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Effects of Fish Meal Replacement with Full-Fat Soy Meal on Growth and Tissue Fatty Acid Composition in Atlantic Cod (*Gadus morhua*)

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Atlantic cod of initial mean weight ~220 g were fed a control diet and three diets in which fish meal (FM) was replaced with increasing levels of full-fat soybean meal (FFS) supplied at 12, 24, and 36% of dry diet, for 12 weeks. There were no significant differences in final weights, but the specific growth rate (SGR) was significantly higher in fish fed the control (FFS0) diet compared to fish fed the FFS12 and FFS36 diets, and the feed conversion ratio (FCR) was significantly lower in fish fed the FFS0 diet compared to the other three treatments. The fatty acid (FA) compositions of the cod muscle and liver were highly affected by dietary treatment, and linear relationships between dietary and tissue FA concentrations were shown for some of these. Moreover, selective utilization or accumulation in the tissues of specific FA was suggested by the results.

KEYWORDS: Full-fat soybean meal; polyunsaturated fatty acids (PUFA); Atlantic cod; sustainable aquafeeds

INTRODUCTION

In recent years there has been a continuous decline in cod commercial fisheries, resulting in an increasing interest in cod culture. Specifically, global cod culture production has increased from 169 t in 2000 to 3812 t in 2004, showing a trend for further future increase (*1*). Cod require high-protein/low-oil diets, with high dependence on marine fish meal (FM) and fish oil (FO) (*2*−*6*). Regarding the estimated stable supplies of FM and FO (*7*) and the increased demand and price for these commodities for aquafeeds in the next decade (*8*, *9*), improvements in feeds that use alternative sustainable protein and lipid sources are vital for the long-term future of the cod industry.

Full-fat soybean meal (FFS), along with other soy products, is considered to be a good potential protein substitute for FM in aquafeeds. These products have been used to replace FM in diets for various fish species such as Atlantic salmon and rainbow trout (*10*−*12*), sea bream and sea bass (*13*), and Atlantic halibut (*14*). However, the results of these studies are contradictory as they vary from positive to negative effects on growth and other parameters. These variant results could be explained by the antinutritional factors (ANF) that soybeans contain (*15*); hence, the effect of the dietary inclusion of these products on the fish depends on the treatment and the process of the soybeans, the level of inclusion, the species and the age/size of the fish, etc.

In cod, very little is known about the replacement of FM with soy products. A study by Von der Decken and Lie (*16*) showed that FFS may replace up to 200 g kg^{-1} of the FM protein with no negative results on growth or feed intake. However, negative results were shown in the same study at the 300 g kg^{-1} level of FFS protein. In a more recent study (*17*) plant proteins, including solvent-extracted soybean meal, corn gluten meal, and a mix of soy protein concentrate and wheat gluten meal, was used to replace FM at levels up to 440 g kg^{-1} of plant ingredients in the diet. Hansen et al. (*17*) concluded that high growth rates and feed conversion ratios (FCR) were shown irrespective of the dietary treatment and, although the apparent digestibility coefficients (ADC) of protein and fat were reduced with high inclusions of plant proteins, fish maintained growth by increasing feed intake. These results are in line with a study by Refstie et al. (*18*), in which extracted soybean meal and a

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bioprocessed soybean meal, with reduced ANF, were used to replace FM in diets for on-grown cod. The authors concluded that cod showed high tolerance for these soy products, as there were no negative effects on growth and the fish compensated for the reduced ADC of amino acids and lipid by increased feed intake.

Although FFS is included in dietary formulations largely as a protein source, with a crude protein content of ~38%, it also contributes to the dietary fat, containing about 18% lipid (*19*). More than 50% of the total fatty acids (FA) of the soybeans is 18:2n-6, more than 25% is 18:1n-9, less than 10% is 16:0, and around 6% is 18:3n-3, whereas it does not contain any highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (*20*, *21*).

Fish, including cod, require essential FA (EFA), that is, arachidonic acid, EPA, and DHA, for normal growth (*6*, *22*, *23*). Fish tissue FA compositions largely reflect that of the diet and, as previous studies have shown, cod is no exception to this general principle, although selective utilization or retention of specific FA may occur (*3*, *23*−*27*). In cod the main adipose tissue is liver. The fat stored in this tissue could be used for the production of cod liver oil and, provided that its FA composition satisfies the basic commercial quality standards, it could be a potentially important byproduct of cod culture (*3*). Cod muscle is lean, with a very low lipid content (~1% of wet weight) that is mainly membrane lipids. However, the muscle represents the edible part of the fish, and therefore its nutritional value is of importance for the consumer; in particular, high concentrations of HUFA are desirable for promoting human health (*28*−*30*).

Hence, any inclusion of plant ingredients at the expense of FM and FO in cod diets should ensure that the consequent changes in the dietary FA composition will not be detrimental either to fish growth and health or to the quality of the final product to the consumer. The aim of this study was to investigate the effects of the partial replacement of FM and FO with FFS on growth and tissue FA composition in cod.

MATERIALS AND METHODS

Fish and Facilities. The experiment was conducted at the Scottish Association for Marine Science (SAMS) (Ardtoe, Scotland) from February to May 2004. Twelve tanks (4 × 3 × 1.6m2) were each stocked with 30 fish (Atlantic cod, *Gadus morhua*) of initial average weight of 220 g. The fish were PIT tagged, allowing individual measurements of biological characteristics to be obtained at each sampling at 6 and 12 weeks. Fish were held in 1600 L volume circular black polypropylene tanks supplied with UV-treated seawater filtered to 30 μm on a flow-through system at 15 L min^{-1}. The tanks were housed indoors, subjected to a photoperiod regime of 24 h light, and the water temperature over the experimental period was 8.3 ± 0.1 °C.

Experimental Diets. The fish were fed four extruded diets, produced by Fiskeriforskning, Norway, for 12 weeks. The control diet did not contain FFS (FFS0), whereas the experimental diets were formulated to contain 120, 240, or 360 g of FFS (FFS12, FFS24, and FFS36, respectively) per kilogram of diet at the expense of FM and FO (**Table 1**). All diets were isoenergetic and isonitrogenous, containing approximately 500 g kg^{-1} protein and 160 g kg^{-1} fat. With regard to amino acids and minerals the diets were balanced for methionine (4.2 g kg^{-1} feed protein), lysine (7 g kg^{-1} feed protein), and digestible phosphorus (7 g kg^{-1} feed protein). In all diets mineral and vitamin premixes were added, and the diets were formulated in accordance with all of the known nutritional requirements of cold-water fish (*6*, *19*). The analyzed proximate composition of the experimental diets is shown in **Table 1**, and the FA compositions are shown in **Table 2**. The fish were fed to satiation once a day, in the morning. Mortalities and daily feed intake and feed wastage were recorded.

Table 1. Feed Ingredients (Grams per Kilogram) and Analyzed Feed Composition

diet	FFS0	FFS12	FFS24	FFS36
fish meal[a]	617	559	501	445
fish oil[b]	114	94	75	56
full-fat soy[c]	0	120	240	360
wheat[d]	200	154	106	57
wheat gluten[e]	50	50	50	50
DL, 99% Met[f]	0	1	2	3
Lys-HCl, 80[g]	0	0.9	1.7	2.4
dicalcium phosphate	0	2.5	5	7.5
premixes[h,i]	19	19	19	19
Y_2O_3[j]	0.1	0.1	0.1	0.1
analyzed proximate composition (%)				
moisture	8.8	8.7	10.0	9.6
protein	51.0	50.8	50.5	50.5
fat	16.2	16.1	16.3	16.5
ash	8.2	8.1	8.0	8.3
fiber	0.8	1.3	1.3	1.6

[a] LT fish meal, SILFAS, Bergen, Norway. [b] NorsalOil, Norsildmel AL, Fyllingsdalen, Norway. [c] Full-fat soy; SOYAXAQUA, Shouten Industries B.V., Giessen, The Netherlands. [d] Wheat, Norgesmøllene, Bergen, Norway. [e] Wheat gluten, provided by EWOS Innovation, Dirdal, Norway. [f] DL-Methionine (minimum 98% Met); Degussa, Antwerpen, Belgium. [g] L-Lysine-HCl (minimum 78% lysine); Ainomoto Euro-Lysine, Paris, France. [h] Provided per kg of feed: vitamin D_3, 3000 IE,160 mg; vitamin E, 136 mg; thiamin, 20 mg; riboflavin, 30 mg; pyrodoxine-HCl, 25 mg; vitamin C, 200 mg; calcium pantothenate, 60 mg; biotin, 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B_{12}, 0.05 mg; menadion bisulfite, 20 mg. [i] Provided per kg of feed: magnesium, 500 mg; potassium, 400 mg; zinc, 80 mg; iron, 50 mg; manganese, 10 mg; copper, 5 mg. [j] Yttrium trioxide, included as an inert marker to determine apparent digestibility of nutrients.

Table 2. Fatty Acid Compositions (Percent by Weight of Total Fatty Acids) of the Experimental Diets Containing Increasing Levels of Full-Fat Soybean Meal (FFS)

fatty acid	FFS0	FFS12	FFS24	FFS36
14:0	5.4	4.8	3.8	3.2
16:0	14.6	14.7	14.1	14.0
18:0	2.0	2.4	2.7	3.1
total saturated[a]	22.5	22.5	21.3	20.9
16:1n-7	5.6	4.9	4.0	3.3
18:1n-9	8.5	10.0	11.8	13.9
18:1n-7	2.0	2.0	1.9	1.9
20:1n-9	9.9	8.4	6.7	5.4
22:1[b]	13.8	12.1	9.7	7.7
24:1n-9	0.8	0.6	0.5	0.5
total monoenes[c]	41.5	38.6	35.3	33.3
18:2n-6	4.4	12.0	19.4	26.1
20:2n-6	0.2	0.2	0.2	0.2
20:4n-6	0.4	0.4	0.2	0.2
22:5n-6	0.1	0.1	0.1	0.0
total n-6 PUFA[d]	5.3	13.0	20.1	26.6
18:3n-3	1.5	2.2	3.0	3.6
18:4n-3	4.3	3.5	2.9	2.3
20:4n-3	0.6	0.5	0.4	0.3
20:5n-3	10.2	8.4	7.1	5.4
22:5n-3	0.8	0.5	0.5	0.4
22:6n-3	11.6	9.5	8.3	6.2
total n-3 PUFA[e]	29.1	24.6	22.3	18.3
total PUFA	36.0	38.9	43.4	45.8
(n-3)/(n-6)	5.4	1.9	1.1	0.7

[a] Includes 15:0, 20:0, and 22:0. [b] Includes 22:1n-11 and 22:1n-9. [c] Includes 16:1n-9 and 20:1n-7. [d] Includes 18:3n-6, 20:3n-6, and 22:4n-6. [e] Includes 20:3n-3 and 22:4n-3.

Sampling Procedure. Samples were taken from all diets and stored at −20 °C until analyzed. After 6 and 12 weeks, the fish were weighed individually and specific growth rate (SGR), thermal growth coefficient

(TGC), and feed conversion ratio (FCR) were determined for each individual. Fish with TGC < 1 for the 0−6 week experimental period were not considered to be representative of the whole population and of the effects of the dietary treatments and, hence, were excluded from all further calculations. At the end of the trial (12th week), five fish per tank were sampled at random from the population in each cage for lipid and FA composition of liver and muscle. The samples from each tank were pooled to one sample, providing three samples per treatment for each tissue. Fish were killed with a sharp blow to the head, and samples of liver were dissected and frozen immediately in dry ice and then stored in a freezer at −20 °C pending analyses. A muscle sample, representative of the edible portion, was obtained by cutting a steak between the dorsal and ventral fins. This section was then skinned, deboned, homogenized, and stored at −20 °C until analyzed.

Proximate Analysis of the Diets. Moisture was measured gravimetrically by thermal drying to constant weight in an oven at 110 °C for 24 h. Crude protein contents were determined by Kjeldahl analyses (nitrogen × 6.25, Kjeltec Autoanalyser, Tecator). Crude fat was determined by acid hydrolysis using a Soxtec System 1047 hydrolyzing unit (Tecator application note 92/87) followed by exhaustive Soxhlet extraction using petroleum ether (40−60 °C, boiling point) on a Soxtec System HT6 (Tecator application note 67/83). Ash content was determined by dry-ashing in porcelain crucibles in a muffle furnace at 600 °C overnight. Extraction of crude fiber was conducted using a Tecator 1020 extraction apparatus at 550 °C for 2 h according to the method described in Tecator application note 01/78. All methods are based on those described in AOAC (*31*) and modified as described by Bell et al. (*32*).

Lipid Extraction and Fatty Acid Analyses. Total lipids of feed, muscle, and liver samples were extracted according to the Folch method (*33*) and, specifically, by homogenization in 20 volumes of chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant. Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification using 2 mL of 1% H_2SO_4 in methanol plus 1 mL of toluene as described by Christie (*34*). The extraction and purification of the FAME were carried out as described by Tocher and Harvie (*35*). FAME were separated and quantified by gas−liquid chromatography (Fisons 8160, Carlo Erba, Milan, Italy.) using a 30 m × 0.32 mm capillary column (CP-wax 52CB; Chrompak Ltd., London, U.K). Hydrogen was used as carrier gas, and temperature programming was from 50 to 150 °C at 40 °C/min and then to 225 °C at 2 °C/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (*36*).

Statistical Analysis. Data are presented as means ± standard deviation (SD) ($n = 3$). Significant differences between dietary treatments were determined by one-way ANOVA followed by Tukey's post-hoc test to rank the groups ($P = 0.05$). Percentage data and data that were identified as nonhomogeneous (Lavene's test) were subjected to square-root, log, or arcsine transformation before analysis. Differences were regarded as significant at $P < 0.05$ (*37*). ANOVA and regression analyses were performed using SPSS 13 (SPSS Inc., 2004). The Δ values represent the difference between diet and muscle or liver FA concentrations and were calculated on the basis of the percentages of total FA in diet and tissues. Negative Δ values indicate lower values in the tissue compared with diet, whereas positive values indicate accumulation in muscle or liver relative to diet.

RESULTS

Diets. Proximate analysis of the four experimental diets showed that the dietary protein and fat levels were approximately 500 and 160 g kg^{-1}, respectively, and constant between the dietary treatments (**Table 1**). However, there was an increase in dietary fiber with graded inclusion of FFS from 8.4 g kg^{-1} in the FFS0 diet to 16.4 g kg^{-1} in the FFS36 diet.

The increased inclusion of FFS at the expense of FM and FO had a direct effect in the FA compositions of the diets (**Table 2**). In particular, 14:0 decreased and 16:0 remained stable, whereas 18:0 increased, resulting in a slight decrease for the total saturates (from 22.5% in FFS0 to 20.9% in FFS36). Total monoenes were reduced from 41.4% in the control group to 33.3% in the FFS36, mainly due to the respective decrease of 16:1n-7, 20:1n-9, and 22:1 (including 22:1n-11 and 22:1n-9) almost by half. However, there was a major increase in 18:1n-9 from 8.5% to 13.9%, for FFS0 and FFS36, respectively. The high content of 18:2n-6 in FFS resulted in an increase of that FA by almost 6-fold and a subsequent increase in total n-6 PUFA, although the rest of the n-6 PUFA remained stable, totalling less than 1%. 18:3n-3 was increased by >2-fold between FFS0 and FFS36. Nevertheless, EPA and DHA were decreased by half. In addition, total n-3 also decreased, from 29.1% to 18.3%, for FFS0 and FFS36, respectively. The n-3/n-6 ratio decreased from 5.4 in FFS0 to 0.7 in FFS36.

Table 3. Growth and Performance of Cod Fed the Experimental Diets for 12 Weeks[a]

	FFS0	FFS12	FFS24	FFS36
length (cm)				
start	276.1 ± 6.1	273.5 ± 2.6	270.8 ± 11.5	269.8 ± 7.3
6 weeks	305.6 ± 6.1	298.8 ± 3.4	298.4 ± 11.7	295.4 ± 7.4
12 weeks	343.0 ± 8.4	330.5 ± 3.7	333.1 ± 12.1	364.7 ± 55.8
weight (g)				
start	226.4 ± 6.9	229.6 ± 9.9	219.1 ± 30.2	212.5 ± 17.0
6 weeks	349.9 ± 11.6	327.0 ± 16.8	324.3 ± 42.5	300.3 ± 20.1
12 weeks	562.2 ± 35.9	489.2 ± 23.9	488.5 ± 51.2	466.7 ± 23.6
SGR[b]				
0–6 weeks	1.03 ± 0.01a	0.85 ± 0.01bc	0.94 ± 0.03b	0.82 ± 0.05c
6–12 weeks	1.12 ± 0.08	0.94 ± 0.10	0.97 ± 0.08	1.05 ± 0.07
overall	1.08 ± 0.04a	0.89 ± 0.05b	0.95 ± 0.04ab	0.94 ± 0.06b
TGC[c]				
0–6 weeks	2.98 ± 0.02a	2.42 ± 0.11bc	2.65 ± 0.13b	2.28 ± 0.09c
6–12 weeks	3.09 ± 0.26a	2.49 ± 0.22b	2.53 ± 0.13ab	2.70 ± 0.17ab
overall	3.09 ± 0.14a	2.49 ± 0.18b	2.63 ± 0.04b	2.52 ± 0.13b
FCR[d]				
0–6 weeks	0.71 ± 0.03	0.88 ± 0.10	0.77 ± 0.02	0.92 ± 0.13
6–12 weeks	0.66 ± 0.01b	0.83 ± 0.03a	0.77 ± 0.04a	0.77 ± 0.03a
overall	0.68 ± 0.02b	0.84 ± 0.05a	0.77 ± 0.01ab	0.82 ± 0.01a
K[e]				
start	1.07 ± 0.04	1.12 ± 0.03	1.09 ± 0.02	1.07 ± 0.05
6 weeks	1.21 ± 0.04	1.22 ± 0.04	1.21 ± 0.03	1.15 ± 0.03
12 weeks	1.38 ± 0.02	1.34 ± 0.03	1.33 ± 0.05	1.28 ± 0.03
mortalities	0	1	0	0

[a] Values are mean ± SD. Values within a row with a different letter are significantly different ($P < 0.05$). [b] Specific growth rate (%/day) = 100 × [ln(final *W*) − ln(initial *W*)]/days. [c] Thermal growth coefficient (× 1000): $1000 \times [(\text{final } W)^{1/3} - (\text{start } W)^{1/3}] \times (\text{days} \times °C)^{-1}$. [d] Feed conversion ratio = feed intake (g)/weight gain (g). [e] Condition factor = 100 × (BW, g) × (fork length, cm)$^{-3}$.

Growth, Feed Efficiency, and Mortality. All groups showed good performance results regarding weight gain, FCR, SGR, and TGC at 6 and 12 weeks (**Table 3**). Fish weights varied from 300 to 350 g and from 467 to 562 g at 6 and 12 weeks, respectively, with the fish fed the control diet having the highest weight gain, although the differences between the groups were not statistically significant. The same trend was shown in SGR (ranging between 0.89 and 1.08) and TGC (varying from 2.49 to 3.09) over the whole experimental period. Fish fed on FFS0 had significantly higher SGR than fish fed on FFS12 and FFS36 and significantly higher TGC than all of the other treatments. In both cases there were no significant differences between the groups fed the FFS. No significant differences were observed in condition factor (*K*), which varied between 1.28 and 1.38 over the 12 weeks of the trial. With regard to FCR the range, over the whole experimental period, was from 0.68 for the FFS0 group to 0.84 for the FFS12. The fish fed the FFS0 diets had significantly lower FCR than the fish fed diets containing FFS.

Fatty Acid Composition of Muscle and Liver. The FA compositions of the cod muscle and liver (**Tables 4** and **5**) were

Table 4. Total Lipid (Milligrams of Lipid per Gram of Tissue) and Fatty Acid Compositions (Percent by Weight of Total Fatty Acids) of Muscle from Cod Fed the Experimental Diets for 12 Weeks[a]

	FFS0	FFS12	FFS24	FFS36
total lipid	9.6 ± 1.6	8.7 ± 1.3	9.9 ± 1.8	10.2 ± 0.5
fatty acid				
14:0	1.9 ± 0.3a	1.6 ± 0.1a	1.2 ± 0.1b	0.8 ± 0.1b
16:0	22.4 ± 2.3	23.3 ± 1.8	20.8 ± 1.5	19.7 ± 2.5
18:0	4.9 ± 1.2	4.4 ± 0.4	4.5 ± 0.8	5.0 ± 0.7
total saturated[b]	30.1 ± 4.7	30.4 ± 2.9	27.2 ± 2.6	26.2 ± 4.0
16:1n-7	2.7 ± 0.0a	2.4 ± 0.3ab	2.0 ± 0.2bc	1.5 ± 0.1c
18:1n-9	9.8 ± 0.3	9.8 ± 1.0	10.6 ± 0.4	9.5 ± 0.9
18:1n-7	2.3 ± 0.1a	2.2 ± 0.2ab	2.3 ± 0.1ab	1.9 ± 0.1b
20:1n-9	3.5 ± 0.4a	3.2 ± 0.4a	2.6 ± 0.2ab	2.0 ± 0.3b
22:1[c]	1.7 ± 0.0a	1.8 ± 0.0a	1.4 ± 0.1b	1.1 ± 0.1c
24:1n-9	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.2
total monoenes[d]	21.0 ± 1.1a	20.0 ± 2.0ab	19.7 ± 1.0ab	16.7 ± 1.4b
18:2n-6	3.8 ± 0.1c	8.3 ± 0.6b	13.4 ± 0.2a	15.0 ± 1.0a
20:2n-6	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.3 ± 0.3
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
20:4n-6	0.9 ± 0.1	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.1
total n-6 PUFA[e]	5.4 ± 0.1d	10.0 ± 0.7c	15.0 ± 0.2b	16.7 ± 0.8a
18:3n-3	0.9 ± 0.0b	1.2 ± 0.1b	1.6 ± 0.1a	1.6 ± 0.2a
18:4n-3	1.5 ± 0.1a	1.3 ± 0.1ab	1.2 ± 0.1b	0.9 ± 0.1c
20:4n-3	0.6 ± 0.0a	0.5 ± 0.0b	0.5 ± 0.0b	0.4 ± 0.0c
20:5n-3	14.7 ± 0.7a	12.9 ± 0.5b	12.5 ± 0.6b	11.8 ± 0.7b
22:5n-3	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.0	1.1 ± 0.1
22:6n-3	24.6 ± 2.9	22.5 ± 1.2	21.3 ± 0.7	24.8 ± 2.6
total n-3 PUFA[f]	43.5 ± 3.5	39.6 ± 1.8	38.1 ± 1.5	40.5 ± 3.2
total PUFA	49.0 ± 3.6b	49.7 ± 1.7ab	53.1 ± 1.6a	57.2 ± 2.9a
(n-3)/(n-6)	8.0 ± 0.4a	4.0 ± 0.4b	2.5 ± 0.1c	2.4 ± 0.3c

[a] Values are mean ± SD ($n = 3$). Values within a row with a different letter are significantly different ($P < 0.05$). [b] Includes 15:0, 20:0, and 22:0. [c] Includes 22:1n-11 and 22:1n-9. [d] Includes 16:1n-9 and 20:1n-7. [e] Includes 18:3n-6, 20:3n-6, and 22:4n-6. [f] Includes 20:3n-3 and 22:4n-3.

Table 5. Total Lipid (Milligrams of Lipid per Gram of Tissue) and Fatty Acid Compositions (Percent by Weight of Total Fatty Acids) of Liver from Cod Fed the Experimental Diets for 12 Weeks[a]

	FFS0	FFS12	FFS24	FFS36
total lipid	575.7 ± 55.4	604.0 ± 53.2	592.9 ± 24.2	529.3 ± 33.0
fatty acid				
14:0	3.7 ± 0.2a	3.2 ± 0.2a	2.7 ± 0.2b	2.3 ± 0.1b
16:0	17.6 ± 0.9a	16.9 ± 0.5a	15.7 ± 0.5a	13.5 ± 0.8b
18:0	4.3 ± 0.5	4.4 ± 0.4	4.7 ± 0.1	3.8 ± 0.4
total saturated[b]	26.1 ± 1.8a	25.2 ± 0.9a	23.8 ± 1.0a	19.9 ± 1.3b
16:1n-7	5.6 ± 0.1a	5.1 ± 0.1b	4.3 ± 0.2c	3.9 ± 0.1d
18:1n-9	16.0 ± 0.9b	16.7 ± 0.7b	18.6 ± 0.1a	19.4 ± 0.4a
18:1n-7	3.4 ± 0.3	3.3 ± 0.2	3.0 ± 0.3	3.1 ± 0.1
20:1n-9	10.7 ± 0.2a	9.6 ± 0.3b	7.8 ± 0.2c	6.9 ± 0.4d
22:1[c]	9.6 ± 0.3a	8.3 ± 0.2b	6.4 ± 0.2c	4.9 ± 0.1d
24:1n-9	0.6 ± 0.0a	0.5 ± 0.0ab	0.4 ± 0.0bc	0.3 ± 0.1c
total monoenes[d]	46.4 ± 1.0a	43.9 ± 1.3b	40.8 ± 0.1c	39.0 ± 0.4c
18:2n-6	4.6 ± 0.1d	10.2 ± 0.5c	16.2 ± 0.3b	22.8 ± 0.5a
20:2n-6	0.3 ± 0.0a	0.3 ± 0.0b	0.4 ± 0.0c	0.4 ± 0.0d
20:3n-6	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
20:4n-6	0.3 ± 0.0a	0.3 ± 0.0ab	0.2 ± 0.0b	0.2 ± 0.0b
total n-6 PUFA[e]	5.5 ± 0.1d	11.1 ± 0.5c	17.0 ± 0.3b	23.6 ± 0.5a
18:3n-3	1.3 ± 0.1d	1.7 ± 0.1c	2.3 ± 0.0b	3.0 ± 0.1a
18:4n-3	3.0 ± 0.3a	2.6 ± 0.2ab	2.3 ± 0.0bc	1.9 ± 0.1c
20:4n-3	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1
20:5n-3	7.4 ± 0.9a	6.5 ± 0.6ab	5.8 ± 0.2b	5.3 ± 0.3b
22:5n-3	0.7 ± 0.1a	0.6 ± 0.0ab	0.5 ± 0.0ab	0.5 ± 0.1b
22:6n-3	9.0 ± 1.1a	7.9 ± 0.7ab	7.0 ± 0.4b	6.4 ± 0.5b
total n-3 PUFA[f]	22.0 ± 2.6a	19.9 ± 1.8ab	18.4 ± 0.7ab	17.6 ± 1.0b
total PUFA	27.5 ± 2.7c	30.9 ± 2.3bc	35.4 ± 1.0ab	41.1 ± 0.9a
(n-3)/(n-6)	4.0 ± 0.4a	1.8 ± 0.1b	1.1 ± 0.0c	0.7 ± 0.1c

[a] Values are mean ± SD ($n = 3$). Values within a row with a different letter are significantly different ($P < 0.05$). [b] Includes 15:0, 20:0, and 22:0. [c] Includes 22:1n-11 and 22:1n-9. [d] Includes 16:1n-9 and 20:1n-7. [e] Includes 18:3n-6, 20:3n-6, and 22:4n-6. [f] Includes 20:3n-3 and 22:4n-3.

highly affected by the dietary treatment. The main effect on both tissues was the significant increase in the concentration of linoleic acid, with increasing FFS level. In particular, 18:2n-6 in muscle increased 4-fold, from 3.8 to 15%, for the FFS0 and FFS36 groups, respectively, whereas in liver it increased 5-fold, from 4.6 to 22.8%.

In muscle (**Table 4**), further to the increase in 18:2n-6 with graded inclusion of FFS, the concentration of total n-6 PUFA also increased (from 5.4 to 16.7%, for FFS0−FFS36, respectively). Moreover, 18:3n-3 significantly increased from 0.9 to 1.6% between the control group and FFS36. However, there were significant reductions in 20:1n-9 and 22:1 (including 22:1n-11 and 22:1n-9) (from 3.5 and 1.7% in FFS0 to 2.0 and 1.1% in FFS36, respectively) and the total monoenes (from 21 to 16.7% for FFS0 and FFS36, respectively). The EPA concentration was also decreased from 14.7% in FFS0 to 11.8% in FFS36, although there were no significant differences between the three groups fed the FFS. The inclusion of FFS resulted in a significant reduction in the n-3/n-6 ratio, from 8.0 to 2.4 for the FFS0 and FFS36 groups, respectively. Finally, no significant differences were shown in the concentrations of 16:0, 18:0, total saturated FA, 18:1n-9, 20:4n-6, DHA, and total n-3 PUFA.

Similarly, in liver (**Table 5**), the dietary inclusion of FFS and the consequent major increase in the concentration of 18:2n-6 resulted in a significant increase in total n-6 PUFA (from 5.5 to 23.6% for FFS0 and FFS36, respectively). Moreover, 18:1n-9 and 18:3n-3 were also significantly increased from 16.0 and 1.3% to 19.4 and 3.0%, respectively. In contrast, significant reductions were observed between FFS0 and FFS36 in 16:0 (17.6−13.5%), total saturated (26.1−19.9%), 20:1n-9 (10.7−6.9%), 22:1 (including 22:1n-11 and 22:1n-9) (9.6−4.9%), 24:1n-9 (0.6−0.3%), total monoenes (46.4−39.0), 20:4n-6 (0.3−0.2%), EPA (7.4−5.3%), DHA (9.0−6.4%), total n-3 PUFA (22.0−17.6%), and the n-3/n-6 ratio (4.0−0.7).

The concentrations of specific FA in muscle were plotted against the respective dietary FA concentrations. The plots of muscle FA against dietary FA concentrations are shown in **Figure 1**, and the correlation coefficients (r), slopes, Y-axis intercepts, and F and P values from these plots, including the difference (Δ) between diet and muscle FA values for the FFS0 and the FFS36 groups, are shown in **Table 6**. There was a significant linear correlation between dietary and muscle 18:2n-6 and 20:5n-3 (panels **a** and **e** of **Figure 1**, respectively; the correlation coefficients, r, were 0.99 and 0.96, respectively). Moreover, a similar trend, although not significant, was shown for 18:3n-3 and 22:1 (panels **b** and **d** of **Figure 1**, respectively), with r values of 0.95 and 0.94, respectively. However, the slopes and the intercepts of the linear plots of these FA differed, indicating a different relationship between the dietary and muscle concentrations for each individual FA. This is also supported by the Δ values shown in **Table 6**, where negative Δ values indicate lower values in muscle compared with diet and positive values indicate accumulation in tissues relative to diet. Hence, EPA and DHA were present in higher concentrations in muscle compared to diet in both the FFS0 and FFS36 diets, although at a higher level in the latter. In contrast, the inclusion of FFS in the diet (FFS36) resulted in lower concentrations of 18:2n-6, 18:3n-3, and 18:1n-9 in muscle lipid relative to diet lipid. It is noteworthy that the regression analysis of 18:1n-9 and 22:6n-3 (panels **c** and **f** of **Figure 1**, respectively) resulted in almost zero r and slope values and not significant correlations.

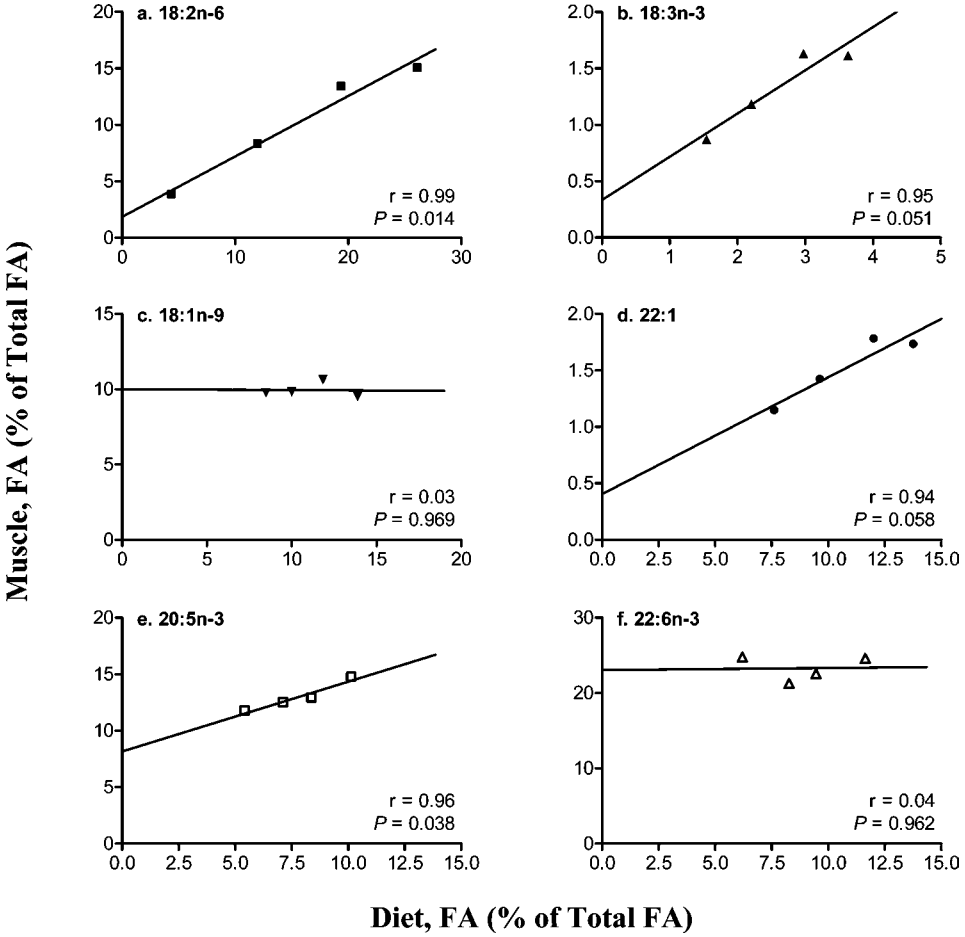

Figure 1. Relationship between dietary fatty acid concentrations and muscle fatty acid concentrations of 18:2n-6 (**a**), 18:3n-3 (**b**), 18:1n-9 (**c**), 22:1 (**d**), 20:5n-3 (**e**), and 22:6n-3 (**f**) in total lipids of cod fed diets containing increasing levels of full-fat soybean meal (FFS).

Table 6. Correlation Coefficients (*r*), Slopes, *Y*-Axis Intercepts, and *F* and *P* Values from Plots of Dietary Fatty Acid Concentrations versus Muscle Fatty Acid Concentrations for Cod Fed the Four Experimental Diets, Including the Difference (Δ)[a] between Diet and Muscle Fatty Acid Values for the Full-Fat Soybean Meal (FFS0) and the FFS36 Groups[b]

fatty acid	*r*	slope	*Y*-axis intercept	*F*	*P*	Δ FFS0	Δ FFS36
18:2n-6	0.99	0.534	1.860	70.563	0.014	–0.6	–11.1
18:3n-3	0.95	0.383	0.332	18.088	0.051	–0.7	–2.0
18:1n-9	0.03	–0.006	10.011	0.002	0.969	1.3	–4.4
22:1[c]	0.94	0.103	0.405	15.768	0.058	–12.1	–6.6
20:5n-3	0.96	0.617	8.164	24.605	0.038	4.6	6.3
22:6n-3	0.04	0.028	23.043	0.003	0.962	13.0	18.6

[a] Negative Δ values indicate lower values in muscle compared with diet, whereas positive values indicate accumulation in muscle relative to diet. [b] Fatty acid concentrations are percentages of total fatty acids in diet and muscle. [c] Includes 22:1n-11 and 22:1n-9.

The plots of liver FA concentrations against the dietary FA concentrations are shown in **Figure 2**, and the correlation coefficients (*r*), slopes, *Y*-axis intercepts, and *F* and *P* values from these plots, including the difference (Δ) between diet and liver FA values for the FFS0 and the FFS36 groups, are shown in **Table 7**. The regression analysis and the plots of the concentrations of 18:2n-6, 18:3n-3, 18:1n-9, 22:1, 20:5n-3, and 22:6n-3 in the diet against their concentration in the liver showed significant linear correlations for all of them (correlation coefficients *r* ranging from 0.98 to 1.00). Similarly to muscle, the linear relationships between dietary and liver FA concentration differ between the individual FA as the slope values ranged from 0.5 to 0.8 and the intercepts from −1.0 to 10.4. This is also shown by the Δ values shown in **Table 7**. The positive Δ values of 18:1n-9 both for FFS0 and for FFS 36 indicate a higher concentration of this FA in the liver compared to the diet. On the contrary, hardly any other FA was in a higher concentration in the liver than in the diet. It should be mentioned, though, that EPA and DHA had higher Δ values in FFS36 than in FFS0, showing a lower utilization and/or increased retention of these FA with the inclusion of FFS.

DISCUSSION

All groups showed good performance, for cod of this size, with regard to weight gain, FCR, SGR, and TGC at 6 and 12 weeks (**Table 3**). However, in this study a negative effect of the FFS inclusion was clearly shown. In particular, the fish fed the FFS diets showed poorer growth (expressed as SGR and TGC) compared to the control group (FFS0). Both SGR and TGC were significantly lower between the FFS0 and the rest of the groups in the first period of the trial (0−6 weeks), whereas in the second period (6−12 weeks) no significant differences were shown in SGR and only FFS0 and FFS12 differ significantly with regard to TGC. Nevertheless, the differences of the first period probably affected the overall growth of the fish, and significant differences between the fish fed the control diet and the fish fed the FFS diets were observed.

These results are in agreement with previous studies in Atlantic cod fed diets containing soybean meal (*16*) and could be due to the presence of ANF in FFS (*15*). However, Von der

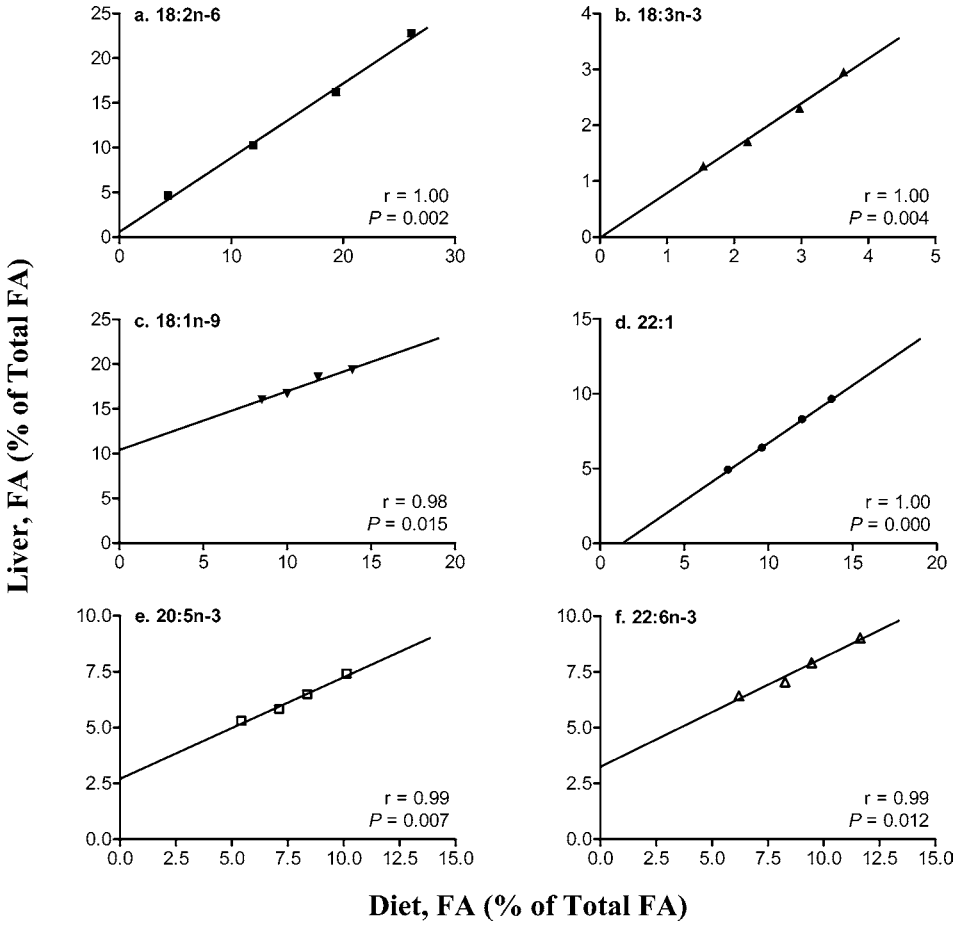

Figure 2. Relationship between dietary fatty acid concentrations and liver fatty acid concentrations of 18:2n-6 (**a**), 18:3n-3 (**b**), 18:1n-9 (**c**), 22:1 (**d**), 20:5n-3 (**e**), and 22:6n-3 (**f**) in total lipids of cod fed diets containing increasing levels of full-fat soybean meal (FFS).

Table 7. Correlation Coefficients (*r*), Slopes, *Y*-Axis Intercepts, and *F* and *P* Values from Plots of Dietary Fatty Acid Concentrations versus Liver Fatty Acid Concentrations for Cod Fed the Four Experimental Diets, Including the Difference (Δ)[a] between Diet and Liver Fatty Acid Values for the Full-Fat Soybean Meal (FFS0) and the FFS36 Groups[b]

fatty acid	*r*	slope	*Y*-axis intercept	*F*	*P*	Δ FFS0	Δ FFS36
18:2n-6	1.00	0.831	0.566	482.934	0.002	0.2	–3.4
18:3n-3	1.00	0.801	–0.014	270.867	0.004	–0.3	–0.7
18:1n-9	0.98	0.657	10.407	63.421	0.015	7.5	5.5
22:1[c]	1.00	0.773	–1.021	5662.108	0.000	–4.2	–2.8
20:5n-3	0.99	0.454	2.706	132.046	0.007	–2.8	–0.2
22:6n-3	0.99	0.489	3.248	82.646	0.012	–2.6	0.2

[a] Negative Δ values indicate lower values in muscle compared with diet, whereas positive values indicate accumulation in muscle relative to diet. [b] Fatty acid concentrations are percentages of total fatty acids in diet and muscle. [c] Includes 22:1n-11 and 22:1n-9.

Decken and Lied (*16*) concluded that only inclusion of FFS above 300 g kg^{-1} negatively affected the growth of the fish. In the present study the negative effects were shown even with 120 g kg^{-1} inclusion. It is noteworthy that there were hardly any significant differences between the FFS diets. This indicates that the negative effect was unrelated to the inclusion level and was probably related to a general effect of the FFS on the physiology and consequently the growth of the fish. This is likely due, at least in part, to a change in diet that would probably affect the palatability and therefore the consumption rate by the fish in the initial few weeks. Fish, especially cod, are often very sensitive to a dietary change, and this could have occurred here, although changes in digestibility related to ANF, etc., may also have been a factor.

Previous studies in cod have demonstrated that tissue FA compositions reflect the dietary FA composition (*3*, *23*−*27*, *38*). This was clearly shown in the present study, both in muscle and in liver (**Tables 4** and **5**). Specifically, in muscle linear relationships between dietary and tissue FA concentrations were shown for 18:2n-6 and 20:5n-3 and a similar tendency for 18:3n-3 and 22:1 (**Figure 1**). In liver, 18:2n-6, 18:3n-3, 18:1n-9, 22:1, 20:5n-3, and 22:6n-3 were linearly related to dietary FA (**Figure 2**). Linear relationships between dietary and tissue FA compositions have been previously demonstrated in salmonids (*32*, *39*). In agreement with these studies, the linear correlations obtained in the present trial revealed differences between the relationships of dietary and tissue FA for each individual FA, as slopes and intercepts and also Δ values differed between FA (**Tables 6** and **7**). In particular, EPA and DHA were present in higher concentrations in muscle compared to diet in both the FFS0 and FFS36 diets, although in a higher level in the latter, whereas in liver the concentrations were higher than in the diet only in the FFS36 group. In line with previous studies, these results suggest that when these FA were provided to the fish in low concentrations they were selectively retained in the tissues (*39*). The differences in retention and utilization of specific FA in muscle and liver are related to the different functions of FA in the two tissues. The liver is the primary lipid storage organ in cod, and thus most of the FA are stored as triacylglycerols, whereas the lean muscle tissue has most of its FA contained in membrane phospholipids. Because HUFA, especially EPA and DHA, are vital for the function of cell membranes, the retention

of these HUFA in phospholipids is generally more efficient than in triacylglycerols (*22*).

It is important to point out that no significant difference was shown between treatments in muscle DHA, whereas EPA was only moderately decreased. This is important from the perspective of the nutritional quality of the product for the human consumer, as long-chain HUFA play an important role with regard to human health (*28*−*30*). However, although lipid levels in muscle are low, the high levels of HUFA present in phospholipids of lean fish are an important source of FA for the consumer. Nevertheless, in liver, EPA and DHA were significantly reduced between fish fed the FFS0 and the FFS36 diets, although the reductions were still moderate.

In muscle and liver, 18:2n-6 and 18:3n-3 were found in much lower concentrations than in the diet when the fish were fed the FFS diets, indicating that when these FA were abundant in the diet, they were selectively utilized for metabolism, probably for energy production (*40*, *41*). However, these FA are the precursors of HUFA, through Δ6 desaturation and elongation (*22*), and numerous studies in salmonids have shown that the replacement of FO with vegetable oils significantly enhances these pathways (*42*, *43*). Hence, the utilization by the fish of these FA demonstrated in the present study could also be for the synthesis of HUFA, in addition to the oxidation for energy production. However, the desaturation and elongation activities in Atlantic cod, even when fed high levels of vegetable oils, are very low (*44*) and, as was observed in rainbow trout, >90% of dietary 18:3n-3 is used for energy production (*45*).

Similarly, 22:1 was also utilized in all treatments; however, the inclusion of FFS resulted in lower dietary concentrations of this FA and a subsequent lower utilization compared to the FFS0 group (*40*, *41*). Interestingly, the concentration of 18:1n-9 in liver was higher than in the diet, suggesting a preferential retention and/or endogenous synthesis, whereas in muscle it presented a negative Δ value for the fish fed the FFS36 diet. Because no significant differences were shown by ANOVA in muscle 18:1n-9, it is clear that only the excess of this FA provided by the FFS diets was used by the fish for metabolism, whereas a specific amount was constantly retained in muscle. These results suggest that in cod 22:1, 18:2n-6, and 18:3n-3 are preferred substrates for oxidation, in agreement with studies in Atlantic salmon (*39*).

It is worth mentioning that the effects of the dietary FA composition on tissue FA composition were clearer in liver than in muscle; for instance, in muscle the regression analysis of 18:1n-9 and 22:6n-3 resulted in nonsignificant correlations. This is obviously due to the different lipid class composition of these two tissues; in cod, and gadoid fish in general, muscle is mainly constituted by polar lipids of the cell membranes, whereas the major lipid class of the liver is triacylglycerols (*22*).

The results of this study showed that FFS can be used to partially replace FM and FO in diets for cod with no major detrimental effects on growth and tissue FA compositions, although specific FA of muscle and liver are significantly affected at high FFS inclusion levels.

ABBREVIATIONS USED

FFS, full-fat soybean meal; FM, fish meal; FO, fish oil; DHA, docosahexaenoic acid (22:6n-3); EPA, eicosapentaenoic acid (20:5n-3); HUFA, highly unsaturated fatty acids (carbon chain length ≥ C_{20} with ≥3 double bonds); PUFA, polyunsaturated fatty acid.

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