronounced for EPA than for docosahexaenoic acid. The *n*-3:*n*-6 fatty acid ratio reached the lowest values in fish fed the SO diets; this was associated with a higher liver lipid deposition. No differences were found in fillet texture and pH. However, under conditions of forced peroxidation, muscles from fish fed the SO diets had lower peroxidation levels. Vegetable oil substitution decreased lipogenesis in liver and this effect was greatest at the highest substitution level. In contrast, muscle β -oxidation enzymes had increased activities with vegetable oil substitution. Thus, the lower hepatic lipogenesis was correlated with an increased lipid utilisation in muscle. It is concluded that growth and lipid metabolism were affected by experimental diets.

Sea bream: Vegetable oil: Lipid metabolism: Flesh quality: Essential fatty acids

As the preferred lipid source in commercial diets for cultured fish, the demand for fish oil (FO) for aquatic feeds is increasing. Despite the most optimistic projections forecast, a global demand for aquaculture of about 1.90 million tonnes for the year 2015, production of FO seems to have reached a maximum at about 1.20 million tonnes per year, this value decreasing markedly during El Niño years (Food and Agriculture Organization, 2002). Since that production has to be shared with other uses, such as feeds for broilers and piglets and for pharmacology, there is a great interest in searching for alternative lipid sources in commercial fish feeds.

It has been shown in salmonids that FO can be replaced by certain types of vegetable lipid sources by up to 80–100% without compromising fish growth, but affecting tissue lipid composition and metabolism (Torstensen *et al.* 2000; Rosenlund *et al.* 2001; Bell *et al.* 2001, 2002; Caballero *et al.* 2002). The ability of marine fish to synthesize highly unsaturated fatty acids, such as arachidonic (AA),

EPA and docosahexaenoic (DHA) acids (which are essential for these fish species; Watanabe *et al.* 1983; Izquierdo *et al.* 2000), from their C₁₈ precursors present in vegetable oils is more limited than that of salmonids; thus, FO substitution levels in marine fish diets are frequently lower (Alexis, 1997). Substitution of up to 60% FO by vegetable oils does not seem to affect sea-bream (*Sparus aurata*) growth, although alterations in several immune variables, post-stress cortisol response (Montero *et al.* 2004) and histological alterations in the hepatic tissue (Caballero *et al.* 2004) suggest a modification in certain aspects of the lipid metabolism in fish.

Lipogenesis processes in fish are regulated by two main enzymes: glucose-6-phosphate dehydrogenase (G6PD), which is involved in the generation of reductor equivalents (NADPH), and fatty acid synthetase (FAS), an enzymatic complex that needs NADPH as a cofactor to synthesize fatty acids. A low-fat diet stimulates NADPH consumption in rainbow trout (*Onchorynchus mykiss*) and seabass

Abbreviations: AA, arachidonic acid; CPT, carnitine palmitoyltransferase; DHA, docosahexaenoic acid; EGTA, ethylene glycol-*O*,*O'*-bis(2-amino-ethyl)-*N,N,N',N'*-tetraacetic acid; FAS, fatty acid synthetase; FO, fish oil; G6PD, glucose-6-phosphate dehydrogenase; LO, linseed oil; L3HOAD, L-3-hydroxyacyl-CoA dehydrogenase; OA, oleic acid; SO, soyabean oil.

* **Corresponding author:** Dr José M. Bautista, fax +34 91 394 3824, email jmbau@vet.ucm.es

(*Dicentrarchus labrax*), whereas a high-fat diet inhibits FAS activity by regulation of the NADPH generation by G6PD (Alvarez *et al.* 1998). In general, high-lipid diets depress lipogenesis in many fish species (Likimani & Wilson, 1982; Shimeno *et al.* 1993; Corraze *et al.* 1999) including gilthead sea bream (Bonamusa *et al.* 1992), but there is little information about the effects of alternative lipid sources on the lipogenic processes in fish, and particularly in gilthead sea bream. Changes in the activities of G6PD and FAS have not only been shown to be indicative of the lipogenic state, but also of the fish flesh susceptibility to peroxidation both in sea bass and rainbow trout (Alvarez *et al.* 1998). Although the important contribution of white muscle to the overall fatty acid β -oxidation reported for Atlantic salmon (*Salmo salar*; Frøyland *et al.* 1998, 2000) and haddock (*Melanogrammus aeglefinus* L.; Nanton *et al.* 2003) red muscle has the highest specific activity expressed per mg soluble protein or per g wet tissue (Frøyland *et al.* 1998, 2000; Nanton *et al.* 2003). Mitochondrial β -oxidation is regulated through the inhibition of carnitine palmitoyltransferase (CPT)-I activity by malonyl-CoA (Frøyland *et al.* 1998). However, the interactions among the dietary fatty acid profiles, lipid catabolism and flesh quality in sea bream are unknown.

The current study investigates the effect of feeding FO with high levels of vegetable oil substitution on the lipid metabolism and the fillet quality variables of market-sized gilthead sea bream.

Materials and methods

Experimental fish

Sea-bream juveniles were provided by a local fish farm (ADSA, Las Palmas, Spain). Fish (n 975) of average initial weight 85 g were randomly distributed into fifteen 500-litre polyethylene circular tanks (sixty-five fish per tank) supplied with continuous seawater (36‰) flow and aeration. Fish were fed under natural photoperiod conditions (12 h light–dark cycle). Water temperature was 19.5–23.8°C

and dissolved O₂ 6.2–7.4 μ l/l. After 2 weeks of acclimation to the experimental tanks and the control diet, the fish were fed the experimental diets until apparent satiation three times per d at 09.00, 12.00 and 15.00 hours, 6 d per week, until commercial size (400–500 g) was reached (204 d).

Diet formulation

Five isoenergetic and isoproteic experimental diets were formulated with a constant lipid content of about 220 g/kg. Diets were manufactured and supplied by ARC, Stavanger, Norway. FO was the only added lipid source in the control diet, representing the zero substitution level for the two lipid sources soyabean oil (SO) and linseed oil (LO) in the factorial arrangement, whereas the other diets contained either SO (60%SO and 80%SO) or LO (60%LO and 80%LO) oils at two different substitution levels: 60 and 80% of the FO contained in the control diet. FO was included in all diets at a level high enough to maintain n -3 PUFA levels >3 g/100 g total fatty acids in order to provide the essential fatty acid requirements for this species (Ibeas *et al.* 1994) (Tables 1 and 2).

Biological variables and sample collection

At the end of the growth trial, fish were individually weighed and samples of muscle and liver from ten fish per tank were collected, pooled and stored at –80°C until biochemical analysis. Fish for flesh-quality studies were starved for 24 h before being slaughtered in a small tank with ice and seawater. Nine fish from each treatment were chosen at random, gutted, filleted and frozen at –70°C until analysis.

Biochemical analysis

Lipids from the experimental diets, muscle, liver and faeces were extracted with chloroform–methanol (2:1, v/v), as

Table 1. Basal composition of experimental diets

| | Control | 60% SO | 80% SO | 60% LO | 80% LO |
|-------------------------------------|---------|--------|--------|--------|--------|
| Ingredients (g/kg) | | | | | |
| Fish meal-LT* | 381 | 381 | 381 | 381 | 381 |
| Maize gluten | 260 | 260 | 260 | 260 | 260 |
| Fish oil | 176 | 70.4 | 35.2 | 70.4 | 35.2 |
| SO | – | 105.6 | 140.8 | – | – |
| LO | – | – | – | 105.6 | 140.8 |
| Wheat | 150.6 | 150.6 | 150.6 | 150.6 | 150.6 |
| Premix** | 25 | 25 | 25 | 25 | 25 |
| Lysine (990 g/kg) | 7.23 | 7.23 | 7.23 | 7.23 | 7.23 |
| Yttrium oxide | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Proximate composition (g/kg wet wt) | | | | | |
| Moisture | 60.5 | 62.0 | 70.2 | 87.2 | 75.9 |
| Ash | 62.6 | 63.2 | 63.9 | 63.3 | 63.6 |
| Protein | 446.4 | 456.4 | 459.6 | 443.0 | 450.1 |
| Fat | 216.7 | 215.2 | 210.0 | 211.7 | 241.9 |

SO, soyabean oil; LO, linseed oil.

*Fish meal LT containing 71.8% protein and 8.4% lipid.

**Premix (g/kg): vitamin and mineral premix 15.6 (National Research Council, 1993; proprietary composition, Nutreco ARC, Stavanger, Norway), Lysine-HCl 9.0 (BASF, Ludwigshafen, Germany), Lutavit C 0.4 (BASF).

described by Folch *et al.* (1957). The fatty acid methyl esters were obtained by transesterification with H₂SO₄ (10 ml/l methanol; Christie, 1982). Fatty acid methyl esters were purified by absorption chromatography on NH₂ Sep-pack cartridges (Waters, S.A., Milford, MA, USA) as described by Fox (1990), and separated and quantified by GLC as described by Izquierdo *et al.* (1989).

Induced lipid peroxidation

Stimulated lipid peroxidation analysis was carried out by a modification of the method of Kornbrush & Mavis (1980). Muscle homogenates (approximately 0.1 g/ml KCl (11.5 g/l)) were incubated at 37°C in a solution containing 40 mM-Tris-maleate buffer (pH 7.4) and 0.4 mM-ascorbic acid. At fixed intervals (0, 10, 20, 30, 45, 60, 90 and 120 min), 0.4 ml aliquots were removed for measurements of thiobarbituric acid-reactive substances. Thiobarbituric acid-reactive substances were expressed as nmol malondialdehyde/mg soluble protein.

Mitochondrial preparations, soluble extracts and enzyme analyses

Red muscle was dissected from one side of the fish and homogenized with an Ultra turrax T18basic (IKA®; Labor-technik, Staufen, Germany) in 10 vol. ice-cold buffer (10 mM-Tris-HCl, 0.25 M-sucrose, 1 mM-ethylene glycol-*O*,*O*'-bis(2-amino-ethyl)-*N,N,N',N'*-tetraacetic acid (EGTA), 10 mg bovine serum albumin/l, pH 7.4). Centrifugation conditions were set to perform three sequential spins at 4°C for 1 min each, following the method of Harper & Saggerson (1975). The homogenate was first spun at 3000 g, the resulted supernatant fraction was collected and spun again at 20 000 g. Finally, the resulting pellet was washed once and centrifuged under the same conditions in 10 mM-Tris-HCl (pH 7.4) containing 0.25 M-sucrose and 1 mM-EGTA. The mitochondrial extract was resuspended in 10 mM-Tris-HCl (pH 7.4) containing 0.3 M-sucrose and 1 mM-EGTA. The activity of L-3-hydroxyacyl-CoA dehydrogenase (L3HOAD) was measured according to Bradshaw & Noyes (1975) on mitochondrial isolates disrupted by sonication and a Triton X-100 solution (10 ml/l). The activity of CPT-I was assayed on intact mitochondrial extracts as previously described (Sanz *et al.* 2000).

Liver homogenates and the activities of G6PD and FAS were performed as described by Dias *et al.* (1998). The soluble protein content of liver homogenates and muscle mitochondrial preparations was determined by the method of Bradford (1976) using bovine serum albumin as the standard. All the enzyme activity assays were conducted at 30°C. Care was taken to ensure that initial rates were being measured in all assays. Control experiments established that the enzyme was stable in the buffer used during the time period of the assay and at the temperature required (Alvarez *et al.* 1998). All enzyme assays were performed in duplicate or triplicate. The enzymatic activity units (IU), defined as μmol substrate converted to product per min at the assay temperature, were expressed per mg hepatic soluble protein (specific activity). (¹⁴C(acetyl-Co A (2035 MBq/mmol) and L-[methyl-³H]carnitine hydrochloride

(3034 × 10³ MBq/mmol) were supplied by Amersham Biosciences (Uppsala, Sweden), while the remaining reagents were supplied by Sigma Aldrich (Madrid, Spain).

Texture

A model 4465 Instron UTM texture analyser (Instron Corporation, Canton, MS, USA) was used to measure the texture of both the whole fish and individual fillets. All these tests were carried out at refrigeration temperature (0–5°C), and the whole and the filleted fish were kept in constant contact with ice. The whole fish were compressed using a cylinder of 12 mm diameter at a speed of 50 mm/min and the force required to penetrate the dorsal zone of the right-hand side of the fish to a depth of 4 mm was measured. The tests attempted to imitate the pressure that a person carrying out a sense evaluation would apply, and 4 mm was chosen because at this depth the fish skin is not destroyed. The tests carried out on the fillets were of the puncture and compression type using the methodology that has previously been described by Borderías *et al.* (1983), although slight modifications were incorporated. In our present study, a cylindrical piece of fillet of 53 mm diameter and 12 mm thickness was cut from the left-hand flank of the fish and used for the puncture. Similarly, a cylindrical piece of fillet with 26 mm wide and 12 mm thickness was used for the compression test. The flesh puncture was measured as the force required to completely penetrate the fillet by a 8 mm diameter cylinder at a speed of 80 mm/min. The flesh compression was measured as the force required to compress the thickness of the fillet by 30 % using a 36 mm diameter cylindrical piston at a speed of 50 mm/min.

Colour

Instrumental colour analysis was performed with a Minolta chromameter CR200 (Minolta, Osaka, Japan) giving results in Commission Internationale de l'Eclairage (1976) values for lightness (L*), redness (a*) and yellowness (b*). Skin measurements were taken along a lateral line at the level of the fore insertion of dorsal fin and fillets measurements were taken in the same area after fish were skinned.

pH

The pH of the flesh was determined using a Crison penetration electrode (accurate to 0.01 pH unit, model 507; Crison Instruments S.A., Barcelona, Spain) after carrying out an incision on the skin.

Statistical analysis

Data were submitted to a two-way ANOVA with fat source, vegetable oil inclusion level and their interactions as main effects by using the General Linear Model procedure contained in the SAS computer software (SAS Institute Inc., Cary, NC, USA). Bonferroni tests were used when ANOVA main effects were significantly different. A repeated-measure mean test was used to compare differences in malondialdehyde concentrations between

groups during stimulated lipid peroxidation (Morris, 1999). Pearson correlation coefficients between intramuscular total lipids and texture variables were calculated by using the 'proc corr' procedure of SAS. Differences were considered significant at the level of $P < 0.05$.

Results

Final body weight and specific growth rate ($(100 \times (\ln \text{ final fish wt} - \ln \text{ initial fish wt}))/d$) were significantly reduced by the increase in the vegetable oil inclusion level with fish fed the 80% SO diet having the lowest values (Table 3). Vegetable oil inclusion affected feed conversion index (dry feed intake/wet wt gain) with the fish fed the 80% SO diet having the highest values (Table 3). Both the dietary fat type and the level of FO substitution affected the liver weight and hepatosomatic index ($(100 \times \text{ liver wt})/\text{ fish wt}$), while inclusion level affected liver total lipids (Table 3).

The fatty acid composition of liver lipids showed a reduction in saturated fatty acid content with the gradual inclusion of vegetable oils ($P < 0.001$; Table 4). Conversely, oleic acid (OA) content was elevated by the increase in the vegetable-oil inclusion level ($P < 0.001$), but there was a reduction in the other monoenes when dietary vegetable oils were included. Thus, both type and inclusion level of dietary fat had a significant effect on total

MUFA present in the liver ($P < 0.001$). As expected, both linoleic and linolenic acids in liver lipids were significantly ($P < 0.001$) affected by dietary fat source and inclusion level and by the combination of both dietary factors. Although EPA content was only reduced with the increase in the inclusion level of vegetable oils, AA and DHA contents in liver lipids were also affected by the type of dietary fat (Table 4). Accordingly, *n*-3 PUFA content decreased and the OA:*n*-3 PUFA and OA:DHA ratios increased with the progressive substitution of FO, regardless to the type of vegetable oil (Table 4). The EPA:DHA ratio, which was lower in the initial fish liver lipids in comparison with the final ones, decreased with the inclusion of vegetable oils (Table 4). Conversely, the AA:EPA ratio in liver significantly decreased in fish fed the experimental diets compared with initial values: the higher inclusion of dietary vegetable oils, the higher AA:EPA ratio in liver lipids. Finally, the *n*-3:*n*-6 fatty acid ratio (Table 4) was reduced by the gradual inclusion of vegetable oils, particularly by SO.

The inclusion of vegetable oils significantly reduced the content of saturated fatty acid in muscle lipids, irrespective of the fat source (Table 5), mainly due to the reduction in stearic and palmitic acids ($P < 0.001$). Regarding MUFA, the gradual inclusion of vegetable oils significantly reduced the palmitoleic acid content in muscle lipids ($P < 0.001$), but increased OA content ($P < 0.001$), particularly when

Table 2. Selected fatty acid composition (g/100 g total fatty acids) of sea-bream (*Sparus auratus*) diets containing 100% fish oil (control), 40% fish oil + 60% soyabean oil (60% SO), 20% fish oil + 80% soyabean oil (80% SO), 40% fish oil + 60% linseed oil (60% LO), 20% fish oil + 80% linseed oil (80% LO)*

| Fatty acid | Control | 60% SO | 80% SO | 60% LO | 80% LO |
|---------------------------------------|---------|--------|--------|--------|--------|
| 14:0 | 6.80 | 3.11 | 1.99 | 3.03 | 1.99 |
| 16:0 | 17.51 | 14.26 | 13.45 | 11.48 | 9.58 |
| 18:0 | 2.93 | 2.96 | 3.07 | 3.21 | 3.25 |
| Total saturated fatty acids† | 27.38 | 20.25 | 18.35 | 18.06 | 14.82 |
| 16:1 <i>n</i> -7 | 7.10 | 3.29 | 2.17 | 3.18 | 2.02 |
| 18:1 <i>n</i> -9 | 13.65 | 17.07 | 18.24 | 16.32 | 15.99 |
| 18:1 <i>n</i> -7 | 2.51 | 1.80 | 1.66 | 1.51 | 1.12 |
| 20:1 <i>n</i> -9 | 1.72 | 1.39 | 1.36 | 1.41 | 1.66 |
| 22:1 <i>n</i> -11 | 2.00 | 1.61 | 2.36 | 1.38 | 2.10 |
| 22:1 <i>n</i> -9 | 0.21 | 0.20 | nd | 0.23 | nd |
| Total monoenes | 28.50 | 25.34 | 25.35 | 24.51 | 22.86 |
| 18:2 <i>n</i> -6 | 4.70 | 30.42 | 38.52 | 11.48 | 13.24 |
| 20:2 <i>n</i> -6 | 0.11 | 0.10 | 0.11 | 0.08 | 0.08 |
| 20:4 <i>n</i> -6 | 0.74 | 0.42 | 0.30 | 0.32 | 0.25 |
| Total <i>n</i> -6 fatty acids‡ | 7.72 | 31.27 | 38.18 | 12.87 | 14.28 |
| 18:3 <i>n</i> -3 | 0.64 | 3.42 | 4.39 | 28.09 | 36.67 |
| 18:4 <i>n</i> -3 | 1.96 | 0.92 | 0.63 | 0.87 | 0.66 |
| 20:4 <i>n</i> -3 | 0.58 | 0.32 | 0.08 | 0.26 | 0.26 |
| 20:5 <i>n</i> -3 | 16.16 | 7.85 | 5.02 | 6.94 | 4.30 |
| 22:5 <i>n</i> -3 | 1.87 | 0.25 | 0.52 | 0.80 | 0.09 |
| 22:6 <i>n</i> -3 | 7.01 | 4.24 | 2.44 | 3.99 | 3.31 |
| Total <i>n</i> -3 Fatty acids | 30.52 | 17.76 | 13.27 | 41.32 | 44.73 |
| <i>n</i> -3 : PUFA§ | 25.31 | 12.53 | 7.83 | 11.97 | 7.99 |
| AA : EPA | 0.05 | 0.05 | 0.06 | 0.05 | 0.06 |
| EPA : DHA | 2.31 | 1.85 | 2.06 | 1.74 | 1.30 |
| OA : DHA | 1.95 | 4.03 | 7.49 | 4.09 | 4.84 |
| OA : <i>n</i> -3 PUFA§ | 0.52 | 1.30 | 2.23 | 1.33 | 1.94 |
| <i>n</i> -3 : <i>n</i> -6 Fatty acids | 3.96 | 0.57 | 0.35 | 3.21 | 3.13 |

AA, arachidonic acid (20:4*n*-6); DHA, docosahexaenoic acid (22:6*n*-3); OA, oleic acid (18:1*n*-9); nd, not detected.

*For details of diets and procedures, see Table 1 and p. 000.

†Includes 15:0 and 17:0.

‡Includes 18:3*n*-6, 18:4*n*-6, 20:3*n*-6 and 22:5*n*-6.

§PUFA include 18:3*n*-6, 18:4*n*-6, 20:3*n*-6 and 22:5*n*-6.

||EPA 20:5*n*-3.

Table 3. Growth performance, liver weight and hepatosomatic index in sea bream (*Sparus aurata*) fed fed diets containing 100% fish oil (control), 40% fish oil + 60% soyabean oil (60% SO), 20% fish oil + 80% soyabean oil (80% SO), 40% fish oil + 60% linseed oil (60% LO), 20% fish oil + 80% linseed oil (80% LO) (Mean values and standard deviations for nine fish per group)

| | Experimental diets | | | | | | | | | | | | |
|-------------------------------|--------------------|------|---------------------|------|--------------------|------|---------------------|------|---------------------|------|--------------------------|--------|----------|
| | Control | | 60% SO | | 80% SO | | 60% LO | | 80% LO | | Probability of contrasts | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Fs | InL | Fs × InL |
| SGR | 0.68 ^a | 0.01 | 0.68 ^{ab} | 0.03 | 0.62 ^b | 0.01 | 0.67 ^{ab} | 0.01 | 0.66 ^{ab} | 0.01 | NS | 0.009 | NS |
| Feed conversion index† | 1.38 ^b | 0.01 | 1.42 ^{ab} | 0.03 | 1.55 ^a | 0.01 | 1.43 ^{ab} | 0.07 | 1.46 ^{ab} | 0.02 | NS | 0.001 | NS |
| Final body weight (g) | 463.9 ^a | 56.1 | 460.8 ^{ab} | 48.5 | 441.1 ^c | 53.4 | 459.8 ^c | 54.0 | 440.9 ^{bc} | 56.9 | NS | 0.0001 | NS |
| Muscle total lipids (g/kg DM) | 285.9 | 3.20 | 259.2 | 4.35 | 319.6 | 20.6 | 237.9 | 54.2 | 274.0 | 42.7 | NS | NS | NS |
| Liver weight (g) | 6.15 ^a | 0.35 | 5.40 ^{bc} | 0.53 | 5.70 ^{ab} | 0.54 | 5.33 ^{bc} | 0.13 | 5.04 ^c | 0.21 | 0.05 | 0.0001 | NS |
| Liver total lipids (g/kg DM) | 393.6 ^b | 44.4 | 484.7 ^{ab} | 54.7 | 537.1 ^a | 55.2 | 480.7 ^{ab} | 49.6 | 494.7 ^{ab} | 29.1 | NS | 0.002 | NS |
| HSI* | 28.99 ^a | 0.88 | 24.00 ^b | 1.33 | 22.11 ^c | 0.39 | 23.72 ^b | 0.68 | 21.23 ^c | 0.48 | 0.05 | 0.02 | NS |

SGR, specific growth rate ((100 × (ln Final Fish wt - ln Initial Fish wt) / (d)); HSI, hepatosomatic index ((100 × liver wt) / fish wt); Fs, fat source; InL, inclusion level.

^{a, b} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and p. 42.

† Feed conversion index = dry feed intake/wet wt gain.

LO was included ($P < 0.001$). Accordingly, total monoenes were only slightly, but significantly ($P < 0.001$), reduced by the inclusion of vegetable oils (Table 5). Both the type of dietary fat and the inclusion level had a significant effect on linoleic and linolenic contents. In addition, an interaction effect between both dietary factors was observed ($P < 0.001$) in the concentration of these fatty acids. The EPA content of fish muscle was significantly affected by both type and level of inclusion of dietary fat, whereas concentrations of AA and DHA were only influenced by the inclusion level (Table 5). Thus, *n*-3 PUFA content in muscle was significantly reduced by the inclusion level of the vegetable oil ($P < 0.05$) regardless of the type of vegetable oil used (Table 5), but in a lower proportion than it was reduced in the liver lipids. Hence, OA:*n*-3 PUFA and OA:DHA were increased by the inclusion of vegetable oils. The initial values of EPA:DHA were lower than those found in fish fed the experimental diets and were reduced by the inclusion of vegetable oils (Table 5). Conversely, AA:EPA ratios were significantly increased by the inclusion of vegetable oils. Finally, the *n*-3:*n*-6 fatty acid ratio was higher in the muscle of fish fed the fish-oil diet, while no differences were found between initial values and fish fed the diets containing LO, the percentage of oil substitution having no effect. The lowest values corresponded to the fish fed the SO (Table 5).

No dietary effects were found in fillet pH and texture variables (Table 6); however, a significantly negative correlation was found between whole fish puncture values and total intramuscular lipids ($R^2 = 0.36$; $P < 0.02$). Fillet *a** (redness) and *b** (yellowness) were affected by the inclusion level of the vegetable oil in the feed (Table 6) and the L* (lightness) of the skin was also affected by the percentage of FO substituted. The effects of experimental diets on the susceptibility of fish filets to peroxidation are shown in Table 7. A significant effect of incubation time was observed on malondialdehyde concentration ($P < 0.001$). In addition, a significant interaction effect between time and the fat source was observed ($P < 0.01$). The order of fillet peroxidation level after 120 min incubation as affected by main dietary fat source was FO > LO > SO (Table 7). The results provided in Table 7 shows an interaction effect of time × inclusion level ($P < 0.001$). However, dietary lipid type, but not level of substitution, influenced the concentration of malondialdehyde. The highest FO level was significantly different for 20 v. 40% FO substitution, but there was no significant difference between 20 and 40% FO for either the SO- or the LO-treatment groups. No correlations were found between intramuscular lipid levels and induced lipid peroxidation.

Dietary vegetable oil inclusion level significantly reduced the activity of G6PD activity in the liver ($P < 0.001$). FAS activity was more markedly reduced by the LO than by the SO inclusion ($P < 0.001$), thus also being affected by the type of vegetable oil used (Table 8). Both type and level of dietary fat had significant effects on CPT-I and L3HOAD activities. Moreover, an interaction effect was observed in the activity of enzymes involved in lipid catabolism between dietary fat source and inclusion level (Table 8).

Table 4. Fatty acids(g/100 g total fatty acids) of liver lipids of sea bream (*sparus auratus*) fed diets containing 100 % fish oil (control), 40 % fish oil + 60 % soyabean oil (60%SO), 20 % fish oil + 80 % soyabean oil (80%SO), 40 % fish oil + 60 % linseed oil (60%LO), 20 % fish oil + 80 % linseed oil (80%LO)* (Mean values and standard deviations for nine fish per group)

| Fatty acids | Experimental diets | | | | | | | | | | | | | | | | | | |
|------------------------------|--------------------|------|--|--------------------|------|--|---------------------|------|--|---------------------|------|--|--------------------|------|--|--------|--------|--------|--------|
| | Control | | | 60% SO | | | 80% SO | | | 60% LO | | | 80% LO | | | | | | |
| | Mean | SD | | Mean | SD | | Mean | SD | | Mean | SD | | Mean | SD | | | | | |
| 14:0 | 4.48 ^a | 0.31 | | 2.51 ^b | 0.18 | | 1.75 ^d | 0.03 | | 2.81 ^b | 0.16 | | 2.08 ^c | 0.10 | | 0.0015 | 0.0001 | 0.0001 | 0.043 |
| 16:0 | 19.30 ^a | 0.64 | | 16.35 ^b | 0.90 | | 14.71 ^c | 0.41 | | 15.93 ^b | 0.61 | | 14.39 ^c | 0.50 | | NS | 0.0001 | 0.0001 | NS |
| 18:0 | 6.15 ^a | 0.35 | | 5.40 ^{bc} | 0.53 | | 5.70 ^{ab} | 0.54 | | 5.33 ^{bc} | 0.13 | | 5.04 ^c | 0.21 | | 0.0177 | 0.0001 | 0.0001 | 0.0229 |
| Total saturated fatty acids† | 28.99 ^a | 0.88 | | 24.00 ^b | 1.33 | | 22.11 ^c | 0.39 | | 23.72 | 0.68 | | 21.23 ^c | 0.48 | | NS | 0.0001 | 0.0001 | NS |
| 16:1 n-7 | 7.52 ^a | 0.34 | | 4.25 ^b | 0.10 | | 3.40 ^c | 0.14 | | 4.27 ^b | 0.11 | | 3.04 ^d | 0.22 | | 0.0287 | 0.0001 | 0.0001 | 0.0285 |
| 18:1 n-9 | 22.26 ^b | 0.15 | | 25.83 ^a | 2.62 | | 27.00 ^a | 0.81 | | 25.67 ^a | 0.66 | | 25.67 ^a | 0.85 | | NS | 0.0001 | 0.0001 | NS |
| 18:1 n-7 | 3.82 | 0.07 | | 2.69 | 0.00 | | nd | | | nd | | | nd | | | 0.0001 | 0.0001 | 0.0001 | NS |
| 20:1 n-9 | 1.44 ^a | 0.04 | | 1.27 ^b | 0.05 | | 1.14 ^c | 0.04 | | 1.14 ^c | 0.02 | | 1.39 ^a | 0.05 | | 0.0013 | 0.0001 | 0.0001 | 0.0001 |
| 22:1 n-11 | 0.75 ^{ab} | 0.08 | | 0.59 ^{bc} | 0.09 | | 0.51 ^c | 0.05 | | 0.58 ^{bc} | 0.01 | | 0.87 ^a | 0.27 | | 0.0019 | 0.0013 | 0.0001 | 0.0001 |
| Total monoenes | 35.59 ^a | 0.16 | | 32.85 ^b | 0.95 | | 31.86 ^{bc} | 0.83 | | 31.45 ^{cd} | 0.75 | | 30.52 ^d | 0.92 | | 0.0001 | 0.0001 | 0.0001 | 0.0069 |
| 18:2 n-6 | 5.36 ^e | 0.18 | | 24.70 ^b | 0.65 | | 28.08 ^a | 0.71 | | 10.38 ^d | 0.04 | | 12.07 ^c | 0.11 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| 20:2 n-6 | 0.20 ^c | 0.00 | | 0.70 ^a | 0.05 | | 0.77 ^a | 0.10 | | 0.25 ^{bc} | 0.01 | | 0.31 ^b | 0.05 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| 20:4 n-6 | 0.67 ^a | 0.06 | | 0.32 ^b | 0.02 | | 0.25 ^{cd} | 0.02 | | 0.29 ^{bc} | 0.02 | | 0.21 ^d | 0.01 | | 0.0172 | 0.0001 | 0.0001 | NS |
| Total n-6 fatty acidst | 7.51 ^e | 0.15 | | 26.61 ^b | 0.61 | | 30.65 ^a | 0.41 | | 11.50 ^d | 0.04 | | 13.00 ^c | 0.04 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| 18:3 n-3 | 0.60 ^e | 0.10 | | 2.30 ^d | 0.15 | | 3.84 ^c | 1.79 | | 19.60 ^b | 0.83 | | 24.56 ^a | 0.36 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| 18:4 n-3 | 1.02 ^{ab} | 0.04 | | 0.54 ^c | 0.06 | | 0.52 ^c | 0.05 | | 1.09 ^a | 0.03 | | 0.93 ^b | 0.11 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| 20:4 n-3 | 0.81 ^a | 0.03 | | 0.46 ^c | 0.05 | | 0.41 ^c | 0.04 | | 0.67 ^b | 0.05 | | 0.64 ^b | 0.05 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| 20:5 n-3 | 8.20 ^a | 0.49 | | 3.58 ^b | 0.34 | | 2.34 ^c | 0.10 | | 3.48 ^b | 0.20 | | 2.05 ^c | 0.16 | | NS | 0.0001 | 0.0001 | NS |
| 22:5 n-3 | 5.28 ^a | 0.14 | | 2.46 ^b | 0.32 | | 1.65 ^d | 0.05 | | 2.01 ^c | 0.25 | | 1.34 ^c | 0.16 | | 0.0001 | 0.0001 | 0.0001 | 0.0033 |
| 22:6 n-3 | 9.11 ^a | 0.19 | | 5.16 ^b | 0.79 | | 4.55 ^{bc} | 0.34 | | 4.76 ^b | 0.35 | | 4.07 ^c | 0.20 | | 0.0031 | 0.0001 | 0.0001 | NS |
| Total n-3 | 24.67 ^c | 1.03 | | 14.50 ^d | 1.62 | | 13.53 ^d | 1.55 | | 31.56 ^b | 1.35 | | 33.73 ^a | 0.43 | | 0.0001 | 0.0024 | 0.0001 | 0.0001 |
| n-3 PUFA\$ | 22.42 ^a | 0.87 | | 11.43 ^b | 1.42 | | 9.16 ^c | 0.56 | | 11.28 ^b | 0.81 | | 8.97 ^c | 0.09 | | NS | 0.0001 | 0.0001 | NS |
| AA: EPA | 0.08 ^b | 0.00 | | 0.09 ^b | 0.01 | | 0.11 ^a | 0.01 | | 0.08 ^b | 0.00 | | 0.10 ^a | 0.00 | | NS | 0.0001 | 0.0001 | NS |
| EPA: DHA | 0.92 ^a | 0.07 | | 0.70 ^b | 0.05 | | 0.52 ^c | 0.05 | | 0.73 ^b | 0.03 | | 0.50 ^c | 0.02 | | NS | 0.0001 | 0.0001 | NS |
| OA: DHA | 2.44 ^c | 0.06 | | 5.18 ^b | 1.30 | | 5.95 ^{ab} | 0.39 | | 5.42 ^{ab} | 0.43 | | 6.32 ^a | 0.50 | | NS | 0.0001 | 0.0001 | NS |
| OA: n-3 PUFA\$ | 0.94 ^c | 0.03 | | 2.24 ^b | 0.50 | | 2.87 ^a | 0.18 | | 2.21 ^b | 0.19 | | 2.76 ^a | 0.05 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| n-3: n-6 fatty acids | 3.29 ^a | 0.10 | | 0.54 ^c | 0.05 | | 0.44 ^c | 0.06 | | 2.74 ^b | 0.11 | | 2.59 ^b | 0.03 | | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

AA, arachidonic acid (20:4 n-6); DHA, docosahexaenoic acid (22:6 n-3); OA, oleic acid (18:1 n-9); Fs, fat source; InL, inclusion level; nd, not detected.

^{a,b,c,d}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and p. 42.

† Includes 15:0 and 17:0.

‡ Includes 18:3 n-6, 18:4 n-6, 20:3 n-6 and 22:5 n-6.

\$ PUFA include 20:3, 20:4, 20:5, 22:5 and 22:6.

|| EPA 20:5 n-3.

Table 5. Some fatty acids (g/100 g total fatty acids) of muscle lipids of sea bream (*sparus auratus*) fed diets containing 100 % fish oil (control), 40 % fish oil + 60 % soyabean oil (60 % SO), 20 % fish oil + 80 % soyabean oil (80 % SO), 40 % fish oil + 60 % linseed oil (60 % LO), 20 % fish oil + 80 % linseed oil (80 % LO)* (Mean values and standard deviations for nine fish per group)

| Fatty acids | Experimental diets | | | | | | | | | | | | |
|-------------------------------|--------------------|------|--------------------|------|---------------------|------|---------------------|------|---------------------|------|--------------------------|--------|----------|
| | Control | | 60 % SO | | 80 % SO | | 60 % LO | | 80 % LO | | Probability of contrasts | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Fs | InL | Fs × InL |
| 14:0 | 5.71 ^a | 0.07 | 3.24 ^b | 0.06 | 2.22 ^c | 0.55 | 3.15 ^b | 0.14 | 2.50 ^c | 0.27 | NS | 0.0001 | NS |
| 16:0 | 20.06 ^a | 0.18 | 17.70 ^b | 0.53 | 17.04 ^{bc} | 1.49 | 16.84 ^{bc} | 1.14 | 15.56 ^c | 1.45 | 0.0055 | 0.0001 | NS |
| 18:0 | 3.50 ^c | 0.09 | 3.62 ^{bc} | 0.24 | 3.79 ^{bc} | 0.27 | 4.13 ^a | 0.22 | 3.92 ^{ab} | 0.30 | 0.0007 | 0.0001 | 0.0033 |
| Total saturated fatty acids † | 29.80 ^a | 0.10 | 25.08 ^b | 0.84 | 23.62 ^{bc} | 1.97 | 24.71 ^b | 1.48 | 22.47 ^c | 2.03 | NS | 0.0001 | NS |
| 16:1 n-7 | 8.16 ^a | 0.18 | 4.84 ^b | 0.09 | 3.92 ^c | 0.34 | 4.61 ^b | 0.16 | 3.76 ^c | 0.21 | 0.0079 | 0.0001 | NS |
| 18:1 n-9 | 19.79 ^c | 0.36 | 23.44 ^b | 1.58 | 24.93 ^a | 0.50 | 24.83 ^a | 0.78 | 24.09 ^{ab} | 0.70 | NS | 0.0001 | 0.001 |
| 18:1 n-7 | 3.26 | 0.14 | 2.52 | 0.00 | nd | | nd | | nd | | 0.0055 | 0.0001 | NS |
| 20:1 n-9 | 1.77 ^a | 0.11 | 1.56 ^b | 0.02 | 1.62 ^b | 0.04 | 1.58 ^b | 0.11 | 1.80 ^a | 0.12 | 0.0136 | 0.0001 | 0.0119 |
| 22:1 n-11 | 1.22 ^{ab} | 0.11 | 1.02 ^b | 0.02 | 1.24 ^{ab} | 0.24 | 1.22 ^{ab} | 0.27 | 1.51 ^a | 0.48 | 0.0333 | 0.017 | NS |
| Total monoenes | 36.02 ^a | 0.78 | 32.81 ^b | 0.88 | 32.42 ^b | 1.09 | 33.04 ^b | 1.16 | 31.67 ^b | 1.29 | NS | 0.0001 | NS |
| 18:2 n-6 | 5.58 ^e | 0.13 | 23.71 ^b | 0.20 | 28.31 ^a | 1.66 | 10.36 ^d | 0.15 | 12.21 ^c | 0.15 | 0.0001 | 0.0001 | 0.0001 |
| 20:2 n-6 | 0.15 ^a | 0.01 | 0.45 ^b | 0.00 | 0.55 ^a | 0.03 | 0.21 ^d | 0.01 | 0.26 ^c | 0.01 | 0.0001 | 0.0001 | 0.0001 |
| 20:4 n-6 | 0.53 ^a | 0.01 | 0.31 ^b | 0.02 | 0.26 ^c | 0.05 | 0.31 ^b | 0.02 | 0.23 ^c | 0.04 | NS | 0.0001 | NS |
| Total n-6 fatty acids ‡ | 7.63 ^a | 0.20 | 25.42 ^b | 0.22 | 29.94 ^a | 1.70 | 11.62 ^d | 0.19 | 13.23 ^c | 0.26 | 0.0001 | 0.0001 | 0.0001 |
| 18:3 n-3 | 0.82 ^d | 0.19 | 2.34 ^d | 0.08 | 4.37 ^c | 2.17 | 17.71 ^b | 0.72 | 22.14 ^a | 1.84 | 0.0001 | 0.0001 | 0.0001 |
| 18:4 n-3 | 1.32 ^a | 0.04 | 0.67 ^c | 0.03 | 0.49 ^d | 0.09 | 0.76 ^b | 0.03 | 0.71 ^{bc} | 0.09 | 0.0001 | 0.0001 | 0.0001 |
| 20:4 n-3 | 0.56 ^a | 0.16 | 0.40 ^{bc} | 0.02 | 0.31 ^c | 0.04 | 0.47 ^{ab} | 0.02 | 0.43 ^{bc} | 0.06 | 0.0305 | 0.0001 | NS |
| 20:5 n-3 | 9.14 ^a | 0.34 | 4.11 ^b | 0.34 | 2.44 ^c | 0.54 | 3.48 ^b | 0.60 | 2.31 ^c | 0.45 | 0.0193 | 0.0001 | NS |
| 22:5 n-3 | 3.10 ^a | 0.06 | 1.79 ^b | 0.18 | 1.06 ^d | 0.26 | 1.40 ^c | 0.25 | 0.98 ^d | 0.20 | 0.0022 | 0.0001 | 0.0094 |
| 22:6 n-3 | 7.37 ^a | 0.28 | 5.09 ^b | 0.76 | 3.88 ^c | 0.95 | 4.60 ^{bc} | 0.72 | 4.00 ^c | 0.70 | NS | 0.0001 | NS |
| Total n-3 | 23.41 ^b | 0.61 | 14.84 ^c | 1.42 | 12.87 ^c | 3.47 | 29.22 ^a | 2.27 | 31.35 ^a | 3.19 | 0.0001 | NS | 0.0001 |
| n-3 PUFAS | 20.15 ^a | 0.35 | 11.44 ^b | 1.30 | 7.85 ^c | 1.84 | 10.49 ^b | 1.50 | 8.44 ^c | 1.37 | NS | 0.0001 | NS |
| AA: EPA | 0.06 ^c | 0.00 | 0.08 ^{bc} | 0.00 | 0.11 ^a | 0.02 | 0.09 ^{ab} | 0.01 | 0.10 ^a | 0.02 | NS | 0.0001 | 0.02 |
| EPA: DHA | 1.24 ^a | 0.04 | 0.82 ^b | 0.06 | 0.63 ^c | 0.04 | 0.76 ^b | 0.09 | 0.58 ^c | 0.02 | 0.005 | 0.0001 | NS |
| OA: DHA | 2.69 ^c | 0.15 | 4.71 ^b | 0.88 | 6.91 ^a | 2.17 | 5.54 ^{ab} | 1.09 | 6.25 ^{ab} | 1.40 | NS | 0.0001 | NS |
| OA: n-3 PUFAS | 0.97 ^d | 0.04 | 2.06 ^c | 0.31 | 3.37 ^a | 1.00 | 2.41 ^{bc} | 0.42 | 2.92 ^{ab} | 0.58 | 0.0001 | 0.0001 | 0.0001 |
| n-3/n-6 Fatty acids | 3.07 ^a | 0.16 | 0.58 ^c | 0.05 | 0.45 ^c | 0.14 | 2.51 ^b | 0.19 | 2.37 ^b | 0.22 | 0.0001 | 0.0001 | 0.0001 |

AA, arachidonic acid (20:4 n-6); DHA, docosahexaenoic acid (22:6 n-3); OA, oleic acid (18:1 n-9); Fs, fat source; InL, inclusion level.

a,b,c Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and p. 42.

† Includes 15:0 and 17:0.

‡ Includes 18:3 n-6, 18:4 n-6, 20:3 n-6 and 22:5 n-6.

§ PUFA include 20:3, 20:4, 20:5, 22:4, 22:5 and 22:6.

|| EPA 20:5 n-3.

Table 6. Texture variables pH, and tristimulus color characteristics values of raw fillets and skin of sea bream (*sparus auratus*) fed fed diets containing 100% fish oil (control), 40% fish oil + 60% soyabean oil (60% SO), 20% fish oil + 80% soyabean oil (80% SO), 40% fish oil + 60% linseed oil (60% LO), 20% fish oil + 80% linseed oil (80% LO)* (Mean values and standard deviations for nine fish per group)

| | Experimental diets | | | | | | | | | | Probability of contrasts | | |
|-------------------------|--------------------|------|--------------------|-----|--------------------|-----|--------------------|-----|--------------------|-----|--------------------------|--------|----------|
| | Control | | 60% SO | | 80% SO | | 60% LO | | 80% LO | | Fs | InL | Fs × InL |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | | | |
| Whole fish puncture (N) | 11.6 | 2.3 | 11.7 | 2.3 | 10.1 | 4.5 | 14.4 | 3.0 | 12.4 | 3.6 | NS | NS | NS |
| Flesh puncture (N) | 5.7 | 1.3 | 5.7 | 0.8 | 5.4 | 0.4 | 5.9 | 1.3 | 5.9 | 0.8 | NS | NS | NS |
| Flesh compression (N) | 8.9 | 3.8 | 10.8 | 2.0 | 10.4 | 3.0 | 10.0 | 2.6 | 9.7 | 3.5 | NS | NS | NS |
| PH | 6.1 | 0.1 | 6.1 | 0.1 | 6.2 | 0.1 | 6.1 | 0.1 | 6.1 | 0.1 | NS | NS | NS |
| L* (Fillet) | 45.1 | 2.5 | 45.0 | 1.4 | 45.4 | 1.3 | 44.1 | 1.4 | 44.1 | 1.3 | NS | NS | NS |
| a* (Fillet) | -0.01 ^a | 0.6 | -0.85 ^b | 0.3 | -0.72 ^b | 0.5 | -0.71 ^b | 0.4 | -0.98 ^b | 0.2 | NS | 0.0001 | NS |
| b* (Fillet) | -1.3 ^a | 1.0 | -1.8 ^{ab} | 0.6 | -2.4 ^{ab} | 0.3 | -1.8 ^{ab} | 0.6 | -2.3 ^b | 0.4 | NS | 0.003 | NS |
| L* (Skin) | 62.3 ^a | 10.1 | 60.5 ^{ab} | 7.9 | 59.1 ^{ab} | 4.3 | 58.4 ^{ab} | 7.1 | 51.9 ^b | 8.5 | NS | 0.03 | NS |
| a* (Skin) | 0.58 | 1.2 | -0.03 | 1.2 | -0.13 | 0.7 | 0.63 | 0.9 | 0.004 | 1.4 | NS | NS | NS |
| b* (Skin) | 5.3 | 2.8 | 5.8 | 4.3 | 5.0 | 4.0 | 6.2 | 3.7 | 5.0 | 2.4 | NS | NS | NS |

L*, Lightness; a*, redness; b*, yellowness; Fs, fat source; InL, inclusion level.

^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and p. 42.

Discussion

Growth in terms of body weight was good, and only the highest inclusion of vegetable oils produced a significant (about 5%) reduction in body weight in comparison with the control diet. Muscle and liver fatty acid composition was clearly affected by the fatty acid composition of the experimental diets, in accordance with previous reports on sea-bream juveniles (Montero *et al.* 2001). In comparison with fish fed the control diet, accumulation of linoleic acid in muscle and liver was up to 78 and 70% of its contents in diets 60%SO and 80%SO respectively. Linolenic acid reached up to 68 and 65% of its contents in diets 60%LO and 80%LO respectively, thus suggesting a better metabolic utilisation of this fatty acid or a higher digestion and assimilation of 18:2n-6. When FO was replaced by vegetable oils, the DHA and AA contents in the liver were reduced approximately twofold, whereas EPA contents suffered a fourfold decrease at the highest level of fish-oil substitution. The same effect was observed in muscle, thus confirming a preference in the retention of AA and DHA over EPA (Kalogeropoulos *et al.* 1992; Izquierdo, 1996; Koven *et al.* 2001). An increase in liver lipid deposition, OA content, and OA:n-3 PUFA and OA:DHA ratios in polar lipids, together with a reduction in n-3 PUFA contents in liver and muscle, have been described recently as main effects of feeding sea-bream juveniles a diet deficient in essential fatty acids (Montero *et al.* 2001). Although all experimental diets were formulated to provide essential fatty acid requirements of this species, the results of the present study corroborates all these effects. When dietary n-3 PUFA levels decreased due to the FO substitution, liver and muscle n-3 PUFA contents also decreased, regardless of the fat source used. The same effect of dietary fish-oil substitution was observed for increasing OA and OA:n-3 PUFA and OA:DHA ratios in liver and muscle. Conversely, it was also observed that the dietary fat source affected the hepatosomatic index when fish were fed a

blend of SO and FO. This effect may be directly linked with an imbalance in dietary n-3:n-6 PUFA ratio (PUFA > C₁₈) as previously described in sea-bream juveniles (Robaina *et al.* 1998). In our present experiment, a higher hepatosomatic index was observed as the substitution of FO by vegetable oils increased. Moreover, a fat source effect was also observed, in which the hepatosomatic index was greater in sea bream fed a diet containing SO than in groups fed a diet enriched in LO (with a higher content of n-3 fatty acids in tissues).

The effect of dietary fatty acid composition on mitochondrial fatty acid β -oxidation and hepatic lipogenesis is controversial. When menhaden oil was replaced by rapeseed oil in Atlantic salmon, McKenzie *et al.* (1998) found improved exercise performance associated with an increasing efficiency of aerobic ATP production by mitochondria. The authors associated this increase in β -oxidation with the high levels of oleic and linoleic acids found in muscles of fish fed the rapeseed oil. In the same way, we found a positive correlation between dietary MUFA and heart L3HOAD in Atlantic salmon fed at different levels of saturated and n-3 fatty acids (Menoyo *et al.* 2003). Recently, Turchini *et al.* (2003) found higher CPT-I and -II activities in liver of brown trout (*Salmo trutta* L.) fed FO in comparison with different animal and vegetable oils; the authors suggested essential fatty acids sparing and increased fluidity of the mitochondrial outer membrane as the possible effects operating in the higher β -oxidation. However, Torstensen *et al.* (2000) reported no differences in β -oxidation capacity when feeding Atlantic salmon on different vegetable oils. Regost *et al.* (2003b) found no differences in hepatic lipogenesis when replacing FO with SO or LO in feeds for turbot (*Psetta maxima*). However, we found a positive correlation between hepatic malic enzyme activity and dietary MUFA in Atlantic salmon fed at different levels of saturated and n-3 fatty acids (Menoyo *et al.* 2003). Selected enzyme activities were chosen to study possible changes in lipid metabolism produced by the fish-oil replacement in the experimental diets. The

Table 7. Induced lipid oxidation assessed by the concentration of malonyl dialdehyde (nmol/mg soluble protein), in filets of sea bream (*sparus auratus*) fed diets containing 100% fish oil (control), 40% fish oil + 60% soyabean oil (60% SO), 20% fish oil + 80% soyabean oil (80% SO), 40% fish oil + 60% linseed oil + 20% fish oil + 80% linseed oil (80% LO), 20% fish oil + 80% linseed oil (80% LO), incubated at 37°C for up to 120 min (t0–t120)*

| | Malondialdehyde | | | | | | | | | | | | | |
|------------------|-----------------|---------|---------------------|--------------------|--------------------|------|-------------------------|------|----------|-------|------------|-----------|------------|-----------------|
| | Fat source | | Inclusion level (%) | | | | Probability of contrast | | | | | | | |
| | Soyabean | Linseed | 0% | 60% | 80% | SEM† | Fs | InL | Fs × InL | Time | Time × InL | Time × Fs | Time × InL | Time × FS × InL |
| t ₅₀ | 5.31 | 6.11 | 6.58 ^a | 3.47 ^b | 7.08 ^a | 0.82 | NS | 0.01 | NS | 0.001 | 0.01 | 0.01 | 0.001 | NS |
| t ₆₀ | 31.19 | 33.72 | 38.50 ^a | 28.35 ^b | 30.52 ^b | 6.36 | NS | 0.01 | NS | 0.001 | 0.01 | 0.01 | 0.001 | NS |
| t ₆₀ | 38.63 | 42.87 | 47.89 ^a | 37.18 ^b | 37.18 ^b | | | | | | | | | |
| t ₁₂₀ | 45.00 | 51.44 | 58.46 ^a | 43.69 ^b | 42.52 ^b | | | | | | | | | |

Fs, fat source; InL, inclusion level.

a,b Mean values within a row? with unlike superscript letters were significantly different (P < 0.05).

* For details of diets and procedures, see Table 1 and p. 42.

† SEM of the main effects and interactions (n 5).

‡ SEM of the mean of time and interaction time × treatments (n 20).

Table 8. Specific activities of liver glucose-6-phosphate dehydrogenase (G6PD), fatty acid synthetase (FAS), red muscle carnitine palmitoyltransferase (CPT) I and L-3-hydroxyacyl-CoA dehydrogenase (L3HOAD), in sea bream (*sparus aurata*) fed diets containing 100% fish oil (control), 40% fish oil + 60% soyabean oil (60% SO), 20% fish oil + 80% soyabean oil (80% SO), 40% fish oil + 60% linseed oil (60% LO), 20% fish oil + 80% linseed oil (80% LO)*

| | Experimental diets | | | | | | | | | | | | |
|---------|---------------------|------|---------------------|------|---------------------|------|---------------------|------|---------------------|------|--------------------------|--------|----------|
| | Control | | 60% SO | | 80% SO | | 60% LO | | 80% LO | | Probability of contrasts | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Fs | InL | Fs × InL |
| G6PD† | 0.226 ^a | 0.03 | 0.211 ^{ab} | 0.02 | 0.172 ^{ab} | 0.04 | 0.209 ^{ab} | 0.03 | 0.157 ^b | 0.03 | NS | 0.0006 | NS |
| FAS‡ | 0.078 ^a | 0.00 | 0.080 ^a | 0.01 | 0.067 ^{ab} | 0.01 | 0.068 ^{ab} | 0.01 | 0.052 ^b | 0.01 | 0.01 | 0.001 | NS |
| CPT-† | 0.209 ^c | 0.01 | 0.263 ^{bc} | 0.03 | 0.358 ^a | 0.06 | 0.285 ^{ab} | 0.01 | 0.248 ^{bc} | 0.02 | 0.02 | 0.001 | 0.001 |
| L3HOAD† | 0.078 ^{bc} | 0.01 | 0.082 ^{bc} | 0.01 | 0.074 ^c | 0.01 | 0.107 ^a | 0.01 | 0.098 ^{ab} | 0.01 | 0.001 | 0.003 | 0.01 |

Fs, fat source; InL, inclusion level.

* For details of diets and procedures, see Table 1 and p. 42.

† IU/mg soluble protein.

‡ mU/mg soluble protein.

inhibitory pattern of the hepatic lipogenic enzymes G6PD and FAS by the presence of vegetable oils in the diets was followed by an activation tendency in the activities of CPT-I and L3HOAD in muscle, indicating a higher utilisation of fatty acids as energy source. Mitochondrial fatty acid β -oxidation involves a physiological response to meet energy depletion within the cell. The activity of CPT-I is tightly controlled through inhibition by elevated cellular levels of malonyl-CoA. Malonyl-CoA is the product of the reaction catalysed by acetyl-CoA carboxylase, and, as acetyl-CoA carboxylase is stimulated by insulin, an important role in hepatic fatty acid partitioning has recently been postulated for this hormone through the malonyl-CoA–long-chain acyl-CoA axis (Zammit, 1999). Thus, nutritional status is an important determinant of the rate of long-chain fatty acids disposal into β -oxidation or esterification. The significantly higher feed intake found in fish fed the 80%SO diet suggests the need for the fish to eat more to maintain growth; this strategy has been reported in carnivorous fish fed a high starch load in response to an impaired energy usage from the diet (Hemre *et al.* 2002). In the present study, a higher feed intake, together with a more active β -oxidation in fish fed diets containing the higher substitution level of vegetable oil, suggest a negative effect on cell energy availability. This can be related to an unbalanced n -6: n -3 fatty acid ratio in fish tissues. It has been suggested that in mammals CPT-I activity and cell glucose uptake modulation is associated with alterations in membrane lipids (Power & Newsholme, 1997; Clarke, 2000).

Lipid peroxidation is of great importance in terms of fish-flesh quality, because of its negative impact on the flavour, colour and nutritional characteristics (Monahan, 2000). The greatest peroxidation levels are closely related to high PUFA concentrations in tissues (López-Bote *et al.* 2001; Menoyo *et al.* 2002). In the present study, lipid peroxidation products as malondialdehyde in flesh decreased significantly when FO was partially replaced by LO, and even more when the added fat was SO. However, there were no differences when adding vegetable oils at inclusion levels of 60–80%.

Concerning the instrumental texture analysis, no effect of vegetable or FO on firmness of raw fillets was observed, in accordance with the results of Regost *et al.* (2003a) on turbot and Rørå *et al.* (2003) in Atlantic salmon. However, Andersen *et al.* (1997) found a softer fillet when rainbow trout were fed high-lipid diets for 21 weeks, showing a significant negative correlation between force of compression and fat content in muscle. The whole fish puncture value indicates the force needed to penetrate the muscle and is therefore used as a measure of the muscle hardness. According to Andersen *et al.* (1997), a negative correlation was found between puncture values and total intramuscular lipids ($R^2 = 0.36$; $P < 0.02$). Both dietary fat content and dietary fatty acid composition had a significant effect on the flesh colour of Atlantic salmon (Bjerkeng *et al.* 1997, 1999). These authors found a significant positive relationship between the final redness and the total n -3 fatty acids content of the fillet, whereas a negative relationship was found between the redness and the total MUFA content in Atlantic salmon fed different FO. In the present

study, progressive inclusion of vegetable oils, both SO and LO, brought about a decrease in the redness and yellowness of fish flesh. The increased inclusion of both vegetable oils reduced the level of n -3 PUFA by nearly 50% or more in the fillet (Table 5). This is a similar fatty acid response for both oils and may have an effect on fillet colour.

In conclusion, dietary FO can be replaced by LO or SO at a high level of substitution (60%) without compromising marketable-size sea-bream growth. However, lipid metabolism is clearly affected, especially when feeding SO, associated with an imbalance in tissue n -3: n -6 fatty acid ratio, suggesting energy depletion and leading to a mobilization of fatty acids for β -oxidation. It is our opinion that future studies should be carried out to elucidate the effect of dietary n -3: n -6 fatty acids ratio on energy metabolism (lipid *v.* carbohydrate utilisation) in fish.

Acknowledgements

Thanks are due to Dr Grethe Rosenlund (NUTRECO) for diet formulation and Mrs S. Pérez-Benavente and Mr M. Marin for their efficient technical assistance. The research was financed by European Commission Projects QLRT-1999-30058 and QLRT-1999-30360 and FEDER funds 1FD97-1774.

References

- Alexis M (1997) Fish meal and fish oil replaces in Mediterranean marine fish diets. *Cah Options Méditerran* **22**, 183–204.
- Alvarez MJ, López-Bote CJ, Diez A, Corraze G, Arzel J, Dias J, Kaushik SJ & Bautista JM (1998) Dietary fish oil and digestible protein modify susceptibility to lipid peroxidation in the muscle of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*). *Br J Nutr* **80**, 281–289.
- Andersen UB, Thomassen MS & Rørå AMB (1997) Texture properties of farmed rainbow trout (*Oncorhynchus mykiss*): effects of diet, muscle fat content and time of storage on ice. *J Sci Food Agric* **74**, 347–353.
- Bell JG, Henderson RJ, Tocher DR, McGhee F, Dick JR, Porter A, Smullen RP & Sargent JR (2001) Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid composition and hepatocyte fatty acid metabolism. *J Nutr* **131**, 1535–1543.
- Bell JG, Henderson RJ, Tocher DR, McGhee F, Dick JR, Porter A, Smullen RP & Sargent JR (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.
- Bjerkeng B, Refstie S, Fjalestad KT, Storebakken T, Rodbotten M & Roem AJ (1997) Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in diet. *Aquaculture* **157**, 297–309.
- Bjerkeng B, Hatlen B & Wathne E (1999) Deposition of astaxanthin in fillets of Atlantic salmon (*Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils. *Aquaculture* **180**, 307–319.
- Bonamusa L, García de Frutos P, Fernández F & Baanante IV (1992) Features of the lipid transport system of fish as demonstrated by studies on starvation in rainbow trout. *J Comp Physiol B* **156**, 497–502.
- Borderías AJ, Lamua M & Tejada M (1983) Texture analysis of

- fish fillets and minced fish by both sensory and instrumental methods. *J Food Technol* **18**, 85–95.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.
- Bradshaw RA & Noyes BE (1975) L-3-Hydroxyacyl coenzyme A dehydrogenase from pig heart muscle. In *Methods in Enzymology*, vol. 35, pp. 122–128 [JM Lowenstein, editor]. New York: Academic Press.
- Caballero MJ, Izquierdo MS, Kjørsvik E (2004) Histological alterations in liver of sea bream (*Sparus aurata*) caused by feeding for a short- or long-term with vegetable oils. Recovery of normal morphology after feeding fish oil as a solely lipid source. *J Fish Dis* (In the Press).
- Caballero MJ, Obach A, Rosenlund G, Montero D, Gisvold M & Izquierdo MS (2002) Impact of different dietary sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **253**, 253–271.
- Christie WW (1982) *Lipid Analysis*. Oxford: Pergamon Press.
- Commission Internationale de l'Eclairage (1976) *Colorimetry. Commission Internationale de l'Eclairage Publication no. 15*. Vienna: Bureau Central de la CIE.
- Clarke SD (2000) Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. *Br J Nutr* **83**, Suppl. 1, S59–S66.
- Corraze G, Larroquet L & Medale F (1999) Alimentation et dépôts lipidiques chez la truite arc-en-ciel, effet de la température délevage (Nutrition control of lipid deposition in rainbow trout: effect of rearing temperature). *Prod Anim* **12**, 249–256.
- Dias J, Alvarez MJ, Diez A, Arzel J, Corraze G, Bautista JM & Kaushik SJ (1998) Regulation of hepatic lipogenesis by dietary protein/energy in juvenile European seabass (*Dicentrarchus labrax*). *Aquaculture* **161**, 169–186.
- Food and Agriculture Organization (2002) *The State of World Fisheries and Aquaculture*. Rome: FAO.
- Folch J, Lees M & Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.
- Fox C (1990) *Studies on polyunsaturated fatty acid nutrition in the larvae of a marine fish – the herring (Clupea harengus L)*, PhD Thesis, Stirling University.
- Frøyland L, Lie Ø & Berge RK (2000) Mitochondrial and peroxisomal β -oxidation capacities in various tissues from Atlantic salmon *Salmo salar*. *Aquacult Nutr* **6**, 85–89.
- Frøyland L, Madsen L, Eckhoff KM, Lie Ø & Berge RK (1998) Carnitine palmitoyltransferase I, carnitine palmitoyltransferase II, and acyl-CoA oxidase activities in Atlantic salmon (*Salmo salar*). *Lipids* **33**, 923–930.
- Harper RD & Saggerson ED (1975) Some aspects of fatty acid oxidation in isolated fat-cell mitochondria from rat. *Biochem J* **152**, 485–494.
- Hemre GI, Mommsen TP & Kroghdahl Å (2002) Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquacult Nutr* **8**, 175–194.
- Ibeas C, Izquierdo MS & Lorenzo A (1994) Effect of different levels of *n*-3 highly unsaturated fatty acids on growth and fatty acid composition of juvenile gilthead seabream (*Sparus aurata*). *Aquaculture* **127**, 177–188.
- Izquierdo MS (1996) Essential fatty acid requirements of marine fish larvae. *Aquacult Nutr* **2**, 183–191.
- Izquierdo MS, Socorro J, Arantzamendi L & Hernández-Cruz CM (2000) Recent advances in lipid nutrition in fish larvae. *Fish Physiol Biochem* **22**, 97–107.
- Izquierdo MS, Watanabe T, Takeuchi T, Arawaka T & Kitajima C (1989) Requirement of larval red sea bream *Pagrus major* for essential fatty acids. *Bull Japanese Soc Sci Fish* **55**, 859–867.
- Kalogeropoulos N, Alexis MN & Henderson RJ (1992) Effects of dietary soybean and cod-liver oil levels on growth and body composition of gilthead bream (*Sparus aurata*). *Aquaculture* **104**, 293–308.
- Kornbrust DJ & Mavis RD (1980) Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correlation with vitamin E content. *Lipids* **15**, 315–322.
- Koven W, Barr Y, Lutzky S, Ben-Atia I, Weiss R, Harel M, Behrens P & Tandler A (2001) The effect of dietary arachidonic acid (20: 4*n*-6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. *Aquaculture* **193**, 107–122.
- Likimani TA & Wilson R (1982) Effects of diet on lipogenic enzyme activities in channel catfish hepatic and adipose tissue. *J Nutr* **112**, 112–117.
- López-Bote CJ, Diez A, Corraze G, Arzel J, Alvarez M, Dias J, Kaushik SJ & Bautista JM (2001) Dietary protein source affects the susceptibility to lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*) muscle. *Anim Sci* **73**, 433–449.
- Mckenzie DJ, Higgs DA, Dosanjh B, Deacon G & Randall DJ (1998) Dietary lipid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. *Fish Physiol Biochem* **19**, 111–122.
- Menoyo D, López-Bote CJ, Bautista JM & Obach A (2002) Herring vs. anchovy fish oils in salmon feeding. *Aquat Living Resour* **15**, 217–223.
- Menoyo D, López-Bote CJ, Bautista JM & Obach A (2003) Growth, digestibility and fatty acid utilization in large Atlantic salmon (*Salmo salar*) fed varying levels of *n*-3 and saturated fatty acids. *Aquaculture* **225**, 295–307.
- Monahan FJ (2000) Oxidation of lipids in muscle foods: fundamentals and applied concerns. In *Antioxidants in Muscle Foods: Nutritional Strategies to Improve Quality*, pp. 3–24 [EA Decker, C Faustman and CJ Lopez-Bote, editors]. New York: Wiley Interscience.
- Montero D, Kalinowski T, Obach A, Robaina L, Tort L, Caballero MJ & Izquierdo MS (2004) Vegetable lipid sources for gilthead seabream (*Sparus aurata*): Effects on fish health. *Aquaculture* (In the Press).
- Montero D, Robaina LE, Socorro J, Vergara JM, Tort L & Izquierdo MS (2001) Alteration of liver and muscle fatty acid composition in gilthead seabream (*Sparus aurata*) juveniles held a high stocking density and fed an essential fatty acid deficient diet. *Fish Physiol Biochem* **24**, 63–72.
- Morris TR (1999) *Experimental Design and Analysis in Animal Sciences*. Oxford: CAB International.
- Nanton DA, Lall SP, Ross NW & McNiven MA (2003) Effect of dietary lipid level on fatty acid β -oxidation and lipid composition in various tissues of haddock, *Melanogrammus aeglefinus* L. *Comp Biochem Physiol B* **135**, 95–108.
- National Research Council (1993) *Nutrient Requirements of Fish*. Washington, DC: National Academy Press.
- Power GW & Newsholme EA (1997) Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. *J Nutr* **127**, 2142–2150.
- Regost C, Arzel J, Cardinal M, Rosenlund G & Kaushik SJ (2003a) Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*) 2. Flesh quality properties. *Aquaculture* **220**, 737–747.
- Regost C, Arzel J, Robin J, Rosenlund G & Kaushik SJ (2003b) Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*) 1. Growth performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture* **217**, 465–482.

- Robaina L, Izquierdo MS, Moyano FJ, Socorro J, Vergara JM & Montero D (1998) Increase of the dietary *n-3/n-6* fatty acid ratio and addition of phosphorous improves liver histological alterations induced by feeding diets containing soybean meal to gilthead seabream, *Sparus aurata*. *Aquaculture* **161**, 281–293.
- Rørå AMB, Regost C & Lampe J (2003) Liquid holding capacity, texture and fatty acid profile of smoked fillets of Atlantic salmon fed diets containing fish oil or soybean oil. *Food Res Int* **36**, 231–239.
- Rosenlund G, Obach A, Sandberg MG, Standal H & Tveit K (2001) Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar*). *Aquat Res* **32**, 323–328.
- Sanz M, Lopez-Bote C, Menoyo D & Bautista JM (2000) Abdominal fat deposition and fatty acid synthesis are lower and β -oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. *J Nutr* **130**, 3034–3037.
- Shimeno S, Ming DC & Takeda M (1993) Metabolic response to dietary carbohydrate to lipid ratios in *Oreochromis niloticus*. *Nippon Suisan Gakkai Shi* **59**, 827–833.
- Torstensen BE, Lie Ø & Frøyland 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.
- Turchini GM, Mentasti T, Frøyland L, Orban E, Caprino F, Moretti VM & Valfré F (2003) Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). *Aquaculture* **225**, 251–267.
- Watanabe T, Kitajima C & Fujita S (1983) Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture* **34**, 115–143.
- Zammit VA (1999) The malonyl-CoA-long chain acyl-CoA axis in the maintenance of mammalian cell function. *Biochem J* **343**, 505–515.