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Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) juveniles

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Summary

A 12-week feeding trial was conducted to evaluate the effects of fish oil replacement by soybean oil, on lipid distribution and liver histology of two commercially important finfish species: rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*). Sea bass (16.2 ± 0.5 g; mean ± SD) and rainbow trout (52.1 ± 0.5 g) juveniles were fed one of three isonitrogenous (500 g kg^{-1} CP) and isoenergetic (19 kJ g^{-1}) diets, containing 0% (control, diet A), 25% (diet B) and 50% (diet C) soybean oil. At the end of the experiment, lipid deposition was evaluated in muscle, liver and viscera. Cholesterol and triglycerides levels were also determined in plasma. Tissue total, neutral and polar lipid composition (g kg^{-1} total lipids) showed no significant differences within species, regardless the dietary treatment. The same trend was observed for plasma parameters ($P > 0.05$). Viscera were the preferential tissue of lipid deposition, with 252–276 and 469–513 g kg^{-1} total lipid content in trout and sea bass, respectively. Dietary fish oil replacement had no effect on either hepatic lipid droplets accumulation or degree and pattern of vacuolization in the observed liver sections. These data suggest that both sea bass and trout can be fed diets containing up to 50% soybean oil without adverse effects on tissue lipid composition or liver histology.

KEY WORDS: fish oil replacement, lipid classes, lipid distribution, liver histology, plasma–lipid composition, thiobarbituric acid reactive substances concentrations

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Introduction

The annual global supply of fish oils is relatively constant (usually varies between 1.1 and 1.4 million tonnes and may decline to 800 000 tonnes or less in El Ninõ years), whereas the demand for marine fish oil (MFO) in aquafeeds is continually increasing and may exceed 75% of the global supply by the year 2010 (Barlow 2000). In order to sustain the rapid growth of the global aquaculture industry, it is becoming increasingly crucial for the aquafeed industry to evaluate alternatives to fish oils (FAO 1997). Vegetable oils are seen as potential lipid source, as they are virtually free of dioxins and other organic pollutants and can maintain the demand for fish oil at sustainable levels.

The major condition in replacing MFO in aquafeed by alternative lipid sources is to economically supply an optimum level of energy associated with a well-balanced level of essential fatty acids. In such a case, high growth rate, feed efficiency, immune function, disease resistance, survival of fish and flesh quality are likely to be ensured. However, the lack of a well-balanced fatty acid profile (Sargent *et al.* 2002), a lower palatability (Thomassen & Rosjo 1989; Guillou *et al.*

1995; Regost *et al.* 2003) and digestibility, (Caballero *et al.* 2002; Gunasekara *et al.* 2002) and the eventual presence of some anti-nutritional factors may limit the success of vegetable oils as a total animal lipid replacement in fish diets.

Soybean oil is the world's largest source of vegetable oil and contains higher levels of poly-unsaturated fatty acids than others, such as rapeseed oil or palm oil, but lacks eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with linoleic acid ($18:2n-6$) dominating at approximately 51–64% (NRC 1993). It is a rich source of vitamin E (a natural antioxidant) and a cholesterol-free oil. A number of studies have shown that soybean oil can partially replace the fish oils (Hardy *et al.* 1987; Thomassen & Rosjo 1989; Greene & Selivonchick 1990; Guillou *et al.* 1995; Izquierdo *et al.* 2003), without compromising growth and feed efficiency. Nevertheless, besides growth parameters, it is increasingly important to evaluate the effects of fish oil substitution on fat deposition and liver histopathology. The quality, location, distribution, quantitative importance and composition of fats are aspects that strongly influence the nutritional value, organoleptic properties, transformation yield and shelf life of fishery products (Kaushik 1997).

The relative importance of the lipid deposition sites varies markedly among fish species, and include liver, perivisceral adipose tissue or the muscle, which is the only truly edible part (Ackman 1995). Changes in tissue lipids are not reflected in the same way for the different classes of lipids: phospholipids concentration remains almost constant whereas neutral lipids are strongly influenced by dietary fatty acid composition (Corraze 2001). Moreover, in mammals, the use of diets with vegetable oils has affected the cholesterol levels in plasma (Baba *et al.* 2000). In fish, high levels of plant oil as a lipid source have also been previously associated to degenerations in tissue histological structure (Alexis 1997), resulting in an accumulation of large lipid vacuoles in the enterocytes and hepatocytes (Tucker *et al.* 1997; Olsen *et al.* 1999, 2000; Caballero *et al.* 2002, 2004). Histological studies concerning the effects of fish oils substitution are scarce but can provide information on diet quality and metabolism, and can also be used as an indicator of the nutritional status of a fish (Segner & Braunbeck 1988; Caballero *et al.* 2004).

Lipid peroxidation is a serious problem for biological materials containing unsaturated fatty acids. Aquatic animals are characterised by a greater richness in highly unsaturated fatty acids (HUFA), and in particular the ones of the $n-3$ series (Corraze 2001), suffering higher risks of peroxidative attack than others species do (Mourente *et al.* 2002). The use of vegetable oils is proved to reduce lipid peroxidation in mammal's muscle (Lopez-Bote *et al.* 1997) and also in some fish species (Stéphan *et al.* 1995; Alvarez *et al.* 1998), and thereby might enhance the sensorial attributes of the flesh for human consumption.

The aim of the present study was to evaluate the effects of replacing 25 or 50% of the fish oil by soybean oil, during 12 weeks, on tissue fat deposition and general liver histopathology, of two commercially important finfish species: European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) juveniles. The effect of the partial inclusion of dietary soybean oil on plasma triglycerides and cholesterol was also analysed. Because of the high unsaturation of fish muscle, more susceptible to spontaneous oxidation, complementary peroxidation analyses of thiobarbituric acid reactive substances concentrations (TBARS) in muscle samples were also carried out.

Materials and methods

Experimental animals and trial conditions

The feeding trials were conducted at the Fish Culture Experimental Unit of University of Trás-os-Montes e Alto Douro (UTAD), Portugal, from November 2001 to March 2002. Juveniles of European sea bass (*Dicentrarchus labrax*) were obtained at 'Rio Alto' aquaculture station, Póvoa de Varzim, whereas rainbow trout (*Oncorhynchus mykiss*) juveniles were produced in UTAD experimental facilities. The animals were acclimatized to the rearing conditions for 1-week period prior to the feeding trial. During this period, fish were fed the control diet.

Homogeneous groups of 20 sea bass with an average initial body weight of 16.2 ± 0.5 g; (Mean ± SD) were randomly distributed among nine indoor 50 l fibreglass tanks, in a recirculation water system. Each tank was supplied with filtered, heated (20 ± 1 °C), saltwater (33‰), at a water flow of 2 l min^{-1}. Homogeneous groups of 20 rainbow trout with an average initial body weight of 52.1 ± 0.5 g were also randomly distributed into nine 200 l fibreglass tanks, in an open flow-through system, 6 l min^{-1}, at 14 ± 2 °C. The pH, ammonia, nitrites, nitrates and phosphates in the water were monitored during the entire trial and maintained at levels compatible with each species. The sea bass were exposed to an artificial photoperiod of 12-h light, while the trouts were exposed to natural photoperiod.

Feed and feeding

A commercial diet for marine fish, without fish oil, was supplied by Sorgal, and used as a basal diet formulation for the

Table 1 Ingredients and chemical composition of the three experimental diets: Diet A, B and C with 0, 25 and 50% vegetable (soybean) oil, respectively

	Diet		
	A	B	C
Ingredients (g kg^{-1})			
Norwegian fish meal LT 90	700	700	700
Extruded pea seed meal[1]	55	55	55
CPSP Special G70[2]	50	50	50
Wheat meal	50	50	50
Capelin oil	120	60	20
Soybean oil	0	60	100
Vitamin premix[3]	10	10	10
Choline chloride (50%)	5	5	5
Mineral premix[4]	10	10	10
Chemical composition			
Moisture (g kg^{-1})	77	74	73
Crude protein (g kg^{-1} DM)	508	513	508
Lipid (g kg^{-1} DM)	161	161	161
n-6	44	174	165
n-3	192	188	189
EPA + DHA	182	165	167
DHA/EPA	39	20	21
Ash (g kg^{-1} DM)	98	97	97
Gross energy (kJ g^{-1} DM)	190	192	192

[1] Aquatex (20.5 CP; Sotexpro, France).
[2] Soluble fish protein hydrolisate (75.26 CP; Sopropêche, France).
[3] Vitamins (mg or UI/kg diet): Vitamin A, 8000 UI; vitamin D3, 2000 UI; vitamin E, 100 mg; vitamin K, 10 mg; vitamin B12, 0.02 mg; vitamin B1, 15 mg; vitamin B2, 25 mg; vitamin B6, 15 mg; folic acid, 10 mg; biotin, 1 mg; vitamin C, 100 mg; betaine, 500 mg; inositol, 300 mg; nicotinic acid, 100 mg; pantothenic acid, 50 mg; choline chloride, 1000 mg.
[4] Minerals (g or mg/kg diet): Mn (manganese sulphate), 20 mg; I (potassium iodide), 0.6 mg; Cu (copper sulphate), 5 mg; Co (cobalt sulphate), 0.4 mg; Mg (magnesium sulphate), 500 mg; Zn (Bioplex, (Alltech)), 30 mg; Se (Sel-Plex 2000, (Alltech)), 0.3 mg; Fe (iron sulphate), 40 mg; Ca (calcium carbonate), 2.15 g; dibasic calcium phosphate, 5 g; KCl, 1 g; NaCl, 0.4 g.

preparation of the three experimental diets. Feed ingredients and diet composition are summarized in Table 1. Fish oil and soybean oil were then added at the feed laboratory of UTAD using a helicoidal mixer. The final lipid fractions of the diets varied from the sole inclusion of fish oil in diet A (0% soybean oil), to the partial substitution of fish oil by soybean oil at a level of 25% (diet B) and 50% (diet C). Crude fat was kept at a constant level of 161 g kg^{-1} in all diets. The dietary *n*−3 HUFA's (highly unsaturated fatty acid) profile, in particularly EPA (20 : 5*n*−3) and DHA (22 : 6*n*−3), were determined (Table 1) and found to be adequate for optimum growth for both fish species, largely suppressing the species recommendations (NRC 1993). Every experimental diet was randomly assigned to three different tanks. Fish were handfed twice a day to apparent satiety during 12 weeks.

Sampling procedure

At the end of the growth trial data on weight gain were collected from all fish. Prior to sampling, fish were fasted for 24 h and then anaesthetized by immersion in an ethylene glycol monophenyl ether (1 : 2500) bath. Two fish per tank (six fish per treatment) were then withdrawn and blood samples were rapidly taken from the caudal vessel of each fish using heparinised syringes. After centrifugation (1200 × ***g***, 10 min, 5 °C), plasma was separated and frozen at −20 °C for triglycerides and cholesterol determinations. The liver, viscera and a sample of muscle was also removed from the region underneath the dorsal fin, weighed and stored at −20 °C, for fat deposition and TBARS further analyses. For histological analysis, three fish were sampled per tank. Immediately after killing the fish, each liver was quickly removed and sliced into pieces, part of which were subsequently immersed in 10% formol saline and the other part in 10% formol calcium, for 24 h, and routinely processed for embedding in paraffin and for producing frozen sections, respectively.

Analytical methods

Total lipids in liver, viscera and muscle were determined gravimetrically according to Folch *et al*. (1957), with chloroform being replaced by dichloromethane, less toxic than the former. The separation of neutral lipids and phospholipids was performed according to the procedure described by Juaneda & Rocquelin (1985). The total lipids extracts were fractionated on silica cartridges (Sep-Pack, Waters); neutral lipids were eluted by dichloromethane and phospholipids by methanol. Total plasma triacylglycerols and cholesterol concentrations were determined spectrophotometrically using enzymatic kits (Bio-Mérieux reference no. 61218 for cholesterol and Bio-Mérieux reference no. 61236 for triglycerides). Muscle TBARS were measured according to Vyncke (1970).

The formol saline sections were either stained with Haematoxylin and Eosin (H&E) or with Periodic Acid Schiff (PAS). The formol calcium sections were attained with Sudan Black (SB). For each fish, sections were examined for general liver histopathology, cytoplasm vacuolation degree (H&E), glycogen content (PAS), and, finally, lipid content (SB) of the hepatocytes. The SB sections were used to confirm the nature of the hepatocellular vacuolation seen in H&E, and also to help locating any eventual signs of focal steatosis. Several blocks and sections were used for each purpose, and observations were consistently made using a combination of

low magnification (general aspects) and higher resolving power (namely for grading, for which at least 50 randomly sampled fields were analysed per section). Evaluation of the hepatocellular cytoplasm vacuolation degree was made using a semi-quantitative approach, according to the following five grades and criteria: Grade 0 (none) – absence of hepatocellular vacuolation; Grade 1 (low) – on average, <1/3 of the hepatocyte cytoplasm shows vacuolation; Grade 2 (moderate) – on average, $1/3 < \times < 2/3$ of the hepatocyte cytoplasm shows vacuolation; Grade 3 (high) – on average, >2/3 of the hepatocyte cytoplasm shows vacuolation; Grade 4 (extreme) – 3/3 cytoplasm (virtually total) vacuolation.

Assessment of hepatocellular glycogen content also followed a semi-quantitative approach, but based solely in two degrees: 0 (none-low) – when hepatocytes in PAS sections did not present coloration or present a very weakly coloration; 1 (mild) – PAS sections presented a fairly weak coloration. No further degrees were considered because, in practical terms, no overall moderate or strong PAS staining existed.

Statistical analysis

Statistical analyses followed methods outlined by Zar (1996). Data were analyzed by a one-way ANOVA with the STATISTICS 5.0 for Windows package. When *F*-values showed significance, individual means were compared using Tukey's honest significant difference (HSD). The comparison of histological degrees was performed using the Kruskall–Wallis and Mann–Whitney tests. Significant differences were considered when $P < 0.05$.

Results

Growth performance

The inclusion of soybean oil in the diets had no significant effect on final weight or daily growth index (DGI) of any of the studied species ($P > 0.05$), though sea bass final weight and DGI were slightly lower in fish fed the diet with the highest level of soybean oil (Table 2). Similarly, the HSI in sea bass (2.0 ± 0.4) and trout (1.2 ± 0.2) was also not affected by the dietary treatments.

Total, neutral and polar lipids

The total lipid deposition in sea bass and rainbow trout liver, viscera and muscle, is presented in Table 3. Tissue total lipid content within species showed no significant differences between groups ($P > 0.05$), regardless the dietary soybean inclusion level. Sea bass clearly showed a higher lipid deposition in liver and viscera ($P > 0.05$), when compared with rainbow trout, but similar muscle lipid content.

Table 2 Growth performance and hepatosomatic index of sea bass and rainbow trout, fed for 12 weeks with diets containing different incorporations levels of soybean oil, diet A (0%), diet B (25%) or diet C (50%)

Diet	A	B	C
Sea bass			
Initial body weight (g)	16.1 ± 0.6	16.3 ± 0.8	16.3 ± 0.3
Final body weight (g)	37.6 ± 3.2	38.1 ± 4.5	35.5 ± 1.0
Daily growth index (DGI)	1.0 ± 0.0	1.0 ± 0.1	0.9 ± 0.0
HSI	2.0 ± 0.3	1.9 ± 0.3	2.2 ± 0.5
Rainbow trout			
Initial body weight (g)	51.9 ± 0.6	51.9 ± 0.2	52.7 ± 0.3
Final body weight (g)	171.0 ± 14.1	176.4 ± 3.2	171.0 ± 9.0
Daily growth index (DGI)	2.2 ± 0.1	2.2 ± 0.0	2.1 ± 0.1
HSI	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.2

The values are the mean ± SD, $n = 3$.
DGI, Daily growth index: $100 \times [(\text{final body weight})^{1/3} - (\text{initial body weight})^{1/3}] \times \text{days}^{-1}$.
Hepatosomatic index (HSI): $100 \times \text{liver weight} \times \text{body weight}^{-1}$.
Absence of superscripts letters indicates no significant differences between treatments ($P > 0.05$).

There was no evidence that fish oil substitution by soybean oil affected neutral or polar lipid composition in any of the studied species, even at the highest inclusion levels (Table 3). Neutral and polar lipid fraction of liver, muscle and viscera (g kg^{-1} total lipids) did not differ significantly ($P > 0.05$) between groups fed different diets. The tissue neutral lipid fraction was always higher than the polar one, in both species, except in trout liver, where equivalent levels were found.

Lipid peroxidation in muscle – TBARS

Dietary soybean oil incorporation had no significant effect on TBARS values in sea bass and rainbow trout muscle (Table 3). TBARS values were highest in fish fed on diet B (25% soybean oil).

Cholesterol and triglycerides

Sea bass and rainbow trout plasma cholesterol and triglycerides concentrations are presented in Fig. 1a,b. Plasma cholesterol levels were not significantly different among diets A, B or C ($P > 0.05$), and the highest values were always observed in trout. Similarly, no dietary effect ($P > 0.05$) was detected in the triglycerides plasma concentrations in either species, and inversely to cholesterol, sea bass reported the highest concentrations.

Table 3 Composition of liver, viscera and muscle total (TL, g kg^{-1} wet weight), neutral (NL) and polar lipids (PL) (g kg^{-1} total lipids), and thiobarbituric acid reactive substances concentrations (μmol kg^{-1}) in the muscle of sea bass and rainbow trout fed diet A, B or C (fish oil substitution by soybean oil at 0, 25 and 50%, respectively)

Diet	A	B	C
Sea bass			
Liver			
TL	21.2 ± 8.5	21.2 ± 7.1	25.4 ± 7.4
NL	88.7 ± 3.2	87.2 ± 7.6	83.8 ± 3.4
PL	11.3 ± 3.2	12.8 ± 7.6	16.2 ± 3.4
Viscera			
TL	46.9 ± 14.3	49.9 ± 15.4	51.3 ± 20.9
NL	94.0 ± 1.9	95.8 ± 2.7	92.0 ± 6.0
PL	6.0 ± 1.9	42 ± 2.7	8.0 ± 6.0
Muscle			
TL	4.5 ± 1.3	4.0 ± 1.0	5.2 ± 2.3
NL	86.0 ± 1.4	82.0 ± 4.9	86.9 ± 5.3
PL	14.0 ± 1.4	18.0 ± 4.9	13.2 ± 5.3
TBARS	31.1 ± 11.7	35.6 ± 9.7	34.5 ± 11.6
Rainbow trout			
Liver			
TL	6.9 ± 2.1	7.4 ± 3.0	4.9 ± 0.3
NL	56.2 ± 12.3	53.5 ± 17.2	47.6 ± 12.6
PL	43.8 ± 12.3	46.6 ± 17.2	52.4 ± 12.6
Viscera			
TL	25.6 ± 2.4	25.2 ± 5.6	27.6 ± 7.7
NL	91.0 ± 1.4	92.2 ± 1.8	91.1 ± 0.6
PL	9.0 ± 1.4	7.8 ± 1.8	8.9 ± 0.5
Muscle			
TL	5.6 ± 1.6	4.4 ± 0.8	4.6 ± 1.4
NL	84.9 ± 4.6	84.0 ± 5.3	82.6 ± 6.6
PL	15.1 ± 4.6	16.0 ± 5.3	17.4 ± 6.6
TBARS	26.3 ± 9.7	26.8 ± 5.6	25.7 ± 3.0

The values are means ± SD, $n = 6$.
Absence of superscripts letters indicates no significant differences between treatments ($P > 0.05$).

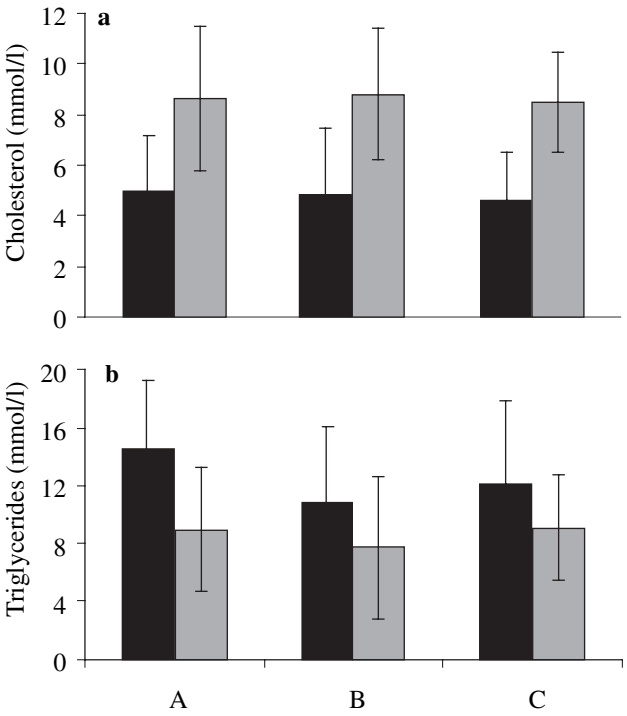

Figure 1 Plasma cholesterol (a) and triglycerides (b) concentrations (mmol l^{-1}) in sea bass (■) and rainbow trout (▒) fed on diet A, B and C (0, 25 and 50% soybean oil, respectively). The values are means ± SD, $n = 6$. No dietary effect was observed ($P > 0.05$).

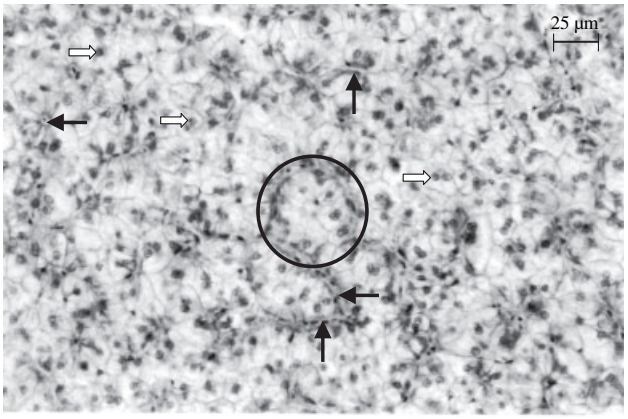

Figure 2 Sea bass liver fed diet C (50% soybean oil) (H&E, 400×). The numerous displayed hepatocytes have the cytoplasm virtually unstained, expect for the basophilic nucleus (white arrows), perinuclear areas, and also for the cytoplasm rim. The vacuolated somewhat 'empty' cytoplasm as seen in H&E is because of the high cell content in lipids (see Fig. 4). Sinusoids (black arrows) appear squeezed amidst the hepatocyte tubules (circle).

Liver histopathology

The microscopy analysis of sea bass livers evidenced a normal histological pattern (Figs 2, 4 and 6), despite the marked hapatocellular vacuolation (median = 3), corresponding to a naturally high lipid content, as shown by SB stain. Moreover, no significant differences were detected among treatments (Table 4). There were no signs of degenerative microvesicular fatty change. In general, the hepatocytes displayed little or no glycogen content, with the highest median score being found in diet C (median = 1).

In trout, microscopy also revealed a normal hepatic histological scenario (Figs 3, 5 and 7). Contrary to the sea bass, as can be observed when comparing Figs 4 and 5, and indirectly derived from the low vacuolization degree (Table 4), the trout hepatocytes showed a relatively poor lipid content, being dispersed in the cytoplasm as small droplets. No significant differences ($P > 0.05$) in the hepatocellular vacuolization degree were observed among dietary treatments. The

Diet	A			B			C		
	Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.
Sea bass									
Vacuolization degree	3	4	3	3	4	3	3	4	3
Glycogen content	0.5	1	0	1	1	0	1	1	0
Rainbow trout									
Vacuolization degree	2	3	1	2	2	1	1	2	0
Glycogen content	1	1	0	0	1	0	0.5	1	0

Table 4 Vacuolization and glycogen content in sea bass and rainbow trout, when fed on diet A, B and C (fish oil substitution by soybean oil at 0, 25 or 50%, respectively)

The values are expressed as median, maximum (Max.) and minimum (Min.); $n = 6$.
Absence of superscripts letters indicates no significant differences between treatments ($P > 0.05$).

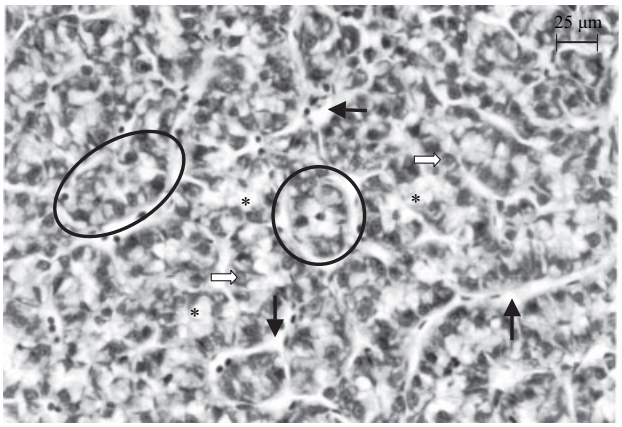

Figure 3 Rainbow trout liver fed diet C (50% soybean oil) (H&E, 400×). The contrast with the previous figure is clear, as the hepatocytes display a much lower vacuolation degree – despite the evident vacuolated areas (asterisks). As the hepatocytes are not so engorged with lipids, as seen in Fig. 1, the sinusoids are more readily seen (black arrow), interspersed within the hepatocytic tubular architecture (circle and oval).

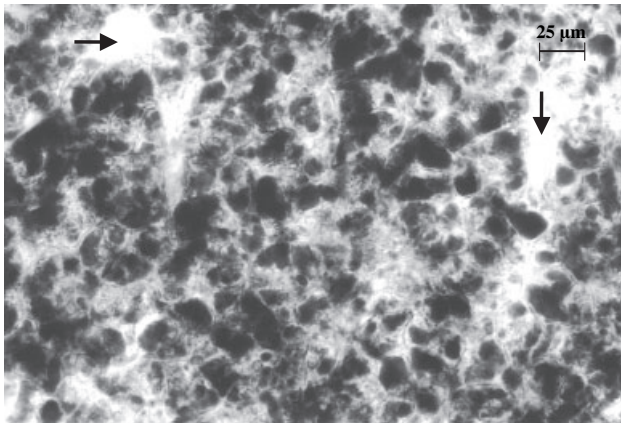

Figure 4 Sea bass liver fed diet C (50% soybean oil) (Frozen section – SB, 400×). The image clearly depicts the high lipid content of hepatocytes. The lipids appear as black stained clumps inside the cells, the limits of which are naturally more difficult to discern when comparing with the observations made in paraffin (see Fig. 1). In this image, a few sinusoidal lumina can be discerned as empty holes (black arrows).

hepatocytes were typically basophilic, showing accordingly little or even no detectable glycogen content, with the highest median being found in diet A, though not significantly different ($P > 0.05$) from the other diets.

Discussion

The results obtained in this experiment for final body weight and daily growth index are in general accordance with earlier observations, when soybean oil was used as partial substitute of fish oil in aquafeeds for sea bass (Alexis 1997; Yildiz & Sener 1997; Izquierdo *et al.* 2003), and for rainbow trout (Greene & Selivonchick 1990; Caballero *et al.* 2002).

There was no evidence that fish oil substitution by soybean oil affected total lipid content in the liver, muscle and viscera, of trout and sea bass, even at the highest inclusion levels, being values within the same range of those observed in these two species for a 180 g kg^{-1} dietary lipid level (Corraze 1997). Viscera were clearly the preferential tissue of lipid deposition, with a total lipid content of 250–280 and 470–510 g kg^{-1} in trout and in sea bass, respectively. Liver was the second preferential tissue for lipid deposition, being values also always higher in sea bass (210–250 g kg^{-1}) than in trout (50–70 g kg^{-1}). Similar results have been reported previously by Corraze & Kaushik (1999) which claimed that lipid deposition in marine fish, such as sea bass, is reported essentially in liver tissue and secondarily in viscera, whereas a fresh water fish (rainbow trout) uses the viscera as the main tissue for lipid depot.

The lipid fraction of fish tissues is most significantly affected by dietary lipids nature (vegetable oils versus fish oils), being triglycerides (neutral lipids) much more affected

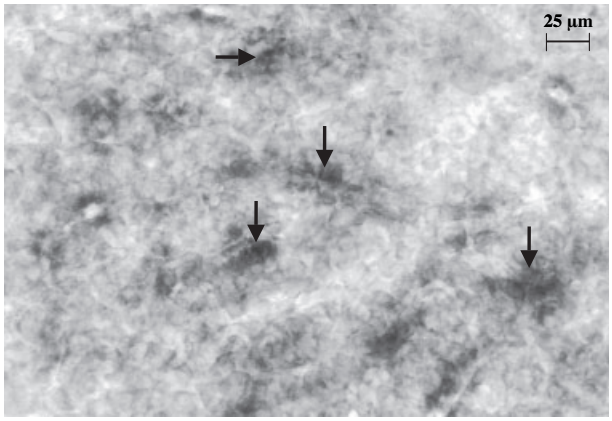

Figure 5 Rainbow trout liver fed diet C (50% soybean oil) (Frozen section – SB, 400×). When compared with the previous image from sea bass, lipid specific staining revealed that the hepatocytes had a quite poor lipid content, further displaying heterogeneity. The positive staining appears as scattered black clumps (arrows), smaller than those seen in Fig. 4. The poorness in lipids mean that the citoplasmic vacuolation observed in H&E (see Fig. 3) is mostly because of glycogen. This was confirmed by PAS staining.

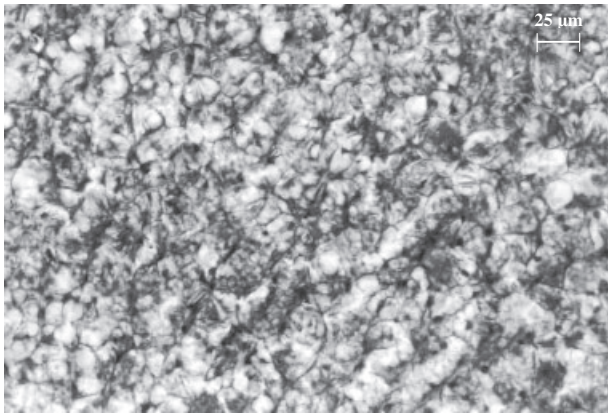

Figure 6 Sea bass liver fed diet C (500 g kg^{-1} soybean oil) (PAS, 400×). When comparing this image with Fig. 2 it can be noticed that certain zones within the parenchymal cells are darkly stained (PAS positive), contrasting with the majority of the remaining vacuolated (lipid rich) cytoplasm. PAS showed that sea bass generally presented scarce glycogen deposits, despite showing some staining heterogeneity among the analysed specimens.

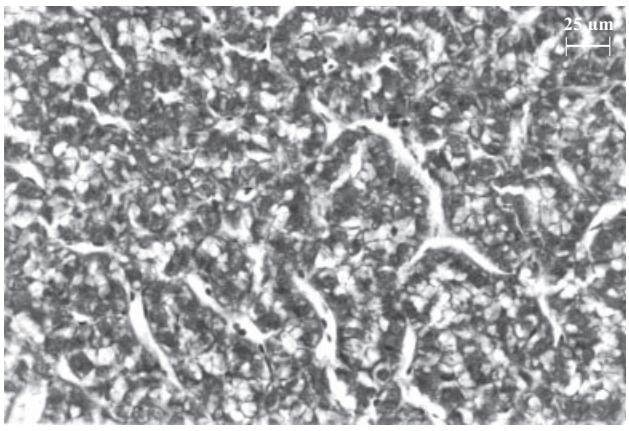

Figure 7 Rainbow trout liver fed diet C (50% soybean oil) (PAS, 400×). The image shows darkly stained areas within hepatocytes, especially around the more or less central nucleus and in the perisinusoidal cytoplasm. The image was taken from a fish that was not so poor in glycogen areas, and in which these deposits (located in the darker areas) counterbalanced the vacuolated (lipid rich) areas. Overall, trout fed 500 g kg^{-1} soybean oil tended to have relatively more glycogen then sea bass with the same substitution level.

than phospholipids (polar lipids) (Corraze 2001; Sargent *et al*. 2002). However, in the present study, no differences were detected in neutral or polar lipid fractions among treatments (Table 4), even at the highest soybean oil incorporation level (50%). Despite the lacking of PUFAs and the richness in linoleic acid (18 : 2–*n*6) of vegetable oils, soybean oil still contains approximately 100 g kg^{-1} of *n*–3 fatty acids (Corraze 2001). Because of the excellent quality of the marine fish oil used in the experimental diets, the soybean diets contained 165–167 g kg^{-1} of EPA + DHA, largely suppressing the recommendations for these species (NRC 1993). When the essential fatty acids requirements are met, the lipids serves essentially as an energy source (Corraze 2001), being variations in fish FA's (fatty acids) composition less pronounced than in feeds. Previous works with vegetable lipids sources (Caballero *et al*. 2002; Izquierdo *et al*. 2003) have also showed good results, without important adverse effects on growth, lipid deposition or flesh quality.

Plasma triglycerides and cholesterol values observed in this experiment are within normal levels reported for rainbow trout (Kaushik *et al*. 1995; Dias 1999) and sea bass (Dias 1999). Baba *et al*. (2000) observed a decrease in plasma cholesterol levels in rats fed vegetable oil diets; however, this trend was not confirmed by the present work. Both triglycerides and cholesterol levels were similar among dietary treatments regardless of fish species. The HSI exhibited by rainbow trout (1.2 ± 0.2) and sea bass (2.0 ± 0.2) were within the range of that reported to the same soybean oil incorporation level (Krajnović-Ozretić *et al*. 1994; Yildiz & Sener 1997) and did not differ among treatments. High HSI were associated with high liver lipid content.

Hu *et al*. (1989) indicated that *n*–3 fatty acids are particularly susceptible to lipid peroxidation. As fish oils are very

rich in *n*−3 fatty acids, stability of feed pellets can be improved with the use of soybean oil (rich in *n*−6 fatty acids, natural source of vitamin E). So, the effect of dietary soybean incorporation level on TBARS was assessed, but no significant differences were found among treatments. This does not correspond with earlier observations in turbot (Stéphan *et al*. 1995) and mammals (Lopez-Bote *et al*. 1997), where the substitution of fish oil by vegetable oils resulted in a decrease in the levels of TBARS, indicating a reduction in the susceptibility to lipids peroxidation. In addition, Alvarez *et al*. (1998) reported a significantly higher susceptibility to muscle oxidation in trout and sea bass fed on diets rich in fish oil than in fish fed on low fish oil diets, but the present results do not support these findings either.

The inclusion of soybean oil had no significant effect on liver histology, independently of the studied species. The rainbow trout hepatocytes evidenced a vacuolised cytoplasm, in all treatments, which correspond with previous findings (Caballero *et al*. 2002) in trout fed diets with 50% incorporation of soybean oil. The marked hepatocellular vacuolation observed in sea bass was caused by the accumulation of very large lipid droplets, which reflect the higher fat content and higher liver/body weight ratios in comparison to salmonids (Table 2). Nonetheless, these results were found normal for this species under typical rearing conditions. Similarly, high degrees of vacuolization because of lipid deposition have also been observed in livers of red drum *Sciaenops ocellatus* fed diets containing soybean oil (Tucker *et al*. 1997), and in livers of turbot *Scophthalmus maximus* fed marine fish oil (Bell *et al*. 1995). Although a few studies have shown that sea bass performance was not affected by diet inclusions of plant oils (soybean or olive oil), a histological examination of liver tissue revealed liver degeneration, being most intense in fish exclusively fed diets with plant oils (Mosconi-Bac 1987; Alexis 1997), however these results were not confirmed by the present work. According to Mosconi-Bac (1990), the accumulation of lipids in the liver of juvenile sea bass is a reversible reaction of the hepatocytes to a new metabolic state, corresponding to reactive adaptations in short-term, but that can lead to liver necrosis if the diet is not corrected.

As feed cost makes up a high proportion of total production cost, the use of diets formulated with lower cost plant oil was shown in the current study to be a viable approach for reducing production cost. Fish were raised on alternative diets without significantly affecting growth, body lipid deposition or liver healthy status. The possibility to use plant oils to replace extensive harvest of marine resources to produce higher quality fish meat is of particular interest in developing more sustainable food production. The increased demand for plant feed sources, together with new insights in plant genetics and genomics opens up the possibility of exploring new potential plant lipid sources in aquaculture of new species. Although plant lipids may be more available and cheaper than fish oil in some countries, considerable research is still required to evaluate the long-term use in aquafeeds and possible effects on fillet quality.

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