Dietary Rapeseed Oil Affects the Expression of Genes Involved in Hepatic Lipid Metabolism in Atlantic Salmon (Salmo salar L.)


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ABSTRACT Supplies of marine fish oils (FO) are limited, and sustainable production in aquaculture dictates that alternatives that do not compromise fish health and product quality, such as vegetable oils, must be found. Nutrigenomics will increase our understanding of how nutrition influences metabolic pathways and homeostatic control, and may be used to measure and validate subtle changes in organ-specific, metabolic gene expression signatures. We compared 2 groups of Atlantic salmon fed diets containing 100% FO or 75% rapeseed oil (RO) for 42 wk. A small-scale cDNA microarray was constructed to screen for changes in the expression of lipid metabolism genes in the liver resulting from this partial substitution of RO for FO. Δ5 fatty acid desaturase gene expression was significantly greater in fish fed 75% RO than in fish fed the control diet; this was confirmed by quantitative real time PCR analysis. In addition, several genes, among these mitochondrial proteins, peroxisome proliferator-activated receptor γ, as well as other transcription factors, coactivators, and signal transducers, showed significant differential regulation. This partially validated microarray may be used for further gene expression profiling using other dietary comparisons, and for further characterization of selected genes. J. Nutr. 135: 2355–2361, 2005.

KEY WORDS: fatty acid, microarray, quantitative PCR, lipid metabolism, Atlantic salmon

Partial substitution of marine fish oils (FO) by vegetable oils in fish diets has become increasingly common in aquaculture, due to limited supplies of FO (1). These dietary changes are thought to influence lipid metabolic gene expression patterns in liver of Atlantic salmon, as in mammals, where changes in quantity or composition of dietary fat affect hepatic de novo lipogenesis, VLDL synthesis and secretion, fatty acid oxidation, and cholesterol metabolism (2). Several genes involved in these metabolic pathways are regulated in mammals through response elements for nuclear receptors. Hepatic nuclear factor-4α (HNF-4α) positively regulates apolipoprotein (apo) C-II (3) and apo A-I gene expression (3).

The (n-3) and (n-6) fatty acids and other peroxisome proliferator-activated receptor (PPAR)α activators induce transcription of liver X receptor (LXR)-α through DR1 elements and regulate the expression of apo C-II (5) and sterol regulatory element binding protein (SREBP)-1c (6). In addition, Δ9-fatty acid desaturase (SCD) (2,7), Δ6-, and Δ5- fatty acid desaturases (FADs) were induced by elevated SREBP-1c and PPARα ligands. PPARα is required for the transcription of mitochondrial enzymes such as long-chain acyl CoA synthetase (long-chain ACS) (2) and peroxisomal bi-functional enzyme containing enoyl CoA hydratase (9).

The influences of dietary lipids on hepatic de novo lipogenesis, lipoprotein metabolism, fatty acid oxidation, and cholesterol metabolism (10–12) as well as nutritional effects on fatty acid desaturation and elongation activities (13,14) in Atlantic salmon (Salmo salar) have been thoroughly assayed. However, little is known about the expression of hepatic genes involved in lipid metabolism in salmonids. Fatty acids regulate the expression of genes involved in fatty acid desaturation and elongation (15). Leaver and co-workers (16) characterized PPARs from 3 fish species, including Atlantic salmon (unpublished results). All PPARs were detected in all tissues studied, and it was suggested that fish PPARs bind a wide range of fatty acids. PPARα, involved in the induction of mammalian FADs (2,7,8), was particularly responsive to unsaturated fatty acids.

Hepatic mitochondrial β-oxidation exhibits substrate specificity in fish (17–19), and carnitine palmitoyltransferase (CPT)-II, which is induced by 20:5(n-3) and 22:6(n-3) in rat

1 All funding was provided through “RAFOA, Researching Alternatives to Fish Oils in Aquaculture,” Q5RS-2000–30058 funded by the European Commission.

2 Supplemental Table 1 is available as Online Supporting Material with the online posting of this paper at www.nutrition.org.

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5 Abbreviations used: ACS, acyl-CoA synthetase; apo, apolipoprotein; CLA, conjugated linoelic acid; CPT, carnitine palmitoyltransferase; Cy, cytochromes; EST, expressed sequence tag; FAD, fatty acid desaturase; FO, fish oil; HNF-4, hepatic nuclear factor-4; HUFA, highly unsaturated fatty acid; (CPT)-II, which is induced by 20:5(n-3) and 22:6(n-3) in rat

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liver (20), has been used as a marker for mitochondrial $\beta$-oxidation activity in the liver of brown trout (Salmo trutta L.) (21) and Atlantic salmon (22,23). However, to date, no one has studied mitochondrial $\beta$-oxidation gene expression patterns in salmon.

In the present study, we examined whether nutrigenomics could be used as a tool for the analysis of nutrient-gene interactions in aquaculture nutrition. Because large-scale fish-specific arrays were not available at the time of the study, a small-scale lipid metabolism cDNA microarray was designed using amplicons from available sequenced cDNAs. This microarray was then used to determine the effects of dietary oil on hepatic gene expression in salmon fed either a 100% FO or a 75% rapeseed oil (RO) diet in a large-scale nutritional trial. Samples were pooled to ensure low variability and a normal distribution within the population. In accordance with this, an experimental design was chosen using a high number of technical replicates to validate the array itself for use in further studies. To further validate some of the result on the array quantitative real time PCR (Q-PCR) analyses were performed to confirm some of the results from the microarray profiling research.

**MATERIALS AND METHODS**

**Dietary experiment**

The dietary experiment was performed at Gildeksaal Research Station, Inndyr, Norway as described in more detail in Torstensen et al. (24). In wk 21, 2001, ~600 postsmolt Atlantic salmon, mean body weight 0.142 kg, were randomly distributed to 7 net pens, of 125 m$^3$ each, and fed a control diet containing 100% FO (2 replicates) or experimental diets in which FO was replaced by 0, 25, 50, 75, and 100% RO or 50% olive oil (OO) (one replicate for each oil concentration) in a linear regression design for 42 wk (Table 1). Diets, produced by Nutreco ARC, differed only in their oil composition. The chemical compositions of diets were (g/kg): lipid, 300; protein, 450; moisture, 60; ash, 70; and nitrogen-free extract, 120.

Due to low expected differences in gene expression between groups at low RO or OO inclusion, lower mean body weight (bulk weighing) in the 100% RO group after 22 wk and the high analytical cost of microarray construction and analysis, the 100% FO and 75% RO diets were selected as candidates for technical validation of microarrays and microarray analysis of a number of hepatic lipid metabolism genes.

**Sampling procedure**

Samples were collected during wk 22 and 42 for Q-PCR analysis, and during 42 wk for microarray analysis. The fish were deprived of food for 24 h before sampling. Randomly sampled fish from each tank, (i.e., dietary treatment) were anesthetized with methomidate (7 g/L; Norsk Medisinaldepot) before being killed with a blow to the head. Liver samples taken from 30 individual fish from each dietary group were pooled and preserved in RNAlater® (Ambion) according to the manufacturer’s recommendations. For RT-PCR analysis, liver samples were immediately removed from 6 individual fish from each dietary group, snap-frozen in liquid nitrogen, and stored at −80°C. The protocol was approved by the local representatives for the Norwegian State Board of Biological Experiments with Living Animals.

**TABLE 1**

**Dietary fatty acid composition of the experimental diets**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>100% FO</th>
<th>25% RO</th>
<th>50% RO</th>
<th>75% RO</th>
<th>100% RO</th>
<th>50% OO</th>
<th>75% OO</th>
<th>100% OO</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>6.7</td>
<td>5.0</td>
<td>3.6</td>
<td>2.1</td>
<td>0.4</td>
<td>3.4</td>
<td>14.4</td>
<td>10.8</td>
</tr>
<tr>
<td>16:0</td>
<td>11.6</td>
<td>10.0</td>
<td>8.9</td>
<td>7.5</td>
<td>5.7</td>
<td>11.7</td>
<td>25.0</td>
<td>21.6</td>
</tr>
<tr>
<td>18:0</td>
<td>1.0</td>
<td>1.2</td>
<td>1.4</td>
<td>1.6</td>
<td>1.7</td>
<td>1.9</td>
<td>2.1</td>
<td>2.6</td>
</tr>
<tr>
<td>SFA</td>
<td>20.4</td>
<td>17.4</td>
<td>14.9</td>
<td>12.5</td>
<td>9.1</td>
<td>18.0</td>
<td>43.9</td>
<td>37.5</td>
</tr>
<tr>
<td>16:1(n-9)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>—</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>11.2</td>
<td>22.5</td>
<td>32.4</td>
<td>42.4</td>
<td>53.6</td>
<td>37.3</td>
<td>24.1</td>
<td>48.5</td>
</tr>
<tr>
<td>FO</td>
<td>75%</td>
<td>100%</td>
<td>RO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:1(n-11)</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>17.1</td>
<td>13.3</td>
<td>9.8</td>
<td>6.1</td>
<td>2.1</td>
<td>9.0</td>
<td>36.9</td>
<td>28.5</td>
</tr>
<tr>
<td>22:1(n-11)</td>
<td>13.3</td>
<td>10.1</td>
<td>7.3</td>
<td>4.3</td>
<td>1.0</td>
<td>5.4</td>
<td>28.6</td>
<td>21.8</td>
</tr>
<tr>
<td>22:1(n-9)</td>
<td>2.0</td>
<td>1.6</td>
<td>1.3</td>
<td>0.9</td>
<td>0.5</td>
<td>1.1</td>
<td>4.4</td>
<td>3.5</td>
</tr>
<tr>
<td>MUFAs</td>
<td>57.1</td>
<td>58.4</td>
<td>59.6</td>
<td>60.4</td>
<td>61.0</td>
<td>61.2</td>
<td>123.0</td>
<td>125.6</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>3.5</td>
<td>7.6</td>
<td>11.5</td>
<td>15.4</td>
<td>19.5</td>
<td>19.7</td>
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<td>16.3</td>
</tr>
<tr>
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<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.2</td>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.5</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>4.1</td>
<td>8.1</td>
<td>11.5</td>
<td>15.4</td>
<td>19.5</td>
<td>7.9</td>
<td>8.3</td>
<td>16.4</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>1.1</td>
<td>3.0</td>
<td>4.7</td>
<td>6.6</td>
<td>8.6</td>
<td>2.6</td>
<td>2.3</td>
<td>6.4</td>
</tr>
<tr>
<td>20:4(n-3)</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>—</td>
<td>0.2</td>
<td>0.2</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>5.9</td>
<td>4.5</td>
<td>3.4</td>
<td>2.1</td>
<td>0.7</td>
<td>3.2</td>
<td>12.6</td>
<td>9.7</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>—</td>
<td>0.3</td>
<td>0.3</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>4.6</td>
<td>3.7</td>
<td>3.0</td>
<td>2.1</td>
<td>1.0</td>
<td>2.9</td>
<td>10.0</td>
<td>8.0</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>15.8</td>
<td>14.2</td>
<td>13.3</td>
<td>11.9</td>
<td>10.6</td>
<td>10.9</td>
<td>34.1</td>
<td>30.6</td>
</tr>
<tr>
<td>20:1(n-11)</td>
<td>3.9</td>
<td>1.8</td>
<td>1.2</td>
<td>0.8</td>
<td>0.5</td>
<td>1.4</td>
<td>3.9</td>
<td>1.8</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>97.5</td>
<td>98.6</td>
<td>99.4</td>
<td>100.0</td>
<td>100.0</td>
<td>98.0</td>
<td>210.1</td>
<td>212.0</td>
</tr>
</tbody>
</table>

1 Values are means, n = 2. Values < 0.1 are denoted by —. The remaining constituent in vegetable oil diets was FO. All diets, produced by Nutreco ARC were composed (g/kg diet) of fish meal (338.3), maize gluten (200.0), soybean meal (HP; 100.0), wheat (79.0), oil (257.7) and premixes (25.0). Diets were supplemented with vitamins and minerals according to the nutritional requirements of fish (25).

2 Includes 14:1(n-9), 15:0, 16:1(n-7), 17:0, 16:2(n-4), 16:3(n-3), 18:1(n-11), 18:1(n-7), 16:4(n-3), 18:4(n-3), 20:0, 20:1(n-7), 20:3(n-3) and 24:1(n-9).
Fatty acid composition

Lipids from livers and diets were extracted by homogenization in chloroform:methanol (2:1, v/v). After extraction of total lipid, an aliquot was saponified and methylated using 12% BF3 in methanol. The fatty acid composition of total lipids was analyzed using methods described previously by Lie and Lambertz (26). Briefly, methyl esters were separated using a trace gas chromatograph 2000 ("cold on column" injection, 60°C for 20 s, 25°C/min; 160°C for 28 min, 25°C/min; 190°C for 17 min, 25°C/min; 220°C for 9 min), equipped with a 50-m CP-sil 88 (Chrompack) fused silica capillary column (i.d., 0.32 mm). The fatty acids were identified by retention time using standard mixtures of methyl esters (Nu-Chek-Prep), and quantified using Totalchrom software (version 6.2, Perkin Elmer). The amount of fatty acid per gram tissue was calculated using 19.0 methyl ester as an internal standard.

Microarray analysis

Minimum information about a microarray experiment (MIAME). MIAME standards (27) were used to define the content and the structure of the information for this microarray experiment. In accordance with these standards features used (Online Supplemental Table 1), the experimental conditions (Microarray hybridization protocol), the experiment (Experimental design), and array design (Microarray construction) as well as a description of samples, hybridizations, and normalizations controls (RNA isolation and preparation, Microarray hybridization protocol, and Normalization and statistical analysis) were reported.

Experimental design. Two arrays (i.e., technical replicates) were scanned using cihybridized labeled total RNA from control and experimental samples labeled with cyanine (Cy3) and Cy5, respectively. Each of these experiments was replicated with Cy dyes switched to control for labeling bias. The same experimental design was used for cihybridized mRNA samples from fish fed the 2 diets.

Microarray construction. PCR ampiclons from selected Atlantic salmon expressed sequence tags (ESTs) (28) were printed on bar-coded CMT-GAPS coated slides (Corning Microarray Technology) using a manual pin caster microarray printing system (Micro Caster 8 pin system, Schleicher & Schuell). cDNA clones were selected on the basis of their similarity [BLAST search of EST sequence (29)] to features involved in lipid metabolism. The Microarray Assistant Clone Organizer and Array Simulator (30) was used to organize clones and ampiclons. A total of 73 features, mainly from Atlantic salmon, were spotted on the array in quadruplicate (4 subarrays with 30 pooled livers using the RNAwiz protocol™ (Ambion). RNA was isolated from total RNA using the Oligotex® mRNA kit (Qiagen). RNA quality and quantity were determined as described above.

RNA isolation and preparation. Total RNA was purified using TRIZOL® (Invitrogen) and subjected to DNase treatment (DNA-free, Ambion). RNA quality and quantity were determined as described above.

RT-PCR. cDNA (125 ng) was synthesized following a modified protocol from the Taq Man Reverse Transcription Reagents kit (Applied Biosystems). Modifications included increasing the total reaction volume to 30 μL and accordingly increasing the volume of all reagents used. Oligo d(T)16 primers were used for ΔΔ FAD and liver basic fatty acid binding protein (LB-FABP), and random hexamers were used for 18S rRNA. The reactions were incubated at 25°C for 10 min and 48°C for 60 min, and the reverse transcriptase enzyme was inactivated at 95°C for 5 min followed by a decrease to 4°C. RT-PCR efficiency was monitored using a 4-step, 2-fold dilution curve of RNA.

Q-PCR. BLAST searches (29) for Atlantic salmon 18S rRNA, ΔΔ FAD, and LB-FABP were performed to identify highly conserved regions for primer design. Selected sequences were translated using the Expasy translate tool (33) and intron-exon borders were identified from alignments of genomic and cDNA sequence using ClustalW software (34). Primer and probe sequences were constructed using the assayed by Design service (Applied Biosystems). The primer and probe sequences (5’ to 3’) for 18S rRNA (AJ476292) were: CCGCCTTAATGGGATGACATCTTT(F), ACGCATTTGGACTCGAATTACC(R), FAM-CACCAGACTCGTTCGCC-CTC-MGBNFQ (reverse probe), for ΔΔ FAD (AF478472.1) GGAACACAAACTGCAACAAGT(F), GTGCTGGAGTACGATGCTG GTT(R) and FAM-CACGGAGCCACGTATGCTG-MGBNFQ (reverse probe), and for LB-FABP (BG395075) CGGACATCCACCACTAGGA(F), GCTTCCTCCCTCCAGTTGTR and FAM-CAGTGCACTGGACGT-MGBNFQ (reverse probe).

P-PCR was performed using FAM fluorescent chemistry on an ABI prism 7000 (Applied Biosystems). The reaction mixture (25 μL) contained primers (900 nmol/L each), FAM probe (200 nmol/L), 1X TaqMan universal PCR master mix (Applied Biosystems) and 40 μL cDNA. All samples were assayed in triplicate with nontemplate controls on the same plate. The reaction was incubated at 50°C for 2 min followed by 95°C for 10 min and 50 cycles of 95°C for 10 s and 60°C for 15 s. Liver RNA samples from 6 fish from each diet were tested.

Normalization and statistical analysis. 18S rRNA levels were used to normalize the results and to calculate relative expression levels using the Q gene method (37). The normalized relative expression data were analyzed using the nonparametric Kruskal-Wallis method (Statistic 6.1, Statsoft; n = 6 and n = 5) due to heterogeneity in variance within the 75% RO group. Differences were considered significant at P < 0.05.
Statistical analysis of dietary and liver fatty acid composition

For fatty acid composition in the experimental diets (Table 1, n = 2 analytical parallels) and in liver samples (Table 2, n = 3 and 6 pooled samples), means and medians (ranges) were calculated, respectively, using descriptive statistics (Statistica 6.1, Statsoft). Data were not analyzed for significant differences.

RESULTS

The body weight of Atlantic salmon fed the experimental diets increased from 0.142 kg initially to 1.463 kg after 42 wk. The body weight data were based on bulk weighing and counting of all fish. The body weight in the 100% RO-fed group after 22 wk (0.926 kg) was notably lower than that for groups fed FO (1.026 ± 0.020 kg) (mean ± SD, n = 2). Mortalities were negligible throughout the experimental period. More details on data for growth and feed efficiency are reported elsewhere (24).

The concentration of 20:5(n-3) was 60% lower in the 75% RO diet compared with the control (FO) diet (Table 1). The concentrations of 22:5(n-3) and 22:6(n-3) were 56 and 50% lower, respectively. The total highly unsaturated fatty acid (HUFA) content, the sum of SFA, and the sum of (n-3) fatty acids were 58, 32, and 16% lower, respectively, whereas the sum of (n-6) fatty acids was 3-fold greater in the 75% RO diet compared with control. In addition, the amount of total (n-9) fatty acids was almost 2-fold greater in the 75% RO diet compared with control. The concentrations of 18:1(n-9) (40%) and 18:2(n-6) (15%) fatty acids were high in the 75% RO diet. Those of 18:3(n-3) and 18(3n-3) were ~4- and 6-fold greater, respectively, in the 75% RO diet. The (n-3):(n-6) ratio in the 75% RO diet was 79% less than the control.

Irrespective of diet, 22:6(n-3) dominated in the salmon liver total lipid (Table 2). The concentrations of 18:1(n-9) (18.2(n-6) were ~2-fold greater, and that of 18:3(n-3) > 6-fold greater (median values) compared with livers from salmon fed the control diet. In contrast, concentrations of 22:5(n-3), 20:5(n-3), and 22:6(n-3) in liver of salmon fed the 75% RO diet were 46, 34, and 31% lower, respectively, than those of controls. Further details on fatty acid composition in livers of salmon fed different RO diets were published (11).

Microarray analysis demonstrated that expression of hepatic fatty acid Δ5 desaturase was significantly greater in livers of 75% RO-fed salmon than FO-fed salmon, whether total RNA or mRNA was analyzed (Table 3). Expression of apo A1 precursor was upregulated in analyses using labeled total RNA, and the expression of apo C-II was upregulated in analyses using combined and mRNA datasets. Long-chain ACS3 was among several genes that were downregulated in livers of 75% RO-fed salmon in all analyses.

SAM analysis of combined and mRNA datasets identified SCD and acyl carrier protein (mitochondrial precursor) as genes that were downregulated in livers of 75% RO-fed salmon, whereas SAM analysis of the combined and total RNA datasets identified translocase of outer mitochondrial membrane (tom70), outer mitochondrial membrane translocase, and CPT-II as genes that were downregulated in livers of 75% RO-fed salmon. A single analysis from arrays hybridized with total RNA from fish fed control and experimental diets identified enoyl CoA hydratase, PPARγ, and catalase as downregulated genes in livers of 75% RO-fed salmon. Data from Q-PCR analysis confirmed results from the microarray screening showing an ~2-fold increased gene expression of Δ5-FAD in livers of 75% RO-fed salmon after 42 wk of feeding (Fig. 1). Gene expression of Δ5-FAD in livers of 75% RO-fed salmon after 22 wk of feeding, was higher (P < 0.05, nonparametric statistics, n = 5) than in the control (n = 6).

DISCUSSION

Microarray analysis can be used as a tool for studying homeostatic metabolic systems (38) and can potentially reveal small but significant changes in gene expression if reliable controls are used. The internal control and reference genes, used only to ensure quality, yielded expected values of expression, validating this approach for studying the expression of genes involved in liver lipid metabolism in Atlantic salmon. Therefore, microarray analysis gave a preliminary indication of the metabolic processes that may be responding to changes in dietary oil and thus changes in tissue fatty acid composition. Because a primary objective was to validate the suitability of the array for further studies, technical replicates of several types were selected (mRNA, total RNA, and fluor flips plus replicates within these groups). Additional quality assurance was also prioritized through duplicate feature spotting and quadruplicate subarrays. Pooling of livers before RNA isolation and microarray analysis was performed to control for individual genetic variation, similar to the strategy described by Douglas and co-workers (39). The degree of technical replication allows a thorough technical validation of the array as well as examination of variation between treatment groups (40).

Q-PCR analysis, performed to validate expression data from microarray analysis, using essays for Lb-FABP (results not shown) (Online Supplemental Table 1) confirmed the lack of gene specific variation between dietary treatments. However,
both the microarray and Q-PCR results demonstrated that \( \Delta 5 \) FAD gene expression was higher in livers of 75% RO-fed salmon (Table 3 and Fig. 1). This is in agreement with Zheng and co-workers (15) who observed an increase in the expression of hepatic \( \Delta 5 \) desaturase and fatty acid elongase genes in salmon fed increased levels of dietary linseed oil for 20 wk. The values for expression of fatty acid elongase obtained in the present microarray study were too low to be deemed reliable. Bell and co-workers (13,14) suggested that the stimulation of hepatic desaturation and elongation by vegetable oils presumably reflects increased substrate availability \( [18:2(n-6) \text{ and/or } 18:3(n-3)] \) coupled with reduced end product concentration \( [20:5(n-3) \text{ and } 22:6(n-3)] \), with high concentrations of the latter inhibiting the pathway.

In contrast, SCD gene expression was reduced in livers of 75% RO-fed salmon compared with control salmon. Supporting these data, Berge et al. (41) demonstrated that SCD enzyme activity was reduced in salmon fed conjugated linoleic acid (CLA), and gene regulation of SCD was shown to differ from regulation of \( \Delta 5 \)- and \( \Delta 6 \)-FAD (42). However, SCD has not been cloned in salmon, and further studies are required.

Dietary \( 20:4(n-6) \), \( 20:5(n-3) \), and \( 22:6(n-3) \) were reported to affect hepatic gene expression (3). Therefore, this long-term dietary fatty acid change may reduce mRNA transcript synthesis in livers of 75% RO-fed salmon.

The HFUA, 20:5(n-3) and 22:6(n-3), were reported to be 2- to 4-fold more potent inducers of fatty acid \( \beta \)-oxidation than \( 18:2(n-6) \) and \( 18:3(n-3) \) in mammals (43). Although there was an increase in the amount of \( 18:2(n-6) \) and \( 18:3(n-3) \), the reduced dietary \( 20:5(n-3) \) and \( 22:6(n-3) \) in the 75% RO diet compared with control correlated with a significant downregulation of several mitochondrial genes. These include [38x271]compared with control. 

Supporting these data, Berge et al. (41) demonstrated that SCD gene expression was reduced in livers of 75% RO-fed salmon compared with control salmon. Bell and co-workers (13,14) suggested that the stimulation of hepatic desaturation and elongation by vegetable oils presumably reflects increased substrate availability [18:2(n-6) and/or 18:3(n-3)] coupled with reduced end product concentration [20:5(n-3) and 22:6(n-3)], with high concentrations of the latter inhibiting the pathway.

In contrast, SCD gene expression was reduced in livers of 75% RO-fed salmon compared with control salmon. Supporting these data, Berge et al. (41) demonstrated that SCD enzyme activity was reduced in salmon fed conjugated linoleic acid (CLA), and gene regulation of SCD was shown to differ from regulation of \( \Delta 5 \)- and \( \Delta 6 \)-FAD (42). However, SCD has not been cloned in salmon, and further studies are required.

Dietary \( 20:4(n-6) \), \( 20:5(n-3) \), and \( 22:6(n-3) \) were reported to affect hepatic gene expression (3). Therefore, this long-term dietary fatty acid change may reduce mRNA transcript synthesis in livers of 75% RO-fed salmon.

The HFUA, 20:5(n-3) and 22:6(n-3), were reported to be 2- to 4-fold more potent inducers of fatty acid \( \beta \)-oxidation than \( 18:2(n-6) \) and \( 18:3(n-3) \) in mammals (43). Although there was an increase in the amount of \( 18:2(n-6) \) and \( 18:3(n-3) \), the reduced dietary \( 20:5(n-3) \) and \( 22:6(n-3) \) in the 75% RO diet compared with control correlated with a significant downregulation of several mitochondrial genes. These include [38x271]compared with control. 

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Long-chain ACS3, enoyl CoA hydratase, 2 mitochondrial outer membrane proteins, CPT-II, and mitochondrial acyl carrier protein (precursor). Furthermore, several additional mitochondrial genes (malate dehydrogenase, translocase of inner mitochondrial membrane, and mitochondrial solute carrier protein) were identified as significantly downregulated in fish fed 75% RO compared with fish fed a FO diet, with a downregulation in livers from 75% RO-fed fish. The former showed a significant change in mRNA levels due to the rat long-chain ACS1 gene (NM_012820). Only the former showed a significant change in mRNA levels due to diet, with a downregulation in livers from 75% RO-fed fish. Rat long-chain ACS3 and ASC1 are from different subfamilies with distinct functions, and share only 30% amino acid sequence identity (46). Rat long-chain ACS3 activates 20:5(n-3) and 20:4(n-6) most efficiently and has a 4-fold higher capacity for utilization of C20 HUFA than rat ASC1 (47)

The apo A-I precursor [sequence similarity to apo A-I-I (BE 518583)] and apo C-II were affected by the dietary oil source, showing upregulation in livers of 75% RO-fed salmon compared with control. Apo A-I-like protein was associated with HDL, and apo-C-like protein with LDL in rainbow trout (52). In contrast to the apolipoprotein expression results in the present study, the amount of HDL was significantly decreased in plasma of 75% RO-fed salmon, compared with fish fed FO (11).

In conclusion, the current thoroughly validated and quality controlled microarray study (technical replicates, internal controls, and PCR) showed differential, although relatively minor, regulation of several genes related to hepatic lipid metabolism in Atlantic salmon in response to changes in dietary fatty acids. These included ∆5 FAD, PPARγ, and several genes whose gene products are involved in mitochondrial functions. Salmon have a partially tetraploideic genome, and genes may be present in more than one isotype, which may differ in expression patterns (56,57). Thus, there is an argued need for a more thorough examination of genes and gene expression patterns. However, the effects of nutrients are under strict homeostatic control (58), and microarray analysis is a powerful tool with which to screen for nutritional effects on a gene-expression level in Atlantic salmon, and should be further pursued.

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LITERATURE CITED


FIGURE 1 Q-PCR analysis of ∆5 FAD mRNA expression in livers of salmon fed 75% RO and 100% FO diets after 22 and 42 wk of feeding. Values are presented as relative to control, 100% FO (set to 1), for each sampling. Data were normalized to 18S rRNA levels. Values are means ± SD, n = 5 or 6 individual samples. *Different from control (FO), P < 0.05.