PAPER III

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Running title: LIVER AND PLASMA LIPIDS IN SALMON

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Abstract

Atlantic salmon (*Salmo salar* L.) were fed either 100% fish oil (FO) or 100% vegetable oil blend (55% rapeseed oil, 30% palm oil and 15% linseed oil) (VO) based diets from start feeding and onwards for 22 months. Liver and plasma lipoprotein lipid class levels and lipoprotein fatty acid composition were analysed through the sea water phase, whereas liver fatty acid composition, plasma cholesterol, triacylglycerol and protein levels were analysed through both freshwater and sea water stages. Further, enzyme activity of liver Fatty acid synthetase (FAS), NADH-isocitrate dehydrogenase (ICDH), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) and expression of the gene Peroxisome proliferator activated receptor γ (PPARγ) was analysed during both fresh water and sea water stages through the experiment.

Dietary VO significantly increased salmon liver TAG and hence total liver lipid stores after 14 and 22 months of feeding. Further, after 22 months of feeding, plasma lipid levels and plasma LDL levels were significantly decreased in VO fed salmon compared to FO fed fish. The same trend, however not statistically significant, was seen for plasma VLDL. Plasma HDL levels was not affected by dietary VO replacement. The fatty acid composition of liver and lipoproteins reflected the dietary fatty acid composition, however with 22:6n-3 being relatively high in both liver and HDL also in the VO fed fish. The activity of FAS was generally low throughout the experiment with the VO group having significantly lower activity after 16 months of feeding. For the other lipogenic enzymes, replacing FO with VO gave either no effect or significantly increased activity. The expression of PPARγ in livers increased prior to sea water transfer followed by a decrease, and then another increase towards the final sampling (22 months). Dietary vegetable oil replacement had no impact on PPARγ expression in salmon liver.

Introduction

Atlantic salmon (*Salmo salar* L.) in aquaculture are increasingly being fed diets based on vegetable oils as a replacement for fish oil. In contrast to fish oil, vegetable oils do not contain fatty acids longer than 18 carbon and three double bonds and thus lacking the long chain n-3 PUFAs such as EPA; 20:5n-3 and DHA; 22:6n-3. Some of the most frequently used vegetable oils contain high levels of 18:1n-9 which has been shown in different cell systems (Ranheim *et al.*, 1994; Vegusdal *et al.*, 2005) and rats (Halvorsen *et al.*, 2001) to affect liver lipid and lipoprotein metabolism. Further, both EPA and DHA are reported to affect hepatic triacylglycerol (TAG) metabolism and β-oxidation capacity (Nossen *et al.*, 1986; Willumsen *et al.*, 1996; Berge *et al.*, 1999; Madsen *et al.*, 1999) with especially EPA having a plasma lipid lowering effect in rats (Frøyland *et al.*, 1996; Frøyland *et al.*, 1997). Further, n-3 PUFA is suggested to inhibit the secretion of TAG-rich VLDL particles by inhibiting the rate limiting enzyme diacylglycerol acyltransferase (DGAT, EC 2.3.1.20) (Berge *et al.*, 1999; Madsen *et al.*, 1999) and by inhibiting the assembly of VLDL particles in the liver (Lang and Davis, 1990; Brown *et al.*, 1999; Kendrick and Higgins, 1999). Several studies in humans have shown that dietary EPA and DHA decrease plasma TAG (Harris *et al.*, 1983; Nestel, 1990) and protect against coronary heart diseases (Bang *et al.*, 1971; Seierstad *et al.*, 2005).

The high dietary lipid levels used for Atlantic salmon are thought to result in low hepatic lipogenic enzyme activity, as demonstrated by negative correlation between dietary lipid and lipogenic enzyme activity (Arnesen *et al.*, 1993). Further, dietary PUFA is reported to inhibit lipogenesis in both rainbow trout (Alvarez *et al.*, 2000) and rat (Zampelas *et al.*, 1995). The NADPH produced by lipogenic enzymes can be used for, in addition to the lipogenic pathways, the maintenance of the cellular red-ox state (reviewed by Kletzien *et al.* (1994)),

and PUFA is reported to affect the requirement for antioxidants such as NADPH (Benzie, 1996).

Peroxisome proliferator activated receptor γ (PPARγ) is characterised in mammals and rodents as the nuclear transcription regulator correlating with lipid storage (reviewed by Desvergne and Wahli (1999)). Two forms of PPARγ have previously been described in Atlantic salmon liver (Ruyter *et al.*, 1997; Andersen *et al.*, 2000). Recent studies performed in knock out mice suggest a role for liver PPARγ2 in lipid homeostasis as an important regulator of genes involved in lipogenesis, and fatty acid storage, but also fatty acid transport and oxidation (Gavrilova *et al.*, 2003; Wolf, 2004).

Reports on dietary vegetable oil replacement show contradictory results regarding hepatic lipid storage. By replacing fish oil with oleic acid enriched-sunflower oil and rapeseed oil, respectively, slight increases in hepatic total lipid storage was reported (Torstensen *et al.*, 2000; Bell *et al.*, 2001), whereas no effects on hepatic lipid was found when replacing with palm oil (Torstensen *et al.*, 2000; Bell *et al.*, 2002) or a 1:1 rapeseed oil: linseed oil mix (Tocher *et al.*, 2001).

The aim of the present study was to examine through liver lipid levels, lipogenic enzyme activities, PPARγ expression and plasma lipoproteins how hepatic lipid turnover and lipid transport are affected by complete replacement of dietary fish oil with a vegetable oil blend from start feeding until adult Atlantic salmon.

Materials and Methods

Animals and diets

The dietary trial was started April 5th 2002 at Lerang Research Station, Nutreco ARC, Stavanger, Norway, and with final sampling in January 2004 (a total of 22 months). In April 2002 approximately 2000 juveniles with a mean weight of 0.160 ± 0.052 g were distributed equally into each of six 100 L tanks followed by 2x2x1 m freshwater tanks after 3 months of feeding. Sea water transfer was performed in two steps; the first step (10th February 2003, after 11 months of feeding) was done by changing the fresh water supply to sea water supply with the smolts remaining in the same tanks with the same temperature. The second step (May 2003) was performed when sea water temperature had reached the same temperature as on shore tank sea water, and 600 Atlantic salmon from each tank were randomly taken and transferred to individual 125m^3 seawater net pens. From start feeding onward, fish in 3 randomised tanks or net pens were fed on either diets fully based on fish oils (100% FO), or diets fully based on vegetable oils (100% VO) (n=3 per experimental diet), using fish meal as protein source and as n-3 Polyunsaturated Fatty Acids (PUFA) source in the 100% VO diet. Diets were produced by Nutreco ARC, Stavanger, and proximate and fatty acid feed composition of the diets is given in Table 1. For the 100% FO-diet two different seasonal batches of capelin oil (Norsildmel, Norway) were used, one batch for the start to 4mm pellet size FO-diets and the other batch for the 6 and 9 mm pellets size FO-diets. For the 100% VO-diet a mixture of 55 % rapeseed oil (Oelmuhle, Germany), 30 % palm oil (Denofa, Norway) and 15 % linseed oil ((NV Oliefabriek, Belgium) was used to obtain a lipid profile of saturated, monounsaturated and PUFA as similar as possible to capelin oil. The two experimental diets were fed throughout the entire life-cycle, with both diets changing in pellet size and lipid content and with samplings performed according to the changes in pellet lipid content and salmon life stage. Fish were fed restricted by automatic feeders, followed by hand feeding

until satiation. After 0, 6, 9, 14, and 16 months fish were weighed, and the amount of feed given was adjusted in accordance with biomass. Temperature was recorded daily, and water oxygen content was measured twice a week. Temperature followed natural variation during the 2 years feeding period, with lowest and highest mean monthly temperatures of 7.6 and 17.5 ° C. From the start (April 2002) of the experiment until seawater transfer (February, 2003) fish were reared under constant light, except for a short period just before seawater transfer (24 October to 13 December, 2003) where fish were reared under a 12 hour light: 12 hour dark regime to ensure smoltification. During the entire seawater growth phase fish were reared under natural light conditions.

Sampling

Samples were taken from all diets and stored at -20°C. Fish was not fed during the 24 h prior to sampling. Samplings were done when pellet size and hence dietary lipid level changed and during smoltification, (i.e. 6, 9, 14, 16 and 22 months after start of feeding), pooled samples were taken of 10 fish from each tank for tissue. Ten randomly sampled fish from each tank were anaesthetised with MS222 (7 g/l). Blood was collected from the caudal vein using EDTA vacutainers for lipoprotein samples and heparin for the nutrient plasma samples and the fish killed by a blow to the head, followed by dissection of tissues including liver. At each sampling, pooled samples were taken of 10 fish from each tank for tissue and plasma analyses. Liver was sampled from 10 fish, flash frozen in liquid nitrogen and pooled to one sample for each tank for lipid class composition analysis and lipogenic anzyme activity measurements obtaining n=3 for each dietary treatment. For total lipid and fatty acid composition analysis, 10 livers from each tank were pooled and homogenised and frozen on dry ice. All samples were stored at -80°C.

For PPARγ Q-RT-PCR analysis, approximately 1 g liver from 3 fish from each tank were dissected out and immediately transferred to liquid nitrogen (snap freezing protocol), before stored at -80°C.

Proximate composition of diets

Dry matter in the diets was measured gravimetrically after freeze-drying of homogenised samples for 48 hours. Total nitrogen was determined on homogenised, freeze-dried samples using a nitrogen determinator (LECO, FP-428 system 601-700-500; Perkin Elmer Coop., Ct, USA). Protein was calculated as N x 6.25. Total lipid of the diets and fillet was measured gravimetrically after ethyl acetate extraction and after acid hydrolysis of the diet samples.

Fatty acid composition

Fatty acid composition was analysed in the diets, liver, plasma and lipoproteins. Lipids from the samples were extracted by adding chloroform/methanol (2:1, v/v) and 19:0 methyl was added as internal standard. After extraction of lipids, the samples were filtered, an aliquot was removed for determining lipid class composition as described below, and then the remaining samples were saponified and methylated using 12 % BF_3 in methanol. Fatty acid composition of total lipids was analysed using methods described by (Lie and Lambertsen, 1991) where the methyl esters were separated using a Trace gas chromatograph 2000 (Fison) ("cold on column" injection, 60°C for 1 minute $^{25°C/min}$ 160°C for 28 minutes $^{25°C/min}$ 190 °C for 17 minutes $^{25°C/min}$ 220°C for 10 minutes), equipped with a 50m CP-sil 88 (Chromopack) fused silica capillary column (id: 0.32 mm). The fatty acids were identified by retention time using standard mixtures of methylesters (Nu-Chek, Elyian, USA), and the fatty acid composition (weight %). All samples were integrated using the Totalchrom software

(ver. 6.2, Perkin Elmer) connected to the GLC. Amount of fatty acid per g tissue was calculated using 19:0 methyl as internal standard.

Total protein in lipoproteins and plasma as well as plasma TAG and total cholesterol were analysed on a Technicon RA-1000 clinical analyser system (Bayer) according to standard Technicon methods, also described by (Sandnes *et al.*, 1988).

Separation of lipoproteins

Very low density lipoproteins (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) in plasma were obtained by sequential centrifugal flotation (Havel *et al.*, 1955; Aviram, 1983) as described by (Lie *et al.*, 1994) using Beckman Optima^{TM}XL-100K Ultracentrifuge equipped with a SW41Ti rotor. The centrifugation was done at 197 600 x g_{av} and 4°C. The density intervals were obtained by addition of solid KBr (Warnick *et al.*, 1979), and run time for separation of lipoproteins was: VLDL, d<1.015 g ml $^{-1}$ for 20 h; LDL, 1.015 g ml $^{-1}$ < d < 1.085 g ml $^{-1}$ for 20 h and HDL, 1.085 g ml $^{-1}$ < d < 1.21 g ml $^{-1}$ for 44 h. The remaining fraction of non-lipoprotein plasma is named NLP (non lipoprotein) in the following. The lipoprotein and NLP fractions were stored at -80 °C until further analyses.

Lipid class composition

The quantification of lipid class composition in diets, liver, plasma and the different lipoprotein fractions was performed using high-performance thin-layer chromatography (HPTLC) as described by (Bell *et al.*, 1993). 10 µg total lipid was applied to a 10 x 20 cm HPTLC plate that had been pre-run in hexane:diethyl ether (1:1 v/v) and activated at 110°C for 30 min. The plates were developed at 5.5 cm in methyl acetate: isopropanol: chloroform: methanol: 0.25% (w/v) aqueous KCl (25:25:25:10:9, by volume) to separate phospholipid classes with neutral lipids running at the solvent front (Vitello and Zanetta, 1978). After drying, the plates were developed fully in hexane: diethyl ether: acetic acid (80:20:2, v/v/v) to

separate neutral lipids and cholesterol. Lipid classes were visualised by charring at 160°C for 15 min after spraying with 3% copper acetate (w/v) in 8% (v/v) phosphoric acid identified by comparison with commercially avaliable standards. Lipid classes were quantified by scanning densiometry using a CAMAG TLC Scanner 3 and calculated using an integrator (WinCATS-Planar Chromatography, Version 1.2.0). Further, quantitative determination (mg lipid class/g tissue) of lipid classes was performed by establishing standard equations for each lipid class within a linear area, in addition to including a standard mix of all the lipid classes at each HPTLC plate for corrections of between plate variations.

Lipogenic enzyme activity

Enzyme activity of liver NADH-isocitrate dehydrogenase (ICDH, EC 1.1.1.42), malic enzyme (ME, EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) was analysed in both fresh water (October 02 and January 03) and sea water (June 03, August 03 and January 04) stages through the life cycle. The method is described in detail by Sanden (Sanden, 2001). Briefly, for determination of lipogenic enzyme activities the crude enzymes were prepared by homogenising liver samples by a mortel that was kept on dry ice, using homogenising buffer with mercaptoethanol. Liver samples from ten fish were pooled from each tank. The homogenates were centrifuged (High performance Avanti J-25 centrifuge), and enzyme extracts (infranant) were carefully collected and kept one ice until analysis. The enzyme activities were measured at 24°C for 2 minutes using a Shimadzu spectrofotometer and Quartz semi-micro cuvettes. Total protein in homogenates were analysed on a Technicon RA-1000 clinical analyser system (Bayer) according to standard Technicon methods, also described by (Sandnes *et al.*, 1988). The specific lipogenic enzyme activities are expressed as nmol/min/mg protein. Fatty acid synthetase (FAS, EC 2.3.1.38) activity was analysed by

isotopic methods according to Dias et al. (Dias *et al.*, 1998) and Hsu et al. (Hsu *et al.*, 1969). Enzyme activity units (IU), defined as µmoles of substrate converted to product per minute at assay temperature, were expressed per g liver tissue (wet weight).

Q-PCR assays

Primer and probe sequences for elongation factor 1-A (EF $1A_A$) {Moore, 2005 #91}was published earlier. For more details on the use of normalisation controls.{Olsvik, 2005 #100}

The exon-exon specific primer and probe sequences (exon 3-4) borders (5'-3') were for PPARγ (GenBank accession nos. AJ416952, AJ416951, AJ292963, AJ292962) GCCTCAGGCTTCCACTATGG (F), CAGTTTTAACCGCACGGTTCTG (R) and FAM-CTGCAAGGGATTCTTC-MBGNFQ (probe).

Before cDNA synthesis, total RNA was purified from 3 fish per replicate (3 per diet) using TRIZOL (Invitrogen, California, USA), before subjected to DNase treatment (DNA-*free*™, Ambion, Austin, USA). RNA quality and quantity were determined by denaturing gel electrophoresis and spectrophotometry ($A_{260/280}$).

cDNA (125 ng) was synthesized following a modified protocol from the Taq Man Reverse Transcription Reagents kit (Applied Biosystems, New Jersey, USA). Modifications included increasing the reaction volume to 30 µL, using 1X TaqMan RT buffer, 5.5 mmol/L $MgCl_2$, 500 µmol/L dNTP each, 0.4 U/µL RNase Inhibitor,1.67 U/µL Multiscribe™ Reverse Transcriptase. The reactions (triplicates) were incubated at 25 °C for 10 min and 48 °C for 60 min, and the reverse transcriptase was inactivated at 95 °C for 5 min followed by a decrease to 4 °C. RT-PCR efficiency was monitored using a four-step two-fold dilution curve of RNA (from 250 ng total RNA).

Quantitative PCR was performed using FAM fluorescent chemistry on an ABI prism 7000 (Applied Biosystems, New Jersey, USA). The reaction mixture (25 µL) contained primers

(900 nmol/L each), FAM probe (200 nmol/L), 1X TaqMan universal PCR master mix (Applied Biosystems art. nr. 430 4437) and 5 µL cDNA. All samples were run in triplicate with non-template controls (NTC) on the same plate. Reactions were incubated at 50 °C for 2 min and followed by 95 °C for 10 min and 50 cycles of 95 °C for 10 s and 60 °C for 15 s.

EF-1α was used to calculate relative expression levels for PPARγ using the Q gene method (Simon, 2003).

Statistics

The relative fatty acid composition data of diets, liver, plasma and lipoproteins was analysed using SIRIUS for Windows (Version 6.5). Principal component analysis (PCA) (Wold *et al.*, 1987) was performed in each data matrix of the relative fatty acid compositions. The purpose of PCA is to express the main information in the variables by a lower number of variables, the so-called principal components (PC1, PC2, ...). A high positive or negative loading reveals a significant variable in the actual PCA model. Score plots from the PCA explore the main trends in the data, and their respective loading reveal variables with a significant loading. The samples with similar relative fatty acid and lipid class composition are located in the same area in the score plot. These classes are indicated in Figure 3 by arrow drawn on freehand. Since samples with the same composition will be located on top of each other, to ease interpretation the samples the classes contain are written beside the arrow. Differences between the two dietary treatments were analysed by Breakdown & One-way ANOVA followed by Tukeys HSD test, using CSS:Statistica (version 6.1; StatSoft Inc.). The significance level was set to $P \leq 0.05$, and data are presented as mean ± STD (n=3).

Results

The salmon weight increased from 0.16 g at start feeding to 103 g by the time of smoltification and sea water transfer in February 2003, and further to 470 g in June 2003 and to 890 g in August 2003. No differences were found mean fish weight, SGR or feed conversion ratio between the two dietary groups from start feeding to August 2003. However, from August 2003 until January 2004 the fish weight increased from mean bulk weight of 890 g in both dietary groups to mean bulk weight of 2.3 kg and 2.7 kg in the 100% FO and 100% VO groups, respectively. Mortalities were low and with no difference between the dietary groups.

Dietary fatty acid composition and proximate composition show that diets with 100% FO and 100% VO blend have similar levels of total saturated, total monoene fatty acids and total n-3 fatty acids (Table 1). However, within each fatty acid class the individual fatty acids are different between the two diets with 100% VO having shorter chain fatty acids (18 carbon or less) and 100% FO having high levels of the 20 and 22 carbon fatty acids. Fish meal was used as dietary protein source, thus contributing with 20:5n-3 and 22:6n-3 in the 100% VO diet. To ensure always optimal dietary conditions, the dietary lipid level increased with increasing pellet sizes given to the fish (Table 1). With increasing dietary lipid level, the relative contribution of fatty acids from the oils increased. Further, due to logistics a new batch of capelin oil was used for the larger pellet sizes (6mm and 9mm). This capelin oil was a winter capelin oil with relatively higher levels of PUFAs (Table 1). As a consequence, with increasing dietary lipid level and winter capelin oil, the differences in fatty acid composition between the 100% FO and 100% VO diet increased with increasing pellet size through the experiment.

Most fatty acids in liver reflected the dietary fatty acid composition shown by both fatty acid levels (Table 2) and relative fatty acid composition (Figure 3). However the amount (mg g^{-1}) of 22:6n-3, 16:0, total monoene fatty acids, 20:4n-6 and total n-3 fatty acids was not significantly different in livers from the two dietary groups in most samplings (Table 2). Further, total amount of fatty acids in liver were significantly higher in the 100% VO group after 14 and 22 months of feeding compared to the 100% FO group, whereas in the other samplings no differences were found in total fatty acid levels in liver (Table 2).

Liver lipid class composition (Table 3) showed significantly lower relative levels of phosphatidyl choline (PC), phosphatidyl serine (PS) and phosphatidyl inositol (PI) and significantly higher relative levels of cholesterol and borderline significant higher levels of triacylglycerol (TAG) in the 100% VO group in June 2003. The absolute amount of lipid classes, however, show a major increase in TAG in livers from the 100% VO group compared to the 100% FO group, and thus neutral lipids which consequently resulted in relative decreases in the polar lipids (Table 3). Also total liver lipid was higher in the 100% VO group compared to the 100% FO after 14 and 22 months of feeding. In the sampling in August 2003 (after 16 months of feeding), however, there were no significant differences between the two dietary groups regarding liver lipid class composition. When comparing the two sampling points, there was a significant increase in TAG and cholesterol in both dietary groups with 100% FO having the highest increase in neutral lipids in liver (Table 3). The levels of phospholipids either significantly decreased from June to August 2003 or remained unchanged (Table 3). From 16 to 22 months of feeding the difference in liver TAG between the two dietary treatments progressed further, and salmon fed 100% VO had more than two-fold higher TAG levels compared to livers from salmon fed 100% FO. No other lipid classes were affected by dietary fatty acid composition after 22 months of feeding (Table 3).

The lipogenic enzymes either produce or regenerate NADPH which may be used for fatty acid synthesis, reduction of glutathione, synthesis of cholesterol and reductive biosynthesis. Except for malic enzyme (ME) (Figure 1), the enzyme activity was higher in livers from salmon fed 100% VO diet compared to the 100% FO group (Figure 1), although not always statistically significant. After 16 months of feeding (August 2003) the between-tank variation was higher for all the measured enzymes except FAS. Generally lipogenic enzyme activities were significantly induced in salmon fed 100% VO compared to 100% FO. However, the FAS activity in August 2003 was significantly lower in the 100% VO group ($p=0.009$) compared to the 100% FO group (Figure 1).

Plasma TAG, total cholesterol (cholesterol and cholesterol esters combined) and protein levels are presented in Figure 3 with two sampling points before (6 and 9 months of feeding) and three after smoltification and sea water transfer, which was 10 months after start feeding. Plasma protein levels were relatively stable throughout the experimental period. Plasma TAG and total cholesterol, however, showed different pattern and response to dietary treatment in the fresh water and sea water stages (Figure 2). Plasma TAG was significantly higher in fish fed 100% VO at the October 2002 sampling, but not at the January 2003 sampling. Further, after sea water transfer there was a significant drop in plasma TAG concentrations and an increasing trend towards lower plasma TAG levels in fish fed 100% VO, however not statistically different (Figure 2). Plasma cholesterol was also significantly higher during the fresh water stages compared to the sea water stages. Further, salmon fed 100% VO had significantly lower plasma cholesterol levels before sea water transfer compared to salmon fed 100% FO (Figure 2).

Principal component analysis (PCA) of the relative fatty acid composition in very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), non-lipoprotein fraction (NLP), plasma, liver and diets from all sea water samplings showed that plasma VLDL clearly reflects dietary fatty acid composition (Figure 3) followed by LDL, liver and plasma, HDL and NLP. The samples from the two dietary groups form a gradient from plasma and HDL to the diet for each dietary group (indicated by arrows the score plot) (Figure 3 upper).

The relative amount of TAG gradually decreased from VLDL to LDL to HDL, whereas the amount of cholesterol ester (CE) and phospholipids (PL) were high in HDL compared to VLDL (Table 4). The lipid class composition of plasma reflected that of HDL, concomitantly almost all plasma lipids were recovered in HDL presented as sum lipid in HDL g^{-1} plasma after 14 months of feeding (Table 4). Comparing lipid class composition after 14 and 16 months of feeding, the relative TAG levels were higher in VLDL and HDL after 16 months whereas LDL TAG and all other lipid classes in lipoproteins and plasma was stable (Table 4). After 14 and 16 months of feeding there were no statistically significant differences in either relative or absolute lipid class composition between the 100% VO and FO fed fish (Table 4). There was, however, a trend towards decreased lipid levels in plasma and decreased TAG and total lipid in LDL after 14 and 16 months of feeding (Table 4). Comparing the lipid level in plasma, HDL and NLP after 14 and 16 months of feeding, recovery of all lipid classes was significantly lower after 16 months, and NLP contributed with almost 50% of the plasma lipid (Table 5). Further, the HDL protein levels were almost identical in the two sampling points, whereas the amount of protein recovered in NLP was approximately 12 mg g^{-1} plasma higher after 16 months compared to 14 months of feeding (Table 4). Concomitantly, the plasma protein levels were above 50 mg g^{-1} plasma after 16 months compared to 34 mg g^{-1} plasma

after 14 months (Table 4 and Figure 2). After 22 months of feeding (January 2004), however, there were statistically significant lower levels of both plasma neutral and total lipid, and a range of LDL lipid classes in plasma from fish fed 100% VO compared to fish fed 100% FO (Table 5). However not statistically significant, there was a clear trend towards less VLDL lipid also in plasma from 100% VO fed salmon (Table 5). Total plasma protein intermediate compared to the two previous samplings, and about 60% of plasma lipid was recovered in HDL. There was no difference in HDL lipid level in plasma between the dietary treatments (Table 5).

Similarly as in the diets, lipoprotein and plasma levels of 18:1n-9 increased in all fractions (Table 6). Further, 18:2n-6 levels increased and n-3/n-6 ratio decreased in all fractions except in LDL. Plasma and lipoprotein 22:6n-3 remained relatively unchanged, especially in the HDL fraction (Table 6, Figure 3 lower). The total amount of fatty acids per g plasma was significantly higher the 100% VO group LDL and significantly lower in the 100% VO NLP compared to the 100% FO fractions (Table 6). The amount of protein constituting each lipoprotein fraction per g plasma showed no significant differences between the two dietary groups in neither of the samplings after 14, 16 and 22 months of feeding. However, although not statistically significant there was a tendency towards lower LDL protein levels after 16 and 22 months of feeding (Table 4 & 5). Further, the amount of HDL protein was higher in the January 2004 sampling compared to the two previous samplings. Also the amount of VLDL and LDL protein per g plasma gradually increased with increasing fish size (Table 4 & 5).

No difference in PPARγ expression between the dietary treatments was observed (Figure 4).

However, PPARγ mRNA transcripts from fish fed the VO diet were on average higher in SW phase, and lower than transcripts in the FO diet in FW. Further, the transcription of PPARγ was highest in January 2003 which was during the smoltification period prior to sea water transfer. From August 2003 to January 2004 the PPARγ expression increased more than two-fold in both dietary groups (Figure 4).

Discussion

Atlantic salmon liver lipid and plasma lipoprotein levels were significantly affected by replacing dietary fish oil with a 100% VO blend. Liver TAG stores increased whereas plasma lipid, and especially plasma LDL, decreased in VO fed fish. *In vitro* experiments using rat hepatocytes and CaCo-2 (enterocyte model) cells (Nossen *et al.*, 1986; Ranheim *et al.*, 1994; Berge *et al.*, 1999) have shown that 18:1n-9 increase liver TAG synthesis and secretion as well as intestinal chylomicron synthesis. In contrast, 20:5n-3 inhibits the synthesis and secretion of hepatic TAG (Nossen *et al.*, 1986; Ranheim *et al.*, 1994; Berge *et al.*, 1999). In rats, it has been reported that dietary n-3 fatty acids decreased the levels of serum TAG, PL, free fatty acids and total cholesterol compared to long-chain monoenoic fatty acid feeding (Halvorsen *et al.*, 2001). In feeding experiments with Atlantic salmon both slight increases in hepatic total lipid storage (Torstensen *et al.*, 2000; Bell *et al.*, 2001) by replacing 100% of fish oil with either oleic acid enriched sunflower oil and rapeseed oil, respectively, and no effects (Torstensen *et al.*, 2000; Bell *et al.*, 2002) of replacing with palm oil or a 1:1 rapeseed oil: linseed oil mix (Tocher *et al.*, 2001) have been reported. However, previous reports have used total liver lipid results or relative lipid class composition data, and not quantified each of the lipid classes which give more in depth results on hepatic liver storage. Thus, this is the first report showing detailed data on increase in *in vivo* hepatic TAG storage when Atlantic salmon were fed high 18:1n-9 (43 % of total dietary FA) and low 20:5n-3 (0.6 % of total dietary FA) levels showing the opposite trends of published *in vitro* studies in Atlantic salmon (Vegusdal, 2004; Stubhaug *et al.*, 2005; Vegusdal *et al.*, 2005) and rat (Nossen *et al.*, 1986; Ranheim *et al.*, 1994; Berge *et al.*, 1999) hepatocytes. However, other dietary fatty acids than 18:1n-9 and 20:5n-3 in the tested salmon VO diet may also have an effect on the hepatic *in vivo* TAG storage in addition to the fact that a feeding experiment is more balanced regarding nutrient

composition and under constant homeostatic control compared to an *in vitro* experiment with cultured heaptocytes being exposed to single fatty acids.

Liver TAG levels were significantly higher in VO fed fish in two of the three time points analysed, and especially profound at the final sampling after 22 months of feeding. During the final experimental period also the growth was significantly higher in the VO group resulting in a final mean fish weight of 2.7 kg in the VO group compared to 2.3 kg in the FO group, as reported in greater detail by Torstensen and co-authors (2005), possibly related to increased digestibility of the VO dietary fatty acids at lower water temperatures. Thus, the large difference in liver TAG stores in the final sampling may be related to the difference in growth rate. However, since the increase in liver TAG in VO fed fish also were seen at the earlier stage (after 14 months of feeding) when no differences in growth were observed, growth alone can not be the explanation for increased liver TAG levels in the VO fed fish.

In addition to increased liver TAG stores in the 100% VO fed salmon, also plasma total lipid was decreased significantly. Often in *in vitro* studies hepatic TAG stores have been related to secretion of TAG through VLDL into the medium (Nossen *et al.*, 1986; Ranheim *et al.*, 1994; Vegusdal *et al.*, 2005), with increased TAG stores being correlated with decreased TAG secretion from hepatocytes (Vegusdal *et al.*, 2005). In the current experiment, in addition to the significantly reduced plasma LDL levels in salmon fed 100% VO, although not statistically significant, plasma VLDL lipid levels were increasingly more reduced through the sea water period in salmon fed 100% VO compared to fish fed 100% FO. Plasma VLDL sampled 24 hours after the last meal is predominantly endogenously synthesised VLDL excreted from liver (Babin and Vernier, 1989; Torstensen, 2000; Torstensen *et al.*, 2001). Also in fish, plasma LDL originates from plasma VLDL after lipid uptake by peripheral

tissues and LDL is the transporter of cholesterol from liver to peripheral tissues (Babin and Vernier, 1989). However, endogenous levels of VLDL and LDL is very low in post-absorptive fish plasma, and VLDL almost disappear in fish plasma 24 hours after feeding (Santulli *et al.*, 1997; Torstensen *et al.*, 2000; Torstensen *et al.*, 2001), as was also observed in the current study. Thus, the trend of reduced plasma VLDL levels and significantly reduced plasma LDL may be a result of decreased hepatic synthesis of VLDL and secretion of TAG from livers in salmon fed 100% VO. In contrast, salmon *in vitro* studies (Vegusdal *et al.*, 2005), however, showed that high 18:1n-9 levels increased both hepatic TAG synthesis and excretion, whereas high 20:5n-3 gave reduced TAG secretion. Further, Frøyland et al (1997) reported decreased plasma TAG induced by 20:5n-3, whereas high 22:6n-3 levels had no effect on plasma TAG levels in rats. Compared to *in vitro* studies and feeding studies varying one fatty acid at the time and often using up to pharmacological fatty acid concentrations, the current feeding experiment vary a range of dietary fatty acids concomitantly probably giving a more complex and balanced response.

Another possible explanation for the reduced plasma lipid and LDL in VO compared to FO fed salmon is increased uptake of plasma LDL by peripheral tissue in VO fed fish by up-regulating of the LDL receptors. However, no differences in cholesterol or TAG levels were observed in either whole fish or muscle (data not shown) lipid class levels, and LDL receptor studies were not included in this experiment. Thus, further elucidations of LDL metabolism when fish oil is replaced by vegetable oils through the sea water period Atlantic salmon is necessary to elucidate the possible effects on LDL metabolism.

Further, in the fresh water phase plasma cholesterol was significantly lower in VO fed fish, however after smoltification and sea water transfer the plasma cholesterol levels were similar

in the two dietary groups. In contrast to fish oils, vegetable oils contain variable levels of plant sterols (phytosterols) which has previously been reported to reduce LDL cholesterol and TAG in male brook trout (*Salvelinus fontinalis*) (Gilman *et al.*, 2003) and LDL cholesterol in humans (Ketomaki *et al.*, 2005). Phytosterols are reported to compete with intestinal cholesterol uptake and thereby reduce the uptake of dietary cholesterol (reviewed by Orzechowski *et al.* (2002)). Further, phytosterols themselves are absorbed at very low levels (about 10%) in humans (Ketomaki *et al.*, 2005). Especially β-sitosterol, which is the dominating phytosterol in rapeseed oil (Gordon and Miller, 1997) which contributed with 55% of the oil in the 100% VO diet, are known to reduce plasma LDL cholesterol in humans (Pollak and Kritchevsky, 1981). Dietary rapeseed oil replacing up to 100% of fish oil have also previously been reported to affect plasma lipoproteins (Torstensen *et al.*, 2004). Thus, the significant effects of replacing FO with 100% VO blend in salmon on plasma total cholesterol and TAG in early freshwater stages and on plasma LDL levels in later seawater stages may be due to an effect of plant sterols on dietary cholesterol uptake and metabolism. However, the effect of reduced intestinal cholesterol uptake being most obvious in early freshwater life stages may be reduced during the seawater stages due to a compensatory increase in cholesterol synthesis.

Liver and lipoprotein fatty acid composition was, in line with previously reported results (Lie *et al.*, 1994; Torstensen *et al.*, 2000; Torstensen *et al.*, 2004), highly influenced by dietary fatty acid composition. As also previously reported, HDL and liver have generally high levels of 22:6n-3 irrespective of dietary level, which may reflect also the high phospholipid levels in liver and high phosphatidyl choline (PC) and sterol ester (SE) levels in HDL compared to other tissues and lipoproteins, which is reported to be less influenced by dietary fatty acid composition compared to neutral lipids (Brodtkorb *et al.*, 1997; Olsen and Henderson, 1997).

Of the hepatic lipogenic enzymes measured ME, G6PDH, 6PGDH and ICDH are all NADPH producing or regenerating enzymes whereas FAS is a multienzyme complex which together with acetyl-CoA carboxylase catalyses de novo fatty acid synthesis. The activity of several lipogenic enzymes have previously been shown to be depressed by dietary lipid level in Atlantic salmon (Arnesen *et al.*, 1993), juvenile seabass (Dias *et al.*, 1998) and trout (Henderson and Sargent, 1981; Brauge *et al.*, 1995) already at dietary lipid levels of about 10 %. Compared to juvenile sea bass (Dias *et al.*, 1998) the FAS activity was about two-fold lower in livers from salmon in the current experiment, and may be biologically insignificant in the contribution of fatty acids for the salmon lipid storage and metabolism as has also previously been suggested by Sargent *et al.* (1989). A general trend, though, for all lipogenic enzymes, except for ME, was a significantly higher enzyme activity in livers from fish fed 100% VO compared to 100% FO. A similar effect has been reported in rats where high dietary 18:3n-3 levels affected FAS activity resulting in altered plasma TAG levels (Kim *et al.*, 2004). However, the quantitative impact of effects on lipogenic enzyme activity, especially due to the overall low FAS activity, on liver and plasma lipid levels is considered to be minor in the current study.

NADPH is used for reductive synthesis, such as fatty acid biosynthesis, and for maintenance of the cellular red-ox state (reviewed by (Kletzien *et al.*, 1994)). It is reported that dietary PUFA inhibit expression of the lipogenic enzyme glucose-6-phosphate dehydrogenase (G6PDH) in rat hepatocytes (Stabile *et al.*, 1998), and lipogenesis in rat (Zampelas *et al.*, 1995) and rainbow trout (Alvarez *et al.*, 2000). Dietary PUFA further affect the requirement for antioxidants (Benzie, 1996), including endogenous antioxidants such as NADPH. The degree of unsaturation was significantly higher in membranes of fish fed 100% FO compared

to 100% VO. However, the activity of NADPH producing and regenerating enzymes was generally higher in livers from fish fed 100% VO or no significant difference between the two dietary groups. Thus, feeding salmon 100% VO through the life cycle did not have any strong inducing or inhibiting effect on the activity of the liver lipogenic enzymes at any of the measured life stages.

The expression of PPARγ increased prior to sea water transfer followed by a decrease, and then another increase towards the final sampling, and with no effect of dietary treatment. Thus, there was no relationship between increased liver TAG stores in VO fed fish and expression of PPARγ in liver. However, the increase in PPARγ expression measured in both dietary groups after 22 months of feeding coincides with the significant increase in liver lipid storage during this period. PPARγ is considered to play a critical role in tissue lipid storage and especially fat accumulation in adipocytes in mammals (reviewed by Desvergne and Wahli (1999)). It has, however, recently been suggested a different role of PPARγ in fish compared to mammals and rodents based on both tissue expression patterns, lack of nutritional regulation and differences in ligand activation (Leaver *et al.*, 2005). PPARγ was first described in Atlantic salmon by Ruyter et al (1997), and this short gene transcript form (Gen Bank Acc. No AJ292962) has been suggested to be associated with peroxisomal β-oxidation (Ruyter *et al.*, 1997) *in vitro*. Whereas the function of the long form, expressed at highest transcripts levels in liver (Andersen *et al.*, 2000) and ubiquitously expressed in salmon (Gen Bank Acc. No AJ292963) remains uncharacterised. PPARγ has recently also been characterised in liver of zebrafish (Ibabe *et al.*, 2002; Ibabe *et al.*, 2005), gray mullet (Ibabe *et al.*, 2004), sea bass (Boukouvala *et al.*, 2004), and plaice and sea bream (Leaver *et al.*, 2005).

In summary, this is the first report of liver TAG stores, plasma lipid and LDL levels being affected by dietary vegetable oil replacement in Atlantic salmon during a long term feeding experiment. Current results indicate that high dietary vegetable oil inclusion increase hepatic TAG stores and decrease plasma lipid levels possible through decreased VLDL synthesis.

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Legend to Figures

Figure 1 Liver malic enzyme (ME), NADP-isocitrate dehydrogeanse (ICDH), 6-phosphogluconate dehydrogeanse (6PGDH) and glucose-6-phosphate dehydrogeanse (G6PDH) activity (μmol min^{-1} $protein^{-1}$), and Fatty acid synthetase (FAS) activity (mIU/g liver) from Atlantic salmon fed the experimental diets from April 2002. Data are presented as mean ±std (n=3) Statistical differences between dietary treatments are denoted by *.

Figure 2 Plasma TAG (mM), Total Cholesterol (mM) and Total Protein (g/l) from Atlantic salmon fed the experimental diets from April 2002 to January 2004. Data are presented as mean ± std, n=3. Statistical differences by 1-way ANOVA are denoted by *. The fish were transferred to sea water after 10 months of feeding.

Figure 3 Score plot (upper) and load plot (lower) of the relative fatty acid composition data of the diets (100% FO and 100% VO 6 mm and 9 mm) and of VLDL, LDL, HDL, plasma, NLP and liver from the samplings after Atlantic salmon had been fed the experimental diets for 14, 16 and 22 months. Arrows are drawn by free hand to indicate grouping of classes and correlations between samples and diets. Abbreviation: VLDL=very low density lipoprotein, LDL=low density lipoprotein, HDL=high density lipoprotein, NLP=non lipoprotein, FO=100% fish oil, VO=100% vegetable oil blend.

Figure 4 Mean normalised expression of PPARγ in liver from Atlantic salmon fed 100% VO and 100% FO through their life cycle. Mean normalised expression against was measured after 3, 6, 9, 14, 16 and 22 months of feeding, and is expressed as mean ± std (n=3)

Table 1 Proximate compositions (percentage, w.w.) and fatty acid compositions (wt%, w.w.) of represenatative diets used in freshwater (3mm) and seawater (9 mm pellet)

	Freshwater		Seawater	
	100% FO	**100% VO**	**100% FO**	**100% VO**
Proximate composition				
Protein	51.0 ± 0.4	50.0 ± 0.2	42.7 ± 0.2	42.9 ± 0.2
Lipid	20.7 ± 0.1	20.1 ± 0.1	31.4 ± 0.4	31.3 ± 0.1
Dry matter	93.2 ± 0.2	93.9 ± 0.3	93.9 ± 0.3	93.0 ± 0.1
Ash	8.4 ± 0.1	9.0 ± 0.0	7.3 ± 0.1	7.1 ± 0.1
Fatty acid composition				
14:0	6.1 ± 0.1	1.1 ± 0.1	6.2 ± 0.1	0.6 ± 0.1
16:0	12.4 ± 0.2	16.9 ± 0.4	14.5 ± 0.4	15.3 ± 0.3
18:0	1.5 ± 0.0	2.7 ± 0.0	2.4 ± 0.6	2.7 ± 0.0
Total saturated	20.3 ± 0.3	21.8 ± 0.4	23.6 ± 0.9	19.4 ± 0.3
16:1n-7	7.9 ± 0.1	1.1 ± 0.0	4.9 ± 0.2	0.5 ± 0.1
18:1n-9	11.9 ± 0.4	40.4 ± 0.5	13.2 ± 0.4	43.0 ± 0.2
18:1n-7	3.3 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.0
20:1n-9	19.9 ± 0.4	2.7 ± 0.1	11.1 ± 1.0	1.3 ± 0.1
22:1n-11	15.8 ± 0.3	2.3 ± 0.1	16.5 ± 1.9	0.8 ± 0.0
24:1n-9	0.7 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.0 ± 0.0
Total monoenes	59.4 ± 1.3	50.2 ± 0.5	48.8 ± 2.5	48.2 ± 0.5
18:2n-6	3.9 ± 0.1	13.5 ± 0.2	3.6 ± 0.6	17.1 ± 0.2
20:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.0 ± 0.0
Total n-6 PUFA	4.4 ± 0.1	13.7 ± 0.2	4.6 ± 0.7	17.1 ± 0.2
18:3n-3	0.6 ± 0.0	8.0 ± 0.2	1.2 ± 0.1	13.4 ± 0.5
18:4n-3	1.9 ± 0.1	0.4 ± 0.1	2.5 ± 0.1	0.2 ± 0.0
20:4n-3	0.3 ± 0.0	0.1 ± 0.1	0.7 ± 0.0	0.0 ± 0.0
20:5n-3	5.8 ± 0.6	2.1 ± 0.0	6.5 ± 0.2	0.6 ± 0.0
22:5n-3	0.4 ± 0.1	0.2 ± 0.0	0.9 ± 0.2	0.0 ± 0.0
22:6n-3	5.9 ± 0.6	3.4 ± 0.2	10.0 ± 0.6	1.0 ± 0.0
Total n-3 PUFA	14.9 ± 1.4	14.3 ± 0.6	21.8 ± 0.8	15.2 ± 0.5
Total PUFA	20.3 ± 1.6	28.0 ± 0.8	27.6 ± 1.6	32.3 ± 0.7

Results are means ± SD (n=3 for proximates and n = 2 for for fatty acids)

Table 2 Fatty acid compositions (mg FA g^{-1} liver tissue, w.w.) of livers from Atlantic salmon fed either 100% FO or 100% VO from start feeding (April 2002) Samplings were made after 6, 9, 14, 16 and 22 months of feeding, with sea water transfer after 10 months. Data are presented as mean ±std, n=3

	100% FO					100% VO				
Months of feeding:	**6**	**9**	**14**	**16**	**22**	**6**	**9**	**14**	**16**	**22**
14:0	1.0 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.0	1.0 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
16:0	4.7 ± 0.4*	5.0 ± 0.7*	4.2 ± 0.2	4.5 ± 0.2*	4.6 ± 0.2*	5.0 ± 0.2*	5.2 ± 0.6*	5.0 ± 0.1	4.4 ± 0.1*	5.6 ± 1.0*
18:0	1.2 ± 0.1	1.2 ± 0.3	1.2 ± 0.1	1.9 ± 0.1*	1.5 ± 0.1	1.7 ± 0.0	1.8 ± 0.3	2.2 ± 0.2	2.0 ± 0.1*	3.4 ± 0.6
Saturated FA	*7.2 ± 0.7**	*7.5 ± 1.3**	*6.7 ± 0.4*	*7.5 ± 0.4*	*7.9 ± 0.4*	*7.3 ± 0.2**	*7.3 ± 0.9**	*7.7 ± 0.3*	*6.8 ± 0.2*	*9.6 ± 1.7*
16:1n-7	1.2 ± 0.2	1.6 ± 0.5	0.9 ± 0.1	0.6 ± 0.0	0.9 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.7 ± 0.3
18:1n-7	1.3 ± 0.2	1.1 ± 0.3	0.9 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.5 ± 0.0	1.6 ± 0.4
18:1n-9	4.8 ± 0.6	5.3 ± 1.7	3.2 ± 0.3	3.8 ± 0.5	4.7 ± 0.9	9.1 ± 0.4	10.5 ± 2.3	10.3 ± 1.7	7.3 ± 0.8	25.1 ± 6.7
18:1n-11	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	1.2 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
20:1n-9	2.8 ± 0.5	2.5 ± 0.8	2.2 ± 0.3	1.5 ± 0.2	2.4 ± 0.5	1.4 ± 0.2	0.9 ± 0.3	1.1 ± 0.2	0.6 ± 0.0	2.9 ± 0.7
20:1n-11	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:1n-9	0.3 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
22:1n-11	0.7 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	0.6 ± 0.0	1.4 ± 0.4	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
Monoene FA	*12.1 ± 2.0**	*12.3 ± 3.8**	*8.3 ± 0.8*	*8.1 ± 0.6**	*12.5 ± 2.5*	*12.5 ± 0.8**	*13.2 ± 3.0**	*12.7 ± 2.1*	*8.7 ± 1.0**	*31.1 ± 8.3*
18:2n-6	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.1	2.7 ± 0.1	2.4 ± 0.4	3.2 ± 0.4	2.6 ± 0.2	6.8 ± 1.6
20:4n-6	0.6 ± 0.0	0.5 ± 0.1	0.8 ± 0.0*	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.8 ± 0.1	0.9 ± 0.1*	1.0 ± 0.0*	1.5 ± 0.1
Sum n-6	*1.5 ± 0.2*	*1.4 ± 0.2*	*1.6 ± 0.1*	*1.7 ± 0.0*	*1.9 ± 0.1*	*4.5 ± 0.1*	*4.3 ± 0.7*	*5.2 ± 0.6*	*4.6 ± 0.2*	*10.5 ± 2.2*
18:3n-3	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.0 ± 0.0	0.6 ± 0.1	1.4 ± 0.2	1.0 ± 0.1	2.9 ± 0.8
20:4n-3	0.2 ± 0.1*	0.3 ± 0.0*	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	0.3 ± 0.0*	0.3 ± 0.0*	0.4 ± 0.0	0.2 ± 0.0	0.8 ± 0.1
20:5n-3	1.8 ± 0.1	1.9 ± 0.3	2.1 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.4 ± 0.1
22:5n-3	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.0	1.0 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.7 ± 0.1
22:6n-3	10.1 ± 0.5*	8.1 ± 0.9*	8.5 ± 0.3*	8.6 ± 0.3	8.8 ± 0.3	9.9 ± 0.3*	7.5 ± 0.7*	8.2 ± 0.4*	6.9 ± 0.2	5.6 ± 0.4
Sum n-3	*12.7 ± 0.7**	*10.8 ± 1.3**	*11.6 ± 0.5**	*12.5 ± 0.5*	*13.5 ± 0.1**	*12.9 ± 0.4**	*10.1 ± 1.0**	*12.4 ± 0.5**	*10.7 ± 0.1*	*12.6 ± 1.5**
n-3/n-6	8.3 ± 0.4	8.0 ± 0.4	7.2 ± 0.2	7.2 ± 0.2	7.1 ± 0.5	2.8 ± 0.1	2.4 ± 0.2	2.4 ± 0.2	2.3 ± 0.1	1.2 ± 0.1
Rest FA	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
Total FA	*34.3 ± 3.6**	*32.4 ± 6.6**	*28.7 ± 1.6*	*30.5 ± 1.4**	*36.7 ± 3.0*	*37.8 ± 1.5**	*35.1 ± 5.5**	*38.0 ± 3.5*	*31.0 ± 1.5**	*64.0 ± 13.7*

**denotes not statistically different between 100% FO and 100% VO groups analysed by 1-way ANOVA, n=3.*

Table 3 Lipid class composition (Wt% and mg lipid class g^{-1} tissue, w.w.) and total lipid (g $100g^{-1}$, w.w.) of liver from June 2003 (after 14 months), August 2003 (after 16 months) and January 2004 (after 22 months of feeding) from Atlantic salmon fed 100% FO or 100% VO since April 2002. Data are presented as mean ± std, n=3.

	14 months			16 months		Statistical differences between samplings		22 months		
Wt%	**100%FO**	**100%VO**	**sign diff**	**100%FO**	**100%VO**	**100%FO**	**100%VO**	**100%FO**	**100%VO**	**sign diff**
SM	3.3 ± 0.3	3.7 ± 0.4		3.5 ± 0.2	3.1 ± 0.1			1.8 ± 0.0	1.3 ± 0.3	p<0.05
PC	30.7 ± 1.4	26.2 ± 1.0	p<0.01	28.4 ± 0.6	27.8 ± 0.2	(p=0.059)	(p=0.052)	26.7 ± 1.8	19.0 ± 2.1	p<0.01
PS	3.7 ± 0.1	3.2 ± 0.3	p<0.05	3.8 ± 0.2	3.5 ± 0.3			3.0 ± 0.3	1.9 ± 0.1	p<0.01
PI	5.3 ± 0.3	4.6 ± 0.3	p<0.05	6.0 ± 0.5	5.7 ± 0.2		p<0.01	4.4 ± 0.3	3.1 ± 0.3	p<0.01
PA/CL	2.8 ± 0.2	2.6 ± 0.2		3.4 ± 0.1	3.3 ± 0.1	p<0.01	p<0.01	2.2 ± 0.1	1.6 ± 0.1	p<0.01
PE	12.8 ± 0.6	11.6 ± 0.7		12.8 ± 0.2	12.9 ± 0.2		p<0.05	12.4 ± 0.7	8.8 ± 1.0	p<0.01
CER	4.3 ± 0.3	4.5 ± 0.1		2.4 ± 0.6	3.0 ± 0.6	p<0.01	p<0.01	2.2 ± 0.3	2.6 ± 0.4	
Sum PL	*62.9 ± 2.9*	*56.3 ± 3.1*		*60.2 ± 0.3*	*59.4 ± 0.4*			52.7 ± 2.9	38.3 ± 3.9	p<0.01
MAG	2.5 ± 0.2	2.3 ± 0.1		2.6 ± 0.3	2.6 ± 0.2			n.d. ±	n.d. ±	
CHOL	17.3 ± 0.8	15.7 ± 0.5	p<0.05	15.6 ± 0.5	14.8 ± 0.1	p<0.05	(p=0.056)	16.5 ± 0.3	13.0 ± 1.5	p<0.05
FFA	2.5 ± 0.5	2.9 ± 0.3		2.0 ± 0.6	2.3 ± 0.9			2.6 ± 0.9	2.3 ± 0.7	
TAG	11.9 ± 3.6	19.8 ± 3.8	p=0.059	19.6 ± 1.0	21.0 ± 0.7	p<0.05		29.1 ± 1.8	47.1 ± 6.8	p<0.05
Sum NL	*37.0 ± 3.3*	*43.8 ± 3.0*		*39.8 ± 0.3*	*40.6 ± 0.4*			47.3 ± 2.9	61.7 ± 3.9	p<0.01
Mg g^{-1}										
SM	2.2 ± 0.2	3.1 ± 0.2	p<0.01	2.1 ± 0.1	2.0 ± 0.1		p<0.01	1.3 ± 0.1	1.2 ± 0.2	
PC	29.4 ± 3.3	30.5 ± 0.1		21.3 ± 2.3	22.5 ± 0.2	p<0.05	p<0.01	22.1 ± 0.7	22.6 ± 1.8	
PS	2.4 ± 0.1	2.5 ± 0.1		2.3 ± 0.1	2.2 ± 0.2			2.6 ± 0.1	2.3 ± 0.3	
PE	6.7 ± 0.4	7.5 ± 0.4		6.7 ± 0.7	7.3 ± 0.2			9.2 ± 0.4	9.2 ± 0.6	
Sum PL	*40.7 ± 4.0*	*43.5 ± 0.6*		*32.3 ± 3.1*	*34.0 ± 0.5*	p<0.05	p<0.01	35.2 ± 1.1	35.4 ± 2.5	
CHOL	2.3 ± 0.2	2.6 ± 0.0		2.4 ± 0.1	2.5 ± 0.1			3.1 ± 0.6	3.2 ± 0.8	
FFA	n.d.* ±	n.d. ±		n.d. ±	n.d. ±			±	±	
TAG	2.3 ± 0.8	5.0 ± 1.3	p<0.05	7.3 ± 2.1	8.0 ± 0.6	p<0.05	p<0.05	14.2 ± 2.1	34.7 ± 12.1	p<0.05
Sum NL	*4.6 ± 0.8*	*7.6 ± 1.3*	p<0.05	*9.7 ± 2.1*	*10.5 ± 0.6*	p<0.05	p<0.05	17.3 ± 2.6	37.9 ± 11.8	p<0.05
Sum Lip	*45.3 ± 3.2*	*51.1 ± 1.3*	p<0.05	*42.0 ± 4.7*	*44.5 ± 1.1*		p<0.01	52.5 ± 3.7	73.2 ± 14.4	
Total lipid[1)]	3.8 ± 0.5	4.7 ± 0.4	p=0.075	4.0 ± 0.2	3.5 ± 0.4		p<0.05	3.3 ± 0.6	5.4 ± 3.2	

**n.d. denotes not detected*

[1)]Total lipid analysed by Ethyl Acetate extraction and gravimetric method.

Statistical differences were obtained by testing differences between diets and between samplings within diets by 1-way ANOVA, n=3. No significant differences were found between dietary groups in the August 20003 (after 16 months) sampling.

Table 4 Lipid class composition (wt %) and levels (mg g^{-1}) in plasma and lipoproteins from Atlantic salmon fed 100% VO and 100% FO from April 2002. Due to no significant differences between the groups, dietary treatments are combined (n=6). Data are presented as mean ± std. n.d.: not detected.

			After 14 months (June 2003)			
	Plasma	**VLDL**	**LDL**	**HDL**	**NLP**	**Recovery**
PC	26.8 ± 0.3	9.0 ± 2.4	10.2 ± 2.1	25.3 ± 0.6	22.4 ± 0.8	
PI	1.5 ± 0.4	0.8 ± 0.0	0.8 ± 0.1	2.5 ± 0.6	2.7 ± 0.3	
PE	0.6 ± 0.2	n.d. ±	n.d. ±	0.9 ± 0.2	1.0 ± 0.3	
Sum PL	*34.7 ± 0.7*	*9.7 ± 3.4*	*13.3 ± 4.0*	*35.1 ± 0.6*	*31.7 ± 1.5*	
CHOL	10.9 ± 0.5	19.3 ± 6.9	20.8 ± 4.5	12.0 ± 0.8	11.0 ± 0.6	
FFA	3.2 ± 0.3	n.d. ±	n.d. ±	2.6 ± 0.3	4.3 ± 0.2	
TAG	6.5 ± 0.7	32.0 ± 5.5	20.3 ± 5.8	4.2 ± 0.7	6.4 ± 0.8	
CE	44.7 ± 1.5	39.0 ± 4.5	45.6 ± 8.0	46.0 ± 1.9	46.6 ± 2.3	
Sum NL	*65.3 ± 0.7*	*90.3 ± 3.4*	*86.7 ± 4.0*	*64.9 ± 0.6*	*68.3 ± 1.5*	
Protein (mg g^{-1} plasma)	34.45 ± 3.20	0.00 ± 0.01	0.01 ± 0.01	3.73 ± 0.62	21.88 ± 1.70	90
NL (mg g^{-1} plasma)	3.40 ± 0.34	0.05 ± 0.03	0.10 ± 0.05	4.18 ± 1.09	1.12 ± 0.37	160
PL (mg g^{-1} plasma)	8.16 ± 0.54	0.01 ± 0.01	0.04 ± 0.03	5.77 ± 1.28	1.54 ± 0.47	111
Total lipid (mg g^{-1} plasma)	11.56 ± 0.85	0.06 ± 0.04	0.15 ± 0.07	9.95 ± 2.35	2.66 ± 0.84	74
			After 16 months (August 2003)			
	Plasma	**VLDL**	**LDL**	**HDL**	**NLP**	
PC	24.8 ± 0.5	6.7 ± 1.4	12.3 ± 1.2	25.5 ± 0.3	23.0 ± 0.6	
PI	2.5 ± 0.2	n.d. ±	0.7 ± 0.6	2.4 ± 0.2	3.0 ± 0.2	
PE	0.0 ± 0.0	n.d. ±	n.d. ±	0.4 ± 0.4	0.6 ± 0.1	
Sum PL	*32.8 ± 0.8*	*7.7 ± 2.5*	*18.2 ± 2.3*	*35.1 ± 0.9*	*32.9 ± 0.6*	
CHOL	9.7 ± 0.4	12.6 ± 1.3	16.8 ± 1.3	9.2 ± 0.5	9.4 ± 0.5	
FFA	4.0 ± 0.4	2.7 ± 0.7	0.5 ± 1.3	4.7 ± 0.4	6.7 ± 0.4	
TAG	7.2 ± 0.5	41.5 ± 3.1	17.9 ± 1.3	8.0 ± 0.5	8.2 ± 0.4	
CE	46.3 ± 1.4	35.5 ± 3.5	46.7 ± 2.7	43.0 ± 1.2	42.7 ± 0.7	
Sum NL	*67.2 ± 0.8*	*92.3 ± 2.5*	*81.8 ± 2.3*	*64.9 ± 0.9*	*67.1 ± 0.6*	
Protein (mg g^{-1} plasma)	53.12 ± 5.70	0.02 ± 0.02	0.11 ± 0.02	3.73 ± 0.22	33.35 ± 3.65	70
NL (mg g^{-1} plasma)	4.05 ± 0.39	0.03 ± 0.01	0.18 ± 0.03	1.90 ± 0.14	2.01 ± 0.15	127
PL (mg g^{-1} plasma)	7.16 ± 0.93	0.01 ± 0.00	0.09 ± 0.02	2.77 ± 0.14	2.11 ± 0.13	79
Total lipid (mg g^{-1} plasma)	11.21 ± 1.32	0.04 ± 0.01	0.27 ± 0.05	4.67 ± 0.28	4.11 ± 0.26	96

PL: phospholipids, NL: neutral lipids, PC: phosphatidyl choline, PI: phosphatidyl inositol, PE: phosphatidyl ethanolamine, CHOL: cholesterol, TAG: triacylglycerol, CE: cholesterol ester.

Table 5 Lipid class composition (wt %) and levels (mg lipid class g^{-1} plasma) in plasma and lipoproteins from Atlantic salmon fed either 100% VO or 100% FO from April 2002. Data are presented as mean ± std, n=3.

	After 22 months (January 2004), mg lipid class g^{-1} plasma													
	PLASMA (mg lipid class g^{-1} plasma)		VLDL		LDL		HDL		NLP		Recovery (%)	
	100% FO	100% VO	100% FO	100% VO	100% FO	100% VO	100% FO	100% VO	100% FO	100% VO	100% FO	100% VO
SM	0.5 ± 0.0	0.4 ± 0.0	0.01 ± 0.00	0.00 ± 0.00	0.05 ± 0.01 [a]	0.02 ± 0.00 [b]	0.31 ± 0.07	0.30 ± 0.01	0.12 ± 0.01	0.13 ± 0.03	105	111
PC	5.9 ± 0.4	5.2 ± 0.4	0.05 ± 0.03	0.02 ± 0.01	0.28 ± 0.07	0.16 ± 0.02	3.61 ± 0.58	3.30 ± 0.06	1.24 ± 0.12	1.18 ± 0.23	88	90
Sum PL	6.4 ± 0.5	5.6 ± 0.4	0.05 ± 0.03	0.02 ± 0.01	0.33 ± 0.08 [a]	0.18 ± 0.02 [b]	3.93 ± 0.65	3.61 ± 0.05	1.38 ± 0.13	1.31 ± 0.27	89	92
CHOL	0.5 ± 0.0	0.5 ± 0.1	0.02 ± 0.01	0.01 ± 0.00	0.07 ± 0.01 [a]	0.04 ± 0.00 [b]	0.35 ± 0.07	0.33 ± 0.06	0.13 ± 0.01	0.13 ± 0.04	118	112
FFA	0.1 ± 0.1	0.1 ± 0.0	n.d. ±	n.d. ±	n.d. ±	n.d. ±	n.d. ±	n.d. ±	0.12 ± 0.03	0.12 ± 0.00	85	131
TAG	1.0 ± 0.3	0.7 ± 0.1	0.10 ± 0.08	0.05 ± 0.03	0.13 ± 0.06	0.09 ± 0.02	0.49 ± 0.14	0.38 ± 0.09	0.23 ± 0.07	0.23 ± 0.03	100	107
CE	3.6 ± 0.4	3.0 ± 0.2	0.08 ± 0.05	0.05 ± 0.03	0.35 ± 0.07 [a]	0.20 ± 0.02 [b]	1.99 ± 0.28	1.80 ± 0.11	0.81 ± 0.12	0.76 ± 0.14	89	94
Sum NL	5.2 ± 0.2 [a]	4.2 ± 0.2 [b]	0.20 ± 0.13	0.10 ± 0.06	0.55 ± 0.14	0.33 ± 0.04	2.83 ± 0.20	2.51 ± 0.14	1.30 ± 0.20	1.24 ± 0.21	93	99
Sum Lip	11.6 ± 0.4 [a]	9.8 ± 0.7 [b]	0.25 ± 0.17	0.12 ± 0.07	0.88 ± 0.21 [a]	0.51 ± 0.06 [b]	6.75 ± 0.83	6.12 ± 0.18	2.68 ± 0.33	2.56 ± 0.48	91	95
Protein (mg g^{-1} plasma)	43.9 ± 2.3	41.5 ± 2.3	0.1 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	5.6 ± 0.8	4.9 ± 0.1	27.3 ± 1.4	27.9 ± 2.3	76	79

	After 22 months (January 2004), % distribution of lipid classes in lipoprotein fractions									
	% distribution of lipid classes in plasma		VLDL		LDL		HDL		NLP	
	100% FO	100% VO	100% FO	100% VO	100% FO	100% VO	100% FO	100% VO	100% FO	100% VO
LysoPC	2.1 ± 0.1 [a]	1.5 ± 0.1 [b]	n.d. ±	n.d. ±	0.7 ± 0.1	0.6 ± 0.0	2.0 ± 0.2	1.3 ± 0.1	2.4 ± 0.3 [a]	1.4 ± 0.1 [b]
SM	2.9 ± 0.2	3.1 ± 0.0	1.4 ± 0.2	1.0 ± 0.1	3.5 ± 0.4	3.9 ± 0.1	2.8 ± 0.3	3.1 ± 0.2	2.9 ± 0.1	3.0 ± 0.3
PC	22.5 ± 1.5	24.0 ± 0.5	10.2 ± 0.6	10.0 ± 0.1	13.1 ± 0.4	13.5 ± 0.5	26.4 ± 1.8	27.1 ± 0.9	24.8 ± 1.1	24.6 ± 0.2
PS	n.d. ±	n.d. ±	n.d. ±	n.d. ±	n.d. ±	n.d. ±	n.d. ±	n.d. ±	n.d. ±	n.d. ±
PI	3.6 ± 0.1	3.3 ± 0.2	1.1 ±	0.7 ±	1.9 ± 0.2	1.9 ± 0.2	3.5 ± 0.2	3.3 ± 0.3	4.6 ± 0.4	4.1 ± 0.3
PE	0.8 ± 0.1	0.6 ± 0.1	0.8 ±	n.d. ±	0.6 ± 0.0	0.6 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	1.1 ± 0.1 [a]	0.8 ± 0.1 [b]
Sum PL	*31.9 ± 1.7*	*32.5 ± 0.5*	*12.3 ± 0.5*	*11.2 ± 0.5*	*19.9 ± 0.2*	*20.5 ± 0.4*	*35.6 ± 1.7*	*35.6 ± 0.9*	*35.9 ± 0.5* [a]	*33.9 ± 0.3* [b]
CHOL	9.2 ± 0.6	10.2 ± 0.5	12.6 ± 3.2	14.5 ± 1.9	12.7 ± 0.9	11.8 ± 0.8	10.5 ± 0.7	11.1 ± 1.2	9.2 ± 0.5	9.4 ± 0.6
FFA	4.1 ± 1.0	4.0 ± 0.9	n.d. ±	n.d. ±	1.8 ± 0.5	1.1 ± 0.7	3.3 ± 0.8	3.0 ± 0.5	7.4 ± 1.3	7.6 ± 1.2
TAG	10.8 ± 3.3	9.4 ± 0.9	34.9 ± 4.3	32.7 ± 1.5	17.9 ± 3.7	20.6 ± 2.2	8.0 ± 2.7	6.9 ± 1.1	8.0 ± 1.9	8.1 ± 0.5
CE	44.0 ± 2.8	43.9 ± 0.9	40.3 ± 1.8	41.5 ± 2.8	48.9 ± 3.7	46.7 ± 2.1	42.6 ± 3.0	43.3 ± 0.5	39.5 ± 2.6	40.9 ± 0.8
Sum NL	*68.1 ± 1.7*	*67.5 ± 0.5*	*87.7 ± 0.5*	*88.8 ± 0.5*	*80.1 ± 0.2*	*79.5 ± 0.4*	*64.4 ± 1.7*	*64.4 ± 0.9*	*64.1 ± 0.5* [a]	*66.1 ± 0.3* *b*

*n.d. refers to not detected. All samples were tested for significant differences by 1-way ANOVA.Statistical significant differences between the dietary groups are denoted by upper case letters.

Table 6 Plasma and lipoprotein fatty acid composition (weight %) and total amount of FA (mg g^{-1}) January 2004 from salmon fed the experimental diets since April 2002 (Totally 22 months) Data are presented as mean ± std, n=3.

	Plasma		**VLDL**		**LDL**		**HDL**		**NLP**	
	100% FO	**100% VO**	**100% FO**	**100% VO**	**100% FO**	**100% VO**	**100% FO**	**100% VO**	**100% FO**	**100% VO**
14:0	2.8 ± 0.2	0.6 ± 0.0	3.3 ± 0.7	1.2 ± 0.2	3.1 ± 0.2	0.7 ± 0.1	2.4 ± 0.1	0.5 ± 0.1	3.1 ± 0.0	0.6 ± 0.1
16:0	15.4 ± 0.1	16.7 ± 0.2	10.4 ± 0.4	11.3 ± 0.4	13.1 ± 0.1	14.1 ± 0.2	16.8 ± 0.2	17.5 ± 0.1	16.5 ± 1.4	16.5 ± 0.8
18:0	2.4 ± 0.2	3.3 ± 0.1	2.0 ± 0.3	3.0 ± 0.4	2.1 ± 0.1	3.2 ± 0.3	2.8 ± 0.1	3.9 ± 0.0	2.9 ± 0.8	3.7 ± 0.7
Sum Saturated	*23.7 ± 0.3*	*20.8 ± 0.4*	*18.2 ± 0.4*	*16.1 ± 1.1*	*22.2 ± 1.1*	*18.0 ± 0.5*	*26.0 ± 0.4*	*22.4 ± 0.3*	*25.3 ± 2.4*	*21.4 ± 1.5*
16:1n-7	1.4 ± 0.2	0.5 ± 0.0	2.6 ± 0.5	0.8 ± 0.2	1.7 ± 0.2	0.5 ± 0.0	1.0 ± 0.4	0.4 ± 0.0	1.7 ± 0.1	0.5 ± 0.0
18:1n-7	1.4 ± 0.1	1.4 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.0	1.6 ± 0.3	1.3 ± 0.1
18:1n-9	6.8 ± 0.6	19.4 ± 0.5	12.4 ± 2.8	29.0 ± 1.1	8.8 ± 0.1	24.7 ± 1.1	7.2 ± 0.6	20.3 ± 0.1	7.7 ± 1.8	17.0 ± 1.8
18:1n-11	1.8 ± 0.1	0.0 ± 0.0	2.1 ± 0.2	0.1 ± 0.3	2.3 ± 0.2	0.0 ± 0.0	1.9 ± 0.1	0.0 ± 0.0	1.4 ± 0.4	2.5 ± 0.6
20:1n-9	3.5 ± 0.2	2.0 ± 0.1	6.0 ± 0.8	3.4 ± 1.3	5.0 ± 0.2	3.0 ± 0.2	4.0 ± 0.2	2.4 ± 0.1	4.0 ± 1.5	2.3 ± 0.4
20:1n-11	0.6 ± 0.1	0.0 ± 0.0	1.0 ± 0.1	0.1 ± 0.2	1.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.1	0.0 ± 0.0	0.5 ± 0.1	0.0 ± 0.0
22:1n-9	0.3 ± 0.1	0.1 ± 0.1	0.7 ± 0.3	0.4 ± 0.2	0.7 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.0
22:1n-11	3.3 ± 0.7	0.2 ± 0.2	6.6 ± 2.4	1.3 ± 1.4	5.7 ± 0.6	0.4 ± 0.2	3.5 ± 0.5	0.4 ± 0.1	3.4 ± 1.5	0.3 ± 0.1
Sum Monoene	*19.6 ± 1.3*	*24.1 ± 0.9*	*34.9 ± 2.0*	*38.2 ± 3.2*	*28.0 ± 0.7*	*31.1 ± 1.7*	*21.0 ± 0.6*	*26.1 ± 0.2*	*22.0 ± 4.6*	*25.0 ± 3.0*
18:2n-6	1.3 ± 0.2	8.1 ± 0.2	2.7 ± 1.0	10.0 ± 1.3	1.5 ± 0.2	8.9 ± 0.1	1.2 ± 0.2	7.6 ± 0.1	1.4 ± 0.1	8.1 ± 0.3
20:2n-6	0.4 ± 0.1	1.5 ± 0.1	0.5 ± 0.1	1.6 ± 0.1	0.4 ± 0.0	1.7 ± 0.1	0.4 ± 0.1	1.7 ± 0.1	0.4 ± 0.1	1.6 ± 0.2
20:4n-6	2.1 ± 0.1	2.4 ± 0.3	1.1 ± 0.2	1.1 ± 0.3	1.5 ± 0.2	1.7 ± 0.1	2.1 ± 0.1	2.3 ± 0.2	2.3 ± 0.1	2.8 ± 0.3
Sum n-6	*3.9 ± 0.4*	*14.1 ± 0.4*	*4.6 ± 1.2*	*13.8 ± 1.7*	*3.6 ± 0.4*	*14.1 ± 0.1*	*3.9 ± 0.4*	*13.7 ± 0.4*	*4.2 ± 0.3*	*14.5 ± 0.2*
18:3n-3	0.2 ± 0.2	3.2 ± 0.3	1.0 ± 0.5	4.8 ± 0.9	0.5 ± 0.1	3.9 ± 0.1	0.3 ± 0.0	2.7 ± 0.2	0.4 ± 0.1	3.3 ± 0.4
18:4n-3	0.3 ± 0.1	0.5 ± 0.0	0.7 ± 0.2	0.9 ± 0.0	0.5 ± 0.2	0.8 ± 0.1	0.3 ± 0.1	0.7 ± 0.2	0.3 ± 0.2	0.5 ± 0.1
20:4n-3	1.0 ± 0.0	1.6 ± 0.0	1.5 ± 0.0	1.5 ± 0.1	1.2 ± 0.0	1.5 ± 0.1	0.9 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	1.6 ± 0.1
20:5n-3	13.8 ± 1.1	12.3 ± 0.4	10.9 ± 1.0	8.9 ± 1.1	12.4 ± 1.2	11.0 ± 0.6	11.7 ± 0.3	10.3 ± 0.6	13.5 ± 2.7	11.6 ± 2.7
22:5n-3	3.0 ± 0.1	2.4 ± 0.1	2.8 ± 0.1	1.5 ± 0.1	2.6 ± 0.1	1.5 ± 0.1	3.1 ± 0.1	2.4 ± 0.1	3.0 ± 0.5	3.1 ± 1.0
22:6n-3	30.6 ± 1.0	19.4 ± 0.7	20.9 ± 1.5	12.1 ± 0.9	24.7 ± 0.6	15.4 ± 0.8	29.4 ± 0.6	18.1 ± 0.2	24.0 ± 7.9	14.7 ± 4.9
Sum n-3	*48.9 ± 1.5*	*39.9 ± 0.9*	*37.9 ± 2.2*	*30.4 ± 2.6*	*42.0 ± 1.4*	*34.7 ± 1.2*	*45.7 ± 0.3*	*36.1 ± 0.5*	*42.1 ± 11.5*	*34.9 ± 7.0*
Sum rest FA	3.9 ± 0.7	1.1 ± 0.4	4.4 ± 0.7	1.4 ± 0.1	4.2 ± 0.1	2.1 ± 1.0	3.5 ± 0.7	1.6 ± 0.3	6.4 ± 4.4	4.2 ± 2.5
n-3/n-6	*12.6 ± 1.3*	*2.8 ± 0.0*	*8.5 ± 1.9*	*2.2 ± 0.1*	*11.8 ± 1.0*	*2.5 ± 0.1*	*11.8 ± 1.1*	*2.6 ± 0.1*	*10.1 ± 2.9*	*2.4 ± 0.5*
Sum mg FA g^{-1} fraction	10.0 ± 0.5	9.1 ± 1.0	3.2 ± 1.7	1.7 ± 0.4	7.6 ± 1.7	5.1 ± 0.7	55.0 ± 4.1	54.8 ± 3.0	2.0 ± 0.5	2.0 ± 0.7
Sum mg FA g^{-1} plasma	10.9 ± 0.6	9.9 ± 1.1	0.2 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	3.8 ± 0.3	3.5 ± 0.1	1.6 ± 0.4	1.7 ± 0.6

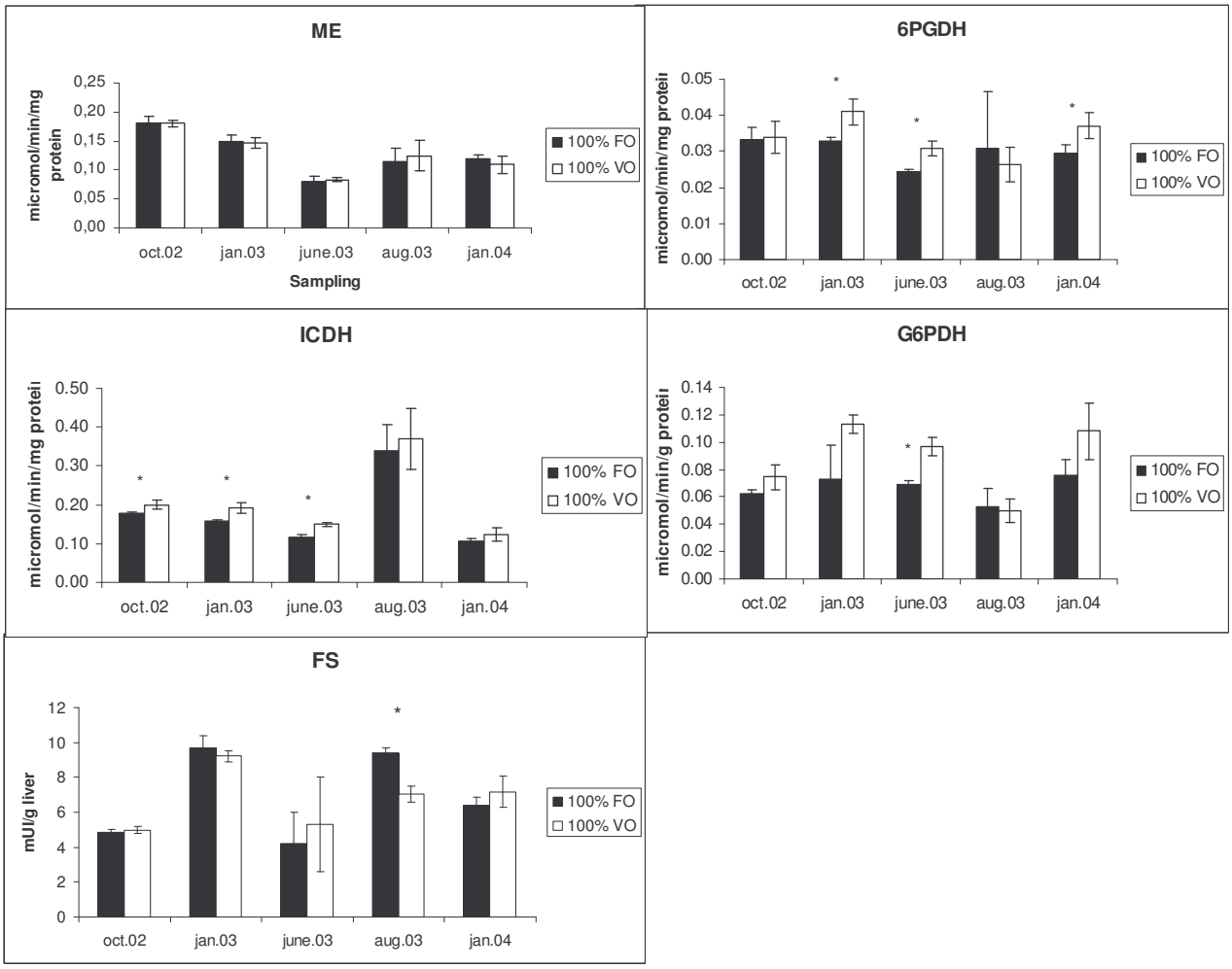
ME
micromol/min/mg protein
0,25
0,20
0,15
0,10
0,05
0,00
oct.02
jan.03
june.03
aug.03
jan.04
Sampling
100% FO
100% VO
6PGDH
micromol/min/mg proteii
0.05
0.04
0.03
0.02
0.01
0.00
ICDH
micromol/min/mg proteii
0.50
0.40
0.30
0.20
0.10
0.00
G6PDH
micromol/min/g proteii
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
FS
mUI/g liver
12
10
8
6
4
2
0

Figure 1

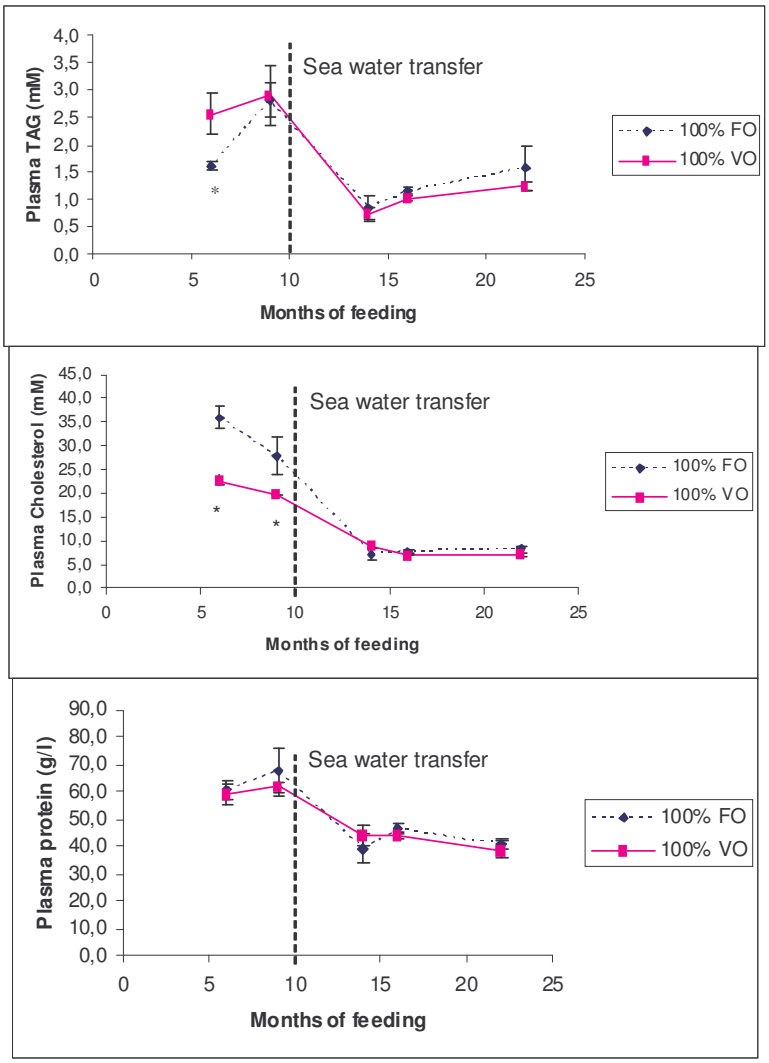
Plasma TAG (mM)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0
5
10
15
20
25
Months of feeding
Sea water transfer
100% FO
100% VO
*
Plasma Cholesterol (mM)
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
Months of feeding
Sea water transfer
100% FO
100% VO
*
*
Plasma protein (g/l)
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
Months of feeding
Sea water transfer
100% FO
100% VO

Figure 2

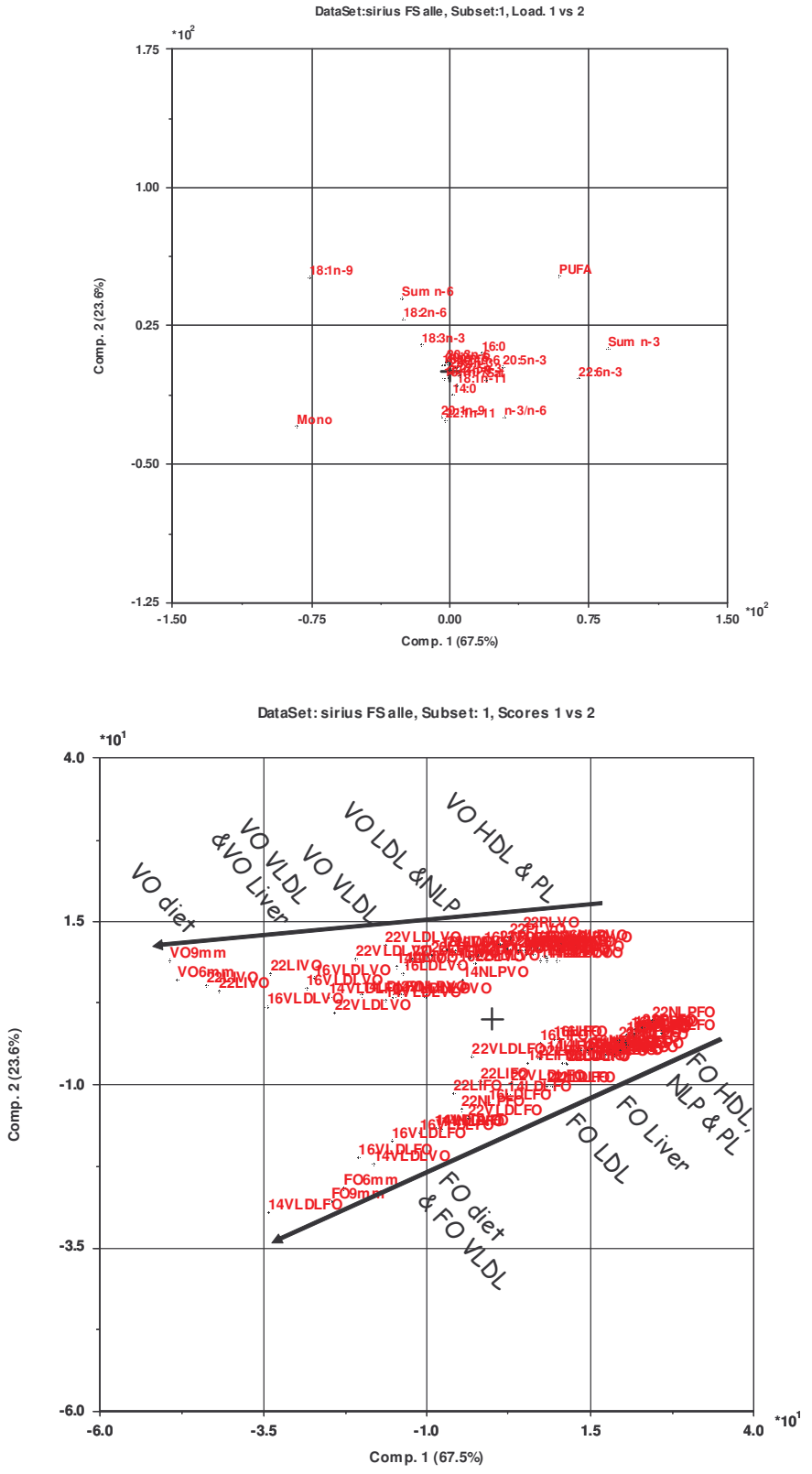
DataSet:sirius FS alle, Subset:1, Load. 1 vs 2
*10^2
1.75
1.00
0.25
-0.50
-1.25
-1.50
-0.75
0.00
0.75
1.50
*10^2
Comp. 2 (23.6%)
Comp. 1 (67.5%)
18:1n-9
PUFA
Sum n-6
18:2n-6
18:3n-3
16:0
20:5n-3
Sum n-3
22:6n-3
18:1n-11
14:0
Mono
n-3/n-6
DataSet: sirius FS alle, Subset: 1, Scores 1 vs 2
*10^1
4.0
1.5
-1.0
-3.5
-6.0
-6.0
-3.5
-1.0
1.5
4.0
*10^1
Comp. 2 (23.6%)
Comp. 1 (67.5%)
VO diet
VO VLDL &VO Liver
VO VLDL
VO LDL &NLP
VO HDL & PL
VO9mm
VO6mm
22LIVO
16VLDLVO
22VLDLVO
14NLPVO
16LDLVO
FO HDL
NLP & PL
FO Liver
FO LDL
FO diet & FO VLDL
22VLDLFO
16VLDLFO
14VLDLFO
FO6mm
FO9mm

Figure 3

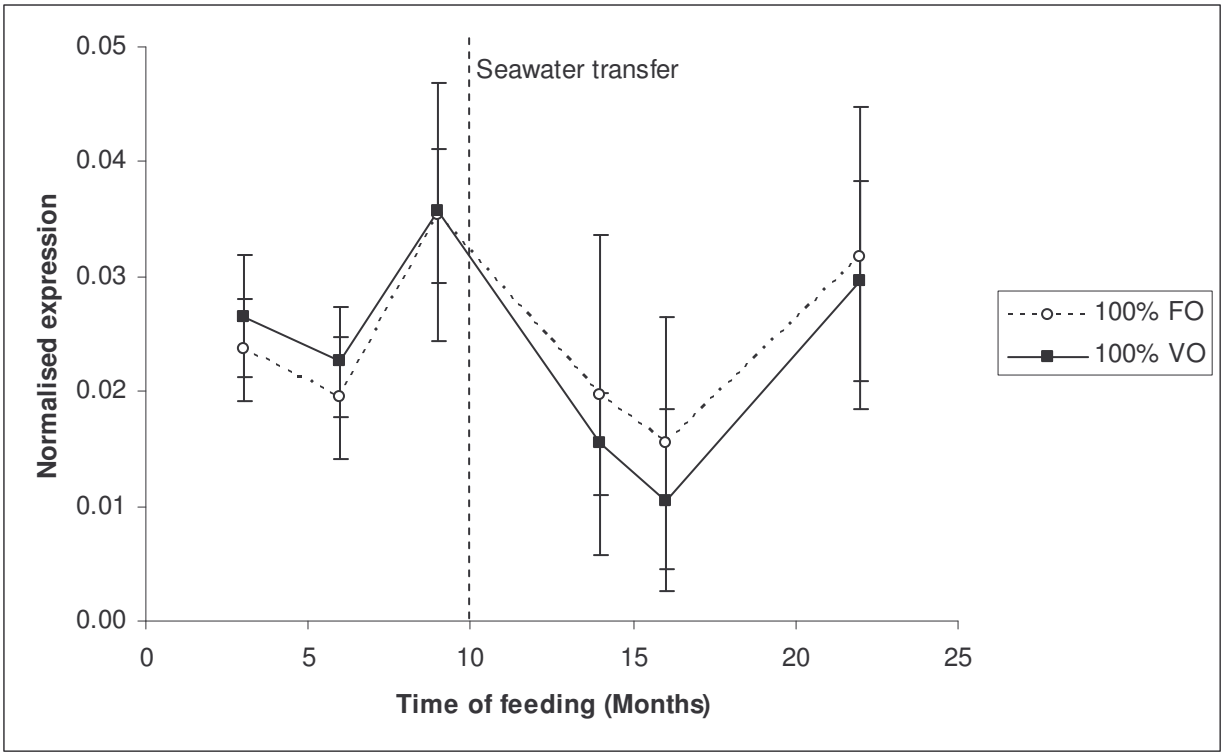
0.05
0.04
0.03
0.02
0.01
0.00
Normalised expression
Seawater transfer
0
5
10
15
20
25
Time of feeding (Months)
100% FO
100% VO

Figure 4