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Tailoring of Atlantic Salmon (*Salmo salar* L.) Flesh Lipid Composition and Sensory Quality by Replacing Fish Oil with a Vegetable Oil Blend

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Atlantic salmon (*Salmo salar* L.) juveniles were fed either 100% fish oil (FO), 75% vegetable oil (VO), or 100% VO throughout their life cycle to harvest weight followed by a finishing diet period when all groups were fed 100% FO. The two experimental VO diets were tested at two different locations (Scotland and Norway) against the same control diet (100% FO). The VO blend was composed of rapeseed oil, palm oil, and linseed oil using capelin oil as a control for fatty acid class compositions. Flesh fatty acid profiles were measured regularly throughout the experiment, with the times of sampling determined by changes in pellet size/lipid content and fish life stage. Growth and mortality rates were not significantly affected by dietary fatty acid compositions throughout the life cycle, except during the seawater winter period in Norway when both growth and protein utilization were increased in salmon fed 100% VO compared to 100% FO. Flesh fatty acid composition was highly influenced by that of the diet, and after the finishing diet period the weekly intake recommendations of very long chain n-3 polyunsaturated fatty acid (VLCn−3 PUFA) for human health were 80 and 56% satisfied by a 200 g meal of 75% VO and 100% VO flesh, respectively. No effect on flesh astaxanthin levels was observed in relation to changing dietary oil sources. Sensory evaluation showed only minor differences between salmon flesh from the dietary groups, although prior to the finishing diet period, flesh from 100% VO had less rancid and marine characteristics and was preferred over flesh from the other dietary groups by a trained taste panel. After the finishing diet period, the levels of typical vegetable oil fatty acids in flesh were reduced, whereas those of VLCn−3 PUFA increased to levels comparable with a 100% FO fed salmon. No differences in any of the sensory characteristics were observed between dietary groups. By blending VOs to provide balanced levels of dietary fatty acids, up to 100% of the fish oil can be replaced by the VO blend without compromising growth or flesh quality. At the same time, 75% of the dietary fish oil can be replaced without compromising flesh VLCn−3 PUFA content, thereby providing a beneficial nutritional profile for human consumption.

KEYWORDS: Fatty acids; lipid level; rapeseed oil; capelin oil; palm oil; linseed oil; sensory analysis; tailoring; flesh quality

INTRODUCTION

Atlantic salmon (*Salmo salar* L.) flesh has a naturally high content of very long chain n−3 polyunsaturated fatty acids (VLCn−3 PUFA) such as EPA (20:5n−3) and DHA (22:6n−3). It has been suggested that these fatty acids are essential for protecting humans against cardiac diseases (*1*) and that these VLCn−3 PUFAs cannot be replaced by the shorter and less unsaturated n−3 fatty acid, 18:3n−3 (LNA) (*1*). Furthermore, salmon flesh lipid is naturally low in n−6 fatty acids and consequently has a high n−3/n−6 ratio, which is recommended for promoting human health (*2*). As capture fisheries decline, an increasing proportion of human fish consumption is provided by aquaculture (*3*−*5*). In 2000 the global production of Atlantic salmon was ∼885 000 metric tonnes, and the production is expected to exceed 1 million metric tonnes by 2005 (*6*). The aquaculture of salmonids [Atlantic salmon and rainbow trout

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Table 1. Composition of the Different Basal Diets (According to Pellet Size; Grams per Kilogram)

	pellet size						
	FW[b]			SW[c]			
ingredient	0.3–2.0 mm[a]	2.0 mm	3.0 mm	3.0 mm	4.0 mm	6.0 mm	9.0 mm
fish meal (LT Nordsildmel, Norway)	672	567	553	147			386
fish meal (Consortio, Peru)				407	506	473	
corn gluten (Cargill, USA)		100	100	100	100	100	100
soybean meal extracted (Denofa, Norway)				54	100	100	100
wheat (Statkorn, Norway)	164	155	166	60	46	80	99
oil[d]	139	153	153	208	223	223	291
vitamins and minerals[e]	25	25	25	25	25	25	25

[a] Includes five different pellet sizes. Here composition of the largest two diet sizes (1.2 and 2.0 mm) is given. [b] Freshwater stage. [c] Seawater stage. [d] Oil is capelin oil (Nordsildmel, Norway) for fish oil based diet or a mixture of 55% rapeseed oil (Oelmuhle, Germany), 30% palm oil (Denofa, Norway), and 15% linseed oil (NV Oliefabriek, Belgium) for vegetable oil based diets. [e] Vitamin and mineral supplementation is estimated to cover requirements according to NRC, 199.

(*Oncorhynchus mykiss*)] currently accounts for 60% of the total fish oil used in aquaculture feeds, with 70% of world supply being used for all aquafeeds (*6*). By 2010, with continuing growth in aquaculture estimates suggest 97% of the global fish oil supply will be used in aquafeeds (*6*). Thus, the decline in fisheries and the increase in aquaculture will make using fish oil (FO) and fishmeal nonsustainable as feed ingredients in the very near future (*7*, *8*). A sustainable alternative to fish oil is vegetable oils (VO), and these are all devoid of VLCn−3 PUFAs, whereas the levels of 18:2n−6 and monoene fatty acids are usually high, giving a low n−3/n−6 ratio. A number of studies have shown that complete or partial replacement of FO with single VOs, such as rapeseed oil, palm oil, linseed oil, or soy oil, in parts of the salmon growth phase does not affect growth but does affect the fatty acid composition of the edible portion (*9*−*16*). Other quality aspects of Atlantic salmon fillet, such as color, liquid holding capacity (*17*), and moisture levels (*14*), have been reported to be affected by dietary VO. However, organoleptic properties of salmonid flesh after VO feeding have been contradictory with reports of both significant effects (*18*−*20*) and no effects (*21*−*23*) of dietary VO feeding on sensory parameters. The visual color and carotenoid concentrations of salmon flesh have also been reported to be both affected (*17*) and unaffected (*9*, *13*) by replacing dietary FO with VO.

Although salmon flesh fatty acid compositions have been shown to be closely correlated with dietary fatty acids (*9*, *12*, *14*, *15*, *24*), other metabolic regulators may influence flesh fatty acid composition. This includes fatty acid digestibility (*15*, *25*, *26*), preferential catabolism of certain fatty acids (*13*, *27*, *28*), and desaturation and elongation of dietary fatty acids (*29*−*32*). Thus, if possible, a FO replacement should aim toward a fatty acid composition favoring high digestibility and retention of VLCn−3 PUFA, high levels of fatty acids preferred for catabolism, and high levels of precursor fatty acids for elongation and desaturation to VLCn−3 PUFA. Concomitantly, n−6 fatty acid levels should be kept low to maintain a high n−3/n−6 ratio and high VLCn−3 PUFA levels beneficial to human health (*33*).

Capelin oil, which is a typical northern hemisphere FO most frequently used in Atlantic salmon aquaculture feeds, generally has high levels of the long-chain monounsaturated and saturated fatty acids and VLCn−3 PUFAs. By using capelin oil as a control, a fatty acid composition formulated by mixing different VOs to provide similar levels of the different fatty acid classes (saturated, monounsaturated, and polyunsaturated n−3 fatty acids) may be better physiologically for salmon health and welfare. Thus, even at 75 or 100% replacement a balanced fatty acid composition should be more beneficial compared to the more extreme fatty acid compositions obtained by replacing FO with a single VO as demonstrated in previous studies (*9*−*16*). The aim of the current study was to investigate the effects of 75 and 100% replacement of FO by a VO blend, based on capelin oil as a control, on Atlantic salmon growth, feed utilization, and final flesh product quality. The dietary trial was conducted through a complete Atlantic salmon life cycle followed by a period with a FO finishing diet and tested at two different locations (Scotland and Norway).

MATERIALS AND METHODS

Animals and Diets. The effect of replacing FO with VO at two replacement levels (75 and 100%) was investigated in Atlantic salmon in a trial conducted over an entire two-year production cycle. As the trial was both large scale and long term, it was carried out as a collaboration between the Institute of Aquaculture, University of Stirling, Scotland, and the National Institute of Nutrition and Seafood Research, Bergen, Norway, with the 75% replacement diet tested in Scotland and the 100% replacement tested in Norway, with the control FO diet replicated at each site. The diets were fed to triplicate tanks/cages from April 2002, and the experiments were performed using identical culture conditions other than the obvious environmental differences such as water temperature and day length. In Scotland, the trial was carried out at Marine Harvest Ltd. facilities at Invergarry, Highland (freshwater), and Loch Duich, Highland (seawater); and in Norway, the entire trial was conducted at the Nutreco Aquaculture Research Centre, Lerang Research Station, Stavanger. At each site, Atlantic salmon fry were distributed randomly into six tanks (3 m × 3 m, depth = 0.5 m) at a stocking level of 3000/tank and weaned onto extruded feeds containing 20% added oil, which was either FO [capelin oil (Norsildmel, Fyllingsdalen, Norway)] or a VO blend, containing rapeseed oil (Oelmuhle, Hamburg, Germany), palm oil (Denofa, Gamle Fredrikstad, Norway), and degummed linseed oil (NV Oliefabriek, Lichterveld, Belgium) in a 3.7:2:1 ratio, replacing 75 or 100% of the FO. Fish were fed the diets described above for around one year until seawater transfer, at which point fish (average weight of ∼50 g in Scotland and ∼120 g in Norway) were transferred into 5 m × 5 m × 5 m net pens at 700 fish/pen. The fish were fed the same diet in seawater as in freshwater, although the dietary oil levels were increased to 25% (3 mm pellet) rising to 32% (9 mm pellets) through the yearlong seawater phase (22 months in Norway and 25 months in Scotland) including the finishing diet phase lasting for an additional 5 months in Norway and 6 months in Scotland, when all dietary groups were fed a 100% FO based diet. The diets aimed to be practical and therefore were formulated according to current practices in the salmon feed industry (**Table 1**) and were manufactured by Nutreco ARC, Stavanger, Norway. All diets were formulated to satisfy the nutritional requirements of salmonid fish (*34*). The measured proximate and fatty acid compositions of the diets are given in **Table 2**.

In Norway, the diets were fed to satiation by hand and the exact amounts consumed recorded. In Scotland, the diets were supplied by automatic feeders controlled by an automated feeding sensor system (Arvo-tec, Sterner AquaTech UK, Inverness, Scotland). Mortalities were recorded and dead fish removed daily.

Table 2. Proximate Compositions (Percentage) and Fatty Acid Compositions (Percentage of Weight of Total Fatty Acids) of Representative Diets Used in Freshwater (3 mm Pellet) and Seawater (9 mm Pellet)

	freshwater			seawater		
	FO	75% VO	100% VO	FO	75% VO	100% VO
			Proximate Composition			
protein	46.5 ± 0.4	45.8 ± 0.3	46.7 ± 0.2	42.1 ± 0.2	41.2 ± 0.4	42.9 ± 0.3
lipid	19.6 ± 0.1	18.2 ± 0.2	18.8 ± 0.1	31.4 ± 0.8	32.8 ± 0.3	31.3 ± 0.1
moisture	8.4 ± 0.0	6.8 ± 0.1	6.6 ± 0.0	7.0 ± 0.3	6.4 ± 0.2	6.5 ± 0.1
ash	7.8 ± 0.0	7.9 ± 0.0	7.5 ± 0.0	7.0 ± 0.1	7.1 ± 0.0	6.6 ± 0.1
			Fatty Acid Composition			
14:0	6.1 ± 0.1	2.7 ± 0.1	1.1 ± 0.1	6.2 ± 0.1	2.2 ± 0.2	0.6 ± 0.1
16:0	12.4 ± 0.2	15.5 ± 0.5	16.9 ± 0.4	14.5 ± 0.4	16.1 ± 0.2	15.3 ± 0.3
18:0	1.5 ± 0.0	2.4 ± 0.1	2.7 ± 0.0	2.4 ± 0.6	3.0 ± 0.5	2.7 ± 0.0
total saturated[a]	20.3 ± 0.3	20.8 ± 0.6	21.8 ± 0.4	23.6 ± 0.9	21.5 ± 0.5	19.4 ± 0.3
16:1n–7[b]	7.9 ± 0.1	3.2 ± 0.0	1.1 ± 0.0	4.9 ± 0.2	1.8 ± 0.3	0.5 ± 0.1
18:1n–9	11.9 ± 0.4	30.6 ± 0.7	40.4 ± 0.5	13.2 ± 0.4	35.2 ± 0.0	43.0 ± 0.2
18:1n–7	3.3 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.5 ± 0.2	2.4 ± 0.0
20:1n–9[c]	19.9 ± 0.4	7.4 ± 0.1	2.7 ± 0.1	11.1 ± 1.0	3.8 ± 0.3	1.3 ± 0.1
22:1n–11[d]	15.8 ± 0.3	6.4 ± 0.1	2.3 ± 0.1	16.5 ± 1.9	5.1 ± 0.4	0.8 ± 0.0
24:1n–9	0.7 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
total monoenes	59.4 ± 1.3	50.6 ± 0.8	50.2 ± 0.5	48.8 ± 2.5	48.6 ± 0.6	48.2 ± 0.5
18:2n–6	3.9 ± 0.1	11.7 ± 0.3	13.5 ± 0.2	3.6 ± 0.6	12.7 ± 1.2	17.1 ± 0.2
20:4n–6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.1	0.0 ± 0.0
total n–6 PUFA[e]	4.4 ± 0.1	12.2 ± 0.3	13.7 ± 0.2	4.6 ± 0.7	13.0 ± 1.1	17.1 ± 0.2
18:3n–3	0.6 ± 0.0	6.8 ± 0.3	8.0 ± 0.2	1.2 ± 0.1	9.0 ± 0.7	13.4 ± 0.5
18:4n–3	1.9 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	2.5 ± 0.1	0.8 ± 0.0	0.2 ± 0.0
20:4n–3	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.7 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
20:5n–3	5.8 ± 0.6	3.6 ± 0.3	2.1 ± 0.0	6.5 ± 0.2	2.4 ± 0.5	0.6 ± 0.0
22:5n–3	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.9 ± 0.2	0.3 ± 0.2	0.0 ± 0.0
22:6n–3	5.9 ± 0.6	4.2 ± 0.4	3.4 ± 0.2	10.0 ± 0.6	3.7 ± 1.1	1.0 ± 0.0
total n–3 PUFA[f]	14.9 ± 1.4	15.9 ± 1.1	14.3 ± 0.6	21.8 ± 0.8	16.5 ± 1.1	15.2 ± 0.5
total PUFA[g]	20.3 ± 1.6	28.6 ± 1.3	28.0 ± 0.8	27.6 ± 1.6	29.8 ± 0.1	32.3 ± 0.7

Sampling. Samples were taken from all diets and stored at −20 °C. Fish were not fed during the 24 h prior to sampling. In Norway 10 fish were sampled randomly from each tank and anesthetized with MS222 (7 g/L, Norsk Medisinaldepot, Oslo, Norway). In Scotland, four fish were sampled per tank/pen until the final and wash out points, at which time 6 fish per pen were sampled. Samplings were done when pellet size and hence dietary lipid level changed and during smoltification, which was, respectively, 6, 9, 14, 16, and 22 months after start of feeding in Norway and 6, 9, 12, 14, 17, 21, and 25 months after start of feeding in Scotland. For total lipid, fatty acid composition, and astaxanthin analysis prior to the finishing diet period, the Norwegian quality cuts (NQC) from salmon sampled from each tank were pooled, homogenized, and frozen on dry ice. During the finishing diet period pooled samples of NQC from 3 fish per net pen (6 fish/pen in Scotland, pooled as two groups of 3) were sampled for analysis. All samples were stored at −80 °C.

Proximate Composition of Diets. Dry matter in the diets was measured gravimetrically after freeze-drying of homogenized samples for 48 h. Total nitrogen was determined on homogenized, freeze-dried samples using a nitrogen determinator (LECO, FP-428 system 601-700-500; Perkin-Elmer Corp., Norwalk, CT). Protein was calculated as N × 6.25. Total lipid of the diets and fillet was measured gravimetrically as described by Lie and co-workers (*35*) after ethyl acetate extraction and after acid hydrolysis of the diet samples.

Lipid Extraction and Fatty Acid Analysis. Total lipid was extracted from salmon flesh NQC by homogenization in chloroform/methanol (2:1, v/v) 19:0 methyl ester as internal standard (Norway), basically according to the method of Folch et al. (*36*). Fatty acid methyl esters (FAME) were prepared from total lipid either by acid-catalyzed transesterification, with FAME extracted and purified as described previously (*37*) (Scotland), or by boron trifluoride following saponification essentially as described by Lie et al. (*38*) and Torstensen et al. (*28*) (Norway). In Scotland FAME were separated and quantified by gas−liquid chromatography with on-column injection using a Thermo Finnegan Trace 2000 GC (Thermoquest, Hemel Hempstead, U.K.) equipped with a fused silica capillary column (ZB wax; 30 m × 0.32 mm i.d.; Phenomenex, Macclesfield, U.K.) with helium as carrier gas; temperature programming was from 50 to 150 °C at 40 °C/min and then to 195 °C at 1.5 °C/min and finally to 220 °C for 2 min. In Norway, a Thermo Finnegan Trace 2000 GC equipped with a fused silica capillary column was used (CP-sil 88; 50 m × 0.32 mm i.d.; Chrompak Ltd.) with temperature programming of 60 °C for 1 min, 160 °C for 28 min, 190 °C for 17 min, and finally 220 °C for 10 min with all intervening temperature ramps being at 25 °C/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (*39*). Data were collected and processed using Chromcard for Windows (version 1.19) computer package (Thermoquest Italia S.p.A., Milan, Italy) (Scotland) or using Totalchrom software (ver. 6.2, Perkin-Elmer) (Norway).

Astaxanthin Analysis. *Norway*. *all-trans*- and 9,13-*cis*-astaxanthin were measured in fish muscle using a procedure modified from that of Bligh and Dyer (*40*) by Bjerkeng et al. (*41*). Briefly, homogenized fish muscle was extracted using a water/methanol/chloroform (1:1:3) solution. An aliquot of the astaxanthin-containing chloroform phase was evaporated under N_2 and redissolved in hexane. Quantitative analysis of astaxanthin from flesh 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), and Xtra Chrom software (HP/Nelson analytical) and eluted isocratically using *n*-heptane/isopropyl acetate/acetone/ethanol (84.5:10:4:1.5) at 2.75 mL min^{-1}. Quantification of astaxanthin was performed using an external standard method by which standard solutions prepared from crystalline *all-trans*-astaxanthin (Hoffman-La Roche, Oslo, Norway) were measured with a spectrophotometer (UV-260, Shimadzu, Kyoto, Japan) using a molar absorptivity in hexane of $E^{1cm}_{1\%} = 2100$ at $\lambda_{max} = 472$ nm for astaxanthin. The retention time for *all-trans*-astaxanthin was 2.9 min, and the detection wavelength was 470 nm.

Scotland. Astaxanthin was extracted from salmon muscle largely by following the method of Barua et al. (*27*). Tissue samples were homogenized in 5 mL of absolute ethanol and 5 mL of ethyl acetate using an Ultra-Turrax tissue disrupter. The homogenate was centrifuged (1000*g*, 5 min) and the supernatant removed to a stoppered glass tube. The pellet was rehomogenized in 5 mL of ethyl acetate and recentrifuged, and the supernatant was combined with the first supernatant. Finally, the pellet was rehomogenized in 10 mL of hexane and recentrifuged, and the supernatant was combined with the pooled supernatant. The pooled supernatant was dried under N_2 and vacuum desiccated for 2 h before the residue was redissolved in 2 mL of hexane containing 0.2% (w/v) BHT. Measurement of astaxanthin (Ax) was carried out using a 5 μm Luna ODS2 column (4.6 × 150 mm, Phenomenex, Macclesfield, U.K.). The chromatographic system was equipped with a Waters model 501 pump, and astaxanthin was detected at 470 nm using a Waters 490E multiwavelength UV−vis detector [Millipore (U.K.), Watford, U.K.]. An isocratic solvent system was used containing ethyl acetate/methanol/water (20:72:8 v/v/v) at a flow rate of 1 mL/min. Ax was detected at 470 nm and quantified using an external standard of Ax obtained from Roche (Heanor, U.K.).

Sensory Analysis—Objective and Subjective Tests Using Taste Panels. Fish was sampled for sensory analysis in January and June 2004 (Norway), that is, before and after the finishing diet period. Furthermore, fish from both Scotland and Norway were sampled in February 2004 (after 23 months of feeding; before the finishing diet period) for sensory analysis performed in Scotland. For the sensory analysis in Scotland fresh-cooked fillets were assessed from the two locations, and smoked salmon was assessed for 100% FO (S) and 75% VO. Two fish from each dietary group were chilled in ice slurry and killed by a blow to the head and cutting the gills. Gutted, chilled salmon were then shipped on ice to the Food Industry Forum (FIF), Queen Margaret University College, Edinburgh. A trained panel performed quantitative descriptive analysis (QDA) methods to profile each salmon in terms of sensory attributes related to smell, appearance, texture, and flavor. For each of the 12 sensory attributes of this QDA test, a two-anchored (e.g., soft and firm) linear scale (0−10) was used, in which the score of 5 is the midpoint. This scale is objective and has nothing to do with the likes or dislikes of a given panelist.

The trained panel was also asked to be subjective and score their degree of like or dislike (preference) for each attribute of each sample (two fish from each dietary group from both Norway and Scotland; see **Table 6**) on another linear scale (0−10). On this scale, scores have the following meanings: 0 = extremely disliked, 10 = extremely liked; 5 = midpoint (which indicates that an attribute was neither liked nor disliked). For example, all scores of >5 are on the liked side of the preference scale. The trained panelists have been taught to be more discerning than the average consumer.

In Norway three salmon from each group of 100% FO and 100% VO (nine in total from each dietary treatment) were chilled in an ice slurry and killed by cutting the gills. Gutted, chilled salmon were then shipped on ice to Matforsk, Ås, Norway, and evaluated on the following day for QDA, ISO 6564:1985 E, by testing 23 sensory attributes with 11 judges. On arrival, the salmon was filleted, and six fillet samples with a thickness of 2.5 cm from each fish fillet were put in coded diffusion-tight plastic bags and vacuum sealed. Prior to the sensory evaluation the fillet samples were heated in a water bath at 75 °C for 30 min. The samples were served to the sensory panel in steel containers on a heated plate at 65 °C. The cooked fillet samples were served to the sensory panel in a randomized manner regarding dietary group of salmon, judge, and replicates. Six samples were served in each session. For each sensory parameter the grading is from 1.0 = no intensity to 9.0 = distinct intensity.

Statistics. The sensory attributes of salmon flesh were analyzed using Sirius for Windows (version 6.5). The purpose of principal component analysis (PCA) is to express the main information in the variables by a lower number of variables, the so-called principal components (PC1, PC2, ...). A high positive or negative loading reveals a significant variable in the actual PCA model. Score plots from the PCA explore the main trends in the data, and their respective loading reveals variables with a significant loading. Samples with similar sensory attributes are located in the same area in the score plot. These classes are indicated in **Figure 5** by circles drawn freehand. Because samples with the same sensory attributes will be located on top of each other, to ease interpretation of the samples the classes contained are written beside the circle. Differences between the dietary treatments through the feeding experiment and after the finishing diet period were analyzed by Breakdown and one-way ANOVA followed by Tukey's HSD test, using CSS:Statistica (version 6.1; StatSoft Inc.). The significance level was set to $P \leq 0.05$, and data are presented as mean ± standard deviation (SD) ($n = 3$ or otherwise stated).

Calculations. Specific growth rate (SGR%) = $\{\exp[\ln W_2 - \ln W_1)/(T_2 - T_1)] - 1\} \times 100$, where W_1 and W_2 are initial and final body mass in g and $T_2 - T_1$ is the sum of experimental days.

Feed conversion ratio (FCR) = g of dry feed eaten/g of live weight gain.

Thermal growth coefficient (TGC) = $[(W_2^{0.333} - W_1^{0.333})/(\sum °C)] \times 1000$, where W_1 and W_2 are initial and final body mass in g and $\sum$ °C is the sum day − degrees in the experiment (*43*).

Protein productive value (PPV) = g of retained protein (g of protein intake)$^{-1}$.

RESULTS

In freshwater, the control diet (FO), formulated with 100% FO, contained ∼20% of the total fatty acids as saturated fatty acids, mainly 16:0 and 14:0, and almost 60% as monounsaturated fatty acids, over half of which were the long-chain monounsaturated fatty acids 20:1 and 22:1. Polyunsaturated fatty acids of the n−6 and n−3 series accounted for 4.4 and 15%, respectively, of the total diet fatty acids. 18:2n−6 was the predominant n−6 fatty acid, and within the n−3 fatty acids 20:5n−3 and 22:6n−3 were present in approximately equal amounts and <1% was 18:3n−3 (**Table 2**). Replacement of FO with the VO blend resulted in increased percentages of 18:3n−3, 18:2n−6, and 18:1n−9 with concomitant decreased proportions of n−3 HUFA, 20:1, 22:1, and total monoenoic fatty acids. These effects were quantitatively greater in the diet with the higher level of FO replacement, diet 100% VO. The FO diet in seawater was characterized by having lower 20:1 and higher 22:6n−3 compared to the freshwater phase due to seasonal variation in batches of capelin oil. However, replacement of FO with the VO blend had similar effects in seawater diets as in the freshwater diets except total levels of monounsaturated fatty acids were unchanged by substitution in seawater diets, whereas there was a 9% point decrease in the freshwater diets (**Table 2**). The VO blend was formulated to mimic FO in total saturated, monounsaturated, and polyunsaturated fatty acid contents, but with no HUFA, and this was largely achieved, particularly in the seawater diets.

Growth, Feed Efficiency, and Mortality. There was no significant difference in SGR, FCR, or TGC between 100% FO and 75% VO in Scotland or between 100% FO and 100% VO in Norway in the freshwater or seawater period (**Table 3**). In Norway the Atlantic salmon fed the experimental diets increased their weights from 0.16 g at the start of feeding to 76 g in October 2002 and to 103 g before seawater transfer (**Figure 1**). After seawater transfer, the salmon fish weight increased to 890 g after 6 months in the sea, and there were no significant differences in growth between salmon fed 100% FO and 100% VO up to this time point (**Figure 1**). However, after almost 1 year in seawater, the 100% VO fed fish had significantly higher weight (2.7 kg) compared to the 100% FO fed fish (2.3 kg) (**Figure 1**). Concomitantly, in the same period when the mean fish weight differences appeared in Norway (from October 2003 to January 2004) the feed efficiencies were poor (100% FO, 2.8 ± 0.3; 100% VO, 1.9 ± 0.4) and the protein productive value (PPV) was generally very low but significantly higher ($p = 0.02$) in the 100% VO group (0.15 ± 0.03) compared to the

Table 3. Experimental Conditions, Feed Conversion (FCR), and Growth[a] for Each Period (FW and SW) of Atlantic Salmon Fed either 100% FO, 75% VO (S), or 100% VO (N) from Start of Feeding until Harvest Size Prior to Finishing Diet Period at Two Different Locations[b]

feeding time (months)	location		FCR		SGR		TGC	
			FO	VO	FO	VO	FO	VO
0–12	S	FW	1.12 ± 0.01	1.17 ± 0.02	1.43 ± 0.00	1.42 ± 0.01	1.00 ± 0.01	1.01 ± 0.01
12–25	S	SW	1.02 ± 0.02	0.98 ± 0.02	1.16 ± 0.02	1.14 ± 0.02	2.45 ± 0.06	2.53 ± 0.06
0–10	N	FW	1.31 ± 0.03	1.34 ± 0.05	2.16 ± 0.03	2.19 ± 0.01	1.23 ± 0.03	1.27 ± 0.01
10–22	N	SW	1.34 ± 0.07	1.22 ± 0.11	0.92 ± 0.06	0.89 ± 0.04	2.13 ± 0.15	2.36 ± 0.17

[a] Specific growth rate (SGR) and thermal growth coefficient (TGC). [b] S, Scotland; N, Norway.

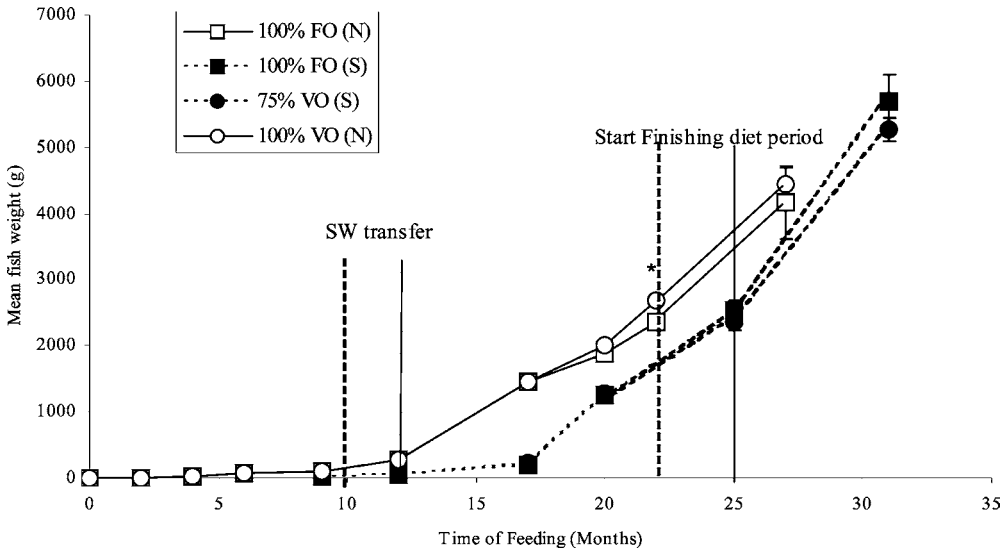

Figure 1. Mean Atlantic salmon weight (grams) from start of feeding to harvest in fish fed either 100% FO, 75% VO, or 100% VO at two different locations (Scotland and Norway). The asterisk (*) indicates statistically significant differences between the dietary groups by one-way ANOVA. Seawater transfer is indicated by dotted (10 months, Norway) and continuous line (12 months, Scotland).

100% FO group (0.09 ± 0.01). Concomitantly, whole fish protein levels were not significantly different between the dietary groups and increased from 31 to 38% from August 2003 to January 2004 (results not presented in detail). It is noteworthy, however, that both PPV and feed efficiencies are merely estimates because feed collection was not conducted in this feeding experiment. The SGR and FCR varied through the experiment with high growth rates in high-temperature periods and lower SGR when temperatures decreased during the winter (detailed data not shown). The mean growth was significantly higher in Norway (where heated water was used in the freshwater period) compared to Scotland (**Table 3**). Thus, the salmon weights at smoltification and seawater transfer were significantly higher in Norway, giving an advantage for growth in the seawater period in Norway (**Figure 1**). There were, however, no significant differences in fish weights (~2.5 kg) between the two locations at the final sampling (after 22 months in Norway and after 25 months in Scotland).

The finishing diet period lasted for 5 months in Norway and for 6 months in Scotland. Furthermore, the finishing diet period in Scotland included the summer of 2004 with higher water temperatures and, hence, high growth, giving a final weight of 5.5 kg compared to ~4.5 kg mean salmon weight after the finishing diet period in Norway (**Figure 1**). Mortalities were low throughout the experiment, and no significant differences were observed between the dietary groups at both locations.

Flesh Lipid Composition. The lipid content of the flesh (**Tables 4** and **5**) after 6 months of feeding was 3.1−4.9% (ww) and was higher in the Scottish fish (4.1−4.9%, ww) compared to the Norwegian fish (3.1−3.2%, ww) (**Table 5**). Thereafter, at all subsequent sampling points during the feeding experiment, salmon from the Scottish location had consistently higher flesh lipid levels compared to salmon from Norway (**Tables 4** and **5**). Flesh lipid level from fish at both locations dropped around smoltification prior to seawater transfer followed by an increase after transfer to seawater. The sampling after 16 (N) and 17 (S) months was an exception, with the fish from Norway having significantly higher flesh lipid levels than their Scottish counterparts (**Table 5**). These data correlate with the highest difference in fish weight at this sampling point between the two locations (about 200 g in Scotland and 1.4 kg in Norway; **Figure 1**). At the final sampling prior to the finishing diet period, at both locations, there were no major differences in flesh lipid content between FO and VO groups (**Table 5**). Although the 100% FO groups from the two locations were significantly different [12.6% (S) and 8.7% (N)], flesh lipid levels from 75% VO and 100% VO fed fish were similar, with ~10% lipid ww (**Table 5**). Throughout the finishing diet period, however, the total lipid level gradually increased in the two dietary groups in Scotland, whereas there was a gradual decrease in the 100% VO fed fish in Norway, resulting in significantly different flesh lipid levels between the dietary treatments at the two locations at the end of the finishing diet periods (**Table 5**; **Figure 2**). In the FO group in Norway, however, flesh lipid levels first increased followed by a decrease toward the final finishing diet sampling (**Figure 2**).

Through both the freshwater and seawater phases the flesh fatty acid composition closely reflected that of the diet (**Tables 4** and **5**). After only 6 months of feeding in freshwater, the flesh fatty acids reflected the dietary fatty acids and the relative levels of all fatty acids, with the exception of 22:6n−3 and the total n−3 PUFA, were significantly different in the 100% FO groups

Table 4. Effect of Feeding Diets Containing Fish Oil or Vegetable Oil on the Fatty Acid Composition (Percentage of Weight) of Total Lipid and Total Fat (Percent) from Flesh of Atlantic Salmon (*S. salar*) at Selected Freshwater and Seawater Stages from Scotland (S) and Norway (N)[a]

	6 months (FW, S)	6 months (FW, N)			9 months (FW, S)	9 months (FW, N)			12 months (FW, S)	12 months (SW, N)		
	100% FO	100% FO	75% VO	100% VO	100% FO	100% FO	75% VO	100% VO	100% FO	100% FO	75% VO	100% VO
total lipid	3.1 ± 0.3	4.9 ± 0.4	3.2 ± 0.3	4.1 ± 0.7	3.1 ± 0.1	4.5 ± 0.6	2.6 ± 0.3 b	3.8 ± 0.6 a	2.0 ± 0.1	na	1.6 ± 0.3	na
14:0	5 ± 0.3	5.3 ± 0.1	2.3 ± 0.1 a	1.7 ± 0.1 b	4.2 ± 0.1	4.8 ± 0.1	1.9 ± 0.1 a	1.6 ± 0.6 b	2.7 ± 0.1	5 ± 0.1	1.1 ± 0.1 b	1.3 ± - a
16:0	14.7 ± 0.5	13.2 ± 0.2	15.8 ± 0.2 a	15.1 ± 0.1 b	14 ± 0.3	13 ± 0.1	15.7 ± 0.5 a	14.7 ± 0.2 b	16.9 ± 1	12.8 ± 0.2	17.8 ± 1.1 a	14.3 ± 0.2 b
18:0	2.3 ± 0.1	2.4 ± 0.1	3.1 ± 0.1	3.6 ± – b	2.3 ± 0.1	2.4 ± 0.1	3.1 ± 0.1 b	3.6 ± – a	2.8 ± 0.1	2.4 ± –	3.8 ± 0.3	3.4 ± 0.1
sum sat.[b]	*22.4 ± 0.8*	*21.6 ± 0.2*	*21.5 ± 0.3* a	*20.6 ± 0.1* b	*21 ± 0.3*	*21* ± -	*21 ± 0.4* a	*20 ± 0.2* b	*23 ± 1*	*21 ± 0.3*	*22.8 ± 1.4* a	*19.7 ± 0.4* b
16:1n–7[c]	6.2 ± 0.1	6.2 ± 0.2	2.8 ± 0.1 a	1.6 ± – b	5.9 ± 0	6 ± 0.1	2.5 ± 0 a	1.5 ± 0.1 b	3.7 ± 0.2	6.2 ± 0.3	1.4 ± 0.1	1.4 ± –
18:1n–7	3.7 ± 0.3	3.4 ± 0.1	2.7 ± – a	2.5 ± – b	3.6 ± 0.3	3.4 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	3.5 ± –	2.1 ± 0.1 b	2.5 ± – a
18:1n–9	15 ± 0.2	13.7 ± 0.5	28.3 ± 0.3 b	31.4 ± 0.4 a	14.0 ± 0.3	14.0 ± 0.3	26.6 ± 1.6 b	33.5 ± 0.5 a	10.2 ± 0.6	13.4 ± 0.2	15.9 ± 2.1 b	34.8 ± 0.4 a
20:1n–9[d]	15.1 ± 0.4	14.6 ± 0.4	6.4 ± 0.2 a	3.8 ± 0.1 b	15.0 ± 0.5	15.0 ± 0.3	5.9 ± 0.5 a	3.8 ± 0.1 b	8.4 ± 0.7	15.3 ± 0.2	3 ± 0.3	3.4 ± –
20:1n–11	0.5 ± 0.1	0.7 ± 0.1	0.4 ± 0.1 a	0.2 ± 0.1 b	0.4 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.3 ± -	0.3 ± –	0.7 ± –	0.2 ± –	0.2 ± –
22:1n–9	1.5 ± 0.2	1.4 ± -	0.7 ± 0.1 a	0.5 ± – b	1.4 ± 0.3	1.3 ± –	0.6 ± 0.1	0.5 ± –	0.6 ± 0.1	1.4 ± –	0.2 ± 0.1	0.4 ± –
22:1n–11[e]	8.7 ± 0.4	9.0 ± 0.2	3.8 ± 0.1 a	2.3 ± – b	7.8 ± 0.3	8.5 ± 0.3	3.3 ± 0.3 a	2 ± – b	4 ± 0.4	8.9 ± 0.1	1.4 ± 0.2 b	1.7 ± – a
sum mono	*52.5 ± 0.3*	*51.7 ± 0.8*	*46.1 ± 0.5* a	*43.2 ± 0.4* b	*50 ± 0.7*	*51 ± 0.6*	*42.8 ± 2.6* b	*45 ± 0.5* a	*31 ± 2*	*52 ± 0.4*	*25.1 ± 2.7* b	*45 ± 0.3* a
18:2n–6	3.3 ± 0.1	3 ± –	9.4 ± 0.1 b	10.4 ± 0.1 a	3.4 ± 0.1	3.0 ± -	9.0 ± 0.3 b	10.4 ± - a	2.4 ± 0.1	3.4 ± –	5.7 ± 0.8 b	10.9 ± 0.2 a
20:2n–6	0.3 ± –	0.3 ± –	0.6 ± –	0.8 ± –	0.3 ± –	0.3 ± –	0.6 ± – b	0.8 ± – a	0.2 ± –	0.3 ± –	0.4 ± 0.1 b	0.9 ± – a
20:4n–6	0.3 ± –	0.5 ± –	0.3 ± – b	0.9 ± – a	0.4 ± –	0.5 ± –	0.4 ± 0.1 b	0.8 ± – a	0.7 ± –	0.5 ± –	0.7 ± 0.1 b	0.9 ± – a
sum n–6[f]	*4.3 ± 0.1*	*4 ± 0.1*	*10.8 ± 0.2* b	*12.5 ± 0.1* a	*4.6 ± 0.1*	*4* ± –	*10.6 ± 0.3* b	*13 ± 0.1* a	*3.8 ± 0.1*	*4.4* ± –	*7.7 ± 0.7* b	*13.3 ± 0.1* a
18:3n–3	0.8 ± 0.1	0.6 ± 0.1	4.9 ± 0.1 b	5.9 ± – a	0.7 ± 0.1	0.6 ± –	4.7 ± 0.2 b	5.4 ± 0.1 a	0.5 ± 0.1	0.6 ± –	3.3 ± 0.3 b	6.2 ± 0.1 a
18:4n–3	1.2 ± 0.1	1.2 ± –	1 ± –	0.9 ± –	1.2 ± 0.1	1.1 ± –	0.9 ± 0.1	0.8 ± –	0.7 ± 0.1	1.2 ± –	0.5 ± 0.1 b	1.2 ± 0.1* a
20:5n–3	3.6 ± 0.1	3.5 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	3.9 ± 0.1	3.5 ± –	2.6 ± 0.3 a	2 ± – b	6.2 ± 0.3	3.8 ± –	4.6 ± 0.4 a	2.1 ± 0.1 b
22:5n–3	1.1 ± 0.1	1.2 ± -	0.7 ± 0.1	0.7 ± –	1.2 ± 0.1	1.2 ± –	0.8 ± – a	0.6 ± – b	1.5 ± 0.1	1.2 ± –	0.9 ± 0.1 a	0.6 ± – b
22:6n–3	12.6 ± 0.5	12.2 ± 0.8	11.2 ± 0.5	11.2 ± 0.7	16 ± 0.6	13 ± 0.2	14.9 ± 2.2 a	11.2 ± 0.5 b	31.3 ± 1.3	12.4 ± 0.4	32.3 ± 2.5 a	10± 0.1 b
sum n–3[g]	*20 ± 0.9*	*19.6 ± 1.0*	*20.9 ± 0.7*	*21.6 ± 0.8*	*24 ± 0.7*	*21 ± 0.2*	*24.8 ± 2.4* a	*21 ± 0.5* b	*41 ± 1.2*	*20 ± 0.4*	*42.6 ± 2.6* a	*21 ± 0.3* b
n–3/n–6	4.7 ± 0.2	4.9 ± 0.2	1.9 ± 0.2	1.7 ± 0.1	5.1 ± 0.3	5.1 ± 0.1	2.3 ± 0.2 a	1.6 ± – b	10.8 ± 0.9	4.6 ± 0.1	5.5 ± 1a	1.6 ± – b

[a] Results are means ± SD. Different letters indicate statistical differences between 75% VO and 100% VO within each sampling period obtained by one-way ANOVA. 100% FO, fish oil diet; 75% VO and 100% VO, diets with 75% and 100% of fish oil replaced by vegetable oil. [b] Totals include 15:0 present at up to 0.3%. [c] Also contains n–9 isomer. [d] Also contains n–11 and n–7 isomers. [e] Also contains n–9 isomer. [f] Totals include 22:5n–6 present at up to 0.5%. [g] Totals include C_{16} PUFA present at up to 0.5%.

compared to either 75% VO or 100% VO, respectively (**Table 4**). Also, the flesh fatty acids in the two VO replacement groups were significantly different for all fatty acids except for the VLCn−3 PUFA. Furthermore, the n−3/n−6 ratio was the same in flesh from salmon fed 75% VO and 100% VO after 6 months of feeding (**Table 4**). From 6 months onward the differences in fatty acid composition between the 100% FO, 75% VO, and 100% VO became more obvious, and the variation, especially in 18:1n−9, 18:2n−6, 18:3n−3, and VLCn−3 PUFA, became greater with length of feeding period and increasing fish weights (**Tables 4** and **5**). During the smoltification period, observed only in the salmon in Scotland, fatty acid compositions showed increasing VLCn−3 PUFA and decreasing 18:2n−6, 18:1n−9, and 18:3n−3 compared to the previous and following sampling points (**Tables 4** and **5**). These alterations in relative fatty acid composition seen during the smoltification period disappeared with further growth and feeding and probably reflect physiological changes induced by the smoltification process (**Table 5**).

Flesh Fatty Acids through the Finishing Diet Period. The relative fatty acid composition in the 75% VO and 100% VO groups changed toward that of the 100% FO flesh fatty acid composition through the finishing diet period (**Table 5**). The amount (milligram of FA per gram of flesh) of 18:1n−9 decreased in the 75% VO and 100% VO fish through the finishing diet period, and the amounts of both 16:0 and 18:1n−9 resulted at the same levels as in the 100% FO group (**Figure 2**). The amounts of 18:2n−6 and 18:3n−3 decreased significantly in both 75% VO and 100% VO fed fish; however, they were still significantly higher than the 100% FO group at the end of the finishing diet period. At relative fatty acid basis, the level of 18:2n−6 decreased by 5% points in both the 75% VO and 100% VO groups, whereas the 100% FO groups were stable at 3% in fillet from fish at both locations (**Table 5**). 18:3n−3 decreased by ∼4% points in both VO groups, being 1.5 and 3.5% higher in the 75 and 100% VO groups, respectively, compared to fillet from fish fed 100% FO through the experiment (**Table 5**). The difference between 100% FO and 75% VO was only 1.4 mg of 18:2n−6 g^{-1} of flesh after the finishing diet period, compared to a difference of 6.5 mg of 18:2n−6 g^{-1} of flesh prior to the finishing diet period (**Figure 2**). The amount of VLCn−3 PUFA (EPA + DHA) in the 100% FO group increased by 3.5 mg g^{-1} of flesh through the finishing diet period (**Figure 2**). Over the same period the levels of VLCn−3 PUFA in the flesh of fish originally fed 75 and 100% VO increased by 6.2 and 4.8 mg g^{-1}, respectively. Consequently, after the finishing diet period with all groups being fed 100% FO based diets, the 75% VO and 100% VO fish still contained significantly lower VLCn−3 PUFA levels (30 and 50% lower, respectively) compared to the 100% FO group (**Figure 2**). Taking all of the n−3 fatty acids together (18:3n−3 + VLCn−3 PUFA) gave a flesh n−3/n−6 ratio that was still significantly higher in the 100% FO fed fish after the finishing diet compared to flesh from the two VO groups (**Figure 2**).

Flesh Astaxanthin. Flesh total astaxanthin increased gradually during growth in seawater (**Figure 3**). No significant differences between 100% FO and VO fed fish were observed at either location. The amount of astaxanthin was generally slightly higher in salmon sampled in Scotland compared to the Norwegian salmon (**Figure 3**). A dramatic increase in flesh astaxanthin levels was observed from 25 to 31 months (finishing diet period) of feeding in Scotland.

Flesh Sensory Quality. The objective sensory taste panels showed no or only minor, but statistically significant, differences

Table 5. Effect of Feeding Diets Containing Fish Oil or Vegetable Oil on the Fatty Acid Composition (Percentage of Weight) of Total Lipid and Total Fat (Percent) from Flesh of Atlantic Salmon (*S. salar*) at Selected Seawater Stages from Scotland (S) and Norway (N)[a]

	17 months (SW, S)	16 months (SW, N)			25 months (SW, S)	22 months (SW, N)			31 months (SW, S)	27 months (SW, N)		
	100% FO	100% FO	75% VO	100% VO	100% FO	100% FO	75% VO	100% VO	100% FO	100% FO	75% VO	100% VO
total lipid	3.1 ± 0.2	8.5 ± 1.5	2.5 ± 0.6 b	8.3 ± 0.3 a	12.6 ± 0.2	8.7 ± 0.7	11.6 ± 1.0	9.8 ± 0.5	15.8 ± 1.4	10.4 ± 2.1	13.1 ± 1.7 a	9.3 ± 0.8 b
14:0	4.4 ± 0.4	5.3 ± –	1.9 ± 0.2 a	1.1 ± – b	5.0 ± 0.1	4.9 ± –	2.2 ± 0.1 a	0.8 ± - b	5.0 ± 0.2	4.8 ± 0.1	4.2 ± 0.1 a	2.7 ± 0.3 b
16:0	13.4 ± 0.3	13.5 ± 0.2	14.8 ± 0.6	14.4 ± 0.1	13.6 ± 0.3	13 ± 0.2	13.3 ± 0.3	12.9 ± 0.1	13.5 ± 0.4	12.9 ± 0.0*	13.4 ± 0.1	12.7 ± 0.0*
18:0	2.4 ± –	2.8 ± –	3.1 ± 0.1 b	3.5 ± – a	2.6 ± 0.1	2.6 ± 0.1	3 ± 0.1	3.1 ± –	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.0	2.9 ± 0.1
sum sat.[b]	*20.4 ± 0.5*	*22.6 ± 0.3*	*20 ± 0.4*	*19.7 ± 0.0*	*21.6 ± 0.4*	*21.1 ± 0.3*	*19 ± 0.4* a	*17 ± 0.1* b	21.5 ± 0.5	*20.9 ± 0.1*	*20.6 ± 0.1* a	*18.6 ± 0.2* b
16:1n–7[c]	5.7 ± 0.6	5.1 ± 0.1	2.3 ± 0.3 a	1.0 ± - b	5.3 ± 0.1	4.4 ± –	2.5 ± 0.1 a	0.9 ± – b	4.9 ± 0.1	4.3 ± 0.2	4.0 ± 0.0 a	2.4 ± 0.3 b
18:1n–7	3.4 ± 0.2	3 ± –	2.6 ± 0.1 a	2.3 ± – b	3.0 ± 0.1	2.7 ± –	2.7 ± 0.1	2.5 ± –	2.8 ± 0.1	2.7 ± 0.0	2.7 ± 0.0	2.7 ± 0.0
18:1n–9	12.9 ± 0.7	13.7 ± 0.1	26.8 ± 2.4 b	39.9 ± 0.1 a	16.7 ± 0.2	14.6 ± 0.3	34.5 ± 0.5 b	41.3 ± 0.2 a	16.8 ± 0.3	15.1 ± 0.3	21.6 ± 0.7 b	29.2 ± 1.9 a
20:1n–9[d]	16 ± 1	12.2 ± 0.2	6.1 ± 0.6 a	2.8 ± 0.1 b	11.2 ± 0.4	10 ± 0.1	5.5 ± 0.1 a	2.7 ± – b	11.1 ± 0.1	9.8 ± 0.1	9.3 ± 0.2 a	6.2 ± 0.5 b
20:1n–11	0.5 ± 0.2	1 ± 0.1	0.2 ± –	0.1 ± –	1.0 ± 0.2	1.4 ± –	0.4 ± 0	– ± –	1.1 ± 0.1	1.6 ± 0.0	0.9 ± 0.1	0.8 ± 0.1
22:1n–9	1.4 ± 0.1	1.1 ± 0.1	0.7 ± 0.1 a	0.4 ± - b	1.0 ± 0.0	0.9 ± –	0.6 ± – a	0.4 ± – b	1.0 ± 0	0.9 ± 0.0	0.9 ± 0.1 a	0.7 ± 0.0 b
22:1n–11[e]	9.2 ± 0.9	10.5 ± 0.1	3.6 ± 0.4 a	1.0 ± – b	11.7 ± 0.5	11.4 ± 0.1	4.1 ± 0.2 a	0.9 ± – b	12.9 ± 0.1	11.7 ± 0.6	10.8 ± 0.3 a	6.6 ± 0.8 b
sum mono	*50.7 ± 3.6*	*48.9 ± 0.5*	*43 ± 3.7*	*47.8 ± 0.0*	*51.4 ± 0.3*	*48.2 ± 0.3*	*51.0 ± 0.6* b	*49.0 ± 0.1* a	52.1 ± 0.4	*49.0 ± 0.4*	*51.6 ± 0.6* a	*50.0 ± 0.1* b
18:2n–6	3.3 ± 0.2	3.4 ± –	8.8 ± 0.6 b	12.7 ± 0.1 a	3.3 ± 0.1	3.3 ± –	10.3 ± 0.1 b	14.4 ± – a	3.1 ± 0.1	3.3 ± 0.2	5.2 ± 0.2 b	9.1 ± 0.8 a
20:2n–6	0.4 ± –	0.4 ± –	0.7 ± 0.1 b	1 ± – a	0.4 ± 0.0	0.4 ± –	1 ± 0	1.2 ± –	0.4 ± 0	0.1 ± 0.2	0.6 ± 0.0 a	0.0 ± 0.0 b
20:4n–6	0.4 ± 0	0.4 ± 0.3*	0.4 ± 0.1 b	0.9 ± –* a	0.4 ± 0.0	0.7 ± –	0.3 ± 0.1 b	0.9 ± – a	0.4 ± 0	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
sum n–6[f]	*4.5 ± 0*	*4.3 ± 0.3*	*10 ± 0.6* b	*15* ± – a	*4.8 ± 0.1*	*4.5* ± –	*12 ± 0.1* b	*17 ± 0.1* a	4.5 ± 0.1	*3.9 ± 0.2*	*6.9 ± 0.2* b	*9.8 ± 0.8* a
18:3n–3	0.6 ± 0.1	0.8 ± –	5.6 ± 0.2 b	8.0 ± 0.1 a	1.0 ± 0.0	1.1 ± –	6.5 ± 0.1 b	8.2 ± 0.1 a	1.0 ± 0	1.1 ± 0.1	2.6 ± 0.2 b	4.6 ± 0.4 a
18:4n–3	1.1 ± 0.1	1.4 ± –	0.7 ± 0.1	0.8 ± -	1.4 ± 0.1	1.5 ± –	0.7 ± 0 b	1.2 ± 0.1 a	1.4 ± 0	1.4 ± 0.0	1.2 ± 0.1	1.2 ± 0.1
20:5n–3	5.3 ± 0.6	4.3 ± 0.1	3.9 ± 0.6 a	1.6 ± – b	4.5 ± 0.1	4.6 ± 0.1	2.2 ± 0.1 a	1.6 ± – b	4.4 ± 0.1	4.6 ± 0.1	3.8 ± 0.2 a	2.9 ± 0.2 b
22:5n–3	1.5 ± 0.1	1.7 ± –	1.1 ± 0.1 a	0.6 ± – b	2.1 ± 0.1	2.1 ± –	1 ± 0.1 a	0.6 ± – b	2.3 ± 0.1	2.3 ± 0.1	1.9 ± 0.1 a	1.5 ± 0.2 b
22:6n–3	14.2 ± 3.3	11.5 ± 0.4	13.2 ± 3.3 a	5.3 ± 0.1 b	11 ± 0.4	12.7 ± 0.2	5.9 ± 0.5 a	3.4 ± 0.1 b	10.6 ± 0.4	12.8 ± 0.4	9.4 ± 0.4	8.4 ± 0.5
sum n–3[g]	*23.7 ± 3.8*	*20.9 ± 0.2*	*26 ± 3.7* a	*17 ± 0* b	*21.7 ± 0.5*	*23.4 ± 0.3*	*18 ± 0.7* a	*16 ± 0.1* b	21.4 ± 0.6	23.9 ± 0.3	20.5 ± 0.7	20.3 ± 0.5
n–3/n–6	5.3 ± 0.7	4.8 ± 0.3	2.5 ± 0.5 a	1.1 ± – b	4.5 ± 0.2	5.2 ± 0.1	1.5 ± 0.2 a	0.9 ± – b	4.8 ± 0.3	6.1 ± 0.2	3.0 ± 0.3 a	2.1 ± 0.2 b

[a] Results are means ± SD. Different letters indicate statistical differences between 75% VO and 100% VO within each sampling period obtained by one-way ANOVA. 100% FO, fish oil diet; 75% VO and 100% VO, diets with 75% and 100% of fish oil replaced by vegetable oil. [b] Totals include 15:0 present at up to 0.3%. [c] Also contains n–9 isomer. [d] Also contains n–11 and n–7 isomers. [e] Also contains n–9 isomer. [f] Totals include 22:5n–6 present at up to 0.5%. [g] Totals include C_{16} PUFA present at up to 0.5%.

between 100% FO and VO fed salmon flesh (**Figures 4**−**6**). The taste panel in Scotland detected no significant differences in any of the sensory attributes between salmon fed 100% FO and 75% VO, with either fresh or smoked Atlantic salmon, after 25 months of feeding (**Figure 4**). The taste panel did, however, find statistically significant differences between salmon fed 100% FO and salmon fed 100% VO for 22 months in Norway [**Figures 5** and **6** (top)]. PCA showed that flesh from salmon fed 100% FO form a separate group from flesh from salmon fed 100% VO for 22 months [**Figure 5** (top)]. Intensities of odor, marine oil odor, color tone, marine oil flavor, and rancid flavor were all given significantly higher scores in fresh-cooked flesh from salmon fed 100% FO compared to 100% VO [**Figure 6** (top)]. Vegetable oil flavor scored significantly higher in the 100% VO fed group compared to the 100% FO salmon [**Figure 6** (top)]. After the finishing diet period (27 months of feeding) when both dietary groups were fed the 100% FO diet, all sensory parameters were equal, demonstrated both by the PCA plot, where no distinct groups were formed [**Figure 5** (bottom)], and by the sensory parameter scores [**Figure 6** (bottom)].

In the preference test with scores from 0 to 10, where 0 was extremely disliked and 10 was extremely liked, no major differences were observed between flesh from the different dietary groups (**Table 6**). However, a ranking test generally showed that flesh from the salmon fed 100% VO was preferred over the other groups.

DISCUSSION

The aim of this study was to replace 75 and 100% of fish oil in diets with a vegetable oil blend throughout the complete life cycle of Atlantic salmon to evaluate the effects of the substitution on growth, flesh nutritional and sensory qualities, consumer acceptance, and consequences as a health-promoting seafood product for humans. For the first time dietary fish oil has been replaced partially and completely through the whole life cycle at two different locations and shown to produce the same results. With both levels of VO replacement examined, growth was high and either not significantly different from that of the 100% FO group or, as in the case of the sampling after 22 months of feeding 100% VO in Norway, gave significantly higher mean fish weight compared to the 100% FO group. This observed higher mean fish weight after 22 months in Norway correlated with higher protein utilization (PPV) in the 100% VO group compared to the 100% FO, indicating that during the late autumn and winter period of the seawater growth phase the fatty acid composition of the 100% VO diet favored protein growth and spared dietary protein from energy production. Previously, dietary lipid content, but not dietary oil source (*15*, *44*), has been shown to affect protein utilization, growth rate, muscle lipid level, and feed conversion (*45*, *46*). The results found in this study, which are supported by Bendiksen and co-workers (*47*), indicate that a dietary fatty acid composition favoring lipid catabolism and sparing dietary protein for muscle growth may manifest itself during certain stages in the growth/life cycle, especially during seasons with generally low growth rates such as the winter time following a high growth rate period. The significantly increased PPV and growth in the 100% VO group may be due to increased digestibility of dietary lipid and protein in the VO diet at low temperatures, as previously shown by Bendiksen et al. (*47*). However, feed intake data and thus PPV

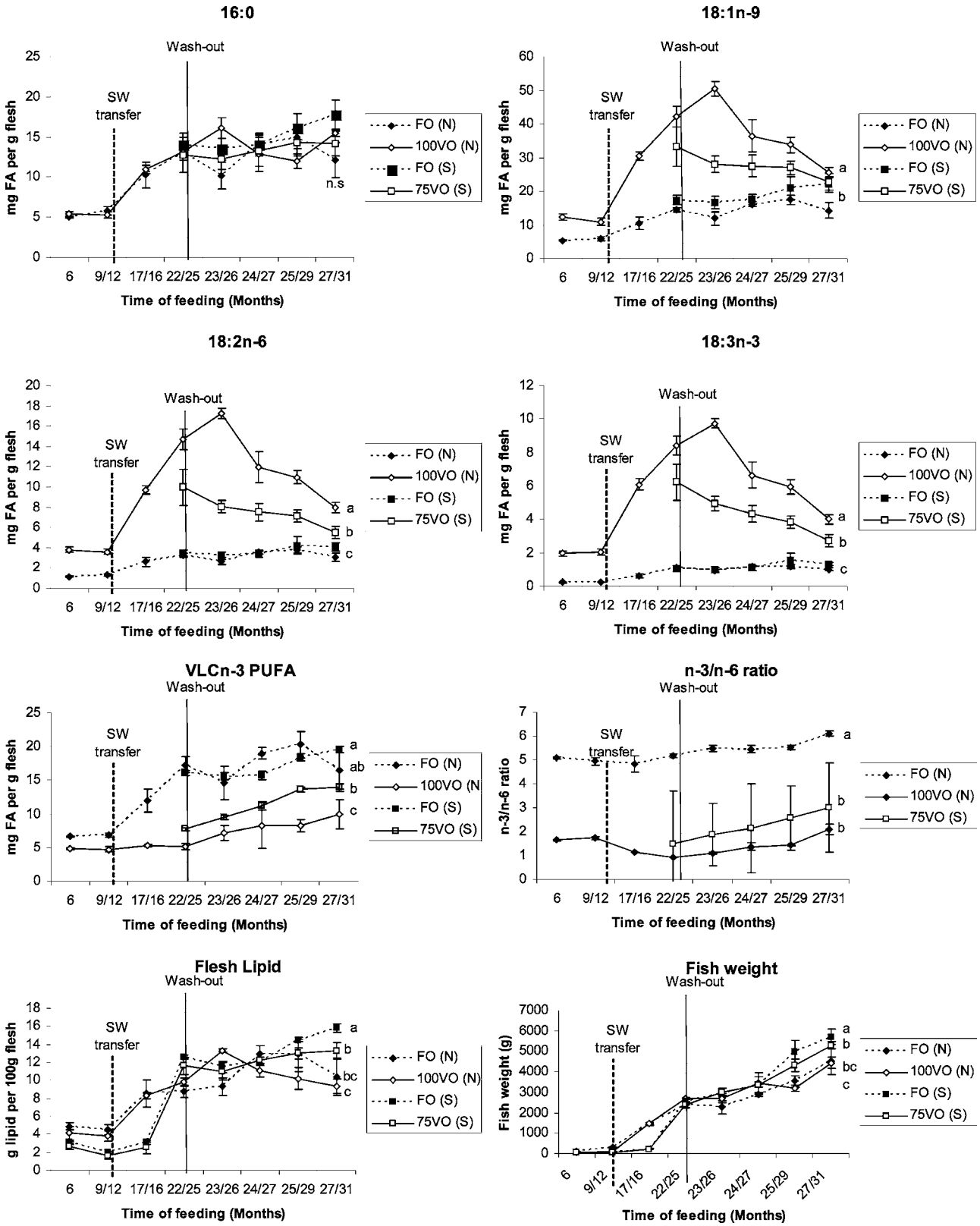

Figure 2. Amounts of 16:0, 18:1n–9, 18:2n–6, 18:3n–3, VLCn–3 PUFA (EPA + DHA), n–3/n–6 ratio (milligrams per gram of flesh, ww), total flesh lipid (grams per 100 grams, ww), and fish weight (grams) through freshwater and seawater periods for Atlantic salmon fed either 100% FO (N, Norway; S, Scotland), 75% VO (Scotland), or 100% VO (Norway) and through the finishing diet period when all groups were fed a 100% FO finishing diet. Time of feeding (*x*-axis) refers to the number of months of feeding after start of feeding, and time points when fish weight and life stage correlate between the two locations are presented together as N/S. Data are presented as mean ± SD. Different letters denote statistically significant differences between the four dietary groups at the final time point of finishing diet period identified by one-way ANOVA.

and feed utilization are estimates and not exact data, due to the lack of feed collection in the current experiment, and can be compared on only a relative basis within this study.

However, when considering growth as a whole through the freshwater and seawater phases, no differences were found here in either growth, feed efficiency, or survival between the dietary

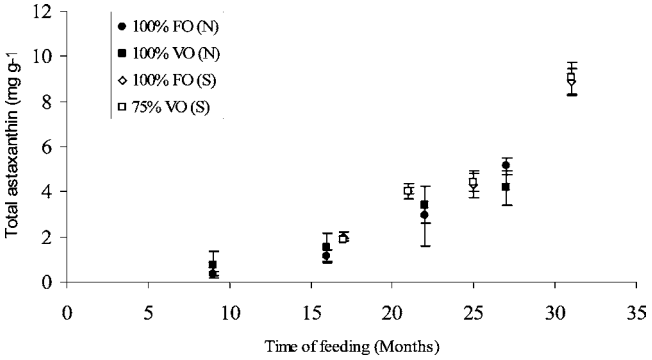

Figure 3. Total astaxanthin (milligrams per kilogram, ww) in flesh from Atlantic salmon fed either 100% FO, 75% VO, or 100% VO from start of feeding to harvest at two different locations (Scotland and Norway). Seawater transfer is indicated by dotted (10 months, Norway) and continuous line (12 months, Scotland).

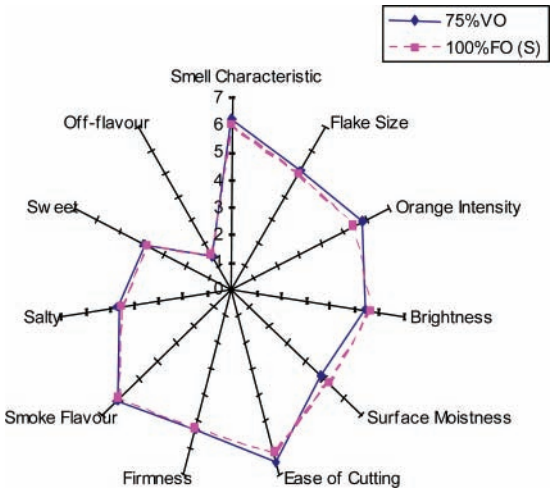

Figure 4. Sensory attributes of smoked salmon (Scotland) after 25 months of feeding Atlantic salmon either 100% FO or 75% VO.

groups examined, which is in line with previously reported results on vegetable oil replacements in Atlantic salmon (*9*, *10*, *12*−*15*, *18*). The higher growth in the freshwater period seen in Norway was mainly due to the use of heated water in the on-shore fish tanks, a system not used in Scotland, where the fish were cultured at ambient water temperatures and photoperiod. Consequently, the mean fish weight was significantly higher during smoltification and seawater transfer in Norway compared to Scotland, in addition to an earlier onset of smoltification in Norway. Furthermore, salmon in Scotland had higher growth rates compared to the Norwegian salmon during the seawater period. Consequently, all samplings were adjusted to life stage and mean fish weight, enabling results from the two locations to be comparable and giving thorough and robust data for investigating effects of the dietary vegetable oil replacements in Atlantic salmon, irrespective of location. In fact, mean fish weight was ∼2.5 kg (**Figure 1**) at the final sampling prior to initiation of the finishing diet period at both locations.

In support of the existing literature (*9*−*11*, *13*, *26*) describing feeding trials with postsmolt salmon, the dietary fatty acid composition resulting from a blend of palm oil, rapeseed oil, and linseed oil used in the current dietary trial had no adverse effects on lipid digestibility at any stage in the life cycle affecting growth or feed efficiency, as has been reported for complete replacement with palm oil (*15*). Furthermore, the contribution of essential n−3 PUFA provided by the LT fishmeal used in the diets was sufficient for meeting the requirement for Atlantic salmon (*48*) even during potentially stressful early life stages and through smoltification. Flesh fatty acids, however, were affected in the salmon sampled in Scotland after 12 months of feeding just prior to seawater transfer with n−3 PUFA being significantly higher. This may be due to a life stage-dependent increase in PUFAs resulting from an increased activity of hepatic desaturase and elongase activity, which has been reported in the same fish in this period (*49*). A similar increase in salmon from Norway may have been obscured due to the use of artificial environmental conditions resulting in early smoltification.

Flesh Lipid Composition through the Whole Life Cycle Including a Finishing Diet Period. The current results confirm the numerous previous reports (*9*−*16*, *50*) of flesh fatty acid composition being highly influenced by dietary fatty acids. By introducing a finishing diet period when all groups were fed a 100% FO diet, the levels of VLCn−3 PUFA increased, whereas the typical VO fatty acids decreased to levels comparable with the 100% FO group. Similar results have been reported previously after feeding of a single level of VO followed by a finishing diet (*9*, *12*). The level of lipid in fillet increased with increasing fish size, which also coincided with increasing lipid content in the feed as pellet size increased. Previously, both dietary lipid content and fish size have been positively correlated with salmon flesh lipid content (*51*, *52*). The slight increase followed by a decrease observed in the salmon flesh in Norway during the finishing diet period was not related to onset of sexual maturation but may be related to the variable flesh lipid content often observed during periods of very low seawater temperatures (*47*). In contrast, the finishing diet period in Scotland started in spring followed by a high-growth period through the summer until September 2004. Thus, these salmon had a higher temperature growth period when flesh lipid gradually increased with increasing fish weight (**Figure 2**).

The latest human n−3 intake recommendation of the International Society for the Study of Fatty Acids and Lipids (ISSFAL; www.issfal.org) is 3.5 g of EPA + DHA per week to provide good cardiac health in adults. After 22/25 months of feeding, the VLCn−3 PUFA (EPA + DHA) contents of a 200 g portion of flesh were 3.2, 1.5, and 1.0 g in salmon from the 100% FO, 75% VO, and 100% VO groups, respectively. After the finishing diet period, the levels of VLCn−3 PUFA were 3.9, 2.8, and 2.0 g, respectively, in the three dietary groups. Thus, only one meal a week of 200 g of flesh from salmon fed 100% FO through the whole life cycle or 75% VO followed by a finishing diet will provide 110 and 80%, respectively, of the weekly recommended intake. By feeding salmon 100% VO for 22 months followed by a finishing diet, 56% of the weekly recommended intake is being met by a 200 g portion. It is generally recommended that an increase in the n−3/n−6 ratio of human diets is achieved largely by increasing the VLCn−3 PUFA intake, but also by decreasing the n−6 intake (*2*, *53*, *54*). After the finishing diet period, the level of 18:2n−6 was still 2-fold higher in the flesh of the 100% VO group than in the 100% FO group and contributed 1.6 g to a 200 g portion. However, a less efficient dilution of 18:2n−6 than that observed in the current study was reported by Torstensen et al. (*9*), who found that the level of 18:2n−6 was 2.6-fold higher in salmon fed 100% rapeseed oil followed by a finishing diet for 6 months (1800 day degrees). Due to the high levels of 18:3n−3 provided by the dietary linseed oil, the n−3/n−6 ratio in flesh from VO fed salmon was not particularly low. However, marine VLCn−3 PUFA provides the health beneficial effects for cardiovascular health, which seem to be lacking, or greatly reduced, with 18:3n−3 (*1*). Therefore, it is important to note that, although n−3/

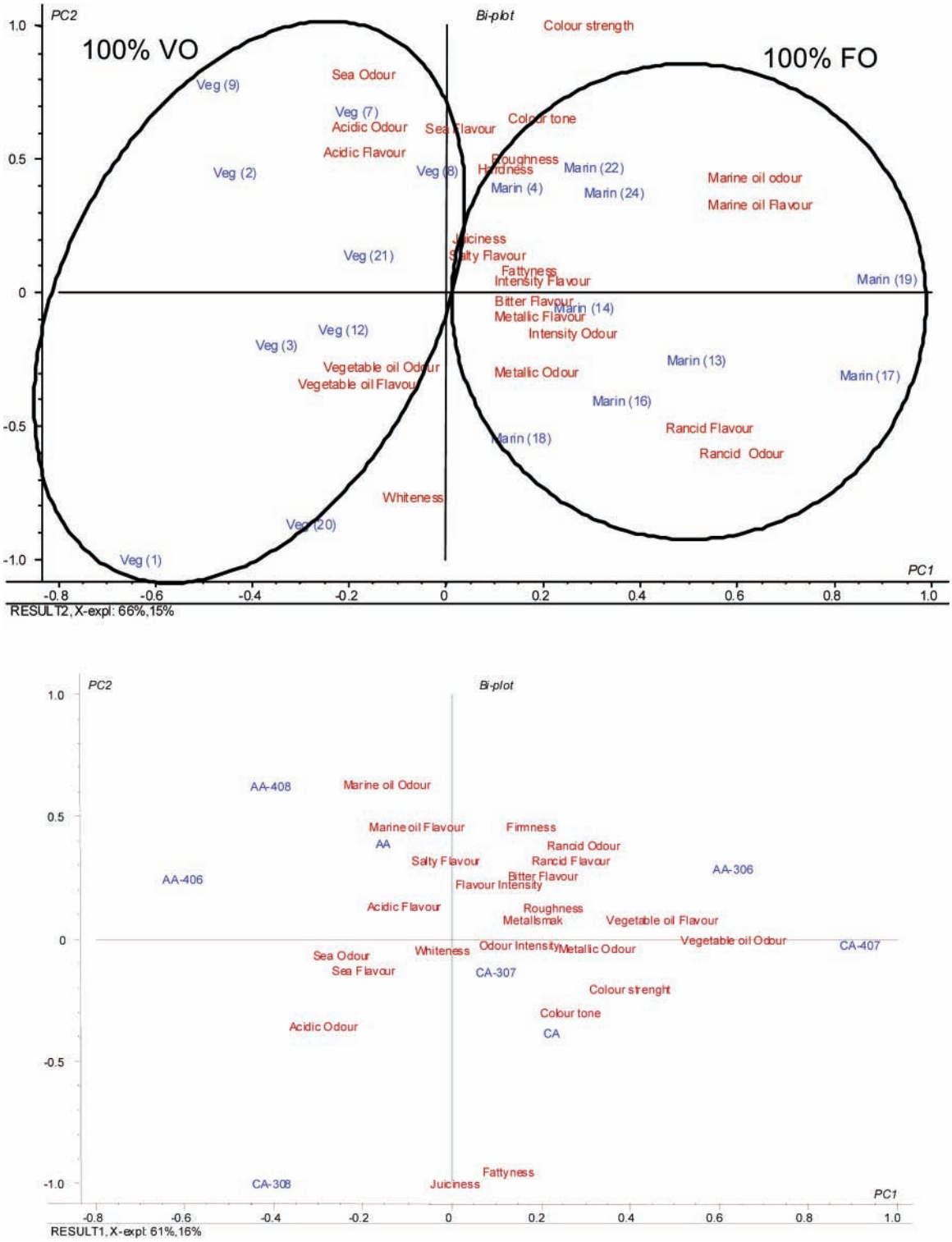

Figure 5. PCA biplot of fillet samples from Atlantic salmon fed either 100% FO or 100% VO from start of feeding and with the sensory attributes before (January 2004; top) and after (June 2004; bottom) finishing diet period with 100% FO.

n−6 did not change more than 2-fold through the finishing diet period, the levels of 18:3n−3 decreased significantly while VLCn−3PUFA levels increased, producing an overall more beneficial product for human consumption.

Flesh pigmentation is an important factor in the perception of flesh quality in salmonids (*55*, *56*). The flesh levels of lipid soluble nutrients, such as astaxanthin, are dependent on the dietary composition (*24*), and it has been shown that both total lipid and type of oil can affect carotenoid absorption (*57*, *58*) and color characteristics in raw and smoked fillets (*17*). However, in this study no negative effects of using up to 100% VO were observed on flesh astaxanthin levels, confirming previous results using single VO replacements (*9*, *10*, *13*). Total flesh astaxanthin content was significantly higher after the finishing diet period in Scotland compared to Norway, which was correlated to, and can be explained by, higher final fish weight and flesh lipid content in Scotland.

Flesh Sensory Quality. Only marginal differences were observed in flesh sensory characteristics between the 100% FO and 100% VO fed fish prior to the finishing diet period, in both

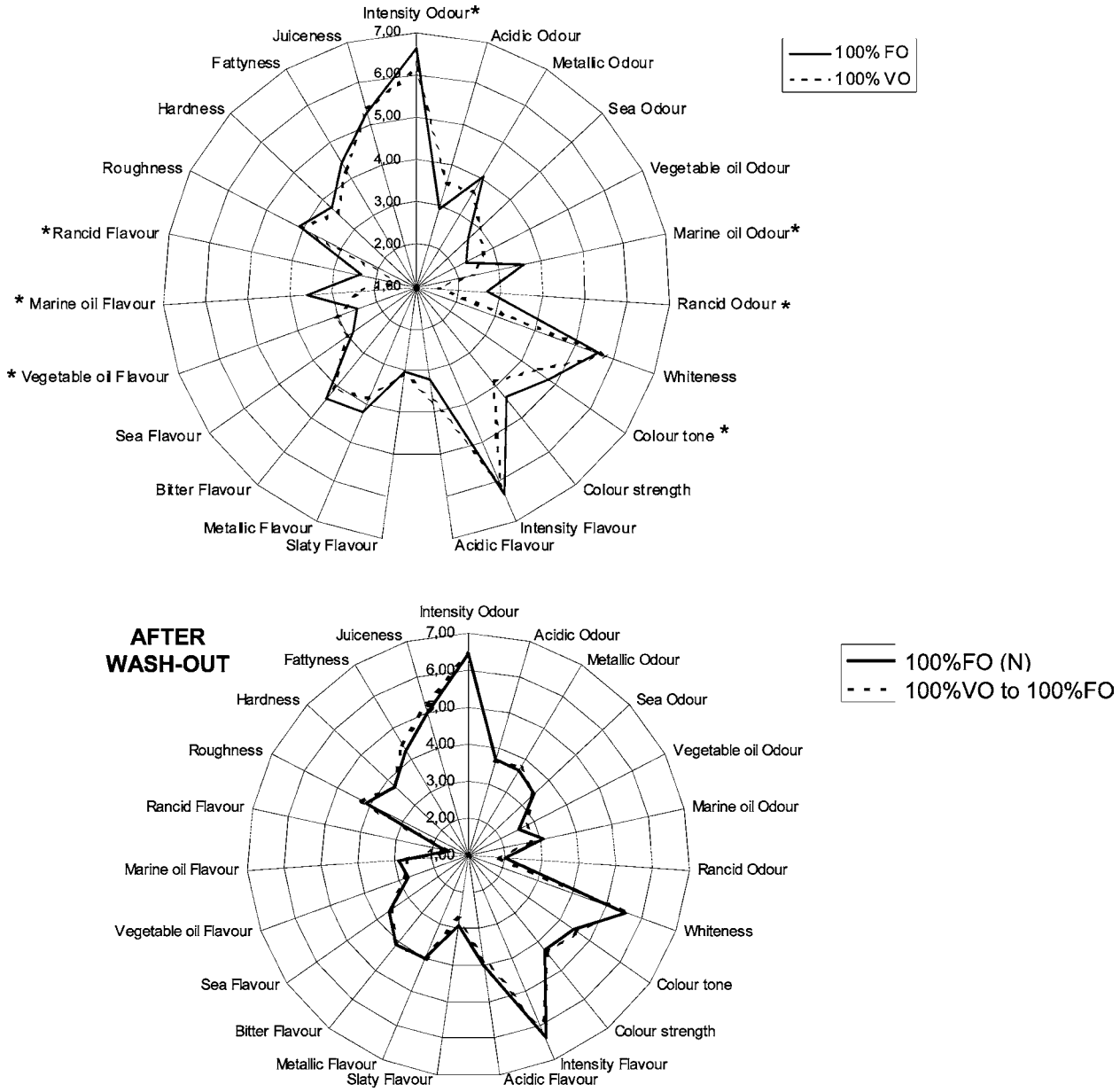

Figure 6. Sensory characteristics score of fillets from Atlantic salmon fed either 100% VO or 100% FO from start of feeding both before (January 2004; top) and after (June 2004; bottom) finishing diet period. Salmon were fed 100% FO or 100% VO for 22 months followed by 5 months of 100% FO diet. Significant differences between the dietary treatments are denoted by asterisks.

fresh and smoked salmon. However, some significant effects on marine and rancid flavor and odor were observed by the Norwegian taste panel in fresh cooked salmon. There was a tendency toward the Norwegian salmon, and especially salmon fed 100% VO, being preferred over the Scottish salmon; however, this was not statistically significant. It may be the decreased rancid and marine flavor and rancid odor found in the 100% VO fed salmon, which may have been preferred by the Scottish taste panel. These effects may also have been influenced by the higher lipid content, particularly of fish fed 100% FO from Scotland, compared to fish fed the same diet in Norway. However, after the finishing diet period, even the trained taste panel could not detect any differences between the 100% FO and 100% VO fed salmon. Previous experiments replacing FO with a single level of VO, even at lower replacement levels, have reported differences in "salmon taste and odor" (*19*), taste intensity, fattiness and juiciness (*18*), and "fish aroma" (*20*). In contrast, other reports show no effects of replacing FO with VO on organoleptic properties in other salmonids (*22*, *23*) (also reviewed in ref *59*). Flesh sensory quality for other aquaculture species, such as gilthead seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*), were slightly affected by replacing fish oil with either sunflower oil, rapeseed oil, linseed oil, or a mix of these three, but were very well accepted by the trained sensory panel (*60*). Also, turbot (*Psetta maxima*) flesh sensory quality was affected by replacing FO with soybean oil or linseed oil (*60*). Supporting the current results, all differences in sensory quality disappeared after a finishing diet period was used also in turbot (*61*).

In conclusion, replacing dietary FO with both 75 and 100% VO blend containing balanced levels of saturated, monounsaturated, and polyunsatured fatty acids, from start of feeding to harvest weight, including a finishing diet period, allowed sustainable production of Atlantic salmon with a profile of flesh fatty acids with typical fatty acid profiles that might benefit human nutritional requirements without compromising sensory qualities or fish growth.

Table 6. Sensory Analysis Performed in Scotland on Fillets from Atlantic Salmon Fed either 100% FO (Norway and Scotland) or 75% VO (Scotland) or 100% VO (Norway) for 23 Months[a]

	100% FO (N)	100% FO (S)	75% VO	100% VO
attributes				
fishy aroma	5.7 ± 0.7	5.3 ± 0.8	5.2 ± 1.1	5.2 ± 1.2
color intensity	4.3 ± 1.6	4.2 ± 1.5	3.9 ± 1.4	4.1 ± 1.8
flake size	5.0 ± 1.2	5.0 ± 0.9	4.6 ± 1.6	5.1 ± 1.3
hardness	4.3 ± 1.3	4.9 ± 1.1	4.5 ± 1.5	4.6 ± 1.2
firmness	4.3 ± 1.2	4.6 ± 1.3	4.8 ± 1.5	4.5 ± 1.3
fibrousness	5.2 ± 1.2	4.8 ± 0.9	5.2 ± 1.5	5.1 ± 1.2
chewiness	5.2 ± 1.3	5.1 ± 1.0	5.2 ± 1.4	5.0 ± 1.3
moistness	5.6 ± 1.0	5.0 ± 0.5	5.2 ± 1.2	5.6 ± 1.0
oiliness (texture and flavor)	5.1 ± 0.9	4.8 ± 0.8	4.9 ± 1.3	5.5 ± 1.0
fishy flavor character	5.0 ± 1.2	4.6 ± 1.1	4.8 ± 0.8	4.8 ± 1.2
off-flavor	1.5 ± 0.7	1.3 ± 0.3	1.6 ± 1.0	1.5 ± 0.9
aftertaste	3.7 ± 1.8	3.3 ± 1.5	3.3 ± 1.6	3.2 ± 1.6
preference test				
smell	5.5 ± 1.1	5.1 ± 0.7	5.3 ± 1.5	5.5 ± 1.5
color	5.2 ± 1.5	4.8 ± 1.7	4.9 ± 2.0	5.3 ± 1.7
appearance	5.3 ± 1.4	4.8 ± 1.6	5.0 ± 2.0	5.5 ± 1.8
texture	5.3 ± 1.2	5.3 ± 1.6	5.4 ± 1.8	5.6 ± 1.3
flavor	5.4 ± 1.3	5.4 ± 1.2	5.5 ± 1.4	5.9 ± 1.2

[a] Scores of the attributes are given as 0–10; for the preference test 0 refers to extremely disliked, whereas 10 refers to extremely liked.

ABBREVIATIONS USED

VLCn−3 PUFA, very long chain n−3 polyunsaturated fatty acids (EPA + DHA); VO, vegetable oil blend; FO, fish oil; PCA, principal component analysis; PPV, productive protein value; SGR, specific growth rate; TGC, thermal growth coefficient; FCR, feed conversion ratio.

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