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Total replacement of fish oil by soybean or linseed oil with a return to fish oil in Turbot (Psetta maxima)

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Abstract:

The aim of the study was to investigate the replacement of fish oil by vegetable oils and the effects of a washout with a return to fish oil on growth performances and lipid metabolism. Three experimental fish meal based, isonitrogenous (crude protein content: 57.5%) and isolipidic (crude lipid content: 16.5%) diets, were formulated containing either 9% of added fish oil (FO), soybean oil (SO) or linseed oil (LO). Each diet was distributed to triplicate groups of 25 marketable size turbot (initial body weight of 579 g) grown in seawater at a water temperature of 17°C. Fish were fed once a day to visual satiety. At the end of the growth trial which lasted 13 weeks, all groups of turbot were fed FO diet for 8 weeks. The growth of turbot was high, but the incorporation of vegetable oils in the diets resulted in a slight decrease in growth as compared to those fed the fish oil based diet. Feed and protein efficiency and whole body composition were not affected by dietary lipid sources. Total lipid content was low in the muscle of turbot (below 2%), ventral muscle being fatter than dorsal muscle. Liver and muscle fatty acid (FA) composition reflected dietary FA composition. Liver and muscle of fish fed SO diet were rich in 18:2n-6 whereas those of fish fed LO diet were rich in 18:3n-3. Liver and muscle of fish fed SO and LO diets had lower levels of 20:5n-3 and 22:6n-3 in comparison to those of fish fed FO diet. In turbot, hepatic lipogenic enzyme activities were low and not influenced by dietary lipid source. At the end of the second period, after transfer to FO based diets, muscle FA composition of fish fed previously SO and LO diets was still different to those of fish fed the FO diet. The values of 18:2n-6 and 18:3n-3 respectively were lower than the values found at the end of the growth period but higher than those of fish fed the FO diet. An increase of FA levels, characteristic of fish oil, was observed in the liver and muscle of fish previously fed vegetable oils. Data obtained show that replacement of fish oil by vegetable oils is possible without any significant impact on growth performance of turbot, that dietary lipids are an effective vector to influence the nutritional quality of finished product and that a duration of 8 weeks is not sufficient to bring the FA profile of turbot of this size back to that of fish fed fish oil over the whole period.

1. INTRODUCTION

High dietary fat levels are commonly used in salmonid diets as an important source of energy for protein sparing and to decrease nitrogenous losses (Aksnes et al., 1996). But there is a concern that fish oil and fish meals are becoming more and more scarce (Barlow and Pike, 2001). In the context of research on the substitution of fish oil in diets of fish, several studies (Reinitz and Yu, 1981; Hardy et al., 1987; Thomassen and Røsjø, 1989; Greene and Selivonchick, 1990; Arzel et al., 1994; Guillou et al., 1995) have shown that at least in salmonids, it is possible to totally replace fish oil by plant oil sources such as soybean oil, corn oil, linseed oil and rapeseed oil without affecting growth. However, this kind of substitution is known to modify muscle fatty acid composition (Thomassen and Røsjø, 1989; Greene and Selivonchick, 1990; Arzel et al., 1994). In rainbow trout broodstock, it has also been shown that both egg and milt fatty acid composition is affected by dietary vegetable oils but without affecting reproductive performance (Corraze et al., 1993; Labbé et al., 1993, 1995).

In marine fish, partial substitution of fish oil by vegetable oils has been demonstrated in gilthead sea bream or in European sea bass (Kalogeropoulos et al., 1992; Yildiz and Sener, 1997). However marine fish have a requirement for highly unsaturated fatty acids (HUFA) which must be taken into consideration when vegetable oils are used in the diets. Turbot require a dietary supply of 20:5 or 22:6n-3 fatty acids, since they cannot synthesize these fatty acids from C18 precursors in significant amounts (Léger et al. 1979). Previous work has also shown that the results of fish oil substitution in turbot depend on the n-3 content of the basal diet used (Bell et al., 1994, 1999).

The aims of this study were (1) to evaluate growth performance, chemical composition particularly fat and fatty acid composition and flesh quality of turbot fed diets in which fish oil is replaced by soybean or linseed oil and (2) to evaluate the effects of a return to a diet with fish oil on chemical composition and flesh quality. The flesh quality parameters will be reported separately.

2. MATERIALS AND METHODS

2.1 Experimental diets

Three fish meal based isonitrogenous (digestible protein: 55% of dry matter) and isolipidic (digestible fat: 16% of dry matter) diets were formulated containing 9% of fish oil (FO), 9% of soybean oil (SO) or 9% of linseed oil (LO). Yttrium oxide (0.1%) was added as an indicator for digestibility measurements. Diets were manufactured on an industrial scale by Nutreco (Aquaculture Research Center, Stavanger, Norway), using a twin-screw extruder, in the form of 9-mm diameter pellets. Ingredient and chemical composition of the diets are reported in Table 1 and fatty acid (FA) composition in Table 2.

2.2 Digestibility measurements

Apparent digestibility coefficients (ADC) of the experimental diets were measured using the indirect method. Digestibility and growth trials (described below) were conducted in the experimental facilities of IFREMER (Centre de Brest, France) with identical groups of 25 turbot each. Turbot (*Psetta maxima*) obtained from a commercial farm were allotted to cylindroconical tanks, each of which was equipped with a flat-bottom large-mesh basket and supplied with sea water of constant temperature (17 ± 0.5°C) and with a salinity of 35‰ in a flow-through system. Fish were fed to satiety once a day and faecal samples were collected

using a faeces-settling column. For each treatment, faecal samples were collected once a day each morning over 2 weeks and were centrifuged (3000 × g at 6°C for 20min) and frozen daily at –20°C. After freeze-drying, faeces were analysed for yttrium oxide, crude protein, crude fat and gross energy. The ADC of the experimental diets was calculated as follows:

$$\text{ADC (\%)} = 100 - \left(100 \frac{\text{\% Tracer in Diet}}{\text{\% Tracer in Faeces}} \times \frac{\text{\% Nutrient or Energy in Faeces}}{\text{\% Nutrient or Energy in Diet}}\right)$$

2.3 Growth trial

The growth trial was conducted in the same experimental facilities as the digestibility trial for a period of 13 weeks. Twenty-five turbot from the same source having a mean initial body weight of 579 ± 1 g (mean ± sd) were randomly allotted to each tank (1000 l; flow rate of 14 $l.mn^{-1}$). A 12/12h light/dark cycle was adopted. The three experimental diets were randomly allotted and triplicate groups were fed by hand once a day to visual satiety (visual observation of first feed refusal) over a 90 min period and feed intake was recorded. Each group was weighed every three weeks to follow growth and feed utilisation.

2.4 Washout with fish oil

At the end of growth trial, 13 fish were withdrawn for analyses described below, and all the three groups were fed with a FO diet for a subsequent 8 weeks, which corresponded to 952 degree days. At the end of this period each group of fish was weighed and sampled.

2.5 Samplings

At the beginning of the growth trial, five fish from an initial pool of fish were sampled and stored at -20°C for analyses of whole body composition. At the end of the growth trial, the same protocol of slaughter was followed for each tank. Fish were fasted two days before

slaughter. Fish were stunned, bled in cold water before dissection. Four fish per tank were removed for comparative carcass analyses (water, ash, protein, fat and energy) and nutrient retention calculation. Nine fish per tank were removed to weigh the liver and the digestive tract for calculation of hepatosomatic index (HSI) and viscerosomatic index (VSI). Two fish per tank were removed for lipid and fatty acid analyses in liver and muscle (dorsal and ventral fillets without skin). Six livers were withdrawn from fish for analyses of enzyme activities (glucose-6-phosphate dehydrogenase, acetyl Co-enzyme A carboxylase, fatty acid synthetase and malic enzyme). At the end of the washout period, livers from six fish per tank were removed for calculation of HSI and two fish from each tank for lipid and FA analyses of the liver and muscle (dorsal and ventral fillets without skin).

2.6 Analytical methods

The fish for whole body composition were ground frozen and a representative portion was freeze-dried and homogenised before analysis. Composition analyses of diets, faeces and chemical composition of whole body and tissues were made following standard methods (AOAC, 1984) : dry matter after desiccation in an oven (105°C for 24h), ash (incineration at 550°C for 12h), crude protein (Dumas, Nitrogen Analyser, Fison instrument, N × 6.25), crude fat (dichloromethane extraction by Soxlhet method) and gross energy (IKA Adiabatic Calorimeter C4000A).

For lipid analyses of liver and muscle, extraction was done according to Folch et al. (1957), with chloroform being replaced by dichloromethane. The diets and faecal matter were pre-digested in a solution containing 2% nitric acid and 2 g/L potassium chloride and yttrium was determined with atomic absorption spectrophotometry (Varian, AA-20, wavelength 410 nm) using a nitrous oxide-acetylene flame.

The separation of neutral lipids and phospholipids was performed according to the procedure described by Juaneda and Rocquelin (1985). The total lipid extracts were fractionated on silica cartridges (Sep-Pack, Waters), neutral lipids were eluted by chloroform and phospholipids by methanol. Fatty acids methyl esters (FAME) were prepared following the method of Morrison and Smith (1964) and separated by gas chromatography (Auto-system Perkin-Elmer with a flame ionisation detector, BPX 70 capillary column: 25 m x 0.22 mm i.d. x 0.25 μm d.f.; split-splitless injector, with helium as carrier gas). The injector and detector temperatures were, respectively, 220°C and 260°C. Initial temperature of the oven was 50°C which increased to 180°C by increments of 15°C/min, maintained for 5 min, then increased to 220°C by increments of 3°C/min. Data acquisition and handling were carried out by connecting the GLC to a PE Nelson computer. The individual fatty acid methyl esters were identified by comparing the retention times of authentic standard mixtures. The results of individual FA composition were expressed as percent of total identified FA methyl esters.

For assays of hepatic lipogenic enzyme activities, liver samples were homogenised in three volumes of ice-cold buffer (0.02M Tris-HCl, 0.25M sucrose, 2mM EDTA, 0.1M sodium fluoride, 0.5mM phenyl methyl sulphonyl fluoride, 0.01M β-mercaptoethanol, pH 7.4) and the homogenates were centrifuged at 20 000 × g at 4°C for 20 min. Soluble protein content of liver homogenates was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Selected lipogenic enzyme activities were assayed on supernatant fractions: glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) according to Bautista et al. (1988), malic enzyme (ME; EC 1.1.1.40) according to Ochoa (1955), fatty acid synthetase (FAS, EC 2.3.1.38) according to Chang et al. (1967) and acetyl Coenzyme-A carboxylase (ACoAC; EC 6.4.1.2) as per Holland et al. (1984). Enzyme activity units (IU) defined as

µmol substrate converted to product per min at assay temperature (30°C), were expressed as units per mg hepatic soluble protein and per g liver tissue (wet weight).

2.7. Statistical analysis

All data were subjected to one-way analysis of variance to test the effects of experimental diets. In cases where significant differences occurred (significance level =0.05), the Newman-Keuls test was used to compare means. All statistical tests were performed using the STAT-ITCF (Service des études statistiques, Paris, France) software. Principal component analysis of fatty acid profiles was made using S-Plus software with a specific module.

3. RESULTS

3.1 Growth trial

Apparent digestibility coefficients (ADC %) of protein and fat were very high. The ADC of dry matter was not affected by dietary lipid source. ADC of protein was around 96% and that of energy near 90% for the different treatments. ADC of fat varied from 95.8% for the LO diet to 98.2% for the SO diet, but no significant difference was observed. Growth performance of turbot was high for fish of this size (initial body weight : 579 g). A significant difference was observed in final body weights and growth rates: turbot fed diets SO and LO had final body weights of 912 ± 2 g and 918 ± 4 g, lower than those of turbot fed the FO diet (956 ± 4 g). However no significant differences were observed in feed efficiency (wet weight gain / dry matter intake) which was 1.17 ± 0.02 or in protein efficiency ratio (wet weight gain / crude protein intake) which was 2.04 ± 0.02 in the three groups.

Moisture, ash, protein and fat contents of whole body were also not statistically different among treatments (Table 3). Consequently, protein and energy retention (% of intake) which were very high, were not influenced by dietary treatments ($P > 0.05$). HSI and VSI did not vary significantly among treatments, viscera only representing between 2.5 to 2.6 % of body weight.

No significant difference was observed between dietary treatments on moisture or lipid contents of liver, ventral and dorsal muscles (Table 4). Liver lipid content was high and varied from 18.8 to 22.6%. Muscle protein content was high at around 20%. Total lipid content was low in the muscle, ventral muscle being fatter than dorsal muscle, but represented only 2%. Phospholipids were constant (0.6%) in both muscles.

Within the same treatment, there was no difference between dorsal and ventral muscle FA composition of neutral lipids or phospholipids. Hence, only the FA profiles of dorsal muscle are presented here. FA composition of liver and muscle were clearly influenced by the FA composition of the diets (Tables 5 and 6). In liver and muscle, concentrations of saturated, monounsaturated FA (particularly 22:1n-11) as well as 20:5 and 22:6n-3 fatty acids were significantly higher in fish fed the FO diet than in the other groups. Liver and muscle of fish fed the SO diet were rich in 18:2n-6 and those of fish fed the LO diet were rich in 18:3n-3. In the liver, the quantity of 20:2n-6 was significantly higher in fish fed the SO diet than in the other groups and the quantity of 20:3n-3 was significantly higher in fish fed the LO diet than in the other groups. FA composition of phospholipids were less influenced by dietary FA that of neutral lipids and the concentrations of 20:5n-3 and 22:6n-3 were also higher in phospholipids than in neutral lipids.

Data on the activities of the lipogenic enzymes assayed in turbot liver are reported in Table 7. ACoAC, FAS and the two NADPH-generating dehydrogenases, G6PD and ME had low activities and were not affected by dietary treatments ($P > 0.05$). Turbot fed the LO diet had slightly lower G6PD activity than other groups, but there were no statistically significant differences among treatments.

3.2 Finishing trial

After transfer to FO diets over 8 weeks, the growth performance was similar in all groups ($P>0.05$). Although there was a significant difference in initial body weight at the beginning of the second part (fish fed vegetable oils presented lower weights), no significant difference was observed in final body weight, the values being 1259 ± 54 g for FO group, 1159 ± 39 g for turbot fed previously SO diet and 1172 ± 42 g for those fed previously LO diet. The growth rates were similar to that of the FO group during the previous growth trial. Feed efficiency ratios (1.09 ± 0.06 to 1.17 ± 0.04) and protein efficiency ratios (1.90 ± 0.10 to 2.03 ± 0.07) were not significantly different between treatments ($P>0.05$).

HSI values varied between 2.3 to 2.7% (Table 8). Although, fish previously fed the LO diet had a significantly lower HSI than in the other groups, no significant difference was found in liver lipid level. Dorsal and ventral muscle composition was also not statistically affected by previous treatments, however protein level in dorsal muscle was higher in fish previously fed LO than in the control FO group. Liver fatty acid composition showed few differences among treatments (Table 9). No significant difference was observed for the sum of saturated fatty acids, for 20:5n-3, and 22:6n-3. Fatty acid composition of dorsal muscle was reported in Table 10. The FA compositions of dorsal and ventral muscles were similar. There were

always some differences in the fatty acid profiles among fish maintained on diet FO and those previously fed diets with vegetable oils. This difference remained more marked in neutral lipids than in phospholipids. In fish previously fed the SO diet, the levels of 18:2n-6 was higher than in other groups. Likewise, 18:3n-3 was higher in fish previously fed the LO diet than in the other groups. There were no significant differences among groups in the levels of 18:3n-6, 20:3n-6, 20:4n-6 and 18:4n-3 in either neutral or polar lipids.

The changes that occurred in the muscle neutral and polar lipid profiles before and after the wash out period are depicted in Fig. 1. The polar lipid FA profile is distinct from that of the neutral lipid fraction in all groups, reflecting the high levels of n-3 fatty acids in this fraction.

4. DISCUSSION

Due to the marine fish oil and phospholipids contained in the fish meal itself, the SO and LO diets contained respectively 3.7 and 3.6% of EPA (eicosapentaenoic acid, 20:5n-3) and 5.6 and 5.5% of DHA (docosahexaenoic acid, 22:6n-3) supplying about 1.3% HUFA on a dry matter basis. These amounts are sufficient to meet the EFA requirements of turbot, which is estimated to be 0.8% (Gatesoupe et al., 1977), or even less (0.6%; Léger et al., 1979). The digestibility of the nutrients and of energy was not affected by dietary lipid source and no mortality of turbot was observed. The growth performance of turbot, although high, was affected by dietary lipid sources, i.e. turbot fed diets with vegetable oils had lower growth than those fed the fish oil based diet. Although theoretically, the supply of n-3 HUFA was sufficient, it is possible that the supply of arachidonic acid also considered to be an EFA for turbot (Castell et al., 1994; Sargent et al., 1999), was insufficient. In our study, 20:4n-6 was supplied in low amounts, in the experimental diets both by fish meal and fish oil while linseed

oil as well as soybean oil were deficient in this fatty acid; these two latter oils contain 18:2n-6 (linoleic acid), which is not an available precursor for marine fish having negligible ability for desaturation and elongation (Bell et al., 1986).

In the present study, growth rate of turbot was high and superior to our own earlier data (Regost et al., 2001) on turbot of similar size with the same dietary lipid level at 17°C but inferior to those reported by Devesa (1994) on turbot of 500-1000 g grown at higher temperatures. Feed efficiency and protein efficiency ratio were not affected by dietary treatment. Our data showed clearly that it is possible to replace fish oil by vegetable oil in diets for turbot, considering the relatively low influence observed on growth. Bell et al. (1994) found also no negative effect on growth of juvenile turbot fed a diet with linseed oil. However, with a purified diet containing 19% of linseed oil with no added n-3 and n-6 HUFAs, Bell et al. (1999) obtained a reduction in growth rate of juvenile turbot and an increase of mortality. For salmonids, fish oil can be replaced by soybean oil (Reinitz and Yu, 1981; Hardy et al., 1987; Greene and Selivonchick, 1990; Guillou et al., 1995) and linseed oil (Mugrditchian et al., 1981; Greene and Selivonchick, 1990), as salmonids are known to possess functional fatty acid desaturases (Bell et al., 1997; Seiliez et al., 2001).

No effect of dietary lipid source was observed in whole body or tissue composition of turbot, as reported for other fish (Hardy et al., 1987; Greene and Selivonchick, 1990; Arzel et al., 1994; Dosanjh et al., 1998). The high lipid content of liver is in accordance with Bell et al. (1999) who showed that turbot had the ability to store large amounts of lipid in the liver like other flatfish (Norvedt and Tuene, 1998). On the other hand, muscle lipid content of turbot was low (between 1 to 2%), confirming earlier reports (Sérot et al., 1998; Regost et al., 2001).

In conformity with data for other fish (Reinitz and Yu, 1981, Polvi and Ackman, 1992; Arzel et al., 1994; Bell et al., 1995; Guillou et al., 1995), the fatty acid composition of liver and muscle reflected the FA composition of the diets. The effect of dietary oils was very marked in the liver with 18:2n-6 representing 28% of FA in fish fed diet the soybean oil diet and 18:3n-3 representing 21% of FA in fish fed the linseed oil based diet. In the white muscle, this effect was more pronounced in the neutral lipid fraction than in the polar lipids as generally found in other species (Lie et al., 1986). As phospholipids are the essential component of membranes, the polar lipid FA profile is generally more constant than that of the neutral lipids, which is the fat storage form (Sargent et al., 1989). However, the FA composition of tissues did not exactly reflect the FA profile of diets. The relationship between the polyunsaturated fatty acid (PUFA) levels in the tissues and diets was most likely dependent upon the relative essentiality of the specific FA (Skonberg et al., 1994). Turbot maintained a high proportion of n-3, especially EPA and DHA, particularly in polar lipids, generally richer in PUFA than neutral lipids (Henderson and Tocher, 1987). Polar lipids also had more than 2% of 20:4 n-6, slightly but significantly higher in the FO group than in the SO and LO groups. The latter diets were deficient in 20:4n-6, an important precursor of physiological mediators like prostaglandins and leukotrienes (Bell et al., 1986).

In the present study, an elongation of 18:2n-6 to 20:2n-6 for fish fed SO diet was observed in turbot, particularly in the liver. But the intermediates of 20:4n-6 synthesis, 18:3n-6 and 20:3n-6 remained at very low levels in all groups. For fish fed LO diet, 18:3n-3 was elongated to 20:3n-3 and a slight elongation of 18:2n-6 to 20:2n-6 was observed. Intermediates of n-3 desaturation and elongation process could be observed in both liver and muscle: more of 18:4n-3 and 20:4n-3 were encountered in turbot fed FO diet than in those fed vegetable oil diets. Their relative presence seems dependent of the level of 18:4n-3 in the diets, rather than

that of 18:3n-3. In fact, turbot, as other marine fish, are known to have a very limited capacity to synthesize HUFA from C18 precursors (Owen et al., 1975; Cowey et al., 1976). Desaturases are known to have a better affinity for n-3 FA than for n-6 FA in turbot as in mammals (Tocher, 1993). Our data confirm the observations of Bell et al. (1994) that turbot can elongate C18 to C20 fatty acids in vivo; in vitro, Ghioni et al. (1999) found a limited elongase activity in turbot cell lines. In turbot, a predominance of elongation over that of desaturation of FA and especially the deficiency of Δ5-desaturase was also observed by Bell et al. (1995) and Tocher (1993). Our data confirm that turbot are unable to convert linolenic acid to EPA and DHA (Bell et al., 1999) possibly due to a deficiency of Δ5-desaturase and "Δ4-like"-desaturase (Castell et al., 1994), with no sign of Δ6 desaturation.

Hepatic lipogenic enzyme activities in turbot were low as previously reported (Regost et al., 2001). The activities of G6PD and ME were similar than those found in the earlier study. But ACoAC activity levels in the present study were nearly three times lower than those observed earlier (Regost et al., 2001). No effect of dietary lipid source was observed in hepatic lipogenic enzyme activities. To our knowledge, no study has reported the influence of vegetable oils on lipogenesis in marine fish. According to Shikata and Shimeno (1994), high levels of EPA and DHA tend to inhibit hepatic lipogenic activities. Recently, Alvarez et al. (2000) showed in vitro that the inclusion of EPA and DHA in diets induced a reduction of lipogenic enzyme activities in hepatocytes of rainbow trout.

During the washout period, all turbot were fed the same diet, i.e. FO diet for two months and all groups had comparable growth rates. The increase in HSI in all groups at the end of the finishing trial can be related to an increase in liver fat content during the washout period. Although differences in FA profiles among groups was still present, a decrease in 18:2n-6 and

18:3n-3 in the liver and muscle was observed. These reductions were more apparent in the liver (64% for 18:2n-6 and 73% for 18:3n-3) than in the muscle (around 40% for 18:2n-6 and 18:3n-3 in neutral lipids and 50% for 18:3n-3 in phospholipids). Change to a fish-oil based (FO) diet definitely had a positive effect on both neutral and polar lipids, although they did not reach those of fish fed continuously with fish oil based diets. The ratios of EPA and DHA increased in fish previously fed vegetable oils. In liver, the EPA, DHA and 22:5n-3 values were not significantly different among the various groups. In muscle phospholipids, DHA value of fish fed previously the LO diet no longer differed from that of the FO control. The apparent decrease in the ratios of EPA and DHA in liver of fish fed the FO diet during the two periods could be explained by the relative increase of liver fat content during the latter period. This led to an increase of saturated and monounsaturated FA at the expense of PUFA. The fact that, after two months on the same diet, fish did not fully recover a similar FA profile suggest that time required for adaptation of tissue compositions to a new diet must be longer for turbot of this size. It is also possible that at the end of the three months of growth trial, fish fed vegetable oils had probably not reached a stable FA composition relative to their dietary inputs.

5. CONCLUSION

Three months on diets containing vegetable oils only had a slight effect on growth of turbot as compared to that of a control group fed fish oil. Liver and muscle FA profiles of turbot clearly reflected FA profile of the diets. A switch to a FO-containing diet led to similar growth in all groups. As regards the fatty acid profiled of fish, even after 2 months, the influence of previous diets were still present, although a marked decrease in 18:2n-6 and 18:3n-3 fatty

acids characterising the vegetable oils and a significant increase of EPA and DHA was observed.

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Table 1. Ingredient and proximate composition of the experimental diets.

	Experimental diets		
	FO	SO	LO
Ingredients (g/kg)			
Fish meal (Norseamink), CP 70%	500	500	500
Soluble fish protein concentrate, CP 76%	50	50	50
Scandinavian Marine Fish Oil	90	-	-
Soybean oil (Fredrikstad, Norway)	-	90	-
Linseed oil (Fredrikstad, Norway)	-	-	90
Whole wheat	183	183	183
Wheat gluten, CP 66%	147	147	147
Mineral mix[1]	10	10	10
Vitamin mix[1]	10	10	10
Binder (Sodium alginate)	9	9	9
Yttrium oxide	1	1	1
Proximate composition			
Dry matter (DM),%	89.1	90.2	90.3
Ash (% DM)	9.2	9.1	9.1
Crude protein (% DM)	57.4	57.6	57.6
Crude fat (% DM)	16.6	16.5	16.5
Gross energy (kJ/g DM)	23.5	23.4	23.1
Digestible protein (DP)[2]	55.0	55.4	55.2
Digestible energy (DE)[2]	21.0	21.1	20.5
DP/DE ratio (mg/kJ)	26.2	26.3	26.9

1. Proprietary mixtures (Nutreco, ARC) providing levels meeting requirements as proposed by NRC (1993).
2. DP and DE values are based on determined values of apparent digestibility coefficients (ADC) of protein and energy.

Table 2. Fatty acid composition of the experimental diets (% of methyl esters).

	Experimental diets		
Fatty acids (FA)	FO	SO	LO
∑ saturated FA	22.5	18.5	14.3
22:1n-11	15.1	4.3	3.3
∑ monounsaturated FA	47.1	29.8	25.5
18:2n-6	5.2	35.6	15.5
18:3n-6	0.2	0.1	0.3
20:2n-6	0.3	0.2	0.3
20:3n-6	0.1	0.05	-
20:4n-6	0.5	0.3	0.2
∑ n-6 FA	6.3	36.2	16.3
18:3n-3	1.3	4.6	33.3
18:4n-3	2.9	0.9	0.8
20:3n-3	0.2	0.1	0.1
20:4n-3	0.5	0.2	0.2
20:5n-3	7.9	3.7	3.6
22:5n-3	0.7	0.4	0.4
22:6n-3	10.6	5.6	5.5
∑ n-3 FA	24.1	15.5	43.9
n-3/n-6	3.8	0.4	2.7

Table 3. Whole body composition (in % of wet weight basis), hepatosomatic (HSI) and viscerosomatic (VSI) index, nutrient and energy retention (in % intake) of turbot at the end of growth trial.

		Experimental diets		
	initial	FO	SO	LO
Moisture	75.9	73.9±0.7	73.4±0.2	74.7±0.9
Ash	3.8	3.6±0.1	3.8±0.1	3.6±0.1
Protein	16.9	17.6±0.2	17.7±0.3	17.0±0.3
Fat	2.9	4.8±0.3	4.6±0.3	4.0±0.6
Energy (kJ/g)	5.1	6.1±0.2	6.1±0.1	5.7±0.3
HSI	-	1.8±0.1	1.8±0.1	1.7±0.1
VSI	-	2.5±0.1	2.6±0.1	2.6±0.1
Protein retention	-	38.1±0.8	39.3±1.2	35.4±1.8
Energy retention	-	37.6±2.0	39.8±0.7	34.0±5.0

Values are means ± standard deviations (n=3 except for HIS and VSI where n=9).
Values in the same row with different superscripts are significantly different ($P<0.05$).

Table 4. Composition of liver, dorsal and ventral muscles in turbot at the end of growth trial (in % of wet weight basis).

	Experimental diets		
	FO	SO	LO
Liver			
Moisture	61.1±1.2	58.0±1.5	59.7±2.6
Total lipid	18.8±1.3	22.6±1.3	20.5±2.8
Dorsal muscle			
Moisture	77.6±0.2	77.5±0.2	77.8±0.2
Protein	20.9±0.2	21.0±0.1	21.0±0.2
Total lipid	1.3±0.1	1.2±0.1	1.1±0.1
Phospholipids	0.6±0.0	0.6±0.0	0.6±0.0
Neutral lipid	0.7±0.1	0.6±0.1	0.5±0.1
Ventral muscle			
Moisture	77.0±0.3	77.0±0.1	76.9±0.4
Protein	20.2±0.2	20.6±0.2	20.2±0.1
Total lipid	2.2±0.2	1.8±0.1	2.2±0.3
Phospholipids	0.6±0.0	0.6±0.0	0.6±0.0
Neutral lipid	1.6±0.2	1.2±0.1	1.6±0.3

Values are means ± standard deviations (n=6).
All values were not significantly different ($P>0.05$).

Table 5. Liver fatty acid composition of turbot at the end of growth trial (in % of methyl esters)

	Diets		
	FO	SO	LO
Σ saturated FA	21.2±0.7	18.3±1.7	16.7±2.2
22:1n-11	5.8±0.2[a]	1.8±0.1[b]	1.6±0.1[b]
Σ monounsaturated FA	45.3±0.7[a]	34.1±1.7[b]	29.9±2.1[b]
18:2n-6	5.1±0.3[c]	27.6±2.9[a]	14.0±1.4[b]
18:3n-6	0.2±0.0[a]	0.1±0.0[b]	0.1±0.0[b]
20:2n-6	0.9±0.0[c]	3.1±0.1[a]	1.4±0.1[b]
20:3n-6	0.1±0.0	0.1±0.0	0.1±0.0
20:4n-6	0.5±0.0[a]	0.3±0.0[b]	0.3±0.0[b]
Σ n-6 FA	6.8±0.3[c]	31.2±2.9[a]	15.9±1.5[b]
18:3n-3	0.9±0.0[b]	2.6±0.3[b]	20.8±2.4[a]
18:4n-3	1.1±0.1[a]	0.4±0.0[b]	0.4±0.0[b]
20:3n-3	0.4±0.0[b]	0.8±0.0[b]	5.0±0.4[a]
20:4n-3	1.2±0.0[a]	0.5±0.0[b]	0.4±0.0[b]
20:5n-3	5.5±0.2[a]	2.7±0.1[b]	2.6±0.1[b]
22:5n-3	2.9±0.1[a]	1.6±0.0[b]	1.3±0.0[c]
22:6n-3	14.8±0.6[a]	8.0±0.2[b]	7.0±0.2[b]
Σ n-3 FA	26.8±1.0[b]	16.5±0.5[c]	37.5±2.9[a]
n-3/n-6	4.0±0.1[a]	0.5±0.0[c]	2.4±0.1[b]

Values are means ± standard deviations (n=6).
Values in the same row with different superscripts are significantly different ($P<0.05$).

Table 6. Dorsal muscle fatty acid composition of turbot at the end of growth trial (in % of methyl esters).

	Experimental diets			Experimental diets		
	FO	SO	LO	FO	SO	LO
	Neutral lipid			Polar lipids		
Σ saturated FA	21.7±0.3[a]	19.1±0.2[b]	17.4±0.2[c]	28.8±0.4[a]	27.7±0.1[b]	27.1±0.4[b]
22:1n-11	10.8±0.3[a]	4.6±0.1[b]	4.0±0.1[b]	1.1± 0.1[a]	0.5±0.0[b]	0.4±0.0[b]
Σ monounsaturatedFA	45.3±0.3[a]	34.1±0.3[b]	31.3±0.4[c]	19.4±0.3[a]	15.8±0.3[b]	15.6±0.3[b]
18:2n-6	5.5±0.1[c]	24.2±0.8[a]	12.1±0.2[b]	4.9±0.1[c]	18.7±0.5[a]	10.7±0.2[b]
18:3n-6	0.2±0.0[a]	0.1±0.0[b]	0.2±0.0[b]	0.3±0.0	0.2±0.0	0.2±0.1
20:2n-6	0.5±0.0[c]	1.4±0.1[a]	0.7±0.0[b]	0.4±0.0[c]	1.2±0.1[a]	0.7±0.0[b]
20:3n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.0	0.1±0.0
20:4n-6	0.5±0.0	0.5±0.0	0.5±0.0	2.3±0.1[a]	2.0±0.1[b]	2.0±0.0[b]
Σ n-6 FA	6.9±0.1[c]	26.3±0.8[a]	13.6±0.2[b]	8.1±0.1[c]	22.2±0.5[a]	13.8±0.2[b]
18:3n-3	1.3±0.0[c]	3.0±0.1[b]	18.3±0.9[a]	0.4±0.0[c]	1.1±0.0[b]	8.1±0.3[a]
18:4n-3	2.4±0.1[a]	1.2±0.0[b]	1.1±0.0[b]	0.3±0.1	0.2±0.1	0.2±0.1
20:3n-3	0.3±0.0[c]	0.4±0.0[b]	2.0±0.0[a]	0.0±0.0[b]	0.1±0.0[b]	1.0±0.0[a]
20:4n-3	0.8±0.0[a]	0.5±0.0[b]	0.5±0.0[b]	0.5±0.1[a]	0.3±0.1[b]	0.3±0.1[b]
20:5n-3	6.9±0.1[a]	4.8±0.2[b]	4.5±0.2[b]	10.0±0.2[a]	5.8±0.2[b]	6.1±0.2[b]
22:5n-3	2.0±0.1[a]	1.5±0.1[b]	1.5±0.1[b]	2.6±0.0[a]	1.9±0.0[b]	2.0±0.0[b]
22:6n-3	12.3±0.4[a]	9.2±0.3[b]	9.8±0.7[b]	29.8±0.5[a]	24.9±0.3[b]	25.7±0.5[b]
Σ n-3 FA	26.0±0.4[b]	20.5±0.5[c]	37.7±0.3[a]	43.6±0.2[a]	34.3±0.4[b]	43.5±0.2[a]
n-3/n-6	3.7±0.1[a]	0.8±0.0[c]	2.8±0.0[b]	5.4±0.1[a]	1.6±0.1[c]	3.2±0.0[b]

Values are means ± standard deviations (n=6).
Values in the same row with different superscripts are significantly different ($P<0.05$).

Table 7. Hepatic lipogenic enzyme activities of turbot at the end of growth trial.

	Experimental diets		
	FO	SO	LO
Glucose-6 phosphate			
IU/ g liver	7.25±0.32	7.10±0.44	6.60±0.26
IU/mg protein	0.25±0.01	0.26±0.01	0.21±0.02
Malic enzyme			
IU/ g liver	1.78±0.13	1.87±0.07	1.81±0.08
IU/mg protein	0.06±0.00	0.07±0.00	0.06±0.00
Acetyl-CoA carboxylase			
mIU/ g liver	6.86±0.70	5.28±0.39	5.55±0.47
mIU/mg protein	0.25±0.03	0.20±0.02	0.19±0.02
Fatty acid synthetase			
mIU/ g liver	0.41±0.01	0.66±0.01	0.53±0.02
mIU/mg protein	9.16±2,00	15.11±2.11	11.63±2.92

Values are means ± standard deviations (n=6).
All values were not significantly different (P>0.05).

Table 8. HSI and composition of liver, dorsal and ventral muscles (in % of wet weight) in turbot at the end of finishing trial.

	Experimental diets		
	FO	FO (SO)	FO (LO)
Liver			
HSI	2.7±0.1[a]	2.7±0.1[a]	2.3±0.1[b]
Moisture	52.4±2.6	54.4±1.6	54.8±2.4
Total lipid	28.7±3.2	26.0±1.9	25.1±2.9
Dorsal muscle			
Moisture	77.2±0.4	77.3±0.2	77.2±0.3
Protein	20.8±0.2	20.8±0.1	21.0±0.2
Total lipid	1.5±0.2	1.4±0.1	1.3±0.1
Phospholipids	0.6±0.0	0.6±0.0	0.6±0.0
Neutral lipid	0.9±0.1	0.8±0.1	0.7±0.1
Ventral muscle			
Moisture	76.1±0.7	76.7±0.3	76.5±0.4
Protein	20.3±0.2[a]	20.7±0.2[ab]	21.1±0.1[b]
Total lipid	2.6±0.6	2.1±0.3	1.9±0.2
Phospholipids	0.7±0.0	0.6±0.0	0.6±0.0
Neutral lipid	1.9±0.6	1.5±0.4	1.3±0.2

Values are means ± standard deviations (n=6).
Values in the same row with different superscripts are significantly different ($P<0.05$)

Table 9. Liver fatty acid composition of turbot at the end of finishing trial (in % of methyl esters).

	Diets		
Fatty acids (FA)	FO	FO (SO)	FO (LO)
Σ saturated FA	25.5±0.9	25.0±1.8	22.9±1.2
22:1n-11	6.0±0.3[a]	4.7±0.2[b]	4.7±0.2[b]
Σ monounsaturated FA	52.1±1.1[a]	48.2±1.0[b]	47.5±1.3[b]
18:2n-6	3.6±0.3[c]	10.0±1.5[a]	6.3±0.6[b]
20:2n-6	0.7±0.1[b]	1.3±0.1[a]	0.8±0.0[b]
20:4n-6	0.2±0.0[a]	0.2±0.0[a]	0.0[b]
Σ n-6 FA	4.5±0.4[b]	11.5±1.6[a]	7.1±0.7[b]
18:3n-3	0.6±0.1[b]	1.1±0.2[b]	5.6±0.5[a]
20:3n-3	0.3±0.0[b]	0.4±0.1[b]	2.0±0.1[a]
20:5n-3	4.1±0.3	3.6±0.3	3.9±0.3
22:5n-3	2.2±0.2[a]	1.7±0.1[b]	1.8±0.1[b]
22:6n-3	10.4±0.8	8.5±0.7	9.2±0.7
Σ n-3 FA	17.9±1.4[b]	15.3±1.2[b]	22.5±1.7[a]
n-3/n-6	4.0±0.1[a]	1.4±0.1[c]	3.2±0.1[b]

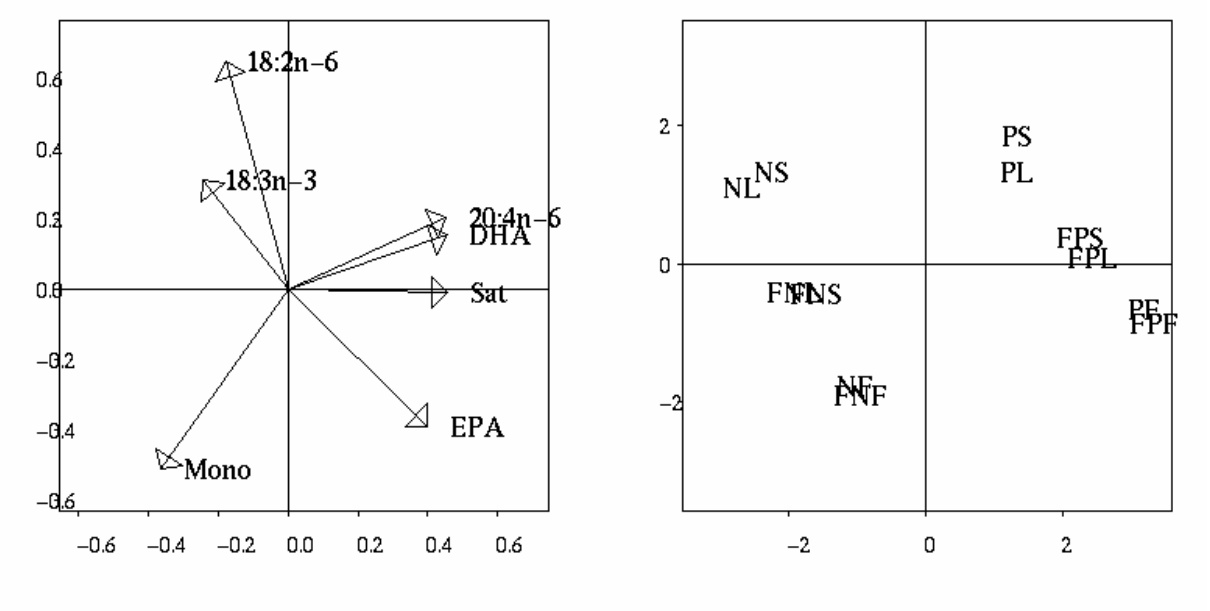
Values are means ± standard deviations (n=6).
Values in the same row with different superscripts are significantly different ($P<0.05$).

Table 10. Dorsal muscle fatty acid composition of turbot at the end of finishing trial

	Experimental diets			Experimental diets		
	FO	FO (SO)	FO (LO)	FO	FO (SO)	FO (LO)
	Neutral lipid			Polar lipids		
Σ saturated FA	23.1±0.1[a]	21.7±0.3[b]	20.4±0.3[c]	30.5±0.4	29.8±0.2	29.4±0.6
22:1n-11	11.3±0.4[a]	8.7±0.2[b]	8.3±0.2[b]	1.3±0.1	1.2±0.1	1.1±0.0
Σ monounsaturated FA	47.0±0.3[a]	42.0±0.5[b]	40.2±0.4[c]	21.7±0.4[a]	20.4±0.5[b]	19.9±0.4[b]
18:2n-6	5.3±0.1[c]	14.1±0.6[a]	8.6±0.1[b]	4.5±0.0[c]	11.8±0.3[a]	7.4±0.2[b]
18:3n-6	0.2±0.0	0.1±0.0	0.1±0.0	0.4±0.1	0.2±0.0	0.3±0.1
20:2n-6	0.5±0.0[c]	0.9±0.1[a]	0.6±0.0[b]	0.4±0.0[c]	0.7±0.0[a]	0.5±0.0[b]
20:3n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:4n-6	0.4±0.0	0.4±0.0	0.4±0.0	2.4±0.1	2.1±0.1	2.2±0.1
Σ n-6 FA	6.5±0.1[c]	15.6±0.7[a]	9.8±0.2[b]	7.8±0.2[c]	14.9±0.3[a]	10.5±0.1[b]
18:3n-3	1.2±0.0[b]	2.0±0.1[b]	10.4±0.6[a]	0.4±0.0[b]	0.7±0.0[b]	3.9±0.2[a]
18:4n-3	2.2±0.1[a]	1.7±0.1[b]	1.6±0.1[b]	0.3±0.1	0.2±0.1	0.2±0.1
20:3n-3	0.3±0.0[b]	0.3±0.0[b]	1.1±0.1[a]	0.0±0.0[b]	0.0±0.0[b]	0.5±0.0[a]
20:5n-3	6.8±0.1[a]	5.7±0.1[b]	5.5±0.1[b]	10.1±0.2[a]	7.9±0.1[b]	8.2±0.2[b]
22:5n-3	1.8±0.0[a]	1.5±0.1[b]	1.5±0.0[b]	2.5±0.0[a]	2.1±0.1[b]	2.0±0.1[b]
22:6n-3	11.1±0.3[a]	9.4±0.1[b]	9.4±0.2[b]	26.6±0.7[a]	24.1±0.6[b]	25.4±0.6[ab]
Σ n-3 FA	23.5±0.2[b]	20.7±0.1[c]	29.6±0.5[a]	40.1±0.4[a]	34.9±0.4[b]	40.3±0.6[a]
n-3/n-6	3.6±0.1[a]	1.3±0.0[c]	3.0±0.1[b]	5.2±0.2[a]	2.3±0.1[c]	3.8±0.1[b]

Values are means ± standard deviations (n=6).
Values in the same row with different superscripts are significantly different (P<0.05).

Figure 1.



Principal component analysis of muscle fatty acid profiles of turbot fed first with diets FO, SO or LO for 13 wks and subsequently transferred to FO for 8 wks. NL, NS and NF represent the FA profiles of neutral lipids of turbot fed linseed oil, soybean oil and fish oil respectively over 13 wks; PL, PS and PF represent the FA profiles of polar lipids of the same groups of turbot; the neutral and polar lipid profiles of the same groups after the washout with fish oil based diets for 8 wks are prefixed with an F, the arrows indicating the change occurring during this period.