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Atlantic Salmon (*Salmo salar* L.) as a Net Producer of Long-Chain Marine ω-3 Fatty Acids

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ABSTRACT: The objective of the present study was to investigate the effects of replacing high levels of marine ingredients with vegetable raw materials and with emphasis on lipid metabolism and net production of long-chain polyunsaturated ω-3 fatty acids (EPA + DHA). Atlantic salmon were fed three different replacement vegetable diets and one control marine diet before sensory attributes, β-oxidation capacity, and fatty acid productive value (FAPV) of ingested fatty acids (FAs) were evaluated. Fish fed the high replacement diet had a net production of 0.8 g of DHA and a FAPV of 142%. Fish fed the marine diet had a net loss of DHA. The present work shows that Atlantic salmon can be a net producer of marine DHA when dietary fish oil is replaced by vegetable oil with minor effects on sensory attributes and lipid metabolism.

KEYWORDS: DHA, EPA, fatty acids, fish oil, net producer, replacement, sensory attributes, vegetable oil

INTRODUCTION

Oily fish such as salmon, tuna, sardines, mackerel, and trout contain long-chain polyunsaturated ω-3 fatty acids (*n*-3 LC-PUFA), which are associated with several health beneficial effects, especially lower cardiovascular disease risk.[1] Furthermore, seafood is low in *n*-6 fatty acids (FAs), resulting in a high *n*-3/*n*-6 ratio, which is associated with lower risks of developing several chronic diseases.[2,3] Farmed fish traditionally fed diets with high inclusions of fish oil (FO) and fish meal (FM) are now fed diets where significant portions of the dietary marine ingredients are replaced with vegetable ingredients. The resulting product has a different FA composition as compared to FO-fed fish.[4−7] Furthermore, when FM is substituted with plant protein (PP), one has to consider the presence of antinutritional factors (ANFs) and an imbalanced supply of indispensible amino acids.[4,8] When it comes to sensory attributes of vegetable oil (VO)-fed fish, results are contradictory.[9]

When high levels of both FM and FO are replaced with plant feed ingredients, dietary levels of total *n*-3 LC-PUFA [eicosapentaenoic acid (EPA), 20:5*n*-3; and docosahexaenoic acid (DHA), 22:6*n*-3] decrease. Recent findings show that a diet high in both PP and VO with a dietary sum of EPA and DHA of 1.3% increase adiposity and plasma lipids in Atlantic salmon.[10] It was suggested that suboptimal levels of taurine and cystathionine combined with decreased levels of dietary EPA and DHA could explain the accumulation of fat in the Atlantic salmon liver.[10] In the same trial, fish fed a diet high in both PP and VO also showed reduced feed intake and final weight.[6]

Atlantic salmon have endogenous capabilities to produce EPA and DHA when FO is replaced by VO (e.g., refs 11 and 12), but an increased endogenous biosynthesis cannot compensate for the lower dietary supply of EPA and DHA in VO-based feeds (e.g., 13 and 14). When replacing 100% FO with a VO blend in a life cycle trial with Atlantic salmon, EPA and DHA decreased from 1.8 to 0.5 g in a 100 g fillet.[5] Nevertheless, the replacement of wild-caught fish with vegetable alternatives for the production of feed ingredients is necessary. How then can we still obtain fish and seafood products with the desired health beneficial FA composition and sensory qualities when using diets high in VO and PP?

There are several approaches that one can take to reduce the use of FO and FM in the diets and still obtain a satisfactory level of EPA and DHA. The nutritional approach is to use VOs naturally high in α-linolenic acid (ALA; 18:3*n*-3) or stearoidonic acid (SDA; 18:4*n*-3) and low in linoleic acid (LA; 18:2*n*-6). Linseed oil, echium oil, or oils from genetically modified (GM) plants high in SDA[15] will provide enough of the right FA substrate for the production of EPA and DHA. Studies show that SDA is readily converted into eicosatetraenoic acid (20:4*n*-3) and EPA but not DHA in cell lines from turbot (TF line) and salmon (AS line).[16,17] Bioactive compounds such as sesamin, genistein, and lipoic acid can be used to promote the ability of fish to convert ALA into EPA and DHA,[18] and selective breeding for heritable traits associated with EPA and DHA composition has been evaluated.[19]

We know that the conversion of LA and ALA to arachidonic acid (AA; 20:4*n*-6), EPA, and DHA in fish is under nutritional regulation.[20,21] It is found that the bioconversion pathway is inhibited by high dietary LC-PUFA and stimulated by high LA and ALA concentrations.[12] We also know that there are substrate preferences for saturated FAs (SFAs) and monounsaturated FAs (MUFAs) over PUFAs in the β-oxidation pathway and that DHA and EPA are relatively spared from β-oxidation[22,23] when dietary levels of these FAs are low.[24,25] One way to quantify PUFA metabolism in fish is to use the whole-body in vivo FA balance method.[26,27] Recently, it was found that rainbow trout, fed a FO-based diet, consumed EPA and DHA while fish fed a linseed oil diet showed a net production.[28] Stubhaug et al.[25] reported that EPA and DHA were selectively stored when given in low concentrations. Atlantic salmon fed a FO-based diet stored approximately 20% EPA and 30% DHA, whereas VO-fed fish

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Table 1. Proximate Composition (g kg^{-1}) and FA Composition (g kg^{-1}) of the Four Experimental Diets[a] at Two Different Pellet Sizes (4 mm Pellet Size, Period T3, and 6 mm Pellet Size, Periods T8 and T12)

	4 mm diet				6 mm diet			
	FMFO	80PP35VO	40PP70VO	80PP70VO	FMFO	80PP35VO	40PP70VO	80PP70VO
				proximate composition				
crude fat	286	281	286	281	343	318	339	328
crude protein	431	442	433	442	422	424	412	426
ash	108	58	72	58	67	54	65	55
starch	86	97	97	101	91	88	81	87
dry matter	928	939	932	943	923	925	924	932
				FA composition				
14:0	10	6	4	4	19	13	8	8
16:0	32	33	39	36	44	40	49	46
18:0	5	5	6	6	7	6	9	8
total SAT[b]	50	46	52	47	74	62	69	65
18:1*n*-7	7	6	6	6	6	6	7	7
18:1*n*-9	25	53	79	76	28	47	91	83
20:1*n*-9	22	14	8	8	19	14	9	10
20:1*n*-11	4	2	1	1	2	1	1	1
22:1*n*-9	4	3	2	2	3	3	4	4
22:1*n*-11	27	17	8	8	30	20	11	12
total MUFA[c]	107	105	109	105	107	105	131	124
18:2*n*-6	4	19	32	29	7	20	39	36
20:4*n*-6	2	1	1	0	3	1	1	1
total *n*-6	6	20	33	30	10	22	40	38
18:3*n*-3	2	12	22	21	4	14	28	27
20:5*n*-3	20	10	7	6	29	20	10	11
22:6*n*-3	26	13	9	7	38	22	15	13
total *n*-3	58	41	40	36	89	69	60	58
total PUFA[d]	64	61	74	65	100	92	101	96
total *n*-3/*n*-6	10	2	1	1	9	3	2	2
total FA	228	215	236	218	290	264	302	287

[a] FMFO, 100% FM and 100% FO; 80PP35VO, 80% PP and 35% VO blend; 40PP70VO, 40% PP and 80% VO blend; 80PP70VO, 80% PP and 70% VO blend [VO blend: mixture of rapeseed, palm, and linseed oil (55/30/15 v/v/v)]. [b] The sum contains 15:0, 17:0, 20:0, and 22:0. [c] The sum also includes *n*-7, *n*-9, and *n*-11 isomers. [d] The sum also includes 20:2, 16:4, 18:4, 20:4, and 22:5 FAs.

stored 70% EPA and 80% DHA. Although the VO-fed fish had a higher retention as compared to the FO-fed fish, no net production of EPA or DHA was reported.[25] The present paper is part of a series of publications that address the combined effects of both FM and FO replacement in feed for fish, as part of an IP-EU project AQUAMAX (016249-2).[6]

The objective of the present study was to investigate the effects of replacing high levels of both FM and FO with vegetable raw materials and with emphasis on lipid metabolism and fatty acid productive value (FAPV) of ingested FAs.

MATERIALS AND METHODS

Fish Trial. This feeding trial has been described previously in detail.[6] In brief, 6000 Atlantic salmon (*Salmo salar* L.) with a mean weight of 355 ± 92 g were distributed into 12 indoor fiberglass tanks in June 2006 at the Institute of Marine Research (IMR), Matre, Norway. The temperature was kept constant at 8.9 ± 0.1 °C, and oxygen was never less than 80% saturation. The fish were acclimated to the experimental conditions before being fed the experimental diets in triplicate tanks for 12 months: (1) FMFO, a diet with high inclusion of FM and FO; (2) 80PP35VO, a diet with high replacement of FM with PP (80% PP) and one-half the high replacement of FO with VO (35% VO); (3) 40PP70VO, a diet with one-half the high replacement of FM with PP (40% PP) and high replacement of FO with VO (70% VO); and (4) a diet with an estimated safe high replacement of both FM and FO with PP (80% PP and 70% VO) (Table 1). FM was replaced with a mixture of corn gluten, wheat gluten, soy protein concentrate, and krill meal. Capelin oil was used as the main source of *n*-3 LC-PUFA. A mixture of rapeseed, palm, and linseed oils (55/30/15 v/v/v) was used as a replacement for the capelin oil. All diets were stored at −20 °C until they were used for feeding to prevent the oxidation of LC-PUFAs and to protect all other nutrients from degradation. The four experimental diets were fed through the whole experiment with all diets changing in pellet size (from 4 to 6 mm) and lipid content (from ∼28 to ∼33%). There were no new oil sources, but there was an increased dietary sum of EPA/DHA (from 1.3 to 2.4%). All diets were formulated to meet nutrient requirements of fish according to NRC recommendations,[29] and the ingredient compositions of the four diets have been provided earlier.[6] The feed consumption per tank was recorded daily throughout the whole feeding trial, giving accurate estimations of feed intake. Samples were collected in June 2006 (start), September 2006 (*T* = 3 months), February (*T* = 8 months), and June

2007 (T = 12 months). Fish sampled for whole body FA composition and lipid content (T3, 10 pooled fish per tank; T8, 3 pooled fish per tank) were unfed 2 days prior to sampling. Fish sampled for fillet FA composition (T12, 5 pooled fish per tank), β-oxidation capacity (T3, T8, and T12, 5 individual fish per tank), and sensory qualities (T8 and T12, 3 fish per tank) were sampled 6 h after feeding. The finishing diet period was implemented in the experiment from February (T8) to June 2007 (T12), and samples were collected for FA composition and sensory evaluations. Fish from each tank were anesthetized with benzocain (7 g L^{-1}) and killed by a blow to the head except fish sampled for sensory qualities. The experiments complied with the guidelines of the Norwegian Regulation on Animal Experimentation and EC Directive 86/609/EEC, and the protocol was approved by the competent person at the laboratory unit at IMR (Bergen, Norway) and the National Animal Research Authority.

Proximate Composition of the Diets. Nitrogen was determined after total combustion using a Nitrogen-Analyzer (Perkin-Elmer, 2410 Ser. II, Norwalk, CT), and the crude protein content was calculated assuming that proteins contain 16% N. The dietary lipid content was determined gravimetrically as the sum of free and bound fat. Fat was extracted with petroleum ether and dried at 103 ± 1 °C. The samples were thereafter hydrolyzed with HCl in a Tecator Soxtec Hydrolyzing unit to release the bound fat, which was extracted with petroleum ether and dried at 103 ± 1 °C. The dry weight and ash content were determined gravimetrically after freeze drying the samples and dried to constant weight in an oven at 550 °C, respectively. Starch in homogenized samples of feed was determined as previously described.[30]

FA Composition. FA compositions were analyzed in the diets, fillet, and whole fish. The lipid from samples was extracted by adding chloroform/methanol (2:1, v/v), and 19:0 methyl was added as an internal standard. After the extraction of lipids, the samples were filtered, saponified, and methylated using 12% BF_3 in methanol. The FA composition of total lipid was analyzed using methods described by Torstensen et al.[23] where methyl esters were separated using a Trace gas chromatograph 2000 (Fison; "cold on column" injection, 60 °C for 1 min 25 °C/min 160 °C for 28 min 25 °C/min 190 °C for 17 min 25 °C/min 220 °C for 10 min), equipped with a 50 m CP-sil 88 fused silica capillary column (Chromopack; i.d., 0.32 mm; Varian Inc., Palo Alto, CA). The FAs were identified by comparison of their retention times with those of standard mixtures of methyl esters (Nu-Chek, Elyian, United States), and the FA composition (area %) was calculated using the Totalchrom software (version. 6.2, Perkin-Elmer) connected to the GLC (Perkin-Elmer Autosystem, XL). The amount of FA per g tissue was calculated using 19:0 methyl as an internal standard.

***β*-Oxidation Capacity in Tissues.** The total β-oxidation capacities in tissues were determined immediately on fresh tissue by measuring the acid-soluble products after adding [1-^{14}C] palmitoyl-CoA as a substrate to the postnuclear fraction as previously described.[24] Further details of measuring of total β-oxidation capacity in red and white muscle and liver are given in refs 24 and 31. Protein in tissue homogenates was measured using BCA protein assay kit (Pierce Biotechnology Inc., Rockford, IL). All samples were stored at −80 °C until required for analysis.

Sensory Evaluation. Fish were sampled for sensory analysis in February (T8) and June 2007 (T12), that is, before and after the finishing diet period from the dietary groups fed either the marine control diet (FMFO) or the high replacement diet (80PP70VO). After 8 months of feeding (T8), when the fish weight was about 2.5 kg, salmon fed 80PP70VO were switched to the FMFO for a 4 months finishing diet period (FD2). Nine salmon from each dietary treatment at T8 and T12 were chilled in 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 Quality−Descriptive−Analysis, ISO 6564:1985 E, by testing 23 sensory attributes by 11 judges. On arrival, the salmon were 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 vacuumed. 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 trained 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. Six samples were served in each session. For each sensory parameter, the grading is from 1.0 = no intensity up to 9.0 = distinct intensity.

Calculations. The FAPV in whole fish (% FA retained in whole fish of total FA eaten in each dietary period) was calculated for all four dietary groups, and the experiment was divided into two periods (T3, from June to October; and T8, from October to February). The calculation was based on the total dietary FA retained in whole fish as % of total FA eaten per kg of whole fish in each dietary period (see the equation and ref 25).

$$\frac{\text{g FA increase per kg whole fish}}{\text{g FA eaten per kg whole fish}} \times 100\%$$

Statistics. Differences between dietary treatments on FAPV, fillet FA composition, whole fish FA composition, total β-oxidation capacity, and sensory qualities through the feeding experiment were performed using Statistica version 7.0 (StatSoft Inc. Tulsa, OK), and differences between the dietary treatments were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. The total β-oxidation capacity was in addition tested using factorial ANOVA with sampling time and diet as the two categorical factors. The significance level was set to $P \leq 0.05$. A nonparametric Krusal−Wallis test was used in cases where data did not follow the general assumptions of homogeneity of variance and normal distribution.[32]

RESULTS

The fish in this trial had a high growth rate, and the fish body weight increased from ~350 g to almost 4 kg during the experimental period (T12). Within each FA family, there were changes in the dietary FA profile when replacing FO with VO (Table 1). The main changes were increased 18:1*n*-9, LA and ALA and decreased 20:1*n*-9, 22:1*n*-11, EPA and DHA in the three replacement diets. These differences were quantitatively greater in the diets with the higher level of FO replacement (40PP70VO and 80PP70VO). As the pellet size increased, the lipid content of all of the feed increased from 28 to 33%, and the dietary protein level decreased equally. The whole fish lipid content increased from an initial 8% in June to around 12% in September (T3) and around 16% in February (T8), but only significant differences were found in T3 between the high replacement group (80PP70VO) and the three other dietary groups.

The dietary FA composition reflected the FA composition in the whole fish and fillet (Tables 2 and 3). The main effects for all periods were an increased level of 18:1*n*-9, LA and ALA and decreased 20:1*n*-9, 22:1*n*-11, EPA and DHA caused by increased VOs in the diets. There was more than a 3-fold increase in total *n*-6 FAs in the high replacement of FO with VO diets (40PP70VO and 80PP70VO) in all of the experimental periods. The total *n*-3 FAs of fillet were significantly lower in all three replacement diet groups during the whole experimental period as compared to fish fed the control diet, and specifically, the amount of EPA and DHA was around 2-fold lower in fillet and whole fish of fish fed the two diets with high replacement of VOs (40PP70VO and 80PP70VO). All of these changes resulted in a

Table 2. Whole Fish FA Composition (g kg $^{-1}$) and Total Lipid (g kg $^{-1}$) of Atlantic Salmon Fed the Four Experimental Diets for 3 (T3) and 8 Months (T8)[a]

	T3				T8			
	FMFO	80PP35VO	40PP70VO	80PP70VO	FMFO	80PP35VO	40PP70VO	80PP70VO
total lipid[b]	125 ± 2 b	121 ± 3 b	124 ± 4 b	113 ± 4 a	167 ± 5	153 ± 7	170 ± 13	160 ± 8
FA composition								
14:0	4 ± 0 a	3 ± 0 b	3 ± 0 c	3 ± 0 c	7 ± 0 a	4 ± 1 b	3 ± 0 c	4 ± 0 cb
16:0	14 ± 1 ab	14 ± 0 ab	15 ± 1 a	13 ± 0 b	21 ± 1 ab	18 ± 2 b	20 ± 1 ab	22 ± 1 a
18:0	3 ± 0 cd	3 ± 0 bd	3 ± 0 a	3 ± 0 ab	4 ± 0 b	4 ± 1 b	5 ± 0 a	5 ± 0 a
total SAT[c]	22 ± 2	21 ± 1	22 ± 1	20 ± 0	34 ± 1 a	28 ± 4 b	30 ± 2 ab	32 ± 1 a
18:1*n*-7	4 ± 0 a	4 ± 0 a	4 ± 0 a	3 ± 0 b	5 ± 0 a	4 ± 0 b	4 ± 0 b	4 ± 0 b
18:1*n*-9	14 ± 1 d	24 ± 1 c	32 ± 2 a	28 ± 2	20 ± 1 c	33 ± 4 b	48 ± 3 a	53 ± 2 a
20:1*n*-9	10 ± 1 a	8 ± 0 b	6 ± 0 c	5 ± 0 c	12 ± 1 a	8 ± 1 b	6 ± 1 c	7 ± 0 cb
20:1*n*-11	1 ± 0	1 ± 0	0 ± 0	1 ± 0	2 ± 0 a	1 ± 0 b	1 ± 0 b	1 ± 0 b
22:1*n*-9	5 ± 0 a	3 ± 0 b	2 ± 0 c	1 ± 1 d	2 ± 0 a	1 ± 0 b	1 ± 0 b	2 ± 0 a
22:1*n*-11	6 ± 1 a	5 ± 1 b	2 ± 1 c	3 ± 1 c	14 ± 1 a	8 ± 1 b	4 ± 0 c	5 ± 0 c
total MUFA[d]	45 ± 4 b	49 ± 2 ab	51 ± 3 a	45 ± 1 b	67 ± 3 b	61 ± 7 b	69 ± 5 ab	77 ± 3 a
18:2*n*-6	3 ± 0 d	9 ± 1 c	12 ± 1 a	10 ± 1 b	4 ± 0 d	12 ± 1 c	18 ± 1 b	20 ± 1 a
20:4*n*-6	1 ± 0 c	1 ± 0 b	1 ± 0 a	1 ± 0 b	1 ± 0 b	1 ± 0 b	2 ± 0 a	2 ± 0 a
total *n*-6	4 ± 1 d	11 ± 1 c	15 ± 1 a	13 ± 0 b	6 ± 0 d	15 ± 2 c	22 ± 2 b	24 ± 1 a
18:3*n*-3	1 ± 0 d	4 ± 0 c	6 ± 3 a	5 ± 0 b	2 ± 0 c	6 ± 1 b	10 ± 1 a	11 ± 1 a
20:5*n*-3	7 ± 1 a	5 ± 0 b	4 ± 0 c	4 ± 0 c	11 ± 1 a	6 ± 1 b	4 ± 0 c	5 ± 0 c
22:6*n*-3	14 ± 1 a	11 ± 0 b	9 ± 0 c	9 ± 1 c	22 ± 1 a	13 ± 1 b	10 ± 0 c	10 ± 0 c
total *n*-3	27 ± 3 a	24 ± 1 ab	23 ± 1 ab	21 ± 1 b	46 ± 2 a	32 ± 3 b	30 ± 2 b	32 ± 2 b
total PUFA[e]	32 ± 3 b	35 ± 1 ab	38 ± 2 a	35 ± 0 ab	52 ± 2 ab	48 ± 5 b	52 ± 3 ab	57 ± 3 a

[a] The pellet size was adjusted according to fish size from 4 (T3) to 6 mm (T8). Data are presented as means ± SDs; n = 3. Different letters denote significant differences between the dietary groups within each experimental period (T3 and T8). [b] Initial lipid content (g kg $^{-1}$) in whole fish 78 ± 3.5 (given as means ± SDs). [c] The sum contains 15:0, 17:0, 20:0, and 22:0. [d] The sum also includes *n*-7, *n*-9, and *n*-11 isomers. [e] The sum also includes 20:2, 16:4, 18:4, 20:4, and 22:5 FAs.

lower *n*-3/*n*-6 ratio in fillets of all the three groups fed the replacement diets (T12) (Table 3).

In the present study, two different finishing diets (FDs) were given in a cross-over design. Fish fed the FMFO-based diet were switched to 80PP70VO (FD1) and opposite for fish fed 80PP70VO that were switched to FMFO (FD2; Table 3). The fillet FA composition in the FMFO group changed toward that of the 80PP70VO (FD1) and opposite in the 80PP70VO group (FD2) that changed toward that of the FMFO group. The fillet level of LA was 2-fold lower in FD2 compared to 80PP70VO and 3-fold higher in FD1 compared to FMFO. There were no differences in fillet levels of LA between the 2 finishing diet groups. The fillet level of EPA and DHA increased 1.4-fold in FD2 compared to 80PP70VO and decreased 1.3-fold in FD1 compared to FMFO. There was a significantly higher level of EPA and DHA in FD1 compared to FD2.

Generally, the FAPV was high for all dietary groups in the first experimental period (T3) when also the growth rate was high (Table 4). The final period (T8) showed a low FAPV, which correlated with a lower growth rate as compared to the earlier period (T3). The retention of 22:1*n*-11 was low and below 45% in all dietary groups in all experimental periods. The retention of SFAs was high in T3 and low in T8 and with no significant differences in FAPV between the diet groups. The same was observed for MUFAs and for the *n*-6 FAs. Fish fed the high replacement diet (80PP70VO) showed a significantly higher FAPV, 142%, for DHA (T3) as compared to the marine control group, FAPV, 80%. The retention of AA increased significantly from around 80% in the marine group to around 600% in the high replacement group (T3 and T8). There were no significant differences on retention of 18:2*n*-6 between the dietary groups at T3 or T8 although the dietary FA composition varied.

Overall, all three replacement groups showed low retention of 18:3*n*-3 as compared to the marine control group at T3 and T8, while at the same time showing a relatively higher retention of EPA and DHA. There were no significant differences in retention of total PUFA between the groups throughout the whole study. Taken together, in periods when fish showed a good growth rate, there was higher retention of EPA and DHA in groups of fish fed the high replacement diet; 80PP70 V0 (T3) as compared to groups of fish fed diets with higher inclusion levels of FO. There was no net production of any FA at T8, which may be explained by an overall higher β-oxidation capacity of red and white muscle as compared to T3 and T12 (Table 5) or related to an increased dietary sum of EPA and DHA from 1.3 to 2.4% when changing the pellet size.

The β-oxidation capacity was not significantly affected by different inclusion levels of plant raw material in the tissues measured (Table 5). However, large variation within the different dietary groups was observed. A significantly ($p < 0.05$) higher β-oxidation capacity was seen in red and white muscle after 8 months of feeding (T8) as compared to T3 and T12, independent of the feed. This coincided with an overall low

Table 3. Fillet FA Compositions (mg g $^{-1}$ Tissue ww) of Atlantic Salmon Fed the Four Experimental Diets for 12 Months (T12) and after the Finishing Diet Period[a]

	T12				finishing diet (FD) period	
	FMFO	80PP35VO	40PP70 V0	80PP70VO	80PP70VO - FD1	FMFO - FD2
14:0	4 ± 1 a	3 ± 0 b	2 ± 0 c	2 ± 0 c	3 ± 0 b	3 ± 0 b
16:0	13 ± 1 bc	14 ± 1 ac	15 ± 1 a	14 ± 1 ac	13 ± 0 bc	11 ± 1 b
18:0	3 ± 0 d	3 ± 0 b	4 ± 0 a	3 ± 0 b	3 ± 0 d	3 ± 0 d
total SAT[b]	21 ± 2 a	21 ± 1 a	22 ± 2 a	20 ± 2 ab	20 ± 1 ab	18 ± 2 b
18:1*n*-7	3 ± 0 ab	3 ± 0 a	3 ± 0 ab	2 ± 0 b	3 ± 0 a	2 ± 0 b
18:1*n*-9	12 ± 1 e	25 ± 1 c	35 ± 2 a	32 ± 3 b	21 ± 1 d	18 ± 1 d
20:1*n*-9	7 ± 1 a	6 ± 0 b	5 ± 1 c	4 ± 1 c	6 ± 0 ab	5 ± 1 c
20:1*n*-11	1 ± 0 a	1 ± 0 a	0 ± 0 b	0 ± 0 b	1 ± 0 a	1 ± 0 a
22:1*n*-9	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
22:1*n*-11	8 ± 1 a	6 ± 0 b	3 ± 1 d	3 ± 0 d	6 ± 0 b	5 ± 1 c
total MUFA[c]	39 ± 4 d	47 ± 2 ab	51 ± 3 a	46 ± 4 abc	44 ± 1 c	35 ± 3 d
18:2*n*-6	2 ± 0 e	10 ± 0 c	13 ± 1 a	12 ± 1 b	7 ± 1 d	6 ± 0 d
20:4*n*-6	1 ± 0 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	1 ± 0 a	0 ± 0 b
total *n*-6	3 ± 0 d	12 ± 0 b	16 ± 1 a	14 ± 1 a	8 ± 1 c	8 ± 0 c
18:3*n*-3	1 ± 0 e	5 ± 0 c	8 ± 0 a	7 ± 1 b	4 ± 0 d	3 ± 0 d
20:5*n*-3	7 ± 1 a	4 ± 0 bc	3 ± 0 d	3 ± 0 d	5 ± 0 b	4 ± 1 c
22:6*n*-3	15 ± 1 a	11 ± 0 bc	8 ± 1 d	7 ± 1 d	11 ± 0 b	10 ± 1 c
sum *n*-3	30 ± 2 a	26 ± 1 bd	24 ± 2 bcde	21 ± 2 ce	26 ± 1 d	22 ± 2 e
total PUFA[d]	34 ± 3 bc	38 ± 1 ab	40 ± 3 a	36 ± 3 ab	34 ± 2 bc	30 ± 2 c
n-3/*n*-6	9 ± 0 a	2 ± 0 c	2 ± 0 c	2 ± 0 c	3 ± 0 b	3 ± 0 b

[a] Finishing diets were fed for 4 months (T8−T12). Switch from FMFO diet to 80PP70VO diet (finishing diet 80PP70VO; FD 1) and switch from 80PP70VO diet to FMFO diet (finishing diet FMFO; FD 2). Data are presented as means ± SDs; $n = 3$. Different letters denote significant differences between all six dietary groups including the finishing diet period. [b] The sum contains 15:0, 17:0, 20:0, and 22:0. [c] The sum also includes *n*-7, *n*-9, and *n*-11 isomers. [d] The sum also includes 20:2, 16:4, 18:4, 20:4, and 22:5 FAs.

FAPV of FAs (Table 4). In liver, the capacity decreased steadily during the experimental period with a significantly lower capacity at the end of the feeding trial (T12) as compared to T3 and T8. No significant interaction effects of sampling time and feed were found on total β-oxidation capacity in the tissues.

The differences between FMFO and 80PP70VO observed in sensory attributes of flesh (T8) were relatively minor for all attributes except flavor intensity, marine oil odor, and marine oil flavor (Figure 1). These attributes were all higher in fillets of salmon fed FMFO. After the finishing diet period, the significant differences detected had all disappeared (Figure 2).

DISCUSSION

The present work suggests that Atlantic salmon can be assigned as a net producer of LC-PUFA and show specifically that it can produce and retain DHA when 70% of the dietary FO is replaced by a VO blend and 80% of the dietary FM is replaced by a PP blend. Close to 1 g of DHA was produced in salmon fed the high replacement diet when growing from 350 to 750 g (Figure 3). The production of DHA was low or negative in the other dietary groups, possibly related to product inhibition as the relative levels of EPA and DHA were higher in these diets. Results from the present study show that it is possible to affect the retention of specific FAs in Atlantic salmon by replacing FO with a blend of VO. The study also shows that fish fed FMFO retained 50% EPA, while fish fed 80PP70VO retained 80% EPA. A higher retention of DHA as compared to EPA is probably related to their different biological functions. Whereas DHA is preferably stored in the phospholipids in the cell membranes,[11] EPA is also a precursor in the eicosanoid production competing with AA. A remarkably high retention of AA was seen in the high replacement diets, and we know that phosphatidylcholin is the lipid class incorporating the largest amounts of AA, constituting up to 19% of the total FAs in gill, heart, and spleen membranes.[33] Although we obtained FAPV values around 600% in the high replacement group, the absolute concentration of AA in whole fish is generally low and was between 0.1 and 0.2% in the present study.

FAPV is a way of determining how much of a FA is retained in the whole body and is related to the intake of the FA and fish growth. Stubhaug et al.[25] reported that fish fed FO-based diets stored less of the dietary EPA and DHA (20 and 30%), whereas VO-fed fish stored 70% EPA and 80% DHA in periods of fast growth. This trend is similar to results in the present study, however, with an overall higher FAPV reported in the current study. Differences in FAPV between studies may be related to recordings of feed intake. In Stubhaug et al.,[25] Atlantic salmon were kept in net pens; therefore, the feed intake used in the calculations was estimated. During the present feeding trial, all of feed spill was collected and accounted for to correct for an overestimation of the feed intake. Another explanation to the higher FAPV values in the present study (T3) may be related to a lower dietary sum of EPA and DHA in the high replacement diet (1.3%) as compared to the levels in the 100% VO diet (2.4%). Although the apparent digestibility of each FA is not accounted for when calculating FAPV as compared to the whole body FA balance method,[27] the apparent FA digestibility was close to

Table 4. Dietary FAPV in Whole Fish during the Period from June to September (T3) and from November to February (T8) Expressed as % of Retained FA of the Total Amount of FA Eaten by Atlantic Salmon Fed Three Different Replacement Diets[a]

	T3				T8			
	FMFO	80PP35VO	40PP70VO[1]	80PP70VO	FMFO	80PP35VO	40PP70VO	80PP70VO
14:0	60 ± 14	73 ± 5	80 ± 12	79 ± 15	41 ± 9 ab	33 ± 13 b	34 ± 3 b	50 ± 4 a
16:0	69 ± 14	72 ± 4	68 ± 7	63 ± 3	45 ± 10	43 ± 17	38 ± 3	51 ± 2
18:0	86 ± 21	105 ± 12	94 ± 13	93 ± 9	60 ± 9	71 ± 30	59 ± 3	86 ± 4
total SAT[b]	69 ± 14	75 ± 5	72 ± 8	68 ± 5	47 ± 9	44 ± 16	41 ± 3	55 ± 2
18:1*n*-7	92 ± 19	106 ± 16	117 ± 16	108 ± 5	61 ± 17	55 ± 22	46 ± 5	64 ± 3
18:1*n*-9	86 ± 16	85 ± 7	83 ± 6	74 ± 2	72 ± 14	69 ± 24	58 ± 6	76 ± 3
20:1*n*-9	79 ± 15 c	99 ± 4 b	130 ± 10 a	117 ± 9 ab	53 ± 13	62 ± 41	68 ± 9	87 ± 2
20:1*n*-11	24 ± 12	37 ± 9	34 ± 30	60 ± 52	63 ± 30	48 ± 29	49 ± 9	86 ± 7
22:1*n*-9	227 ± 29 ab	232 ± 19 ab	285 ± 24 a	164 ± 72 b	53 ± 13	62 ± 41	68 ± 9	87 ± 2
22:1*n*-11	36 ± 11	43 ± 8	33 ± 17	43 ± 26	42 ± 11	34 ± 13	33 ± 4	45 ± 2
total MUFA[c]	72 ± 15	84 ± 6	88 ± 8	79 ± 3	58 ± 13	55 ± 22	54 ± 6	71 ± 3
18:2*n*-6	92 ± 21	89 ± 9	77 ± 7	74 ± 2	63 ± 10	57 ± 18	48 ± 5	62 ± 4
20:4*n*-6	89 ± 16 a	285 ± 20 b	427 ± 22 c	687 ± 29 d	82 ± 13 a	236 ± 42 b	440 ± 33 c	578 ± 40 d
total *n*-6	97 ± 20	104 ± 10	93 ± 8	92 ± 2	72 ± 12	74 ± 21	64 ± 6	81 ± 5
18:3*n*-3	103 ± 23 a	67 ± 6 b	65 ± 4 b	54 ± 1 b	67 ± 12 a	44 ± 12 b	39 ± 4 b	47 ± 3 b
20:5*n*-3	54 ± 14 b	62 ± 5 ab	69 ± 11 ab	80 ± 15 a	41 ± 11 ab	29 ± 10 ab	28 ± 4 b	44 ± 6 a
22:6*n*-3	80 ± 20 c	109 ± 8 bc	119 ± 7 ab	142 ± 23 a	62 ± 9	58 ± 16	60 ± 5	73 ± 6
sum *n*-3	72 ± 17	88 ± 7	86 ± 8	87 ± 8	57 ± 10	45 ± 14	43 ± 3	56 ± 5
total PUFA[d]	74 ± 17	93 ± 7	89 ± 8	89 ± 4	60 ± 10	53 ± 15	52 ± 4	66 ± 6

[a] Feed pellet size 4 (T3) and 6 mm (T8). Data are presented as means ± SDs; $n = 3$. Different letters denote significant differences between the dietary groups within each experimental period. [b] The sum contains 15:0, 17:0, 20:0, and 22:0. [c] The sum also includes *n*-7, *n*-9, and *n*-11 isomers. [d] The sum also includes 20:2, 16:4, 18:4, 20:4, and 22:5 FAs.

Table 5. Total β-Oxidation Capacity in White and Red Muscle and in Liver after Feeding Atlantic Salmon Three Different Diets Replacing FO and FM and One Control Diet with No Replacement (PP, PP Inclusion; VO, VO Inclusion) for 3 (T3), 8 (T8), and 12 Months (T12)[a]

	FMFO	80PP35VO	40PP70VO	80PP70VO
		white muscle[b]		
T3	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
T8	0.05 ± 0.00	0.05 ± 0.01	0.03 ± 0.02	0.05 ± 0.01
T12	0.01 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.01
		red muscle[c]		
T3	0.7 ± 0.2	0.9 ± 0.3	0.6 ± 0.4	1.0 ± 0.3
T8	1.6 ± 0.2	1.2 ± 0.2	1.2 ± 0.1	1.4 ± 0.2
T12	0.6 ± 0.3	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
		liver[d]		
T3	0.37 ± 0.04	0.38 ± 0.03	0.31 ± 0.10	0.40 ± 0.03
T8	0.35 ± 0.04	0.30 ± 0.08	0.23 ± 0.04	0.26 ± 0.06
T12	0.17 ± 0.04	0.19 ± 0.03	0.18 ± 0.03	0.19 ± 0.03

[a] β-Oxidation capacity is expressed as nmol min^{-1} mg $protein^{-1}$. Data are presented as means ± SEMs; $n = 3$. [b] Signficantly higher β-oxidation capacity in T8 as compared to T3 and T12 independent of diet. [c] Signficantly higher β-oxidation capacity in T8 as compared to T3 and T12 independent of diet. [d] Signficantly higher β-oxidation capacity in T3 as compared to T8 and T12 independent of diet.

100% for EPA and DHA and would not affect the FAPV results if included.[6] Our study is the first to our knowledge to show that Atlantic salmon can be a net producer of DHA. A recent study by Turchini et al.[28] shows that rainbow trout fed a linseed oil diet can produce EPA and DHA with a 2-fold net production in the fillet.

The final FA composition in fish is not only dependent on the FA composition of the diet, as dietary FAs are not directly deposited into fish tissues, but has different metabolic fates such as utilization for energy,[14,31] bioconversion (e.g., refs 13 and 34), and de novo FA production. In addition to the known effects of FO replacement, we also know that PP replacements leading to an imbalanced amino acid profile may increase visceral adipose tissue, but it is not known whether this could affect the retention of FAs in whole fish.[10] Our study indicates that the retention of FAs is mainly affected by FA substrate availability and shows that fish fed on the marine control diet (FMFO) stored less EPA and DHA as compared to the high replacement group. In our study, fish fed the high replacement diet (80PP70VO) retained 142% DHA and 80% EPA as compared to 80 and 50% in the FMFO group in a 3 month period.

After 8 months of feeding, FAPV was low, and the fish consumed 56% of the dietary EPA and 27% of the dietary DHA. How can we explain this switch from net production to net consumption? It could be related to the change in feed pellet from 4 to 6 mm and with a concomitant dietary increase in the sum of EPA and DHA. In the high replacement group (80PP70VO), the dietary sum of EPA and DHA increased from 1.3 to 2.4% in this period. We know that EPA and DHA, when given in high concentrations, are β-oxidized in Atlantic salmon tissues during high growth periods.[25] The highest specific β-oxidation capacity for both white and red muscle was found

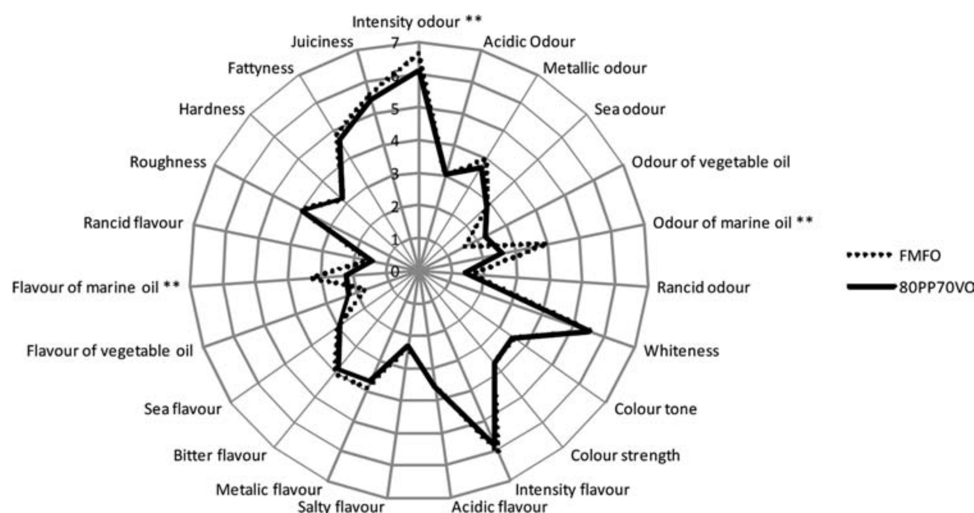

Figure 1. Sensory characteristics score of fillets from Atlantic salmon fed either the control diet (FMFO) or the high replacement diet (80PP70VO) for 8 months (T8) (n = 3). ** indicates significant differences.

Figure 2. Scores of the sensory parameters tested by the trained sensory panel of Atlantic salmon fed FMFO or 80PP70VO for 8 months followed by a 4 month finishing diet period (finishing diet 2; FD2) where all groups were fed FMFO (T12). No significant differences were revealed by one-way ANOVA (n = 3).

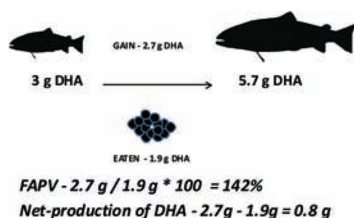

Figure 3. Illustrates data from the trial (T3) for the high replacement diet and shows the content of DHA in whole fish before and after the feeding period. DHA gain is obtained by subtracting the DHA content in whole fish after the feeding period from the DHA content in whole fish before the feeding period. DHA eaten is calculated by using data on feed intake and content of DHA in the feed. FAPV is calculated based on the ratio between DHA gain and DHA eaten (Table 4). Net production of DHA is obtained when the DHA consumption is lower than the gain.

after 8 months of feeding (T8), with no significant differences between the diets, coinciding with an overall low FAPV. Low retention of FA and high β-oxidation capacity indicate that the fish needed the dietary FA to produce energy. This is in accordance to Stubhaug et al.,[25] who also found high β-oxidation and low FAPV values during high growth. Liver had the highest β-oxidation capacity (T3) with no significant differences between the diets and with a steady decrease in FA catabolism with increasing fish size. Similar observations on increasing fish size and decreasing liver β-oxidation capacity have been reported for Atlantic salmon.[14,31,35] It is known that acclimatization to low temperatures may increase the β-oxidation capacity in muscle,[35] but this cannot explain the present increase in red and white muscle β-oxidation capacity as the temperature was kept constant at 8.9 ± 0.1 °C during the whole feeding trial. In the present study, red muscle had the highest specific β-oxidation capacity, whereas white muscle had the lowest, in agreement with previous studies.[14,24] When considering the total mass of these two tissue types, however, white muscle is the largest tissue and hence the most important tissue when it comes to total energy produced by β-oxidation.[11,31]

Despite a change in FA substrate availability in the diets with increased 18:1*n*-9, LA, and ALA and decreased 20:1*n*-9, 22:1*n*-11, EPA, and DHA in the three replacement diets, the β-oxidation capacity was similar between the groups. In a study with Atlantic salmon, a positive correlation between increasing rapeseed oil (RO) in the diet and β-oxidation capacity was found and could be due to the increased level of dietary MUFA in the high RO diets.[24] Despite a 3-fold higher content of 18:1*n*-9 in the two diets with high replacement of VOs (40PP70VO and 80PP70VO) as compared to the control diet, no effect on β-oxidation capacity was found in the present study, which confirms the general conclusion that replacing FO with VO does not significantly affect β-oxidation capacity.[7]

The retention of 18:1*n*-9, LA, and ALA were lower in the 70% VO replacement groups as compared to the FMFO group, although the concentration of these FAs was higher in the three VO replacement diets as compared to the FMFO diet. This indicates that these FAs were used for energy instead of being retained in the fish body. Similar observations have been reported in studies with Atlantic salmon, and here, it was found that MUFA (18:1*n*-9 and 22:1*n*-11) and PUFA (LA and ALA) were oxidized when present in surplus.[25,36] The FA level of 22:1*n*-11 was more than 4-fold higher in the diet (FMFO) as compared to the levels found in whole fish. Despite the high dietary level of 22:1*n*-11, only minor amounts are retained in the phospholipids of fish biomembranes.[33] Previously, it has been found that 22:1*n*-11 is not selectively retained in the fish even at low dietary levels but preferentially oxidized.[25,33] This is well-suited with the present FAPV data, which show low retention of 22:1*n*-11 and 20:1*n*-11 and high apparent digestibility above 95%.[6]

In the first experimental period, when fish doubled their weight (T3), 142% of the ingested DHA was retained in fish fed the high replacement diet, and FAPV was significantly higher as compared to the marine control group. Retention values above 100% indicate bioconversion from ALA into EPA and DHA through alternating succession of desaturation and elongation. As an example, fish fed the high replacement diet (80PP70VO, T3) gained 2.7 g of DHA by eating 1.9 g of DHA resulting in a net production of 800 mg of DHA and a FAPV of 142%. When accounting for the number of fish in this dietary group, more than 1 kg of DHA was produced in this 3 month period. This high FAPV in the 80PP70VO group in T3 coincided with a low retention of ALA. In contrast, fish fed the FMFO diet gained 7.5 g of DHA by eating 9.4 g of DHA resulting in a net loss of 1.9 g of DHA and a FAPV below 100%. The remarkable high retention of AA in the replacement group indicates bioconversion from LA, but the absolute levels of AA were low in the fish tissue. As an example, fish fed the high replacement diet (80PP70VO, T3) gained 0.6 g of AA by eating 0.1 g of AA resulting in a net production of 0.5 g of AA and a FAPV of more than 600%.

Previously, it has been shown that dietary VO enhances the activity of hepatic desaturase and elongase pathway in Atlantic salmon, especially during the seawater stage,[11,24,34,37] and that increased activity correlates with increased mRNA expression of Δ5 and Δ6 desaturase.[38] In the present study, the content of EPA and DHA in whole fish and fillet was approximately 2-fold higher in the FMFO group as compared to the fish fed 70% VO. Hence, a fillet portion of 150 g from salmon fed the high replacement group would provide 1.4 g of EPA and DHA. When considering current recommendations of EPA and DHA intake for healthy individuals,[39] one portion of this fish would provide almost six times the recommended intake of 250 mg per day of sum EPA and DHA.

Although the dietary FA composition was clearly reflected in the fillet and whole fish, the magnitudes of the differences were much lower in the tissues as compared to the differences between the feeds. Specifically, the dietary sum of EPA and DHA was more than 3-fold lower in the two diets with high replacement of VOs (40PP70VO and 80PP70VO) as compared to the marine control diet, but the differences were only 2-fold in the fillet of fish fed these diets (T12). This "dilution" of differences from feed to fillet was not seen for LA; here, the 6-fold difference between the control diet and the VO diets (40PP70VO and 80PP70VO) were reflected in the fillet. It was found previously that LA is not preferentially β-oxidized[5] but follows a dilution model.[40]

During the finishing diet period, the dietary sum of EPA and DHA in the group fed FD2 increased from 9.6 to 13.7 mg g $^{-1}$ fillet but did never reach the levels seen in the group fed only the FMFO diet (21.7 mg g $^{-1}$ tissue). Nevertheless, a fillet portion of 150 g from this group (FD2) would provide 2 g of EPA and DHA. When substituting a significant portion of the oil fraction with VO, the resulting seafood product will not only give a different FA composition but also different sensory qualities as compared to FO-fed fish. Current results of replacing FO and FM correspond to published data where only the FO was replaced by a VO blend (75 and 100%),[5] indicating that it was the dietary oil source rather than the dietary protein source that influenced sensory attributes.

The present work shows that Atlantic salmon can be a net producer of marine protein[6] and marine DHA when dietary FO is replaced by VO. Fish fed the high replacement diet had a net production of 0.8 g of DHA, while salmon fed the marine diet had a net loss of 1.9 g of DHA. The low FAPV seen at the end of the trial may be related to a dietary increase in the sum of EPA and DHA.

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ABBREVIATIONS USED

n-3 LC-PUFA, long-chain polyunsaturated ω-3 fatty acids; FO, fish oil; FM, fish meal; FA, fatty acid; VO, vegetable oil; PP, plant protein; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SDA, stearoidonic acid; ALA, α-linolenic acid; AA, arachidonic acid; LA, linoleic acid; ANFs, antinutritional factors; GM, genetically modified; FAPV, fatty acid productive value; MUFA, monounsaturated

fatty acids; SFA, saturated fatty acids; NIFES, National Institute of Nutrition and Seafood Research Aquamax Sustainable Aquafeeds to Maximise the Health Benefits of Farmed Fish for Consumers; IMR, Institute of Marine Research.

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