Comparison of effects of vegetable oils blended with southern hemisphere fish oil and decontaminated northern hemisphere fish oil on growth performance, composition and gene expression in Atlantic salmon (*Salmo salar* L.)

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Abstract

Replacement of fish oil with sustainable alternatives, such as vegetable oil, in aquaculture diets has to be achieved without compromising the nutritional quality, in terms of n-3 highly unsaturated fatty acid (HUFA) content, of the product. This may be possible if the level of replacement is not too high and oil blends are chosen carefully but, if high levels of fish oil are substituted, a fish oil finishing diet prior to harvest would be required to restore n-3HUFA. However, a decontaminated fish oil would be required to avoid increasing undesirable contaminants. Here we test the hypotheses that blending of rapeseed and soybean oils with southern hemisphere fish oil will have a low impact upon tissue n-3HUFA levels, and that decontamination of fish oil will have no major effect on the nutritional quality of fish oil as a feed ingredient for Atlantic salmon. Salmon (initial weight ~0.8 kg) were fed for 10 weeks with diets in which 60% of fish oil was replaced with blends of soybean, rapeseed and southern hemisphere fish oil (SVO) or 100% decontaminated northern fish oil (DFO) in comparison with a standard northern fish oil diet (FO). Decontamination of the oil was a two-step procedure that included treatment with activated carbon followed by thin film deodorisation. Growth performance and feed efficiency were unaffected by either the SVO or DFO diets despite these having lower gross nutrient and fatty acid digestibilities than the FO diet. There were also no effects on the gross composition of the fish. Liver and, to a lesser extent flesh, lipid levels were lower in fish fed the SVO blends, due to lower proportions of neutral lipids, specifically triacylglycerol. Tissue lipid levels were not affected in fish fed the DFO diet. Reflecting the diet, flesh eicosapentaenoic acid (EPA) and total n-3 fatty acids were higher, and 18:1n-9 lower, in fish fed DFO than FO, whereas there were no differences in liver fatty acid compositions. Flesh EPA levels were only slightly reduced from about 6% to 5% although docosahexaenoic acid (DHA) was reduced more severely from around 13% to about 7% in fish fed the SVO diets. In contrast, the liver fatty acid compositions showed higher levels of n-3 HUFA, with DHA only reduced from 21% to about 18% and EPA increased from under 8% to 9-10% in fish fed the SVO diets. The evidence suggested that increased liver EPA (and arachidonic acid) was not simply retention, but also conversion of dietary 18:3n-3 and 18:2n-6. Increased HUFA synthesis was supported by increased hepatic expression of fatty acyl desaturases in fish fed the SVO diets. Flesh n-3HUFA levels and desaturase expression was significantly higher in fish fed soybean oil than in fish fed rapeseed oil. In conclusion, partial replacement of fish oil with blends of vegetable oils and southern hemisphere fish oil had minimal impact on HUFA levels in liver, but a greater effect on flesh HUFA levels. Despite

lower apparent digestibility, decontamination of fish oil did not significantly impact its nutritional quality for salmon.

1. Introduction

Fish are the most important source of the n-3 highly unsaturated fatty acids (HUFA) in the human diet that have been shown to have beneficial effects in a number of inflammatory and pathological conditions, including cardiovascular and neurological diseases (Brower et al., 2006; Givens and Gibbs, 2006; Eilander et al., 2007). Demand for fish products is increasing such that an increasing proportion of fish are derived from aquaculture production (Tidwell and Allan, 2002). Lipids are a major source of energy, and fish oils are the main source of dietary lipid, providing essential polyunsaturated fatty acids (PUFA), specifically the HUFA, eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids, to promote normal growth and development (Sargent et al., 2002). However, the composition of fish oils varies among sources both in terms of nutrition (n-3HUFA) and also undesirable contaminants (e.g. persistent organic pollutants, POPs) that may affect human health. Fish oil supplies are finite and at their sustainable limit (FAO, 2006) and so continued aquaculture development requires replacement of fish oil with alternative nutritionally suitable and sustainable oils (Tacon, 2004; Pike, 2005).

Presently, vegetable oils (VOs) are the only viable, cost competitive alternative lipid source for aquaculture diets and a number have been used as partial and complete replacements for fish oil (Bell et al., 2005a). As VOs are devoid of n-3HUFA, they have a major impact on the fatty acid composition of the final products if added at high inclusion levels. For that reason, some studies have investigated the use of "finishing diets" containing fish oil to restore levels of n-3HUFA in the flesh (Bell et al., 2003a,b, 2004; Robin et al., 2003; Caballero et al., 2004; Izquierdo et al., 2005; Mourente et al., 2005; Torstensen et al., 2005; Mourente and Bell, 2006). However, the use of FO finishing diets has a conceptual drawback in that, in addition to adding back the valuable and highly desirable n-3HUFA, it could be challenged as a viable solution due to the potential for increasing POPs in a previously low contaminant load product (Bell et al., 2005a). Although the content of POPs in farmed fish, including salmon, has been shown to be below all national and international limits (FDA, UK and European FSA, WHO, EU) (Bell et al., 2005a,b; Berntssen et al., 2005), their presence in farmed fish, especially salmon, has received some negative reporting (Hites et al., 2004). To avoid this conundrum, it may be highly desirable to formulate finishing diets with decontaminated fish oil. Therefore, although availability of decontaminated fish oil is still limited, market demand for this oil may change this situation in the near future, and so it is important to evaluate potential effects of the decontamination process on the nutritional quality of the oil and how it may affect growth performance and product

92 quality.

93 At lower inclusion levels, dietary VOs have correspondingly less impact on flesh fatty acid
94 compositions (Robin et al., 2003). Therefore, an alternative to finishing diets would be the use of lower
95 levels of fish oil substitution, particularly if the oil blends used are carefully chosen to limit HUFA
96 reduction (Torstensen et al., 2004). For instance, southern hemisphere fish oils contain higher levels of
97 n-3HUFA, especially EPA, and so can deliver similar levels of n-3HUFA at lower inclusion levels than
98 the northern hemisphere fish oils traditionally used in salmonid diets (Sargent et al., 2002). On the
99 other hand, the southern fish oils are also lower in C20 and C22 monoenes, traditionally regarded as
100 excellent energy sources for salmonids (Tocher, 2003) as well as containing higher levels of saturated
101 fatty acids (Karalazos et al., 2007). Different VOs also have contrasting nutritional and economic
102 qualities. Rapeseed oil is nutritionally balanced but is relatively expensive, whereas soybean oil is
103 readily available and its price favourable, although it contains high levels of 18:2n-6, which limits the
104 use of this oil due to greater reduction of the n-3/n-6 ratio in the product. In addition, EU legislations
105 limit the commercial use of soybean oils to non-GM products containing a maximum of 1.0 mg kg^{-1}
106 endosulfane (pesticide residue).

107 In the present study, we test two hypotheses. Firstly, that blending of rapeseed and soybean oils with
108 southern hemisphere fish oil is a strategy that may result in lower impact upon tissue n-3HUFA levels
109 in Atlantic salmon. Secondly, that the decontamination (stripping) of fish oil will have no major effect
110 on the nutritional quality of the fish oil as a feed ingredient for Atlantic salmon. Specifically, we
111 investigated the effects of replacement of northern hemisphere fish oil by decontaminated northern fish
112 oil and blends of southern hemisphere fish oil with rapeseed and soybean oils on growth performance
113 and composition of Atlantic salmon. The use of decontaminated fish oil and VO substitution are also
114 two alternative methods for reducing contaminant loads in the flesh of farmed Atlantic salmon. The
115 effects of these diets on flesh contaminant levels will be reported separately.

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117 **2. Materials and methods**

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119 *2.1. Diets and animals*

120 Five diets (9 mm pellets) with the same basal composition but coated with five different oils were
121 prepared at the BioMar TechCentre, Brande, Denmark. The diets were formulated to satisfy the
122 nutritional requirements of salmonid fish (National Research Council 1993), and to contain 33% crude

protein and 34% crude lipid (Table 1). The northern hemisphere and decontaminated northern hemisphere fish oils were both sprat (*Sprattus sprattus*) and were obtained from FF Skagen, Skagen, Denmark. Decontamination of the oil was a two-step procedure that included treatment with activated carbon followed by thin film deodorisation to remove persistant organic pollutants. The southern hemisphere fish oil was from anchovetta (*Engraulis rigens*), and the rapeseed (low in erucic acid and glycosinolates) and soybean oils were both non-GM, degummed quality. The diets produced were 100% northern fish oil (FO) as control, 100% decontaminated northern fish oil (DFO), and three blends of the VOs with southern hemisphere fish oil (collectively termed the SVO diets), specifically 40% southern fish oil/60% rapeseed oil (SRO), 40% southern fish oil/30% rapeseed oil/30% soybean oil (SRO/SO) and 40% southern fish oil/60% soybean oil (SSO). The fatty acid compositions of the experimental diets are given in Table 2. Atlantic salmon (*Salmo salar* L) of initial mean weight 0.78 ± 0.01 kg were fed for 10 weeks one of the five diets in triplicate 5 × 5 × 5 m net pens (n =3 per treatment) with 120 fish/pen at Fjord Research Station, Dønna, Norway. The experiment was performed between July and October under natural photoperiod and when the mean seawater temperature was 11.5 ± 2.7 ^{o}C and salinity was 31.9 ± 0.8 ‰. Feed was supplied manually to apparent satiation twice a day with waste feed collection via an up-lift system. Feed fed, waste feed and the resulting net feed intake were registered daily, as were any mortalities.

2.2. Sampling protocols

At the start and end of the trial, all the fish in each pen were bulk weighed. At the end of the trial, 6 fish per pen (18 per dietary treatment) were anaesthetised by metacaine (50 mg/l) and faeces collected by stripping. Fish were then killed by a blow to the head and samples taken for compositional analyses. The gut cavity was opened on three fish per pen and any remaining contents of the intestine removed before each whole fish was cut into pieces and minced thoroughly and samples collected and stored at -20 ^{o}C prior to proximate analyses. The remaining three fish per pen were used for lipid and fatty acid analyses. Flesh was sampled by taking the Norwegian Quality Cut (NQC) and immediately frozen at -20 ^{o}C. Livers were dissected and three samples collected. Approximately 0.5 g of liver was placed into a glass vial containing ice-cold chloroform/methanol (2:1, by vol.) and then stored at -20 ^{o}C prior to lipid class analysis. A further piece of < 0.5 g was collected into a microcentrifuge tube for RNA analysis, and the remaining liver for fatty acid analysis placed in a plastic test tube, with both these

154 samples being immediately frozen in liquid nitrogen before being stored at -70 ^{o}C and -20 ^{o}C,
155 respectively, prior to analyses.
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157 *2.3. Proximate analyses*
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159 The proximate compositions of diets and minced whole fish were determined by standard procedures
160 (AOAC, 2000). Thus, moisture content was determined after drying to constant weight in an oven at
161 105 ^{o}C for 24h. The samples were then rigorously blended into a homogeneous crumb/meal and used
162 for determination of diet or whole body lipid, protein and ash contents. Lipid content in 1 g samples of
163 dried crumb was determined using the Soxhlet method with extraction in petroleum ether at 120 ^{o}C
164 (Avanti Soxtec 2050 Auto Extraction apparatus; Foss, Warrington, UK). Crude protein content (N x
165 6.25) was determined in the crumb using the automated Kjeldahl method (Tecator Kjeltec Auto 1030
166 Analyser; Foss, Warrington, UK). Ash contents were determined after heating portions of the crumb at
167 160 ^{o}C for 48 h. The gross energy content of the diets was determined by Bomb Calorimetry
168 (Gallenkamp Autobomb System).
169
170 *2.4. Pigment analyses*
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172 Astaxanthin was extracted from salmon muscle largely by the method of Barua et al. (1993). Tissue
173 samples were homogenised in 5 ml of absolute ethanol and 5 ml of ethyl acetate using an Ultra-Turrax
174 tissue disrupter. The homogenate was centrifuged (1000 x *g*, 5 min) and the supernatant removed to a
175 stoppered glass tube. The pellet was re-homogenised in 5 ml of ethyl acetate, centrifuged, and the
176 supernatant combined with the first supernatant. Finally, the pellet was re-homogenised in 10 ml of
177 hexane, centrifuged, and the supernatant combined with the pooled supernatant. The pooled
178 supernatant was dried under N_2 and vacuum desiccated for 2 h before dissolving the residue in 2 ml of
179 hexane containing 0.2% (w/v) BHT. Measurement of astaxanthin was carried out using a 5-µm
180 SYNERGI 4µ MAX-RP column (4.6 x 150 mm, Phenomenex, Macclesfield, UK). The
181 chromatographic system was equipped with a Waters Model 501 pump and astaxanthin was detected
182 at 470 nm using a Waters 490E multiwavelength UV/vis detector (Millipore UK, Watford). An
183 isocratic solvent system was used containing acetonitrile/methanol (95:5, v/v) at a flow rate of 0.8

ml/min. Astaxanthin was detected at 470 nm and quantified using an external standard of astaxanthin obtained from Roche (Heanor, UK).

Carotenoid in diets was extracted using the same solvent mixtures as described above but after enzymatic digestion with Maxatase (International Biosynthetics, Rijswijk, Netherlands). Portions of ground diet (1g) were mixed with 10 ml water and 110 mg Maxatase in a 50 ml stoppered glass tube followed by incubation in a water bath at 50^{o}C for 30 min. Measurement of diet carotenoids was carried out using a 5-µm Lichrosorb 5µ Silica 60 column (4.0 x 125 mm, Phenomenex, Macclesfield, U.K.). The chromatographic system was equipped with a Waters Model 510 pump and astaxanthin was detected at 470 nm using a Waters 486 multiwavelength UV/vis detector (Millipore U.K., Watford). An isocratic solvent system was used containing iso-hexane/acetone (86:14, v/v) at a flow rate 1 ml/min.

2.5. Apparent nutrient digestibility

Yttrium oxide (Y_2O_3) was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). The diet (0.2-0.5g) or faeces (0.1g) were weighed into pre-cleaned beakers and 4 ml of concentrated nitric acid added. The beakers were covered with clean watch glasses and placed in a fume cupboard for 24h. The partially digested samples were placed on a hotplate and boiled for 1h before being transferred quantitatively to pre-cleaned 25 ml volumetric flasks and made to volume with 2% v/v nitric acid. The digested samples were then analysed by ICO-OES using a Varian 725-ES instrument. Standards of between 0.5 and 120 mg/l Y were prepared as calibrants and the Y signal was monitored at two different wavelengths. Apparent digestibility coefficients (ADC) were estimated according to the formula:

$$ADC = 100\text{-}100*((Y_{feed}/Y_{faeces})*(N_{faeces}/N_{feed}))$$

where Y_{feed} = Yttrium oxide in feed, Y_{faeces} = Yttrium in faeces, N_{faeces} = nutrient in faeces, N_{feed} = nutrient in feed. All data were based on calculated dry weight of the samples. For fatty acid digestibilities, data were based on the amount of the individual component in µg mg^{-1} of total lipid.

2.6. Lipid analyses

Liver and skinned and deboned flesh samples from the three fish per pen were prepared as pooled homogenates. Total lipid was extracted from diets or 1g portions of tissue homogenates by homogenising in 20 volumes of ice-cold chloroform/methanol (2:1, v/v) in an Ultra-Turrax tissue

disrupter (Fisher Scientific, Loughborough, U.K.). Total lipid was prepared according to the method of Folch et al. (1957) and non-lipid impurities removed by washing with 0.88% (w/v) KCl. The weight of lipid was determined gravimetrically after evaporation of solvent and overnight desiccation *in vacuo*.

Separation of lipid classes was performed by high-performance thin-layer chromatography (HPTLC). Approximately 10 μg of total lipid was applied as 2 mm streaks and the plate developed to two-thirds distance with methyl acetate/isopropanol/chloroform/methanol/0.25 % aqueous KCl (25:25:25:10:9, by vol.). After desiccation, the plate was fully developed with isohexane/diethyl ether/acetic acid (85:15:1, by vol.). The lipid classes were quantified by charring at 160 ^{o}C for 15 min after spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid, followed by densitometry using a Shimadzu CS-9000 dual-wavelength flying spot scanner and DR-13 recorder (Henderson and Tocher, 1992).

Fatty acid methyl esters (FAME) from diets and tissue total lipid were prepared by acid-catalysed transesterification of total lipid according to the method of Christie (1993). Extraction and purification of FAME was performed as described by Tocher and Harvie (1988). FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-column injection. Hydrogen was used as carrier gas and temperature programming was from 50^{o}C to 150^{o}C at 40^{o}C min^{-1} and then to 230^{o}C at 2.0^{o}C min^{-1}. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980; Tocher and Harvie, 1988). Data were collected and processed using the Chromcard for Windows (version 1.19) computer package (Thermoquest Italia S.p.A., Milan, Italy).

2.7. Gene expression

RNA was extracted from liver samples by homogenising in 5ml of TriReagent using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). First strand cDNA synthesis of total RNA aliquots from pooled biological replicates was conducted using the Reverse-It Max reverse transcriptase kit (ABgene Ltd). Real-time qPCR was performed using a Quantica thermocycler (Techne). Reactions comprised a total volume of 15μl containing 5μl cDNA (10^{-2} dilution of 1st strand synthesis), 10 pmol each PCR primer and 7.5 μl 2 × Absolute QPCR SYBR Green Mix (ABgene Ltd). Primer sequences for the three target genes are given in Table 3. An initial incubation at 95°C for 15

min was employed in order to activate the Thermo-Start® DNA Polymerase. Forty-five cycles of PCR were performed. Each PCR cycle consisted of a denaturation step of 15 s at 95°C; annealing step of 15 s at 60°C and an extension step of 30 s at 72°C. Following PCR melting curve analysis was performed to confirm the production of a single product in these reactions. Standard curves were established using five different dilutions (in triplicate) of cDNA sample solutions. Real-time efficiency was determined for each gene from the slopes given by Quantsoft software, applying the equation $E = 10^{(-1/slope)}$. The relative expression ratio of each gene was calculated using REST© software (http://www.gene-quantification.info/). The relative expression ratio for a considered gene is based on the PCR efficiency (E) and CT of a sample compared with the control, and expressed in comparison to the reference genes (ie normalization with housekeepers), according to Pfaffl's mathematical model (Pfaffl, 2001):

Ratio = $(E_{target})^{\Delta CT?target(control-sample)}/(E_{reference})^{\Delta CTreference(control-sample)}$

for each gene by plotting CT values against the $\log_{10}$ of the serial dilutions.

Statistical differences in gene expression between the control and samples were evaluated in group means by randomization tests (Pfaffl et al., 2002) using REST© software. Five thousand random allocations were performed and differences were considered to be significant at $P < 0.05$.

2.7. Materials

BHT and TriReagent were obtained from Sigma Chemical Co. (Poole, U.K.). HPTLC (10 cm x 10 cm x 0.15 mm) and TLC (20 cm x 20 cm x 0.25 mm) plates, precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK, Loughborough, England.

2.8. Statistical analysis

All data are presented as means ± SD (n value as stated). The effects of dietary treatment were determined by one-way analysis of variance (ANOVA) followed, where appropriate, by Tukey's comparison test (Zar, 1999). Percentage data and data which were identified as non-homogeneous (Bartlett's test) were subjected to arcsine transformation before analysis. Differences were regarded as significant when $P < 0.05$.

3. Results

3.1 Fatty acid composition of the diets

The fatty acid composition of the DFO diet showed some differences compared to the FO diet including lower levels of 18:1n-9 and 18:3n-3, but higher levels of 18:0, 20:1, 22:1, 18:2n-6 and EPA (Table 2). However, most of these differences although significant were relatively minor. The SVO diets all showed increased 18:0, 18:2n-6 and 18:3n-3, and lower levels of 14:0, 16:0, ARA, EPA and DHA compared to the FO and DFO diets. Within the SVO diets, 16:0, 18:0 and 18:2n-6 increased with increasing inclusion of soybean oil so that the rank order for their levels was SSO > SRO/SO > SRO, whereas 18:1n-9 and 18:3n-3 increased with increasing inclusion of rapeseed oil such that the rank order for their levels was SRO > SRO/SO > SS) (Table 2). There was no significant difference between these diets in their levels of n-3HUFA.

3.2. Growth performance, whole body composition and pigment content

There were no significant differences in growth performance (final weights, SGR and TGC) among the dietary treatments (Table 4). There was also no dietary effect on feed efficiency (FCR). Other than slight differences in moisture, the different dietary oils had no effect on the gross composition of the whole salmon or the concentration of astaxanthin (Table 4).

3.3. Apparent digestibility of gross nutrients and fatty acids

The apparent digestibility of the DFO was significantly lower than all the other diets as evidenced by the lower values for crude protein, crude lipid and dry matter digestibility (Table 5). Consistent with this, the apparent digestibility of virtually every fatty acid was lower in fish fed the DFO diet compared to fish fed the FO diet (Table 5). In addition, the digestibility of many fatty acids was often lower in fish fed the DFO diet compared to fish fed the SVO diets. In general, fatty acid digestibility was highest in fish fed the FO diet. There were few differences in fatty acid digestibilities between the fish fed the SVO diets.

3.4. Flesh and liver lipid contents and class compositions

Liver lipid content was significantly lower in fish fed the SVO diets compared to fish fed either the FO or DFO diets (Table 6). There was a similar trend for flesh lipid content to be lower in fish fed the SVO diets compared to fish fed the FO or DFO diets, although this was only significant in the case of fish fed the SRO diet. There were no significant differences in tissue lipid contents between fish fed the FO and DFO diets. Consistent with this, there were no significant effects on flesh or liver lipid class composition between fish fed the FO and DFO diets other than slightly higher cholesterol in liver of fish fed DFO (Table 7). In contrast, the proportions of neutral lipids were reduced and polar lipids increased in both flesh and liver of salmon fed the SVO diets compared to fish fed the FO or DFO diets (Table 7). The lower neutral lipid was primarily due to decreased proportions of triacylglycerol (TAG) in both tissues and also steryl esters in liver. The greater proportions of polar lipids in fish fed the SVO diets were mainly due to increased proportions of the two main phosphoglycerides, phosphatidylcholine and phosphatidylethanolamine, in both flesh and liver. One of the few differences between the fish fed the SVO diets was that the flesh of fish fed the diet with highest level of soybean oil (SSO) had a lower proportion of TAG and a higher proportion of cholesterol (sterol) than fish fed the other SVO diets. The proportion of free fatty acids in the flesh also increased with increasing inclusion of dietary soybean oil.

3.5. Flesh and liver fatty acid compositions

Flesh fatty acid compositions were largely changed corresponding to the fatty acid compositions of the diets with fish fed the SVO diets having reduced 14:0, 16:0, EPA, DHA and arachidonic acid (ARA), but increased 18:2n-6 and 18:3n-3 compared to fish fed the FO and DFO diets (Table 8). The proportions of 18:0 and 18:1n-9 were only increased in fish fed the diets containing soybean oil and rapeseed oil, respectively. Therefore, as with the diets, flesh 16:0, 18:0 and 18:2n-6 increased with increasing inclusion of soybean oil so that the rank order for their proportions was SSO > SRO/SO > SRO, whereas flesh 18:1n-9 and 18:3n-3 increased with increasing inclusion of rapeseed oil such that the rank order for their proportions was SRO > SRO/SO > SSO. Interestingly, the proportions of EPA and DHA were significantly higher in fish fed the diet with highest level of soybean oil (SSO) compared to the other SVO diets. Reflecting the diet, EPA and total n-3PUFA were higher in fish fed DFO than FO, whereas 18:1n-9 was lower (Table 8). In contrast, there were no significant differences in fatty acid composition of liver between fish fed the FO and DFO diets (Table 9). The proportions of

18:0, 18:1n-9, 18:2n-6 and 18:3n-3 in liver of fish fed the SVO diets were similar to those described above for flesh, and reflected the dietary fatty acid compositions. However, it was noteworthy that increased proportions of desaturated and elongated products of 18:2n-6 and 18:3n-3 were clearly observed in liver of fish fed the SVO diets. Thus, proportions of 20:2n-6, 20;3n-6 and 20:4n-6 were all increased in fish fed the SVO diets compared to fish fed the FO and DFO diets. Even more striking was the increased proportions of EPA in the livers of fish fed the SVO diets. In contrast, proportions of 22:5n-3 and, to a lesser extent, DHA were reduced in fish fed the SVO diets compared to fish fed FO or DFO (Table 9).

3.6. Gene expression

The expression of both the Δ6- and Δ5-fatty acyl desaturase genes in liver was increased in fish fed the SVO diets, with the highest expression observed in liver of fish fed the highest inclusion of soybean oil (diet SSO) (Fig.1). In contrast, there was no dietary effect on the hepatic expression of the PUFA elongase gene. There were also no significant differences in expression of any of the genes between fish fed the FO and DFO diets.

4. Discussion

There were no deleterious effects on growth as measured by final weights, SGR or TGC, or on feed efficiency (FCR) of any of the SVO diets. This was consistent with the results of several previous trials in which partial replacement of fish oil by VO, or even 100% replacement, have had no significant effects on growth performance or feed efficiency in Atlantic salmon when using fish meal-based diets (see Bell and Waagbø, 2008). It was also consistent with the analytical data on the digestibilities of gross nutrients and individual fatty acids that were all very similar to those of the FO diet. In contrast, the apparent digestibility of the DFO diet was significantly lower than that of all the other diets, including the FO diet. There were some significant differences in fatty acid composition between the DFO and FO diets, and so some differences in fatty acid and, consequently lipid, digestibility may not be entirely unexpected. However, the lower digestibilities of the DFO diet also included the crude protein and dry matter and so it appeared to be a general effect. It is not known if the decontamination process per se was responsible for these effects, but the fish oil and

decontaminated fish oil used in the diet manufacture were exactly the same oil, simply decontaminated or not. Therefore, it is probable that the decontamination process affected the digestibility, but we cannot be certain without further evidence. Furthermore, it was surprising that the lower digestibilities of the DFO diet did not result in lower growth, as the fish fed the DFO diet attained the same final weight and showed the same SGR and TGC as fish fed the FO diet, and the SVO diets. Feed efficiencies were also unaffected and so the lower digestibilities with the DFO diet were not reflected in any gross growth performance parameter. This apparent dichotomy between nutrient digestibilities, and growth and feed efficiency has been observed before in dietary trials in salmon (Torstensen et al., 2000). Thermal growth coefficient (TGR) was higher and FCR lower in Atlantic salmon held in seawater compared to those in freshwater, despite lower ADC values for dry matter and crude protein (Krogdahl et al., 2004). In comparison to the FO diet, the SVO diets also showed lower lipid digestibility related to lower digestibilities of a number of fatty acids, but crude protein digestibility was not affected by feeding VOs. Lipid and fatty acid digestibility in salmon fed VOs appears to be variable (Torstensen et al., 2000; Bendiksen et al., 2003; Ng et al., 2004), but lower lipid/FA digestibility of diets formulated with VO has been reported previously in trials with salmon (Menoyo et al., 2003) and other fish species including Murray cod (Francis et al., 2007). As with the DFO diet, the lower lipid digestibility did not result in any deleterious effect on growth performance or feed efficiency.

The composition of the fish was unaffected by feeding the DFO diet compared to the FO diet, with whole body proximate compositions, liver and flesh lipid contents and lipid class compositions all being unaffected. Although the SVO diets also had no effect on the gross composition of the fish, their effect in lowering liver lipid was highly significant. Although, not statistically significant, it appeared that the liver lipid lowering effect was greater with the diets containing soybean compared to rapeseed oil. Differences between VOs in their effects on tissue lipid contents have been observed previously. Thus earlier trials on salmon smolts showed that substitution of FO with graded levels of palm oil had no significant effect on liver lipid (Bell et al., 2002), whereas graded substitution with rapeseed oil tended to increase liver lipid (significantly so at 100% replacement) (Bell et al., 2001). Liver lipid was not increased in salmon fed soybean oil at higher water temperatures (Ruyter et al., 2006). Furthermore, liver lipid content was lower in salmon fed a VO blend containing rapeseed, palm and linseed oils, for 16 months but not at 14 and 22 months of feeding (Jordal et al., 2007). In that study, the relative proportions of TAG and cholesterol were increased and decreased, respectively, at all

stages. Thus, the effects of VO diets on liver lipid levels can vary perhaps related to type of oil, water temperature, season and duration of trial. In the present study, the lower liver lipid level in fish fed the SVO diets was associated with decreased neutral lipids, especially TAG and steryl esters whereas the major membrane lipids, phosphatidylcholine, phosphatidylethanolamine and, possibly, cholesterol/sterol were all increased. In Table 7 we have reported sterols rather than cholesterol as the TLC method is not able to separate cholesterol from many plant sterols. The VO diets are likely to contain lower levels of cholesterol but, conversely, may contain a range of other plant sterols (Padley et al., 1986). Plant sterols are generally not absorbed by animals and, indeed, are used to block cholesterol uptake in humans. The situation with fish is not known but it is likely similar. Therefore, it is likely that cholesterol is the predominant sterol in fish tissues, but the presence of plant sterols cannot be excluded completely. However, the higher cholesterol level in liver (and flesh) of fish fed the SVO diets may be a compensatory mechanism, the result of increased cholesterol synthesis in response to reduced dietary cholesterol (Taggart et al., 2008).

Possibly more important in terms of product quality, is the effect that different VOs have on flesh lipid contents in salmon. In the present trial, although there was a trend for flesh lipid content to be lower in fish fed the SVO diets, it was only significantly reduced in fish fed the SRO diet, that is, with 60% rapeseed oil substitution. Previously, graded rapeseed oil substitution in salmon smolts tended to decrease flesh lipid, although this effect was only significant at the 50% replacement level. (Bell et al., 2001). With palm oil substitution in salmon smolts, the flesh lipid lowering effect was very pronounced, with lipid levels decreasing in a graded manner as the level of palm oil substitution increased (Bell et al., 2002). In a study with larger post-smolts, feeding palm oil reduced flesh lipid content compared to fish fed fish, sunflower or a blend of fish and sunflower oil (Torstensen et al., 2000). In a trial feeding salmon a VO blend containing rapeseed, palm and linseed oils over the entire 2-year growth cycle, flesh lipid contents were generally lower at all points in both the freshwater and seawater phases (Torstensen et al., 2005). In that trial, neutral lipid and TAG were reduced in white muscle of salmon fed the VO blend compared to fish fed FO (Nanton et al., 2007). Similarly, in the present trial, the lipid class composition of the flesh in fish fed the SVO diets was characterized by decreased proportions of neutral lipid and TAG. Overall, it is apparent that the effect that substitution of FO with VO has on tissue lipid contents and compositions is dependent upon a number of factors including the specific VO or VO blends used as well as other factors including the specific tissue itself and possibly growth stage and/or season as well as the genetic origin of the stock.

The small, but significant, differences in the fatty acid compositions of the FO and DFO diets cannot be directly attributed to the decontamination process but it is possible. For instance, certain fatty acids could be slightly more adsorbed by the activated carbon than other fatty acids resulting in lower proportions in DFO compared to FO. However, the differences observed, lower levels of 18:1n-9 and 18:3n-3, and higher levels of 18:0, 20:1, 22:1, 18:2n-6 and EPA in DFO compared to FO, did not follow a pattern that could be easily explained by differential adsorption. Some of these differences, particularly the lower level of 18:1n-9 and higher levels of 20:1, 22:1 and EPA were reflected in flesh lipids but not liver lipids, that showed identical compositions in fish fed the FO and DFO diets. The fact that flesh was more affected than liver by differences in dietary fatty acid composition is predictable based on the lipid content of flesh compared to liver in salmon. The higher lipid content is associated with higher neutral (storage) lipid, specifically TAG, that is more readily influenced by diet than phospholipid (Bell and Waagbø, 2008). This simple fact is why changes in dietary lipid have such an impact on salmon flesh composition and why it is, as a consequence, difficult to minimize these effects. This was clearly demonstrated in the present trial where the strategy with VO substitution was to blend them with southern hemisphere fish oil in an attempt to minimize the impact of the VO inclusion on n-3HUFA was, unfortunately only partially successful with flesh. Thus, despite 60% of the FO being replaced by VOs, the EPA levels in the salmon flesh were only slightly reduced from about 6% to 5%. However, DHA was reduced more severely from around 13% to about 6.5 to 7.5%. This simply reflected the diets that also showed quite significantly reduced DHA but relatively less effect on EPA, which was expected as, although southern hemisphere fish oils have higher n-3HUFA than northern hemisphere fish oils, this is due to high percentages of EPA and a high EPA:DHA ratio, but reduced or similar DHA compared to northern FO.

In contrast, the strategy was largely successful in liver as fatty acid compositions of fish fed the SVO diets showed impressive levels of HUFA with ARA and EPA both being significantly increased, and DHA only reduced from around 21% to about 18-19%. EPA actually increased from 7.7% in fish fed the FO and DFO diets to 9-10% in fish fed the SVO diets. The only negative impact being increased 18:2n-6 and reduced 18:1n-9 in the diets containing soybean oil. It is important to emphasize that the increased EPA and ARA were not simply the result of increased deposition and retention, but were also the result of conversion of dietary 18:3n-3 and 18:2n-6, respectively. This contention is supported by other evidence. The SVO diets, although formulated with 40% southern hemisphere fish oil, which is known to be richer in EPA than northern hemisphere fish oils (Sargent et al., 1989), were

still lower in EPA than the FO and DFO diets. Similarly, the ARA levels in the SVO diets were lower than in the FO and DFO diets. Further evidence of active desaturation was provided by the increased level of the intermediate 20:3n-6 in the liver of fish fed the diets containing soybean, although there was also increased production of the dead-end fatty acid 20:2n-6, the direct elongation product of 18:2n-6. Increased proportions of 20:2n-6 and 20:3n-6 have been observed previously in salmon when fed diets containing high levels of 18:2n-6 (Bell et al., 1991, 2001, 2002, 2003b; Lie et al., 1993; Tocher et al., 2003). Thus, the increased proportions of EPA and ARA in liver were the result of enhanced desaturation and elongation of dietary 18:3n-3 and 18:2n-6. The increased hepatic expression of fatty acyl desaturase genes observed in the present trial is entirely consistent with this assertion. We have previously demonstrated that increased expression of fatty acyl genes in liver of salmon fed VOs was associated with increased activity of the HUFA biosynthesis pathway (Zheng et al., 2004, 2005a).

The increased percentages of EPA observed in fish fed the SVO diets was especially noteworthy as it is unusual that dietary VO did not reduce EPA levels, let alone increase them. As mentioned above, it is common to observe increased levels of desaturation products of dietary 18:2n-6 including, most commonly, 20:3n-6, in trials with salmon fed VO (Bell et al., 1991, 2001, 2002, 2003b; Lie et al., 1993; Tocher et al., 2003). Bell et al. (1991) also found increased levels of ARA in phospholipids from liver, heart and retina in salmon fed sunflower oil. Increased ARA was also observed in total lipid from liver of salmon fed a rapeseed/palm/linseed blend (Zheng et al., 2005a). As essentially all VO diets will increase dietary 18:2n-6 in comparison to FO diets, this has been a widely reported observation in salmon tissues, especially liver. However, a similar phenomenon has not been generally observed with 18:3n-3. Therefore, increasing dietary 18:3n-3 levels have not been able to maintain tissue EPA or DHA at levels obtained in salmon fed FO. Indeed, even increased levels of 18:4n-3 or 20:4n-3, intermediates in the pathway from 18:3n-3 to EPA, have been only rarely been reported. In a trial using graded substitution of FO with linseed oil, 18:4n-3 and 20:4n-3 were increased in salmon liver lipids in a graded manner (Tocher et al., 2002). However, EPA, 22:5n-3 and DHA all decreased as the level of linseed oil substitution increased. Direct biochemical measurements of fatty acyl desaturation activities in hepatocytes and enterocytes have conclusively confirmed that HUFA synthesis is up-regulated in salmon fed diets containing VOs, including rapeseed (Bell et al., 2001), palm (Bell et al., 2002) and linseed oils (Tocher et al., 2002). Molecular studies demonstrated that the mechanism includes increased desaturase gene expression (Zheng at al., 2004, 2005a: Jordal et al., 2005). Thus, in

493 previous trials when dietary FOs were substituted by VOs, the reductions in the levels of dietary EPA
494 and DHA have been too great for the conversion of dietary 18:3n-3 by the HUFA synthesis pathway to
495 maintain the tissue levels of EPA or DHA. The precise reason for this observation remains
496 unexplained but it was very interesting that EPA levels in salmon fed the SVO diets were higher than
497 the levels of EPA in fish fed the FO and DFO diets in the present trial.
498 Another interesting factor in the observation above is that there was a difference between rapeseed
499 and soybean oil substitution, with the latter, especially the SSO diet with sole replacement by soybean
500 oil, showing the greatest effect. Thus, the percentages of DHA and EPA in both liver and flesh were
501 higher in fish fed the SSO diet compared to fish fed the SRO diet with rapeseed oil substitution. The
502 difference between fish fed SSO and SRO was highly significant in flesh with total n-3HUFA levels of
503 15.3 versus 13.4%, respectively, with reciprocal differences in substrate 18:3n-3 levels of 3.2 versus
504 5.6%. Consistent with this, the induction of fatty acyl desaturase expression in liver was greatest in the
505 fish fed the SSO diet. It is not clear why this should be. Previous trials on salmonids using substitution
506 of FO with soybean oil have not shown such an effect (Hardy et al., 1987; Guillou et al., 1995; Ruyter
507 et al., 2006). The current paradigm is that it is low dietary HUFA that induces increased desaturase
508 expression (Tocher, 2003), but there was very little difference between the SRO and SSO diets in terms
509 of HUFA content. In contrast, the SSO diet was characterized by very high levels of 18:2n-6 and lower
510 18:3n-3 than the SRO diet. In a study comparing different proportions of 18:1n-9, 18:2n-6 and 18:3n-3
511 (supplied by olive, sunflower and linseed oils, respectively) in diets for Atlantic salmon parr, the
512 authors suggested that the desaturation and elongation of 18:2n-6 and 18:3n-3 may be stimulated by
513 substrate availability as they noted the extent of conversion differed despite dietary HUFA levels all
514 being identical (Rollin et al., 2003). Although it is known that, like most vertebrate desaturases, the
515 salmon desaturases are more active towards n-3 than n-6 substrates (Hastings et al., 2004; Zheng et al.,
516 2005b), how this particular combination of 18:2n-6 and 18:3n-3 could have such a significant effect is
517 unclear. Certainly, previous trials that used VOs blends to produce a better balance of 18:2n-6 and
518 18:3n-3 including rapeseed/linseed oils (Bell et al., 2003b; Tocher et al., 2003) or
519 rapeseed/palm/linseed oils (Zheng et al., 2005a) have not been effective in maintaining EPA. However,
520 these trials were of longer duration and there may have been a transient increase in HUFA, following
521 change of diet, that was not observed due to the frequency of sampling used.
522 In conclusion, we tested the hypotheses that blending VOs with southern hemisphere fish oil is a
523 strategy that may result in lower impact upon tissue n-3HUFA levels, and that the decontamination of

fish oil will have no major effect on the nutritional quality of fish oil as a feed ingredient for Atlantic salmon. Neither the SVO diets nor the DFO diet had any deleterious effects on growth as measured by final weights, SGR or TGC, or on feed efficiency (FCR). Therefore, despite lower apparent digestibility, decontamination of fish oil did not significantly impact on its nutritional quality for salmon. The partial replacement of fish oil with blends of VOs and southern hemisphere fish oil had minimal impact on HUFA levels in liver, but a greater effect on flesh HUFA levels. Soybean oil had significantly less impact than rapeseed oil. Decontamination of fish oil had no effect on the n-3HUFA content of flesh of salmon fed the oil.

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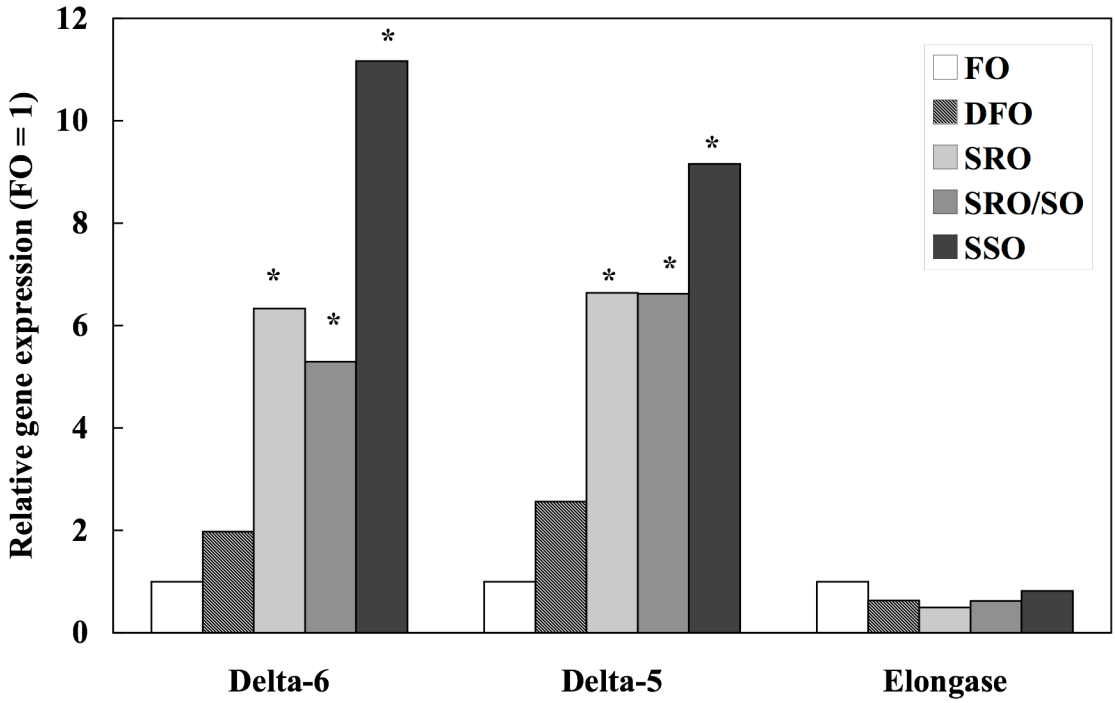

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711 Fig.1. Effects of diet on the hepatic expression of fatty acyl Δ6 (Delta-6) and Δ5 (Delta-5) desaturases,
712 and polyunsaturated fatty acid elongase (Elongase) in Atlantic salmon. Results were calculated by
713 REST 2005 software with β-actin as reference (normalization) gene and expressed relative to
714 expression in fish fed the fish oil diet (FO). Asterisks indicate that the expression of the specific gene in
715 fish fed a particular diet was significantly difference ($P < 0.05$) to its expression in fish fed the FO diet.
716 DFO, decontaminated northern fish oil; FO, northern fish oil; SRO, southern fish oil and rapeseed oil;
717 SRO/SO, southern fish oil, rapeseed oil and soybean oil; SSO, southern fish oil and soybean oil.

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Table 1. Formulations, proximate compositions (g kg^{-1}), energy (kJ g^{-1}) and pigment contents (mg kg^{-1}) of experimental diets fed to Atlantic salmon for 10 weeks

	Diets				
	FO	DFO	SRO	SRO/SO	SSO
Fish meals	378	378	378	378	378
Legume/oilseed meals	149	149	149	149	149
Northern fish oil	325	-	-	-	-
Northern fish oil (decontaminated)	-	325	-	-	-
Southern fish oil	-	-	130	130	130
Rapeseed oil	-	-	195	98	-
Soybean oil	-	-	-	98	195
Binder	140	140	140	140	140
Premixes	9.2	9.2	9.2	9.2	9.2
Composition					
Crude protein	326 ± 1.0^{a}	322 ± 1.1^{b}	314 ± 2.0^{c}	328 ± 1.0^{a}	326 ± 1.0^{a}
Crude lipid	336 ± 1.0^{c}	343 ± 2.2^{ab}	345 ± 3.9^{ab}	342 ± 1.0^{b}	347 ± 1.0^{a}
Moisture	60 ± 1.0^{a}	55 ± 1.0^{b}	63 ± 1.1^{a}	55 ± 0.3^{b}	52 ± 1.0^{c}
Fiber	21 ± 2.0	22 ± 1.9	19 ± 1.4	20 ± 1.0	20 ± 2.1
Ash	78 ± 1.0^{ab}	77 ± 1.0^{b}	76 ± 1.1^{c}	78 ± 0.2^{ab}	78 ± 1.0^{a}
Digestible energy	25.3 ± 0.3	25.3 ± 0.1	25.2 ± 0.1	25.2 ± 0.1	25.3 ± 0.2
Astaxanthin	22.6 ± 0.1^{a}	18.1 ± 1.1^{b}	15.2 ± 0.1^{c}	20.1 ± 1.9^{b}	18.5 ± 0.3^{b}

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different as determined by ANOVA. DFO, decontaminated northern fish oil; FO, northern fish oil; SRO, southern fish oil and rapeseed oil; SRO/SO, southern fish oil, rapeseed oil and soybean oil; SSO, southern fish oil and soybean oil.

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Table 2. Fatty acid compositions (percentage of total fatty acids) of the experimental diets

	FO	DFO	SRO	SRO/SO	SSO
14:0	5.2 ± 0.1^{b}	5.6 ± 0.3^{a}	2.9 ± 0.0^{c}	2.9 ± 0.1^{c}	2.9 ± 0.2^{c}
16:0	19.2 ± 0.4^{a}	18.8 ± 0.7^{a}	10.9 ± 0.0^{d}	13.5 ± 0.2^{c}	15.0 ± 0.7^{b}
18:0	2.2 ± 0.0^{e}	3.3 ± 0.1^{d}	3.8 ± 0.1^{c}	4.4 ± 0.1^{b}	4.8 ± 0.2^{a}
22:0	0.3 ± 0.1^{b}	0.6 ± 0.7^{ab}	1.5 ± 0.5^{a}	1.4 ± 0.2^{ab}	1.4 ± 0.3^{ab}
Total saturated[1]	27.8 ± 0.6^{a}	29.3 ± 0.5^{a}	20.0 ± 0.5^{c}	23.1 ± 0.5^{b}	24.9 ± 1.5^{b}
16:1n-7	6.0 ± 0.1^{a}	5.8 ± 0.1^{b}	2.7 ± 0.0^{c}	2.7 ± 0.0^{c}	2.6 ± 0.1^{c}
18:1n-9	25.9 ± 0.1^{c}	21.6 ± 0.7^{d}	41.2 ± 0.5^{a}	29.5 ± 0.1^{b}	21.2 ± 2.0^{d}
18:1n-7	2.5 ± 0.1^{b}	2.5 ± 0.1^{b}	2.9 ± 0.1^{a}	2.6 ± 0.0^{b}	2.2 ± 0.0^{c}
20:1n-9	2.1 ± 0.0^{b}	3.2 ± 0.2^{a}	1.2 ± 0.0^{c}	0.9 ± 0.0^{d}	0.6 ± 0.0^{e}
22:1n-11	2.4 ± 0.0^{b}	4.1 ± 0.1^{a}	0.3 ± 0.0^{d}	0.3 ± 0.0^{d}	0.4 ± 0.0^{c}
24:1n-9	2.0 ± 0.2^{a}	1.6 ± 0.0^{a}	0.3 ± 0.1^{b}	0.3 ± 0.0^{b}	0.2 ± 0.0^{b}
Total monoenes[2]	42.0 ± 0.1^{b}	40.0 ± 0.8^{b}	48.9 ± 0.7^{a}	36.5 ± 0.1^{c}	27.5 ± 1.8^{d}
18:2n-6	3.8 ± 0.1^{e}	4.3 ± 0.0^{d}	12.6 ± 0.1^{c}	23.1 ± 0.1^{b}	32.0 ± 0.4^{a}
20:4n-6	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}
Total n-6 PUFA[3]	5.6 ± 0.2^{e}	5.9 ± 0.0^{d}	13.4 ± 0.1^{c}	23.9 ± 0.2^{b}	32.7 ± 0.5^{a}
18:3n-3	2.7 ± 0.0^{d}	2.3 ± 0.1^{e}	6.3 ± 0.1^{a}	5.1 ± 0.1^{b}	3.7 ± 0.2^{c}
18:4n-3	2.2 ± 0.0^{a}	2.3 ± 0.1^{a}	0.9 ± 0.0^{b}	0.9 ± 0.0^{b}	0.9 ± 0.0^{b}
20:5n-3	6.7 ± 0.1^{b}	7.2 ± 0.3^{a}	5.4 ± 0.1^{c}	5.2 ± 0.1^{c}	5.1 ± 0.1^{c}
22:5n-3	0.7 ± 0.0	0.7 ± 0.0	0.4 ± 0.4	0.6 ± 0.0	0.6 ± 0.0
22:6n-3	11.6 ± 0.2^{a}	11.4 ± 0.8^{a}	4.3 ± 0.0^{b}	4.3 ± 0.1^{b}	4.2 ± 0.0^{b}
Total n-3 PUFA[4]	24.7 ± 0.4^{a}	24.7 ± 1.3^{a}	17.7 ± 0.2^{b}	16.5 ± 0.4^{bc}	14.9 ± 0.1^{c}
Total PUFA	30.3 ± 0.5^{c}	30.6 ± 1.3^{c}	31.1 ± 0.2^{c}	40.4 ± 0.6^{b}	47.7 ± 0.4^{a}

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different as determined by ANOVA. [1]Includes 15:0 and 20:0 at up to 0.7%; [2]Includes 16:1n-9, 20:1n-11, 20:1n-7 and 22:1n-9 at up to 0.5%; [3]Includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 and 22:5n-6 at up to 0.6%; [4]Includes 20:3n-3 and 20:4n-3 at up to 0.5%. DFO, decontaminated northern fish oil; FO, northern fish oil; PUFA, polyunsaturated fatty acids; SRO, southern fish oil and rapeseed oil; SRO/SO, southern fish oil, rapeseed oil and soybean oil; SSO, southern fish oil and soybean oil.

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Table 3. Primer sequences for target and housekeeping (normalisation) genes used in real-time qPCR

Gene	Primer	Sequence 5' - 3'	Size (bp)
Δ5 Desaturase	F	GTGAATGGGGATCCATAGCA	192
	R	AAACGAACGGACAACCAGA	
Δ6 Desaturase	F	CCCCAGACGTTTGTGTCAG	181
	R	CCTGGATTGTTGCTTTGGAT	
Elongase	F	TGATTTGTGTTCCAAATGGC	219
	R	CTCATGACGGGAACCTCAAT	
Elongation factor-1α	F	CTGCCCCTCCAGGACGTTTACAA	
(Housekeeping)	R	CACCGGGCATAGCCGATTCC	
β-Actin	F	ACATCAAGGAGAAGCTGTGC	141
(Housekeeping)	R	GACAACGGAACCTCTCGTTA	

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Table 4. Growth performance, proximate composition of whole fish (g kg^{-1}) and pigment content (mg kg^{-1}) of Atlantic salmon fed the experimental diets for 10 weeks

	FO	DFO	SRO	SRO/SO	SSO
Growth					
Initial weight (kg)	0.77 ± 0.02	0.78 ± 0.03	0.78 ± 0.03	0.77 ± 0.01	0.79 ± 0.11
Final weight (kg)	2.19 ± 0.05	2.18 ± 0.07	2.18 ± 0.10	2.19 ± 0.05	2.20 ± 0.07
SGR	1.35 ± 0.05	1.34 ± 0.01	1.34 ± 0.01	1.35 ± 0.03	1.33 ± 0.03
TGC	3.92 ± 0.15	3.86 ± 0.04	3.87 ± 0.07	3.89 ± 0.10	3.87 ± 0.10
FCR	0.98 ± 0.00	0.98 ± 0.02	0.98 ± 0.00	0.96 ± 0.02	0.96 ± 0.01
Composition					
Protein	169 ± 3	167 ± 4	167 ± 5	170 ± 7	170 ± 4
Lipid	191 ±10	189 ± 18	188 ± 14	194 ± 20	178 ± 27
Ash	2.8 ± 0.3	3.1 ± 0.2	2.8 ± 0.5	3.1 ± 0.5	2.9 ± 0.4
Moisture	616 ± 11^{a}	611 ± 12^{ab}	614 ± 8^{ab}	601 ± 5^{b}	606 ± 13^{ab}
Astaxanthin	4.7 ± 0.3	4.3 ± 0.3	4.8 ± 0.4	4.8 ± 0.6	4.4 ± 0.6

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different as determined by ANOVA. DFO, decontaminated northern fish oil; FCR, feed conversion ratio; FO, northern fish oil; SGR, specific growth rate; SRO, southern fish oil and rapeseed oil; SRO/SO, southern fish oil, rapeseed oil and soybean oil; SSO, southern fish oil and soybean oil; TGC, thermal growth coefficient.

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Table 5. Apparent digestibility coefficients (ADC) of gross nutrients and fatty acids in salmon fed the experimental diets

Parameters	FO	DFO	SRO	SRO/SO	SSO
Protein	83.2 ± 1.3^{ab}	79.5 ± 0.2^{c}	83.4 ± 0.4^{a}	84.2 ± 0.6^{a}	81.5 ± 1.5^{b}
Lipid	95.0 ± 0.6^{a}	91.2 ± 0.4^{d}	94.1 ± 0.1^{b}	93.6 ± 0.1b^{c}	92.8 ± 0.8^{c}
Dry matter	69.3 ± 1.9^{ab}	62.3 ± 0.5^{c}	70.4 ± 0.8^{a}	70.8 ± 1.4^{a}	67.0 ± 2.7^{b}
Fatty acid					
14:0	98.0 ± 0.3^{a}	94.5 ± 1.1^{b}	94.5 ± 0.1^{b}	94.6 ± 1.0^{b}	94.9 ± 1.2^{b}
16:0	97.2 ± 0.2^{a}	92.4 ± 1.2^{c}	93.9 ± 0.2^{b}	94.7 ± 1.2^{b}	96.2 ± 0.5^{a}
18:0	86.2 ± 1.2^{b}	73.3 ± 4.5^{c}	93.2 ± 0.6^{a}	94.3 ± 1.4^{a}	94.6 ± 2.2^{a}
Total saturated	95.7 ± 0.3^{a}	89.0 ± 1.7^{c}	94.0 ± 0.4^{b}	94.7 ± 1.2^{ab}	95.6 ± 0.8^{ab}
16:1n-7	98.8 ± 0.2^{a}	97.5 ± 0.6^{b}	97.4 ± 0.1^{b}	97.6 ± 0.3^{b}	97.7 ± 0.3^{b}
18:1n-9	99.0 ± 0.1^{a}	97.9 ± 0.5^{c}	98.4 ± 0.2^{b}	98.2 ± 0.3b^{c}	98.3 ± 0.3^{bc}
18:1n-7	98.4 ± 0.3^{a}	96.6 ± 0.6^{c}	97.4 ± 0.2^{b}	97.4 ± 0.4^{b}	97.5 ± 0.4^{b}
20:1n-9	98.5 ± 0.1^{a}	96.6 ± 0.5^{c}	97.4 ± 0.3^{b}	96.7 ± 0.6^{c}	97.0 ± 0.3^{bc}
22:1n11	97.1 ± 3.0	97.2 ± 1.6	96.5 ± 0.3	95.8 ± 1.0	96.6 ± 0.4
Total monoenes	98.7 ± 0.1^{a}	97.3 ± 0.6^{c}	98.2 ± 0.2^{ab}	98.0 ± 0.3^{b}	98.0 ± 0.3^{b}
18:2n-6	99.8 ± 0.1^{b}	99.8 ± 0.1^{a}	99.8 ± 0.1^{a}	99.8 ± 0.1^{a}	99.8 ± 0.1^{a}
20:4n-6	98.8 ± 0.1	97.4 ± 0.5	99.2 ± 1.5	98.3 ± 1.9	99.2 ± 1.5
Total n-6 PUFA	98.8 ± 0.1^{b}	97.6 ± 0.5^{c}	98.8 ± 0.1^{b}	99.1 ± 0.3^{ab}	99.3 ± 0.2^{a}
18:3n-3	99.3 ± 0.1^{a}	98.4 ± 0.4^{c}	98.8 ± 0.2^{b}	98.7 ± 0.2^{bc}	98.6 ± 0.3^{bc}
18:4n-3	99.3 ± 0.1^{a}	98.6 ± 0.4^{b}	98.7 ± 0.6^{b}	99.0 ± 0.1^{ab}	98.8 ± 0.2^{b}
20:5n-3	99.2 ± 0.1^{a}	98.2 ± 0.4^{c}	98.7 ± 0.1^{b}	98.7 ± 0.1^{b}	98.5 ± 0.3^{bc}
22:5n-3	98.9 ± 0.1^{a}	97.5 ± 0.5^{a}	96.0 ± 1.8^{b}	97.8 ± 0.2^{a}	97.8 ± 0.3^{a}
22:6n-3	98.8 ± 0.1^{a}	97.2 ± 0.5^{b}	96.9 ± 0.4^{b}	96.8 ± 0.5^{b}	96.7 ± 0.4^{b}
Total n-3 PUFA	99.0 ± 0.1^{a}	97.8 ± 0.4^{c}	98.3 ± 0.2^{b}	98.2 ± 0.2^{b}	98.0 ± 0.3^{b}
Total PUFA	99.0 ± 0.1^{a}	97.8 ± 0.4^{c}	98.4 ± 0.2^{b}	98.4 ± 0.2^{b}	98.4 ± 0.3^{b}

Results are means ± SD (n = 4). Values within a row with different superscript letters are significantly different as determined by ANOVA. DFO, decontaminated northern fish oil; FO, northern fish oil; PUFA, polyunsaturated fatty acids. SRO, southern fish oil and rapeseed oil; SRO/SO, southern fish oil and rapeseed and soybean oils; SSO, southern fish oil and soybean oil.

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Table 6. Lipid contents (percentage of wet weight) of flesh and liver of salmon at the end of the dietary trial

Diet	Flesh	Liver
FO	11.3 ± 1.3^{a}	8.0 ± 2.8^{a}
DFO	11.2 ± 1.3^{a}	7.0 ± 2.0^{a}
SRO	9.8 ± 0.8^{b}	5.0 ± 1.0^{b}
SRO/SO	10.9 ± 1.1^{ab}	4.7 ± 0.8^{b}
SSO	10.6 ± 1.2^{ab}	4.4 ± 0.7^{b}

Results are means ± SD (n = 3). Values within a column with different superscript letters are significantly different as determined by ANOVA. DFO, decontaminated northern fish oil; FO, northern fish oil; PUFA, polyunsaturated fatty acids. SRO, southern fish oil and rapeseed oil; SRO/SO, southern fish oil and rapeseed and soybean oils; SSO, southern fish oil and soybean oil.

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Table 7. Lipid class composition (percentage of total lipid) of salmon flesh and liver at the end of the feeding trial.

Lipid class	FO	DFO	SRO	SRO/SO	SSO
Flesh					
PC	4.6 ± 0.4[b]	5.0 ± 0.6[b]	6.5 ± 0.7[a]	5.6 ± 0.6[a]	5.4 ± 0.6[a]
PE	3.1 ± 0.4[b]	3.3 ± 0.4[b]	4.1 ± 0.5[a]	4.2 ± 0.6[a]	4.4 ± 0.6[a]
PS	n.d.	n.d.	n.d.	n.d.	n.d.
PI	n.d.	n.d.	n.d.	n.d.	n.d.
SM	n.d.	n.d.	n.d.	n.d.	n.d.
Total PL	7.7 ± 0.7[b]	8.3 ± 1.0[b]	10.6 ± 1.1[a]	9.8 ± 1.2[a]	9.7 ± 1.1[a]
Total NL	92.2 ± 0.5[a]	91.7 ± 1.0[ab]	89.5 ± 1.3[d]	90.9 ± 1.9[bc]	90.3 ± 1.0[
TAG	83.9 ± 1.1[a]	83.2 ± 1.7[a]	80.3 ± 2.5[b]	80.2 ± 2.8[b]	75.4 ± 1.9
Sterol	6.0 ± 0.4[bc]	5.6 ± 0.4[c]	6.1 ± 0.7[b]	6.4 ± 0.6[b]	8.3 ± 2.0[a]
FFA	3.1 ± 0.7[c]	3.0 ± 0.7[c]	3.1 ± 0.7[c]	4.4 ± 0.7[b]	6.6 ± 1.0[a]
Liver					
PC	13.4 ± 0.4[b]	15.3 ± 2.5[b]	19.5 ± 0.2[a]	18.4 ± 1.5[a]	19.9 ± 2.3
PE	8.7 ± 0.7[b]	10.8 ± 2.4[b]	13.5 ± 0.7[a]	13.9 ± 0.8[a]	13.4 ± 1.3
PS	2.4 ± 0.7	2.1 ± 0.0	2.4 ± 0.1	2.8 ± 0.6	2.6 ± 0.4
PI	5.7 ± 0.2[a]	5.5 ± 0.3[a]	5.4 ± 1.3[a]	4.1 ± 0.5[b]	4.2 ± 0.3[b]
SM	2.4 ± 0.3[a]	2.2 ± 0.1[a]	2.3 ± 0.2[a]	1.9 ± 0.3[b]	1.9 ± 0.3[b]
UPL	1.5 ± 0.4[b]	0.6 ± 1.0[b]	1.3 ± 2.3[b]	3.8 ± 0.6[a]	3.4 ± 0.1[a]
Total PL	34.1 ± 1.1[b]	36.4 ± 4.0[b]	44.4 ± 1.4[a]	45.0 ± 3.2[a]	45.7 ± 4.5
Total NL	65.9 ± 1.1[a]	63.6 ± 4.0[a]	55.6 ± 1.4[b]	56.0 ± 3.2[b]	54.3 ± 4.5
TAG	39.6 ± 1.6[a]	37.2 ± 3.8[ab]	32.4 ± 2.7[bc]	32.1 ± 3.4[bc]	28.8 ± 4.5
Sterol	9.1 ± 0.4[d]	10.7 ± 1.2[c]	12.7 ± 0.5[b]	12.3 ± 0.7[b]	14.5 ± 1.0
FFA	1.6 ± 0.3[a]	1.6 ± 0.4[a]	0.5 ± 0.9[b]	1.6 ± 0.6[a]	0.1 ± 0.1[c]
Steryl ester	15.6 ± 0.3[a]	14.2 ± 1.4[a]	10.1 ± 1.1[b]	9.1 ± 1.0[b]	11.0 ± 1.1

Results are means ± SD (n = 9). Values within a row with different superscript letters are significantly different as determined by ANOVA. DFO, decontaminated northern fish oil; FFA, free fatty acid; n.d., not detected; FO, northern fish oil; NL, neutral lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, polar lipids; PS, phosphatidylserine; SM, sphingomyelin; SRO, southern fish oil and rapeseed oil; SRO/SO, southern fish oil, rapeseed and soybean oils; SSO, southern fish oil and soybean oil; TAG, triacylglycerol; UPL, unidentified polar lipid.

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Table 8. Fatty acid composition (percentage of total fatty acid) of salmon flesh at the end of the feeding trial

	FO	DFO	SRO	SRO/SO	SSO
14:0	4.7 ± 0.2^{b}	4.9 ± 0.1^{a}	2.9 ± 0.1^{c}	2.9 ± 0.1^{c}	3.0 ± 0.1^{c}
16:0	17.1 ± 0.6^{a}	16.8 ± 0.3^{a}	10.9 ± 0.6^{d}	12.2 ± 0.4^{c}	13.5 ± 0.5^{b}
18:0	2.8 ± 0.1^{c}	2.7 ± 0.1^{c}	2.7 ± 0.2^{c}	3.2 ± 0.1^{b}	3.6 ± 0.2^{a}
Total saturated[1]	25.3 ± 0.8^{a}	25.1 ± 0.5^{a}	17.2 ± 0.8^{d}	19.0 ± 0.6^{c}	21.1 ± 0.8^{b}
16:1n-7	6.0 ± 0.2^{a}	5.8 ± 0.1^{a}	2.9 ± 1.4^{c}	2.9 ± 1.1^{bc}	3.4 ± 0.1^{b}
18:1n-9	25.1 ± 0.6^{c}	21.9 ± 0.3^{d}	39.0 ± 1.2^{a}	28.9 ± 0.4^{b}	19.9 ± 0.4^{c}
18:1n-7	3.1 ± 0.2^{b}	3.1 ± 0.1^{b}	3.3 ± 0.2^{a}	3.0 ± 0.2^{b}	2.6 ± 0.1^{c}
20:1n-9	2.5 ± 0.1^{b}	3.3 ± 0.1^{a}	1.9 ± 0.1^{c}	1.5 ± 0.1^{d}	1.1 ± 0.1^{e}
22:1n11	1.8 ± 0.1^{b}	3.1 ± 0.2^{a}	0.5 ± 0.1^{d}	0.5 ± 0.1^{d}	0.6 ± 0.1^{c}
24:1n-9	1.3 ± 0.2^{a}	1.2 ± 0.1^{b}	0.4 ± 0.0^{c}	0.4 ± 0.0^{c}	0.4 ± 0.1^{c}
Total monoenes[2]	40.8 ± 0.8^{b}	39.3 ± 0.6^{c}	49.0 ± 1.1^{a}	38.1 ± 0.3^{d}	28.6 ± 0.5^{e}
18:2n-6	5.3 ± 0.2^{d}	5.8 ± 1.0^{d}	11.9 ± 0.3^{c}	20.9 ± 0.6^{b}	28.1 ± 1.1^{a}
20:4n-6	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}
Total n-6 PUFA[3]	7.3 ± 0.3	7.7 ± 1.0	13.8 ± 0.5	23.0 ± 0.6	30.5 ± 1.2
18:3n-3	2.3 ± 0.1^{d}	2.3 ± 0.1^{d}	5.6 ± 0.2^{a}	4.5 ± 0.2^{b}	3.2 ± 0.1^{c}
18:4n-3	1.5 ± 0.1^{b}	1.8 ± 0.1^{a}	0.8 ± 0.1^{d}	0.8 ± 0.0^{d}	0.9 ± 0.1^{c}
20:4n-3	1.2 ± 0.1^{a}	1.1 ± 0.1^{b}	0.7 ± 0.1^{c}	0.7 ± 0.0^{c}	0.7 ± 0.0^{c}
20:5n-3	5.9 ± 0.4^{b}	6.9 ± 0.3^{a}	4.6 ± 0.4^{d}	4.9 ± 0.1^{cd}	5.2 ± 0.3^{c}
22:5n-3	2.1 ± 0.2^{a}	2.0 ± 0.1^{ab}	1.7 ± 0.2^{d}	1.8 ± 0.1^{cd}	1.9 ± 0.2^{bc}
22:6n-3	13.1 ± 0.6^{a}	13.6 ± 0.6^{a}	6.4 ± 0.5^{c}	6.7 ± 0.2^{c}	7.5 ± 0.5^{b}
Total n-3 PUFA[4]	26.6 ± 1.2^{b}	28.0 ± 1.0^{a}	20.0 ± 1.4^{c}	20.0 ± 0.3^{c}	19.8 ± 0.8^{c}
Total PUFA	34.0 ± 1.4^{d}	35.7 ± 0.7^{c}	33.8 ± 1.8^{d}	43.0 ± 0.7^{b}	50.4 ± 1.1^{a}

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different as determined by ANOVA. [1]Includes 15:0, 20:0 and 22:0 at up to 0.5%; [2]Includes 16:1n-9, 20:1n-11, 20:1n-7 and 22:1n-9 at up to 0.6%; [3]Includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 and 22:5n-6 at up to 0.9%; [4]Includes 20:3n-3 at up to 0.3%. DFO, decontaminated northern fish oil; FO, northern fish oil; PUFA, polyunsaturated fatty acids; SRO, southern fish oil and rapeseed oil; SRO/SO, southern fish oil, rapeseed oil and soybean oil; SSO, southern fish oil and soybean oil.

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Table 9. Fatty acid composition (percentage of total fatty acids) of salmon liver at the end of the feeding trial

	FO	DFO	SRO	SRO/SO	SSO
14:0	2.2 ± 0.2^{a}	2.2 ± 0.5^{a}	1.3 ± 0.4^{b}	1.1 ± 0.2^{b}	1.1 ± 0.1^{b}
16:0	11.2 ± 1.5^{ab}	10.7 ± 1.5^{ab}	9.7 ± 2.1^{b}	10.7 ± 1.3^{ab}	12.1 ± 1.1^{a}
18:0	4.1 ± 0.6^{b}	4.0 ± 0.4^{b}	4.4 ± 0.5^{b}	5.5 ± 0.7^{a}	6.0 ± 0.6^{a}
Total saturated[1]	18.0 ± 2.0^{ab}	17.4 ± 2.2^{ab}	15.9 ± 2.8^{b}	17.6 ± 1.4^{ab}	19.4 ± 1.6^{a}
16:1n-7	3.7 ± 0.5^{a}	3.4 ± 0.7^{a}	1.7 ± 0.4^{b}	1.3 ± 0.5^{b}	1.0 ± 0.8^{c}
18:1n-9	23.1 ± 3.3^{b}	24.3 ± 3.8^{b}	28.3 ± 5.9^{a}	20.7 ± 4.0^{b}	15.7 ± 1.8^{c}
18:1n-7	3.8 ± 0.3^{a}	3.7 ± 0.3^{a}	3.0 ± 0.3^{b}	2.6 ± 0.3^{c}	2.2 ± 0.2^{d}
20:1n-9	3.3 ± 0.4^{ab}	3.6 ± 0.4^{a}	3.1 ± 0.5^{b}	2.1 ± 0.4^{c}	1.4 ± 0.2^{d}
22:1n11	0.9 ± 0.3^{a}	1.0 ± 0.5^{a}	0.1 ± 0.3^{b}	n.d.	n.d.
24:1n-9	0.9 ± 0.1^{ab}	1.0 ± 0.2^{a}	0.8 ± 0.2^{ab}	0.7 ± 0.1^{c}	0.7 ± 0.1^{c}
Total monenes[2]	36.7 ± 3.9^{a}	38.2 ± 3.3^{a}	37.6 ± 5.5^{a}	27.8 ± 4.1^{b}	21.6 ± 2.5^{c}
18:2n-6	3.6 ± 0.4^{d}	4.0 ± 1.7^{d}	7.5 ± 2.1^{c}	14.1 ± 2.0^{b}	17.7 ± 2.4^{a}
20:2n-6	1.1 ± 0.1^{c}	1.2 ± 0.3^{c}	1.7 ± 0.4^{b}	3.2 ± 0.4^{a}	3.5 ± 0.4^{a}
20:3n-6	0.4 ± 0.2^{bc}	0.3 ± 0.0^{c}	0.4 ± 0.1^{bc}	0.5 ± 0.1^{a}	0.6 ± 0.1^{a}
20:4n-6	1.7 ± 0.4^{b}	1.6 ± 0.3^{b}	2.0 ± 0.4^{a}	2.3 ± 0.3^{a}	2.3 ± 0.3^{a}
Total n-6 PUFA[3]	7.3 ± 0.3^{c}	7.7 ± 1.9^{c}	12.1 ± 2.0^{b}	20.5 ± 2.0^{a}	24.5 ± 2.2^{a}
18:3n-3	1.6 ± 0.2^{c}	1.7 ± 0.6^{c}	3.0 ± 0.8^{a}	2.4 ± 0.4^{a}	1.6 ± 0.6^{c}
20:4n-3	1.9 ± 0.2^{a}	1.7 ± 0.4^{a}	0.9 ± 0.2^{b}	0.8 ± 0.1^{c}	0.7 ± 0.1^{c}
20:5n-3	7.7 ± 1.4^{b}	7.7 ± 1.1^{b}	9.1 ± 0.9^{a}	10.0 ± 1.3^{a}	9.7 ± 0.9^{a}
22:5n-3	4.3 ± 0.8^{a}	3.7 ± 0.7^{a}	2.5 ± 0.8^{b}	2.7 ± 0.2^{b}	2.6 ± 0.2^{b}
22:6n-3	21.5 ± 1.8^{a}	20.7 ± 3.0^{ab}	17.7 ± 4.6^{b}	17.6 ± 2.1^{b}	19.1 ± 2.5^{ab}
Total n-3 PUFA[4]	38.1 ± 2.3^{a}	36.7 ± 2.8^{ab}	34.5 ± 4.5^{b}	34.1 ± 3.0^{b}	34.5 ±2.9^{b}
Total PUFA	45.4 ± 2.5^{c}	44.4 ± 2.0^{c}	46.6 ± 2.9^{c}	54.6 ± 3.1^{b}	59.0 ± 1.3^{a}

Results are means ± SD (n = 3). Values within a row with different superscript letters are significantly different as determined by ANOVA. [1]Includes 15:0, 20:0 and 22:0 at up to 0.3%; [2]Includes 16:1n-9, 20:1n-11, 20:1n-7 and 22:1n-9 at up to 0.6%; [3]Includes 18:3n-6, 22:4n-6 and 22:5n-6 at up to 0.4%; [4]Includes 18:4n-3 and 20:3n-3 at up to 0.8%. DFO, decontaminated northern fish oil; FO, northern fish oil; PUFA, polyunsaturated fatty acids; SRO, southern fish oil and rapeseed oil; SRO/SO, southern fish

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