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Food Chemistry 87 (2004) 567–580
Food Chemistry
www.elsevier.com/locate/foodchem

Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils

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Received 18 July 2003; received in revised form 8 January 2004; accepted 8 January 2004

Abstract

A feeding experiment was performed to investigate the possibility of feeding vegetable oils to Atlantic salmon, followed by a wash-out period to maintain salmon fillet as a product highly beneficial for human health, due to the high levels of VLC$n-3$ PUFA and high $n-3/n-6$ ratio. Six groups of Atlantic salmon, initial weight 142 ± 1 g, were fed increasing dietary inclusion of rapeseed oil (RO) in a regression design and one group was fed a 50% olive oil (OO)/50% capelin oil (FO) diet for 42 weeks, followed by 25 weeks, of wash-out, when all groups were fed 100% FO. Muscle gross composition, lipid class and fatty acid composition and astaxanthin were measured at the start and after 22 and 42 weeks of feeding. Fillet fatty acid composition was analysed at the start and throughout the wash-out period of 25 weeks and 1788 day degrees. Growth, total lipid, astaxanthin content, and lipid class composition were not affected by dietary oil source. Muscle and fillet fatty acid composition were highly affected by dietary fatty acid composition. Through the wash-out period, the VLC$n-3$ PUFAs EPA and DHA was restored already after 1300 day degrees, whereas the wash-out of 18:2$n-6$, 18:1$n-9$ and 18:3$n-3$ was a slower process, requiring a minimum 1788 day degrees for reaching 100% FO levels. For reducing $n-6$ fatty acids and increasing VLC$n-3$ PUFA, a period of feeding with 100% FO was sufficient for the groups fed 25% RO, 50% RO and 50% OO prior to wash-out. Considering the recommended $n-3/n-6$ ratio and VLC$n-3$ PUFA intake for human consumption, fillets from fish fed 100% FO, 25% RO, 50% RO and 50% OO prior to wash-out, followed by 1788 day degrees of 100% FO diet, can be considered beneficial for human health promotion.

Keywords: Atlantic salmon; Muscle; Astaxanthin; Fatty acids; Lipid class composition; Washout-period; Rapeseed oil; Capelin oil; Olive oil; VLC$n-3$

1. Introduction

The rapid changes in the western diet, particularly during the past 100 years, may be potent promoters of chronic diseases, such as atherosclerosis, hypertension, diabetes, many cancers and lifestyle diseases (e.g., metabolic syndrome and obesity), causing great concern for health authorities worldwide (WHO, 2002).

Of major interest is dietary fat and current research is to a large extent focussed on effects of individual fatty acids related to health. This includes the essential omega-3 and omega-6 fatty acids of plant origin and the very long-chain omega-3 polyunsaturated fatty acids (VLC$n-3$ PUFAs) of marine origin. The individual dietary fatty acids have distinctive functions and physiological effects with consequences for health and disease. With the recent discovery that fatty acids are modulators of nuclear transcription factors influencing genes and gene products this research has attained a new dimension (Clarke & Jump, 1993, 1994; Jump & Clarke, 1999; Sessler & Ntambi, 1998). In a recent workshop report from the UK Food Standards Agency, reviewing current research on whether α-linolenic acid (ALA, 18:3$n-3$) was as beneficial to cardiovascular health as the marine VLC$n-3$ PUFAs, reservations were expressed about the evidence suggesting a beneficial effect ALA on secondary prevention of coronary heart disease (Sanderson et al., 2002). Moreover, the possibility of feeding livestock 18:3$n-3$-rich oils to provide, as a

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0308-8146/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2004.01.009

means of increasing the dietary intake of EPA and DHA in human consumers, was highlighted (Sanderson et al., 2002). Atlantic salmon represent a natural source that generally have high levels $n-3$ PUFAs and low levels of $n-6$ fatty acids, and may be considered as a health-promoting product for human consumption by reducing $n-6$ and increasing $n-3$ intake. However, with the predicted increase in aquaculture and decrease in global supply of fish oil and fish meal (Hardy, 2001), the need for investigating alternative lipid sources in Atlantic salmon diets has increased, in order to ensure a sustainable exploitation of marine resources. Rapeseed oil (RO) has moderate levels of 18:2$n-6$ and 18:3$n-3$ and high levels of 18:1$n-9$, which is considered a preferred substrate for energy production in Atlantic salmon. Further, the 18:2$n-6$/18:3$n-3$ ratio in RO is 2:1, which is beneficial compared to the recommended ratio for human $n-6/n-3$ consumption of 4:1.

The fatty acid composition of salmon muscle is mainly a result of the dietary fatty acid composition (Bell et al., 2002; Bell, McEvoy, Tocher, McGhee, Campbell, & Sargent, 2001; Bell, McGhee, Campbell, & Sargent, 2003; Sargent, Bell, McEvoy, Tocher, & Estevez, 1999; Torstensen, Lie, & Frøyland, 2000; Waagbø, Sandnes, Sandvin, & Lie, 1991). Commercially, salmon diets in the seawater growth phase are lipid rich, with 25% to 40% lipid. The high growth rates of cultured Atlantic salmon are due to the use of high energy diets which are traditionally rich in 20:1 and 22:1 fatty acids, due to the use of northern hemisphere oils. Salmon tissue fatty acid composition also depends on factors other than dietary fatty acid composition. Digestibility (Sigurgisladottir, Lall, Parrish, & Ackman, 1992; Torstensen et al., 2000), fatty acid transport and uptake (Torstensen, 2000; Torstensen et al., 2000), elongation and desaturation processes (Bell et al., 2002, 2001) and β-oxidation of fatty acids (Frøyland, Lie, & Berge, 2000; Frøyland, Madsen, Eckhoff, Lie, & Berge, 1998) will affect the membrane and deposit lipid composition. In vitro studies done on mitochondrial β-oxidation in fish, suggest that there exist substrate preferences for saturated and monounsaturated fatty acids over polyunsaturated fatty acids. Especially, 16:0, 16:1, 18:1$n-9$ and 18:2$n-6$ seem to be preferentially mobilised during starvation, whereas 22:6$n-3$ are found to be oxidised at low rates (Henderson, 1996; Kiessling & Kiessling, 1993). Further, 22:1$n-11$ and 16:0 were found to serve equally well as substrates for mitochondrial β-oxidation in trout liver (Henderson & Sargent, 1985). However, in developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.), 22:6$n-3$ is reported to be the quantitatively most important fatty acid in energy metabolism (Rønnestad, Finn, Lein, & Lie, 1995).

Several studies have shown effects, on Atlantic salmon tissue fatty acid composition and metabolic and physiological parameters, of replacing dietary fish oil with different vegetable oils, such as linseed oil (Bell, Dick, Mc Vicar, Sargent, & Thompson, 1993; Bell, Raynard, & Sargent, 1991; Bell, Sargent, & Raynard, 1992; Rollin, Peng, Pham, Ackman, & Larondelle, 2003; Rosenlund, Obach, Sandberg, Standal, & Tveit, 2001; Thompson, Tatner, & Henderson, 1996; Tocher, Bell, Dick, & Sargent, 1997), sunflower oil (Bell et al., 1993; Bell et al., 1991; Bell et al., 1992; Rollin et al., 2003; Thompson et al., 1996; Tocher et al., 1997), soybean oil (Lie, Sandvin, & Waagbø, 1993; Rosenlund et al., 2001; Waagboe, Sandnes, Joergensen, Engstad, Glette, & Lie, 1993; Waagboe, Sandnes, Lie, & Nilsen, 1993), palm oil (Bell et al., 2002; Rosenlund et al., 2001; Torstensen et al., 2000), medium-chain triglyceride oil (Røsjø, Nordrum, Olli, Krogdahl, Ruyter, & Holm, 2000), RO (Bell et al., 2001, 2003; Bell, Tocher, Farndale, Cox, McKinney, & Sargent, 1997; Rosenlund et al., 2001; Tocher et al., 2000), poultry oil (Rollin et al., 2003) and olive oil (OO) (Rosenlund et al., 2001; Tocher et al., 1997) in different life stages of Atlantic salmon. However, the effects of increasing RO inclusion up to 100% of the lipid in high energy (30% lipid) diets, in the seawater stage up to relevant harvest size, remain to be studied. Further, by feeding salmon a 100% fish oil, diet a limited period prior to harvest (wash-out), one may obtain the desired salmon product with high VLC$n-3$ PUFA levels and low $n-6/n-3$ ratio.

To investigate production of a tailored salmon product for human consumption, giving a health benefit, post-smolt was fed increasing inclusion of RO in a regression design or a 50% inclusion of OO, replacing northern hemisphere capelin oil (Fish oil, FO). Fish growth and feed utilization, as well as tissue lipid and muscle astaxanthin composition were measured through feeding experimental diets for 42 weeks, followed by 25 weeks of wash-out simulating possible production of future commercial Atlantic salmon.

2. Materials and methods

2.1. General

The dietary experiment was started 28th May 2001 at Gildeskaal Research Station, Inndyr, Norway, 67° North, and continued until March 2002 (totally 42 weeks). At that time, 50 salmon from each dietary treatment were individually marked, combined in a single net pen and fed a 100% fish oil diet for another 25 weeks (wash-out period). At the start, approximately 600 post-smolt Atlantic salmon, mean weight 142 g, were distributed to seven net pens of 125 m^3. Five experimental diets, where fish oil was replaced by, respectively, 25%, 50%, 75% and 100% RO and 50% OO, were fed to each group of Atlantic salmon. The control diet, containing 100% fish oil, was fed in duplicate. All

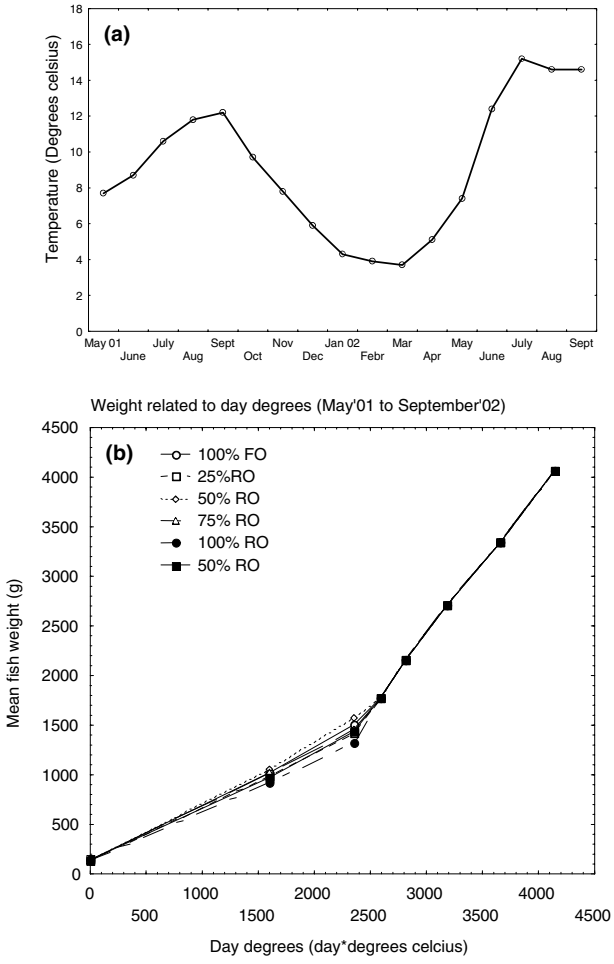

Fig. 1. (a) Sea temperature during the experiment, and (b) mean weight of the Atlantic salmon related to day degrees (days* °C) from May 2001 to September 2002 at each sampling point where all fish were bulk weighed and counted.

diets were formulated to contain 300 g kg^{-1} lipid, 450 g kg^{-1} protein, 60 g kg^{-1} moisture, 70 g kg^{-1} ash and 120 g kg^{-1} NFE (Nitrogen free extract). The diets were fed to satiation by hand and the exact amounts consumed were recorded. Mortalities were recorded and dead fish removed daily. Biomass and average weight were determined by bulk weighing and counting of all fish at each sampling. Mean monthly temperature through the experiment is given in Fig. 1. The fish were exposed to natural light until December 22. From December 22 until May 1 the fish were exposed to continuous light, followed by natural light from May 1 until end of the experiment.

2.2. Sampling procedure

Samples were taken from all diets and stored at −20 °C. Three pooled samples of each five fish were sampled from the whole population at the initial sampling, and from each dietary group at the intermediate sampling time of 22 weeks and at the final sampling time of 42 weeks, for obtaining an expression for within-net pen variation and expressed as $n = 3$ (Riley & Edwards, 1998). The fish were fasted 24 hours prior to sampling. Fifteen randomly sampled fish from each tank were anaesthetised with methomidate (7 g/l). Blood was collected from the caudal vein using EDTA vacutainers and the fish killed by a blow to the head, followed by dissection of red- and white muscle. In addition fillet, as a Norwegian quality cut (NQC) was sampled at the final sampling. The red muscle and white muscle samples from the same 15 fish from each group were homogenised in three batches of 5 fish to obtain a measure of within net pen variation. These samples were immediately frozen on dry ice and stored at −80 °C until further analyses. During the wash-out period, from March 2002 to September 2002, five salmon from each group fed different experimental diets up to March 2002 were individually sampled and fillet (NQC) was homogenised from each fish, frozen on dry ice and stored at −80 °C until further analyses. Samplings during the wash-out period were performed 1, 2, 4 and 6 months after washout.

2.3. Analysis

Fatty acid composition was analysed in the diet, white muscle and fillet. Lipids from the samples were extracted by adding chloroform/methanol (2:1, v/v). After extraction of lipids, the samples were filtered, an aliquot was removed for determining lipid class composition as described below, 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 Carlo Erba gas chromatograph (“cold on column” injection, 60 °C for 20 s 25 °C/min 160 °C for 28 min 25 °C/min 190 °C for 17 min 25 °C/min 220 °C for 9 min), equipped with a 50 m CP-sil 88 (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%) was calculated using an integrator (Chromkvest ver. 2.52, Thermoquest CE Instruments, Milan, Italy), connected to the GLC.

All-*trans* astaxanthin from fish feed was analysed using an *in house* method. Approximately 10 g freshly ground and homogenised fish feed were dissolved in 150 ml acetone and shaken for 30 min. The lipid containing solution was filtered through a vacuum glass filter, the feed residue was washed 3 times with 30 ml diethyl ether and pooled with the acetone fraction. The solution was transferred to a separating funnel, 100 ml of filtered and deionised water was added and the water phase was discarded after mixing and phase-separation. The lipid

containing ether solution was evaporated on a water bath at 80 °C for 30 min and water remnants in the remaining lipid–water emulsion were removed azeotropically by adding and evaporating isopropanol 3 times in a rotavapor. The dry residue was redissolved in *n*-heptane and analysed by HPLC.

Quantitative analysis of astaxanthin from fish feed was performed using an HPLC system consisting of a Spectra-Physics 8810 LC pump, a Gilson 234 Autoinjector, a Spectra-Physics 770 UV–Vis detector coupled with a Spectra-Physics Wavelength drive SFA 339, a Supelcosil 5 μm LC-CN column (25 cm × 4.6 mm), "Xtra Chrom" software (HP/Nelson analytical) and a *n*-heptane:isopropylacetate:acetone:ethanol (84.5:10:4:1.5) solution at 2.75 ml min^{-1} as mobile phase. Quantification of astaxanthin was performed using an external standard method where standard solutions prepared from crystalline all-*trans*-astaxanthin (Hoffman LaRoche, Oslo, Norway) were measured with a spectrophotometer (UV-260, Shimadzu, Japan) using a molar absorptivity in hexane of $E^{1\,cm}_{1\%} = 2100$ at $\lambda_{max} = 472$ nm for astaxanthin. The retention time for all-*trans* astaxanthin was 2.9 min and detection wavelength was 470 nm.

Qualitative analysis of carotenoids from fish feed was performed by evaporation of the sample extract described above and by redissolving the dry sample in the mobile phase used in the system described by Ørnsrud (submitted, Journal of Fish Biology). The identification of peaks was accomplished using retention time and spectrum similarity tests. The relative amounts of the different carotenoids were found by measuring peak height at each individual carotenoid's λ_{max} and dividing this value by the sum of all carotenoid peak heights. All samples were integrated using the Chromquest software (ver. 2.52, Thermoquest CE Instruments, Milan Italy).

All-*trans* and 9 and 13-*cis* astaxanthin were measured in fish muscle using a procedure modified from Bligh and Dyer (1959) by Bjerkeng, Refstie, Fjalestad, Storebakken, Rødbotten, and Roem (1997). Briefly, homogenised fish muscle was extracted using a water:methanol:chloroform (1:1:3) solution. An aliquot of the astaxanthin-containing chloroform phase was N_2 evaporated and redissolved in hexane. The HPLC system used was the same as described in the section for qualitative analysis of carotenoids from fish feed. Quantification of astaxanthin was performed using an external standard method, as described in the section for quantitative analysis of astaxanthin from fish feed.

The quantification of lipid class composition in the muscle was performed using high-performance thin-layer chromatography (HPTLC), as described by Bell et al. (1993).

Dry matter was measured gravimetrically after freeze-drying of homogenised samples for 48 h. 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 $N \times 6.25$. Total lipid was measured gravimetrically after ethyl acetate extraction.

2.4. Statistics

The relative fatty acid composition data of fillet were analysed using SIRIUS for Windows (Version 6.0). Principal component analysis (PCA) (Wold, Esbensen, & Geladi, 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, etc.). A high positive or negative loadings 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 Fig. 3(a) and (c) by circles drawn on freehand. Since samples with the same composition were located on top of each other, to ease interpretation of the samples, the classes they contain are written beside the circle.

3. Results

3.1. General

Atlantic salmon fed the experimental diets increased their weight from 143 g in May 2001 to 1463 ± 82 g (mean ± SD) after 42 weeks in March 2002 (Fig. 1(b)). No major differences were seen in growth between the dietary groups; however, there was a trend of decreased growth in the group fed 100% RO. Feed efficiency, defined as kilos of diet fed per kilos fish weight gain, was estimated to be 0.82–0.84 in all dietary groups from start to 22 weeks, and 1.01–1.12 from 22 to 42 weeks of feeding. The groups fed 100% FO and 100% RO had the highest feed efficacy with 1.1. The mean bulk weight of all the fish in the wash-out period is presented in Fig. 1(b). Further, mean weights based on individual weights ($n = 5$) from each sampling in the wash-out period (Fig. 3(a)) show that salmon fed different diets prior to the wash-out period increased their weight, respectively, 2.6-, 3.3-, 2.1-, 2.3- and 3.1-fold in the 100% FO to the 100% RO group, and the fish fed 50% OO prior to the wash-out period increased their weight 2.3-fold. Further, salmon fed the 100% RO diet had lowest growth throughout the feeding experiment, increased their weight in the last part of the wash-out period and ended up with the highest mean fish weight of all the experi-

Table 1a
Dietary fatty acid composition (weight% and mg fatty acid g^{-1} feed. w.w) of the six experimental diets

	100% FO		75% FO, 25% RO		50% FO, 50% RO		25% FO, 75% RO		100% RO		50% FO, 50% OO	
	(%)	(mg/g)	(%)	(mg/g)	(%)	(mg/g)	(%)	(mg/g)	(%)	(mg/g)	(%)	(mg/g)
14:0	6.7	14.4	5.0	10.8	3.6	8.5	2.1	5.1	0.4	1.0	3.4	7.2
16:0	11.6	25.0	10.0	21.6	8.9	21.0	7.5	17.9	5.7	13.7	11.7	24.9
18:0	1.0	2.1	1.2	2.6	1.4	3.3	1.6	3.8	1.7	4.1	1.9	4.1
Sum saturated	20.4	43.9	17.4	37.5	14.9	35.1	12.5	29.9	9.1	21.7	18.0	38.2
16:1$n-9$	0.2	0.5	0.2	0.5	0.2	0.4	–	–	–	–	0.2	0.4
16:1$n-7$	8.0	17.3	6.1	13.1	4.4	10.4	2.6	6.3	0.6	1.5	4.5	9.5
18:1$n-9$	11.2	24.1	22.5	48.5	32.4	76.2	42.4	102	53.6	129	37.3	79.1
18:1$n-7$	3.4	7.4	3.3	7.2	3.4	7.9	3.4	8.0	3.2	7.7	2.8	5.9
20:1$n-11$	0.4	0.9	0.3	0.6	0.3	0.7	0.2	0.6	–	–	0.3	0.6
20:1$n-9$	17.1	36.9	13.3	28.5	9.8	23.0	6.1	14.7	2.1	5.1	9.0	19.1
20:1$n-7$	0.7	1.5	0.4	0.9	0.3	0.7	0.2	0.5	–	–	0.3	0.7
22:1$n-11$	13.3	28.6	10.1	21.8	7.3	17.1	4.3	10.3	1.0	2.3	5.4	11.6
22:1$n-9$	2.0	4.4	1.6	3.5	1.3	3.0	0.9	2.2	0.5	1.2	1.1	2.3
Sum monoenes	57.1	123	58.4	126	59.6	140	60.4	145	61.1	147	61.2	130
18:2$n-6$	3.5	7.6	7.6	16.3	11.5	27.0	15.4	36.9	19.5	46.9	7.7	16.4
20:4$n-6$	0.3	0.6	0.3	0.5	0.1	0.2	–	–	–	–	0.2	0.4
20:2$n-6$	0.2	0.5	0.2	0.5	–	–	–	–	–	–	–	–
Sum $n-6$	4.1	8.7	8.1	17.3	11.5	27.2	15.4	36.9	19.5	46.9	7.9	16.8
18:3$n-3$	1.1	2.3	3.0	6.4	4.7	11.0	6.6	15.9	8.6	20.7	2.6	5.5
18:4$n-3$	2.8	6.1	2.2	4.7	1.6	3.7	0.9	2.2	0.2	0.4	1.5	3.1
20:4$n-3$	0.4	0.9	0.3	0.6	0.2	0.5	–	–	–	–	0.2	0.5
20:5$n-3$	5.9	12.6	4.5	9.7	3.4	8.0	2.1	5.1	0.7	1.7	3.2	6.9
22:5$n-3$	0.4	0.9	0.3	0.7	0.2	0.6	0.2	0.4	–	–	0.3	0.5
22:6$n-3$	4.6	10.0	3.7	8.0	3.0	7.1	2.1	5.0	1.0	2.5	2.9	6.2
Sum $n-3$	15.8	34.1	14.2	30.6	13.3	31.4	11.9	28.6	10.6	25.4	10.9	23.1
$n-3/n-6$	3.9	3.9	1.8	1.8	1.2	1.2	0.8	0.8	0.5	0.5	1.4	1.4
Sum total FA	100	216	100	215	100	235	100	240	100	241	100	212
Sum identified	97.5	210.1	98.6	212	99.4	234	100	240	100	241	98.0	208

Data are presented as means ($n = 2$). (When result is below 0.1. it is denoted by –).

Table 1b
Total dietary astaxanthin level (mg kg^{-1}), and the relative distribution of astaxanthin, lutein and zeaxanthin in the experimental diets

	Astaxanthin (mg kg^{-1})	Astaxanthin (%)	Lutein (%)	Zeaxanthin (%)
100% FO	54.5	72.0	11.0	4.4
25% RO	64.0	70.9	11.6	4.3
50% RO	70.0	71.5	12.8	5.8
75% RO	66.0	67.0	16.2	6.4
100% RO	66.9	68.0	15.3	7.7
50% OO	70.3	64.5	16.7	9.6

mental groups after 6 months of wash-out. Mortalities were negligible throughout the experimental period.

3.2. Muscle lipid composition

There were no major differences in protein, ash or dry matter in white muscle between fish fed the different experimental diets for 22 or 42 weeks (Table 2). However, after 22 weeks of feeding, white muscle total lipid, in both dry and wet weight, was decreased in fish fed the 100% RO diet compared to the other dietary groups (Table 2). This effect was not seen after 42 weeks of feeding. Total lipid (w.w) and dry matter increased in white muscle with 64% and 62.5%, respectively, from 22 to 42 weeks of feeding.

Lipid class composition of white muscle (Table 3) showed that sum of phospholipids (PL) decreased, whereas sum of neutral lipids (NL) increased from the start to 42 weeks of feeding. Generally, 100% RO and 50% OO fish contained lower relative levels of NL and higher levels of PL in both after 22 (data not shown)

Table 2
White muscle total lipid, protein, ash, dry matter (g 100 g^{-1}) and astaxanthin[a] ($n = 3$ samples from each net pen. $n = 6$ for 100% FO) from initial sampling and from fish fed the experimental diets 22 and 42 weeks

	Initial sampling (May 2001)	100% FO	25% RO	50% RO	75% RO	100% RO	50% OO
After 22 weeks (Intermediate sampling in October 2001)							
Dry matter	23.8 ± 0.5	27.5 ± 0.3	26.8 ± 0.4	26.6 ± 0.3	26.6 ± 0.4	26.2 ± 0.5	25.8 ± 0.2
Total lipid (d.w.)	20.4 ± 0.0	17.0 ± 5.5	16.2 ± 1.6	16.3 ± 0.5	16.5 ± 2.4	11.8 ± 1.5	10.8 ± 2.3
Total lipid (w.w.)	4.9 ± 0.1	4.7 ± 1.5	4.4 ± 0.5	4.4 ± 0.2	4.4 ± 0.7	3.1 ± 0.4	2.8 ± 0.6
Ash		5.1 ± 0.5	5.3 ± 0.6	4.9 ± 0.2	4.9 ± 0.2	5.2 ± 0.2	5.2 ± 0.2
Protein (w.w.)	20.1 ± 0.4	20.6 ± 0.1	20.7 ± 0.1	20.5 ± 0.2	20.4 ± 0.1	20.9 ± 0.2	20.8 ± 0.3
Total astaxanthin[a]	0.2 ± 0.1	3.3 ± 0.3	3.0 ± 0.1	2.8 ± 0.3	2.8 ± 0.3	2.3 ± 0.4	2.9 ± 0.3
After 42 weeks (final sampling in March 2002)							
Dry matter		27.4 ± 0.52	26.8 ± 0.37	27.1 ± 0.7	28.0 ± 0.34	29.0 ± 2.04	26.6 ± 0.1
Total lipid (d.w.)		16.0 ± 1.3	15.8 ± 1.3	16.7 ± 1.2	19.4 ± 0.2	16.0 ± 1.0	15.7 ± 1.9
Total lipid (w.w.)		6.6 ± 0.7	6.6 ± 0.8	7.1 ± 0.5	8.1 ± 0.3	7.0 ± 0.1	6.4 ± 0.8
Ash		4.8 ± 0.1	4.7 ± 0.2	4.8 ± 0.1	5.4 ± 0.6	6.0 ± 0.8	5.9 ± 0.7
Protein (w.w.)		20.9 ± 0.3	20.7 ± 0.2	20.7 ± 0.7	20.7 ± 0.3	21.2 ± 0.3	20.4 ± 0.1
Total astaxanthin[a]		3.6 ± 0.6	3.7 ± 0.7	4.0 ± 0.7	4.0 ± 0.1	2.9 ± 0.6	4.3 ± 0.4

Data are shown as mean ± SD.
[a] Sum all-*trans*,9-og13-*cis* astaxanthin (μg/g).

Table 3
White muscle lipid class composition ($n = 3$ samples from each net pen. $n = 6$ for 100% FO) (wt% of total lipid) from initial sampling and from fish fed the experimental diets for 42 weeks

	Initial sampling (May 2001)	After 42 weeks (final sampling in March 2002)					
		100% FO	25% RO	50% RO	75% RO	100% RO	50% OO
SM	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± –	0.4 ± –	0.5 ± –	0.5 ± 0.1
PC	16.4 ± 1.1	11.1 ± 0.6	10.9 ± 0.6	10.7 ± 1.0	9.6 ± 0.1	11.3 ± 0.6	11.5 ± 0.5
PS	– ± –	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± –	0.8 ± 0.1	0.9 ± 0.1
PI	1.2 ± –	2.0 ± 0.1	2.0 ± 0.2	2.0 ± 0.2	1.8 ± –	2.1 ± 0.2	2.2 ± 0.2
PA/CL	2.3 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
PE	10.2 ± 0.5	5.8 ± 0.4	5.5 ± 0.3	5.6 ± 0.4	5.2 ± 0.2	6.2 ± 0.3	6.3 ± 0.5
Sum PL	30.6 ± 1.9	21.1 ± 1.4	20.4 ± 1.3	20.3 ± 1.7	18.6 ± 0.4	21.8 ± 1.3	22.5 ± 1.5
CHOL	10.7 ± 0.8	2.8 ± 0.2	3.2 ± 0.2	2.8 ± 0.4	2.7 ± 0.2	3.0 ± 0.4	3.5 ± 0.4
FFA	3.3 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± –	1.0 ± 0.1	1.3 ± 0.2	1.1 ± 0.1
TAG	54.8 ± 3.0	72.4 ± 1.8	73.8 ± 1.2	72.4 ± 0.6	73.9 ± 0.3	69.1 ± 2.6	69.0 ± 2.5
Sum NL	68.7 ± 2.0	78.8 ± 1.4	79.6 ± 1.3	79.7 ± 1.7	81.5 ± 0.4	78.2 ± 1.3	77.5 ± 1.5

Data are shown as means ± SD. (When result below 0.1. it is denoted by –).

and 42 weeks of feeding. These differences were typically due to differences in triacylglycerol (TAG) and phosphatidylcholine (PC) levels, respectively, (Table 3). Red muscle lipid was high in TAG (ranged from 90–91.1% of total lipid in the dietary groups after 42 weeks), whereas PL accounted for less than 10% in all dietary groups. No changes were observed in red muscle lipid class composition between the different dietary treatments through the experiment.

White muscle fatty acids (mg/g muscle, w.w.) show a 3-fold increase in 18:1$n-9$ from 100% FO to 75% RO (Table 4). The relative amount (data not presented) of 18:1$n-9$ increased further in the 100% RO group, however, lower total amount of fatty acids in the 100% RO muscle compared to 75% RO resulted in stable 18:1$n-9$ amounts in 75% RO and 100% RO muscle. The increase in dietary 18:1$n-9$ was 5-fold from 100% FO to 100% RO. Based on both relative composition and absolute amounts of fatty acids in muscle (Table 4) the change in 18:2$n-6$, 22:6$n-3$, 22:1$n-11$ and $n-3/n-6$ were, respectively, 3.7-fold increased, 1.9-fold decreased, 7.8-fold decreased and 4.9-fold decreased in muscle from 100% FO to 100% RO. In contrast, dietary 18:2$n-6$, 22:6$n-3$, 22:1$n-11$ and $n-3/n-6$ were, respectively, 6.5-fold increased, 4.6-fold decreased, 13-fold decreased and 7.8-fold decreased from the 100%FO to the 100% RO diet (Table 1a). Further, in all diets, except 100% RO, the amount of 20:5$n-3$ was higher than the 22:6$n-3$.

3.3. Fillet fatty acid composition in the wash-out period

To standardise the time needed for washout regarding time and water temperature, results are presented as

Table 4
White muscle fatty acid composition ($n = 3$ samples from each net pen. $n = 6$ for 100% FO) (mg FA g^{-1} tissue w.w.) from initial sampling and from fish fed the experimental for 42 weeks

	Initial	100% FO	25% RO	50% RO	75% RO	100% RO	50% OO
14:0	1.0 ± 0.1	2.5 ± 0.3	1.8 ± 0.2	1.5 ± 0.1	1.3 ± 0.3	0.4 ± 0.1	1.4 ± 0.1
16:0	2.9 ± 0.2	6.5 ± 0.7	5.4 ± 0.4	5.2 ± 0.3	5.7 ± 0.4	4.1 ± 0.6	5.9 ± 0.4
18:0	0.5 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.0	1.2 ± 0.2	1.2 ± 0.1
Sum sat	*4.6 ± 0.1*	*10.3 ± 1.2*	*8.5 ± 0.7*	*8.0 ± 0.5*	*8.6 ± 0.7*	*5.9 ± 0.8*	*8.8 ± 0.6*
16:1$n-7$	1.1 ± 0.1	3.6 ± 0.5	2.4 ± 0.2	1.8 ± 0.1	1.5 ± 0.3	0.4 ± 0.1	1.8 ± 0.2
18:1$n-7$	0.6 ± 0.0	1.9 ± 0.2	1.7 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	1.6 ± 0.2	1.5 ± 0.1
18:1$n-9$	3.0 ± 0.3	7.3 ± 0.9	10.7 ± 1.0	15.3 ± 0.9	22.0 ± 0.9	22.6 ± 3.3	17.2 ± 1.1
18:1$n-11$	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.0
20:1$n-9$	1.6 ± 0.1	7.8 ± 1.0	5.5 ± 0.4	4.8 ± 0.3	4.3 ± 0.7	1.8 ± 0.3	4.4 ± 0.3
20:1$n-11$	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.0
22:1$n-9$	0.2 ± 0.0	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.0	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
22:1$n-11$	1.7 ± 0.1	4.8 ± 0.6	3.3 ± 0.2	2.6 ± 0.1	2.1 ± 0.4	0.6 ± 0.1	2.5 ± 0.2
Sum mono	*8.9 ± 0.6*	*27.4 ± 3.4*	*25.2 ± 2.3*	*27.6 ± 1.7*	*33.2 ± 1.4*	*27.5 ± 4.1*	*28.7 ± 1.9*
18:2$n-6$	0.7 ± 0.1	2.0 ± 0.2	3.3 ± 0.3	4.9 ± 0.3	7.1 ± 0.4	7.4 ± 1.1	3.2 ± 0.2
20:4$n-6$	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
Sum n − 6	*0.9 ± 0.1*	*2.5 ± 0.3*	*3.9 ± 0.3*	5.8 ± 0.4	*8.4 ± 0.4*	*8.8 ± 1.2*	*3.8 ± 0.3*
18:3$n-3$	0.2 ± 0.0	0.3 ± 0.0	1.0 ± 0.1	1.6 ± 0.1	2.4 ± 0.2	2.5 ± 0.4	0.4 ± 0.0
18:4$n-3$	0.4 ± 0.0	0.7 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
20:4$n-3$	0.3 ± 0.0	0.8 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0
20:5$n-3$	1.2 ± 0.0	2.4 ± 0.3	1.8 ± 0.1	1.5 ± 0.1	1.3 ± 0.2	0.8 ± 0.1	1.4 ± 0.1
22:5$n-3$	0.5 ± 0.0	1.0 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	0.6 ± 0.0
22:6$n-3$	3.4 ± 0.9	4.6 ± 0.3	3.7 ± 0.1	3.4 ± 0.1	3.2 ± 0.2	2.4 ± 0.1	3.3 ± 0.1
Sum n − 3	*6.2 ± 0.8*	*9.8 ± 0.9*	8.2 ± 0.5	*7.9 ± 0.4*	*8.4 ± 0.5*	*6.7 ± 0.7*	*6.5 ± 0.3*
$n-3/n-6$	1.4 ± 0.3	3.9 ± 0.2	2.1 ± 0.1	1.4 ± 0.0	1.0 ± 0.1	0.8 ± 0.0	1.7 ± 0.0
Sum rest FA	0.6 ± 0.2	0.8 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Total FA	21.4 ± 0.0	51.1 ± 5.9	46.1 ± 4.0	49.5 ± 2.9	58.8 ± 2.4	49.1 ± 6.8	47.8 ± 3.1

Data are shown as mean ± SD.

changes in fatty acid content per day degree of feeding. Day degree is defined as day*degrees °C in the sea. Principal component analysis show that fillet fatty acid composition highly reflected the dietary fatty acid composition after 42 weeks (Fig. 2(a) and (b)), whereas, after feeding a 100% FO diet from through washout (totally 1788 day degrees) fillet fatty acid compositions from the 25% RO to the 100% RO groups were increasingly similar to the 100%FO group and 100% FO diet group (Figs. 2(c) and (d)). No changes were seen in relative fatty acid composition in fillet (data not presented) from fish fed 100% FO also prior the 42 weeks of sampling; however, the total lipid content increased and thereby the total fatty acids per g fillet (Table 5). Figs. 3 and 4 show the amounts of 18:2$n-6$, 18:1$n-9$, 22:6$n-3$ and the $n-3/n-6$ ratio during the wash-out period. After 1300 day degrees of wash-out, there were no differences in 22:6$n-3$ content of fillet. Fillet 18:2$n-6$, however, was higher in both 75% RO and 100% RO compared to the other dietary treatments. The $n-3/n-6$ ratio was still increasing after 1788 daydegrees of wash-out feeding. However, the $n-3/n-6$ ratio differed by only 0.6, 0.7 and 0.9 in the 25% RO, 50% OO and 50% RO groups compared to the 100% FO (Table 5).

3.4. White muscle all-trans astaxanthin and carotenoids

Total dietary astaxanthin varied from 54 to 70 mg/kg (w.w.) with no relationship between increased carotenoids with increasing inclusion of RO. The relative distribution of astaxanthin, lutein, zeaxanthin and total dietary carotenoid level is given in Table 1b. Table 2 show that all-*trans* astaxanthin concentration generally increased from the intermediate to the final sampling. White muscle from the 100% RO group had lower astaxanthin levels after 22 weeks of feeding; this difference was, however, not found after 42 weeks (Table 2). This lower astaxanthin level in the 100% RO group coincides with higher relative contribution of zeaxanthin to the total dietary carotenoid level.

4. Discussion

Atlantic salmon, currently available on the market are fed diets containing mainly marine oils, thus being a rich source of VLC$n-3$ PUFA and with a high $n-3/n-6$ ratio, as seen in the control group fed 100% FO. However, with the decreasing world-wide availability of

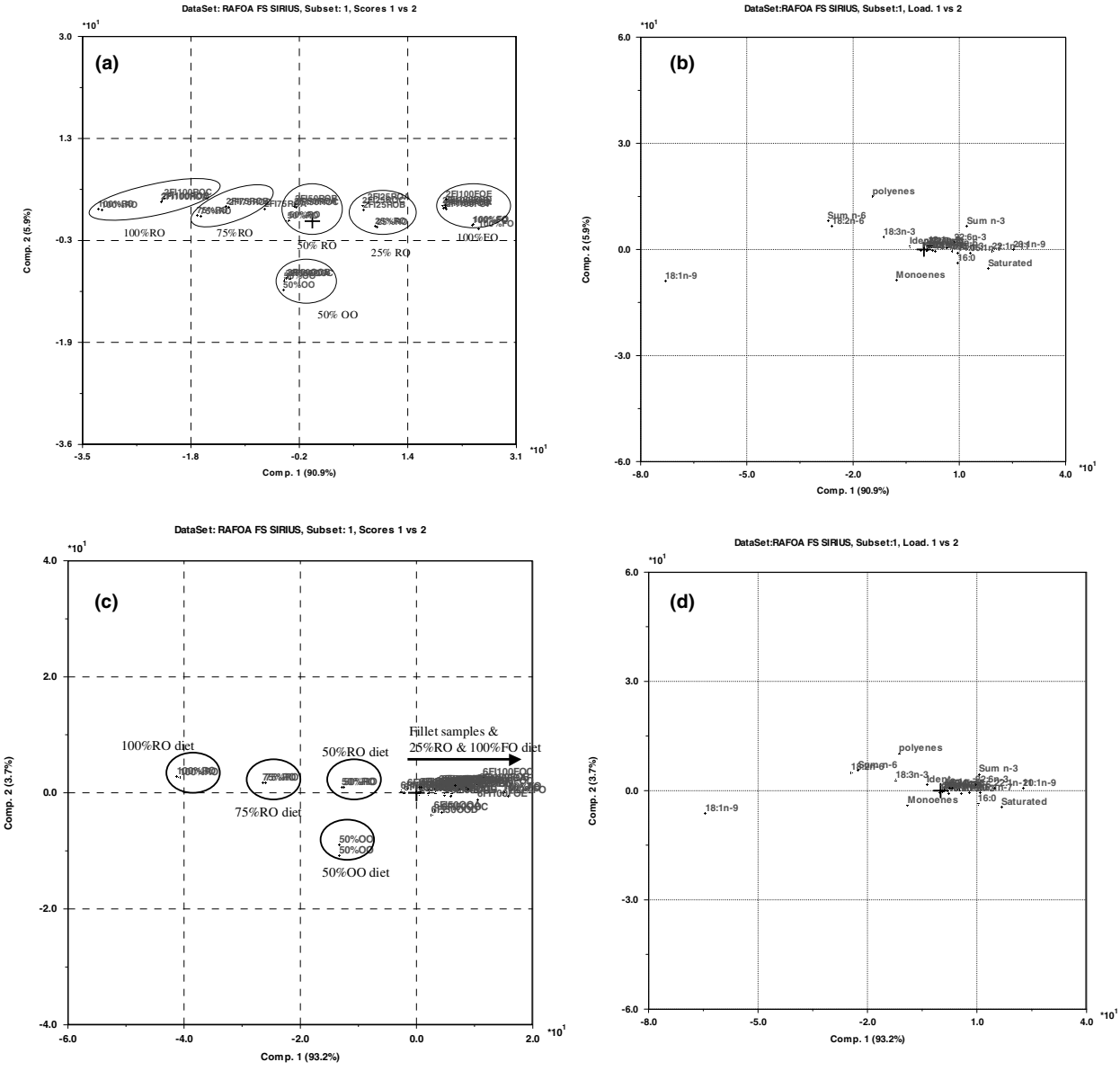

Fig. 2. Score plot (a and c) and load plot (b and d) of the relative fatty acid composition of diets and fillet from fish fed the experimental diets from May 2001 to March 2002 (a and b) and of diets and fillet from fish fed 100% FO diet from March to September 2002 (c and d).

marine oils, with the concomitant increasing demand for dietary oils for aquaculture feeds, the present study shows that replacing fish oils with vegetable oils may be a good alternative from a fish production perspective. Thus, replacing fish oil with RO and OO had no significant effect on growth or survival in accordance with previous reports both on high and low energy salmon diets (Bell et al., 2002, 2001; Rosenlund et al., 2001; Torstensen et al., 2000; Waagbø et al., 1991; Waagbø, Sandnes, Torrisen, Sandvin, & Lie, 1993). In line with Bell et al. (2002, 2001, 2003), the current experiment showed that RO and OO can meet the energy requirements of the fish, concomitant with no apparent negative effects on lipid digestibility, as observed previously with palm oil at low water temperatures (Torstensen et al., 2000). Further, the contribution of EPA and DHA from the dietary fish meal is sufficient for meeting salmon $n-3$ PUFA requirement (Ruyter, 1998; Torstensen et al., 2000). The increase in muscle lipid content with increasing fish weight is in line with previous studies on Atlantic salmon (Aksnes, 1995; Hemre, Sandnes, Lie, Torrisen, & Waagbø, 1995; Johnsen & Wandsvik, 1991; Torstensen, Lie, & Hamre, 2001). However, neither the relative distribution of lipid classes nor the relative fatty acid composition changed from 22 to 42 weeks of feeding the experimental diets, indicating a steady state situation already after 22 weeks, related to muscle fatty acid incorporation with the fish weight below 1 kg. Further, it has been reported that TAG and PL fatty acid incorporation respond differently to die-

Table 5
Fillet fatty acid composition (mg g^{-1} tissue, w.w.) ($n = 3$ samples from each net pen. $n = 6$ for 100% FO in March, $n = 5$ individual fish from each original experimental group) from fish fed 100% FO diet (wash-out period) after 42 weeks of feeding increasing levels of RO and 50% OO

	Start of wash-out (March 2002)						End of wash-out (after 25 weeks and 1788 day degrees)					
	100% FO	25% RO	50% RO	75% RO	100% RO	50% OO	100% FO	25% RO	50% RO	75% RO	100% RO	50% OO
14:0	4.5 ± 0.6	3.6 ± 0.4	3.3 ± 0.4	2.2 ± 0.4	0.7 ± 0.1	2.2 ± 0.3	5.7 ± 1.7	5.0 ± 0.6	4.6 ± 0.6	5.5 ± 0.9	5.3 ± 0.6	4.3 ± 1.3
16:0	11.3 ± 1.4	10.5 ± 1.1	11.2 ± 1.5	9.4 ± 1.0	6.4 ± 0.5	9.4 ± 1.2	13.9 ± 4.1	12.8 ± 1.7	12.0 ± 1.6	14.5 ± 2.2	14.6 ± 1.6	11.8 ± 3.5
18:0	1.7 ± 0.2	1.9 ± 0.2	2.4 ± 0.3	2.2 ± 0.2	1.9 ± 0.1	2.0 ± 0.3	2.3 ± 0.8	2.2 ± 0.4	2.1 ± 0.3	2.6 ± 0.4	2.8 ± 0.3	2.1 ± 0.7
Sum sat	18.0 ± 2.2	16.5 ± 1.9	17.2 ± 2.4	14.2 ± 1.6	9.2 ± 0.7	13.9 ± 1.9	22.3 ± 6.6	20.3 ± 2.6	19.0 ± 2.5	22.9 ± 3.5	22.9 ± 2.5	18.5 ± 5.5
16:1$n-7$	6.6 ± 0.8	4.9 ± 0.6	4.2 ± 0.6	2.6 ± 0.6	0.7 ± 0.1	3.1 ± 0.5	8.2 ± 2.3	7.0 ± 0.9	6.3 ± 0.8	7.5 ± 1.3	7.2 ± 0.8	6.0 ± 1.8
18:1$n-7$	3.6 ± 0.4	3.5 ± 0.4	4.1 ± 0.6	3.5 ± 0.4	2.6 ± 0.2	2.5 ± 0.4	4.3 ± 1.1	4.0 ± 0.5	3.9 ± 0.5	4.6 ± 0.7	4.8 ± 0.7	3.6 ± 1.0
18:1$n-9$	13.5 ± 1.6	22.2 ± 2.6	35.1 ± 4.6	38.5 ± 5.7	37.6 ± 2.5	29.3 ± 4.6	16.8 ± 4.9	18.4 ± 3.4	19.7 ± 3.5	25.9 ± 4.4	30.7 ± 4.0	20.9 ± 5.8
18:1$n-11$	1.0 ± 0.0	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	0.4 ± 0.0	1.3 ± 0.3	1.1 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
20:1$n-9$	14.4 ± 1.5	11.5 ± 1.2	11.0 ± 1.6	7.9 ± 1.2	3.5 ± 0.1	7.7 ± 1.2	17.9 ± 4.4	15.3 ± 1.6	14.0 ± 1.6	16.7 ± 2.7	16.3 ± 1.8	13.3 ± 3.2
20:1$n-11$	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.8 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.0	0.4 ± 0.1
22:1$n-9$	1.6 ± 0.2	1.3 ± 0.1	1.4 ± 0.2	1.1 ± 0.1	0.5 ± 0.0	1.0 ± 0.1	1.8 ± 0.4	1.6 ± 0.2	1.7 ± 0.2	1.8 ± 0.3	1.9 ± 0.3	1.5 ± 0.5
22:1$n-11$	8.7 ± 1.0	6.8 ± 0.7	6.2 ± 0.8	3.7 ± 0.7	1.0 ± 0.1	4.0 ± 0.6	11.0 ± 2.7	9.5 ± 1.0	8.1 ± 1.0	10.1 ± 1.7	9.6 ± 0.9	7.8 ± 2.0
Sum mono	51.0 ± 5.8	52.3 ± 6.0	64.1 ± 8.5	58.9 ± 7.3	46.6 ± 2.9	49.0 ± 7.5	63.2 ± 16.5	58.6 ± 8.0	56.1 ± 7.5	69.4 ± 11.3	73.1 ± 8.2	55.2 ± 14.6
18:2$n-6$	3.7 ± 0.4	6.8 ± 0.8	11.2 ± 1.5	12.5 ± 2.0	12.3 ± 0.8	5.4 ± 0.9	4.5 ± 1.1	5.2 ± 0.8	5.6 ± 1.0	7.5 ± 1.5	9.1 ± 1.1	4.6 ± 1.1
20:4$n-6$	0.3 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.1
Sum $n-6$	4.5 ± 0.6	8.0 ± 0.9	13.3 ± 1.8	14.6 ± 2.3	14.5 ± 0.9	6.3 ± 1.1	5.7 ± 1.3	6.3 ± 1.0	6.8 ± 1.1	9.1 ± 1.7	10.9 ± 1.3	5.6 ± 1.3
18:3$n-3$	0.6 ± 0.1	2.0 ± 0.3	3.7 ± 0.5	4.2 ± 0.7	4.1 ± 0.3	0.7 ± 0.1	0.8 ± 0.2	1.2 ± 0.2	1.4 ± 0.3	1.9 ± 0.4	2.4 ± 0.3	0.7 ± 0.2
20:5$n-3$	4.1 ± 0.5	3.2 ± 0.3	2.9 ± 0.3	2.1 ± 0.3	1.2 ± 0.1	2.1 ± 0.2	4.7 ± 1.3	4.1 ± 0.4	3.8 ± 0.4	4.5 ± 0.7	4.4 ± 0.5	3.6 ± 1.0
22:5$n-3$	1.8 ± 0.2	1.4 ± 0.1	1.4 ± 0.2	0.9 ± 0.2	0.4 ± 0.0	1.0 ± 0.2	2.2 ± 0.5	1.9 ± 0.3	1.7 ± 0.2	2.1 ± 0.3	2.0 ± 0.2	1.6 ± 0.4
22:6$n-3$	7.7 ± 0.5	6.5 ± 0.4	6.5 ± 0.6	4.8 ± 0.7	3.2 ± 0.2	4.8 ± 0.6	7.7 ± 1.2	6.7 ± 0.7	6.6 ± 0.6	7.2 ± 0.8	7.0 ± 0.5	6.2 ± 1.0
Sum $n-3$	16.9 ± 1.5	15.3 ± 1.3	16.4 ± 1.8	13.6 ± 1.7	10.0 ± 0.6	9.6 ± 1.3	18.7 ± 3.9	16.8 ± 1.8	16.0 ± 1.7	18.9 ± 2.8	18.8 ± 1.7	14.4 ± 3.1
$n-3/n-6$	3.8 ± 0.2	1.9 ± 0.1	1.2 ± 0.0	0.9 ± 0.1	0.7 ± 0.0	1.5 ± 0.1	3.3 ± 0.1	2.7 ± 0.1	2.4 ± 0.2	2.1 ± 0.2	1.7 ± 0.1	2.6 ± 0.1
Rest FA	1.4 ± 0.3	0.7 ± 0.1	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.2	2.2 ± 0.6	1.9 ± 0.4	1.3 ± 0.4	1.8 ± 0.3	1.2 ± 0.3	1.2 ± 0.5
Sum identified	92.2 ± 10.3	92.9 ± 9.9	112 ± 14.7	101 ± 12.3	80.4 ± 5.1	79.1 ± 12.0	112 ± 28.8	104.2 ± 13.8	99.4 ± 13.1	122 ± 19.3	127 ± 13.6	95.1 ± 24.9

Data are shown as mean ± SD.

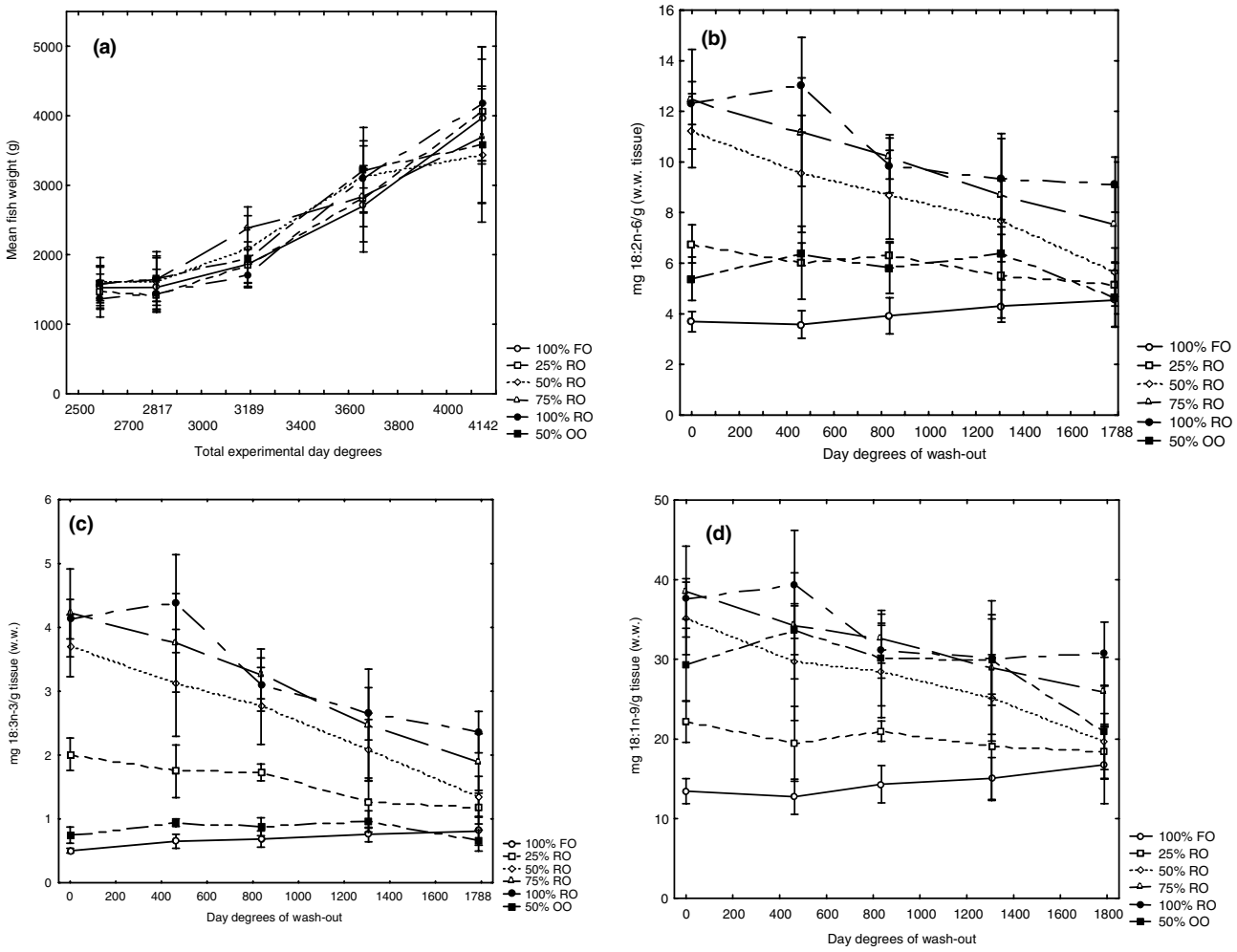

Fig. 3. (a) Mean weight, (b) mg 18:2$n-6$/g fillet, (c) mg 18:3$n-3$/g fillet and d: mg 18:1$n-9$/g fillet of individually sampled Atlantic salmon ($n = 5$) from each of the original experimental groups sampled from March 2002 to September 2002 when all fish were fed a 100% FO diet (wash-out period). Sampling points are related to day degrees (days* °C).

tary fatty acid composition (Olsen & Henderson, 1997). Thus, for the relative fatty acid composition to be in a steady state when the total amount of lipid increases, the relative amount of NL and PL must also be the same, which is the case in salmon muscle from 22 to 42 weeks of feeding. However, during the wash-out period the total amount of lipid increases, whereas relative fatty acid composition changes, indicating a shift in NL/PL ratio.

Feeding Atlantic salmon high levels of dietary vegetable oils profoundly changed the muscle and total fillet fatty acid composition, which is in accordance with previous reports (Bell et al., 2002, 2003; Rosenlund et al., 2001; Waagbø et al., 1991; Torstensen et al., 2000). From a human health perspective, replacing marine oils with vegetable oils may have profound consequences. Characteristic of the marine food chain is the high concentrations of DHA and EPA, whereas the terrestrial food chain is typically rich in 18 carbon $n-3$ and $n-6$ fatty acids. Fish, due to its high VLC$n-3$ PUFA levels, is recommended to patients with risk of coronary heart disease (deDeckere, Korver, Verschuren, & Katan, 1998; Sanderson et al., 2002) and rheumatoid arthritis (reviewed by (deDeckere et al., 1998)), and VLC$n-3$ PUFA affect human immune system, even at low doses (Bechoua, Dubois, Vericel, Chapuy, Lagarde, & Prigent, 2003). The diet of our ancestors (15,000–40,000 years ago) was characterised by being low in fat, high in fibre, and with a $n-6/n-3$ ratio of 1:1 (Simopoulos, 2001). Today, the ratio of $n-6/n-3$ is 16.74 in current western diets (Simopoulos, 2001) which in turn influence membrane composition, eicosanoid production, gene expression, and inter cell-to-cell communication (Simopoulos, 2000). Further, one has to distinguish between requirement of total $n-3$'s and VLC$n-3$ PUFA's, i.e., DHA has been shown to be essential for normal development of brain and retina (Neuringer & Connor, 1986) and EPA is the active component in eicosanoid production, whereas the health beneficial effects of 18:3$n-3$ intake is not yet established (Sanderson et al., 2002).

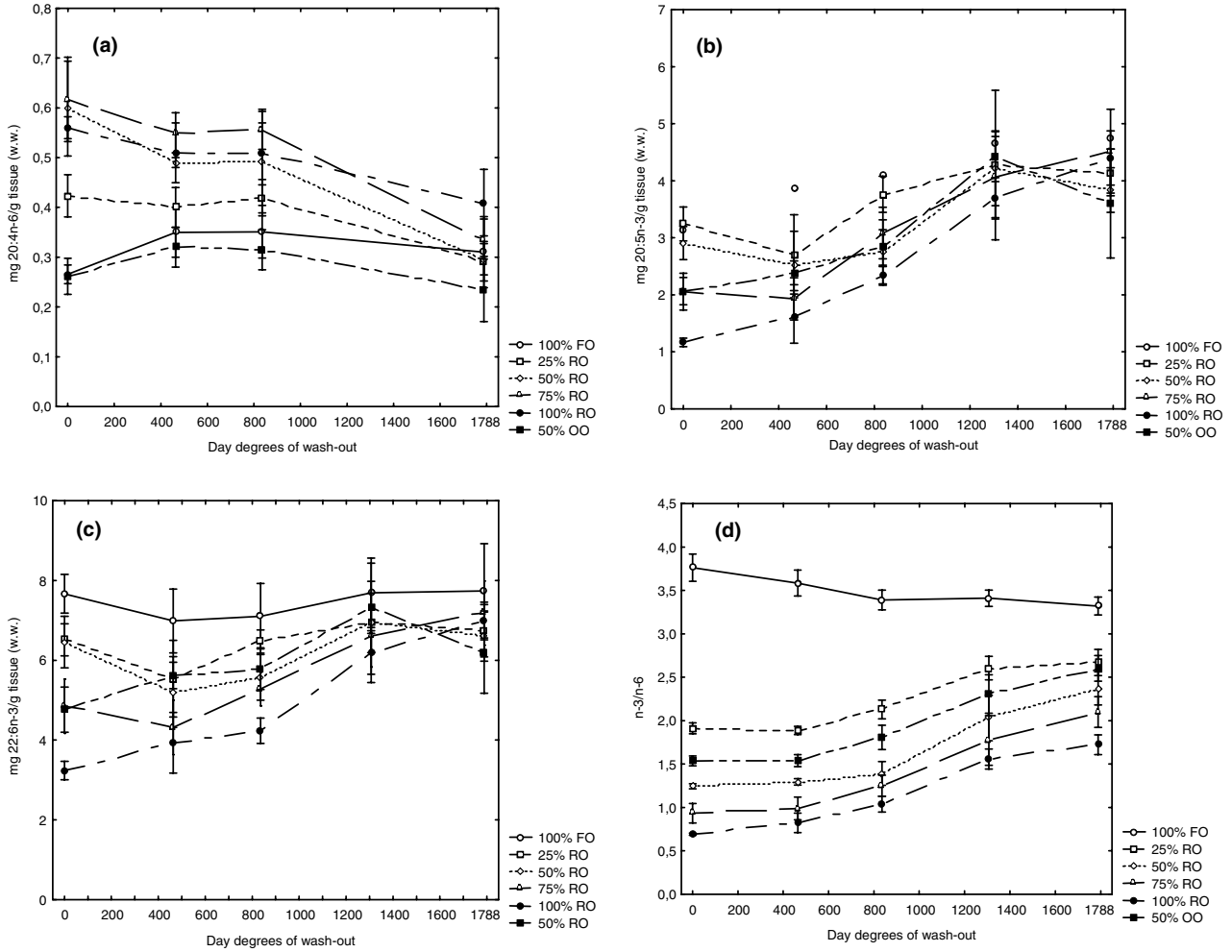

Fig. 4. (a) mg 20:4$n-6$/g fillet, (b) mg 20:5$n-3$/g fillet, (c) mg 22:6$n-3$/g fillet, and (d) $n-3/n-6$ ratio in fillet of individually sampled Atlantic salmon ($n = 5$) from each of the original experimental groups sampled from March 2002 to September 2002 when all fish were fed a 100% FO diet (wash-out period). Sampling points are related to day degrees (days* °C).

Recent recommendations for unsaturated fatty acids (with a 2000 kcal diet) (reviewed by Simopoulos, 2001) give a maximum 18:2$n-6$ of 6.67 g/day, 2.22 g 18:3$n-3$/day, 0.65 g DHA + EPA/day, with at least 0.22 g DHA and 0.22 EPA/day (Simopoulos, 2001). Generally it is recommended to increase the $n-3/n-6$ ratio and especially by increasing marine VLC$n-3$ PUFA's (deDeckere et al., 1998; Simopoulos, 2000; Simopoulos, 2001). Atlantic salmon, as a fatty fish, is considered an excellent source of VLC$n-3$ PUFA's, with high $n-3/n-6$ ratio. Current results showing that muscle fatty acid contents are highly affected by dietary fatty acid composition were further demonstrated by feeding all experimental groups with 100% FO after the experimental feeding period of 42 weeks. For reducing the amount of $n-6$ PUFA and increasing the marine VLC$n-3$ PUFA's, a period of 25 weeks, with 1788 day degrees of feeding 100% FO, was sufficient for the groups fed 25% RO, 50% OO and 50% RO. However, minor differences in $n-3/n-6$ ratio were seen after 25 weeks, with 3.3 in the 100% FO group compared to 2.7, 2.6 and 2.4, respectively, in the 25% RO, 50% OO and 50% RO groups. These ratios are all acceptable within the recommendations of increasing the $n-3/n-6$ ratio (Simopoulos, 2001). In contrast, salmon muscle fed 100% RO and 75% RO through 42 weeks had a $n-3/n-6$ ratio of less than 1, diminishing the positive effects of eating salmon related to the $n-3$ and $n-6$ levels. The $n-3/n-6$ ratio was increased more than twofold through the wash-out period in the 50% RO, 75% RO and 100% RO groups, both by increasing VLC$n-3$ PUFA's and decreasing 18:2$n-6$. However, generally muscle DHA levels were restored sooner (after approximately 1300 day degrees) compared to 18:2$n-6$ which was not reduced to the 100% FO levels until 1788 day degrees. The current study indicates different dynamics of depletion of 18:2$n-6$, 18:3$n-3$ and 18:1$n-9$ compared to the build up of 20:5$n-3$ and 22:6$n-3$. To enable wash-out of fatty acids, there has to be consumption of the fatty acids through

β-oxidation, concurrent with replacement with new dietary fatty acids built into lipid stores and membranes. The decrease in amounts of 18:1$n-9$, 18:3$n-3$ and 18:2$n-6$ in muscle indicates a consumption of both of these fatty acids for energy production. In line with these results, Kiessling and Kiessling (1993) have previously reported that especially 16:0, 16:1, 18:1$n-9$, and 18:2$n-6$ seemed to be preferentially mobilized during starvation whereas 22:6$n-3$ was oxidised at low rates (reviewed by Henderson, 1996). Further, the relative changes in muscle 18:1$n-9$, 18:2$n-6$, 22:1$n-11$ and 22:6$n-3$ increase from the 100% FO to the 100% RO, with fish fed 100% RO, prior to wash-out consuming 18:1$n-9$ and 18:2$n-6$ and replacing these with 22:1$n-11$ and $n-3$ PUFA's to the greatest extent.

Atlantic salmon fillet is also a valuable source of other essential nutrients beneficial for human health, such as lipid-soluble vitamins, minerals and other antioxidants such as astaxanthin, which show strong antioxidant activity (Miki, 1991; Miki, Otaki, Shimidzu, & Yokoyama, 1994). The fillet levels of the lipid soluble nutrients are dependent on the dietary composition (reviewed by Lie, 2001). Further, the red flesh colour caused by astaxanthin in salmonids is an important factor in consumer acceptance of fish quality. It has been shown that both total lipid (Clark & Furr, 2001) and type of oil can influence carotenoid absorption (Clark, Yao, She, & Furr, 2000). Furthermore, additional carotenoids present in the diet, such as zeaxanthin and lutein (Table 1b) may compete with astaxanthin for incorporation into micelles (Borel et al., 1996) and hence, deposition in muscle. However, no differences in astaxanthin deposition were found as an effect of increasing RO in the diets (Table 2), indicating that replacement of FO with RO or OO does not negatively affect colouration of the flesh.

Atlantic salmon fed increasing levels of RO or OO as replacement of fish oil with dietary fish meal all had muscle $n-3$ PUFA levels resulting in a 150 g salmon meal with higher $n-3$ PUFA than recommended levels (Simopoulos, 2001) both before (after 42 weeks of feeding) and after the wash-out period. However, considering the high $n-6$ and saturated fatty acid intake in western diets (Simopoulos, 2001), the contribution of marine $n-3$ PUFA's should be high, ideally, as 100% FO muscle in the current experiment, contributing with 1.18 and 1.24 g marine $n-3$ PUFA's, respectively, in a 100 g serving before and after the wash-out period, and in combination with high $n-3/n-6$ ratio. Actually, after wash-out the contribution of total $n-3$ fatty acids were the same in the 100% FO and the 100% RO group due to also higher levels of 18:3$n-3$ in the RO diets. However, it has been reported that there are different health effects of 18:3$n-3$ compared to 20:5$n-3$ and 22:6$n-3$ (Sanderson et al., 2002) and, based on relative fatty acid values, muscle 100% RO was 2.2% lower in EPA + DHA content compared to the 100% FO group. Previous experiments have shown that replacement of up to 50% of fish oil with vegetable oil give only modest decreases in muscle 22:6$n-3$ (Bell et al., 2002, 2001).

In conclusion, based on contribution of marine VLC$n-3$ PUFA's and $n-3/n-6$ ratios Atlantic salmon muscle from fish fed up to 50% RO and 50% OO during the sea water growth period, followed by a wash-out period of 1780 day degrees, can be presented as equally healthy products for human consumption as the traditionally 100% fish oil-fed salmon, which meets all requirements recommended for human health and protection against cardiovascular diseases. Concomitantly, comparing Atlantic salmon to other farmed animals, salmon having a high growth rate and high fillet lipid level, which highly reflects the dietary fatty acids and lipid soluble nutrients, opens major possibilities for tailoring salmon fillet for the human health functional food market.

Acknowledgements

This experiment was part of the project "RAFOA, Researching Alternatives to Fish Oils in Aquaculture", Q5RS-200-30058 funded by EU, The Fifth Framework Programme. Betty Irgens, Jacob Wessels, Thu Thao Nguen, Kari-Ein Langeland Roed, Vidar Fauskanger and Annbjørg Bøkevold at NIFES are greatly acknowledged for their excellent analytical work. Further, the staff at GIFAS research station is thanked for their assistance with fish husbandry.

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