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Effect of feeding Atlantic salmon (*Salmo salar* L.) a diet enriched with stearidonic acid from parr to smolt on growth and *n*-3 long-chain PUFA biosynthesis

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Abstract

Vegetable oils (VO) have become the predominant substitute for fish oil (FO) in aquafeeds; however, the resultant lower content of *n*-3 long-chain (≥C20) PUFA (*n*-3 LC-PUFA) in fish has put their use under scrutiny. The need to investigate new oil sources exists. The present study tested the hypothesis that in Atlantic salmon (*Salmo salar* L.), a high intake of stearidonic acid (SDA) from *Echium* oil (EO) would result in increased *n*-3 LC-PUFA biosynthesis due to a lower requirement for Δ6 desaturase. Comparisons were made with fish fed on diets containing rapeseed oil (RO) and FO in freshwater for 112 d followed by 96 d in seawater. EO fish had higher whole-carcass SDA and eicosatetraenoic acid (ETA) in freshwater and prolonged feeding on the EO diet in seawater resulted in higher SDA, ETA, EPA and docosapentaenoic acid (DPA) compared with RO fish. Fatty acid mass balance of freshwater fish indicated higher biosynthesis of ETA and EPA in EO fish compared with fish fed on the other diets and a twofold increase in *n*-3 LC-PUFA synthesis compared with RO fish. In seawater, *n*-3 biosynthetic activity was low, with higher biosynthesis of ETA in EO fish and appearance of all desaturated and elongated products along the *n*-3 pathway. SDA-enriched VO are more suitable substitutes than conventional VO from a human consumer perspective due to the resulting higher SDA content, higher total *n*-3 and improved *n*-3:*n*-6 ratio obtained in fish, although both VO were not as effective as FO in maintaining EPA and DHA content in Atlantic salmon.

Key words: Aquafeeds: Fatty acid mass balance: Stearidonic acid: *n*-3 Long-chain PUFA

Aquaculture has expanded rapidly over the past decades with an average growth rate of 8·8 % per year since 1970 compared with only 1·2 % for capture fisheries[1]. This increase in fish production has led to an increase in aquafeed production concurrent with a greater demand for fish oil (FO) and fishmeal[2,3]. Consequently, the rise in FO demand from aquafeed industries has added further pressure on wild fisheries, which are generally considered to be finite, fully exploited and at times unpredictable due to El Niño events[2,4]. It is predicted that the future needs of the aquaculture industry for FO will outstrip the current supply within the next decade[5]. In addition to the predicted shortfall in FO supply, there has been concern about the levels of dioxins and dioxin-like poly-chlorinated biphenyls in some FO depending on the source fishery[6,7], which presents a potential health hazard. Therefore, the aquaculture industry is faced with a major challenge in finding suitable oil sources for the replacement of FO[3,4].

In an effort to sustain Atlantic salmon aquaculture, a wide variety of commercial vegetable oils (VO) have been investigated as FO replacements[3,8]. The use of VO rarely affects fish growth performance[9–11]. However, the low levels of *n*-3 long-chain (≥C20) PUFA (*n*-3 LC-PUFA), in particular EPA and DHA, in fish fed on VO remain a major shortcoming. Generally with increasing increments of VO in diets, there has been a corresponding decrease in *n*-3 LC-PUFA content in fish. In Atlantic salmon fed on 100 % VO, flesh EPA and DHA levels were reduced to 30 and 35 %, respectively[12], because oils derived from vegetable sources lack *n*-3 LC-PUFA, and the capacity for fish, especially of marine origin, to endogenously biosynthesise *n*-3 LC-PUFA from the VO substrates is limited[13]. In addition, VO are usually characterised by high levels of *n*-6 PUFA and low *n*-3:*n*-6 ratios, hence feeding diets rich in VO has the potential to reduce the important health benefits derived from the consumption

Abbreviations: ALA, α-linolenic acid; DPA, docosapentaenoic acid; EO, *Echium* oil; ETA, eicosatetraenoic acid; FA, fatty acid; FAMB, fatty acid mass balance; FO, fish oil; LC-PUFA, long-chain PUFA; NMB, negative mass balance; RO, rapeseed oil; SDA, stearidonic acid; VO, vegetable oil.

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of *n*-3 LC-PUFA obtained by eating fish and other seafoods[14,15].

So far, there has been only one report of the endogenous biosynthesis of *n*-3 LC-PUFA from the metabolic precursors capable of matching levels of EPA and DHA present in fish fed a FO diet. The flesh of Atlantic salmon parr (in freshwater) fed *Echium* oil (EO) rich in stearidonic acid (SDA; 18 : 4*n*-3), a precursor of EPA, contained comparable levels of EPA and DHA with that obtained using a FO diet[16]. However, in a follow-up trial with smolt, high levels of *n*-3 LC-PUFA usually found in seawater Atlantic salmon fed diets rich in FO were not attained via biosynthesis from precursors in the EO diet[13], though gene expression of Δ5 desaturase and elongase enzymes in the liver was up-regulated[13]. Nutritional history might be important, and the smolts had not been fed SDA-rich diets before the EO diet[13]. In the present study, we have attempted to determine whether feeding EO from parr to smolt would result in increased *n*-3 LC-PUFA biosynthesis. A whole-body fatty acid (FA) mass balance (FAMB) approach[17–19] was used to investigate the metabolism of individual FA along the *n*-3 pathway.

Methods

Experimental diets

In the present study, three diets were formulated to compare rapeseed oil (RO), EO and FO (Table 1). Fishmeal (Skretting Australia, Cambridge, TAS, Australia) was defatted using a mixture of hexane and ethanol (400 ml/100 ml fishmeal). EO was provided as Crossential SA14 (Croda Chemicals, East Yorkshire, UK). FO was from Jack mackerel, *Trachurus symmetricus* L. (Skretting Australia), and a domestic RO was used (Steric Trading Pty Limited, Villawood, NSW, Australia). The diets were manufactured using a California Pellet Mill (CL-2), dried and stored at 5°C[20].

Growth experiment

The experiment was conducted at the University of Tasmania (Launceston, TAS, Australia) in accordance with the University of Tasmania Animal Ethics guidelines (Investigation A0009731). Atlantic salmon (*Salmo salar* L.) parr (approximately 25 g) were obtained from Wayatinah Salmon Hatchery (SALTAS, TAS, Australia) and acclimatised for 14 d in a 3000 litre partial recirculation system. Fish were maintained on the FO diet (Table 1) before starting the experiment.

At the start of the experiment, fish were anaesthetised (benzocaine, 50 mg/l) and weighed, and fork length was measured. For the measurement of initial FA and chemical composition of the whole carcass, eight fish were euthanised (100 mg/l) and stored at −20°C. The experiment used a partial recirculation system, equipped with a protein skimmer and physical, UV and biological filters[10]. Water temperature was kept constant at 15°C with continuous daily replacement of approximately 15% volume. Dissolved O_2, pH, NH_3, nitrate and nitrite were monitored daily to ensure that water quality remained within the parameters recommended for Atlantic salmon[21].

A total of forty-four fish were randomly allocated into each of twelve 300 litre tanks, and the three diets were hand-fed in quadruplicate at a fixed ration of 2·0% body weight/d in two equal rations. Every 2 weeks, feed intake was monitored to adjust the feeding ration. Since experimental fish had missed the natural window for smolting, photoperiod was manipulated following normal commercial procedures

Table 1. Ingredient and lipid composition of Atlantic salmon (*Salmo salar* L.) fed the *Echium* oil (EO), fish oil (FO) and rapeseed oil (RO) diets

	Diet		
	EO	FO	RO
Ingredient composition (g/kg)			
Fishmeal (defatted)*	250	250	250
Casein†	50	50	50
Wheat gluten‡	100	100	100
Soyabean meal§	189	189	189
FO*	0	200	0
RO‖	0	0	200
EO¶	200	0	0
Pre-gel starch**	127	127	127
Vitamin mix††	3	3	3
Mineral mix‡‡	5	5	5
Stay C§§	3	3	3
Choline chloride‖‖	2	2	2
Sipernat¶¶	40	40	40
CMC‖‖	10	10	10
Monobasic calcium phosphate‖‖	20	20	20
Yttrium oxide‖‖	1	1	1
Chemical composition			
DM	911·3	905·0	907·7
Crude protein	342·3	346·8	345·8
Total lipid	213·0	215·9	213·7
Energy (MJ/kg DM)	19·7	19·7	19·7
Lipid composition (g/kg DM)			
Total SFA	24·7	52·9	20·9
Total MUFA	36·8	51·1	123·5
18 : 3*n*-3 ALA	41·0	2·0	14·4
18 : 4*n*-3 SDA	15·7	4·3	0·1
20 : 5*n*-3 EPA	0·3	28·3	0·9
22 : 6*n*-3 DHA	0·4	9·8	0·6
Total *n*-3	57·4	50·3	16·1
18 : 2 *n*-6 LA	36·2	9·4	41·4
18 : 3 *n*-6 GLA	14·6	0·6	0·1
Total *n*-6	51·1	13·0	41·5
Total PUFA	108·5	67·5	57·7

CMC, carboxymethyl cellulose; ALA, α-linolenic acid; SDA, stearidonic acid; LA, linoleic acid; GLA, γ-linolenic acid.
* Skretting Australia, Cambridge, TAS, Australia.
† MP Biomedicals Australasia Pty Limited, Seven Hills, NSW, Australia.
‡ Starch Australasia, Lane Cove, NSW, Australia.
§ Hamlet Protein A/S, Horstens, Denmark.
‖ Croda Chemicals, East Yorkshire, UK.
¶ Steric Trading Pty Limited, Villawood, NSW, Australia.
** Penford Limited, Lane Cove, NSW, Australia.
†† Vitamin mix (ASV4) supplied per kg of feed: 2·81 mg thiamin HCl, 1·0 mg riboflavine, 9·15 mg pyridoxine HCl, 25 mg nicotinic acid, 54·32 mg calcium D-pantothenate, 750 mg myo-inositol, 0·38 mg D-biotin, 2·5 mg folic acid, 0·03 mg cyanocobalamin, 2·8 mg retinol acetate, 0·1 mg cholecalciferol, 250 mg α-tocopherol acetate, 5 mg menadione sodium bisulphate (Sigma-Aldrich, Castle Hill, NSW, Australia) and 100 mg Roche Rovimix E50.
‡‡ Mineral mix (TMV4) supplied/kg of feed: 117 mg $CuSO_4.5H_2O$, 7.19 mg KI, 1815 mg $FeSO_4.7H_2O$, 307 mg $MnSO_4.H_2O$, 659 mg $ZnSO_4.7H_2O$, 329 mg Na_2SeO_3, 47.7 mg $CoSO_4.7H_2O$ (Sigma-Aldrich).
§§ Roche Vitamins Australia, Frenchs Forest, NSW, Australia.
‖‖ Sigma-Aldrich.
¶¶ Degussa, Frankfurt, Germany.

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to trigger smoltification. At 28 d intervals, fish were bulk weighed, and sixteen fish per treatment were weighed, and fork length was measured for the calculation of condition factor (*K*). Based on physical characteristics of fish undergoing parr–smolt transformation such as silvering of the body, loss of parr marks and darkening of fin margins[22], fish were transferred to seawater at 112 d. Before seawater transfer, fish were bulk weighed, and two fish per tank were euthanised (benzocaine 100 mg/l) and stored at −20°C for FA and chemical composition analyses of whole carcass. At seawater transfer, one FO replicate tank had less fish than expected (due to escapees). Data from this tank were omitted from analysis due to the different feeding history. After 7 d in seawater, fish were randomly culled to twenty-four fish per tank, and blood from five fish per treatment was taken from below the anal fin with a heparinised syringe for the measurement of plasma osmolality on a Vapro 5250 vapour pressure osmometer (Wescor® Inc., Logan, UT, USA) to confirm smolt status of fish. Blood plasma osmolality values for all groups (314–358 mOsmol/kg) were within the range considered to be normal for Atlantic salmon smolts[23].

At the end of the experiment (196 d), fish were bulk weighed, and three fish per tank were euthanised (benzocaine 100 mg/l) and stored at −20°C for whole-carcass FA and chemical composition analyses.

Specific growth rate was calculated as specific growth rate (%/d) = 100 × (ln BW_f/ln BW_i)/*d*, where BW_f and BW_i are final and initial wet weights (g), respectively, and *d* is the number of days of the experiment. Feed consumption was calculated as the total average amount of feed (g) consumed/fish over the number of days of the experiment. Feed efficiency ratio was calculated as feed efficiency ratio (g/g) = total weight gain (g)/feed consumption (g). *K* was calculated as *K* (%) = 100 × (BW/FL^3), where FL is the fork length (cm).

Digestibility

Diets included yttrium oxide (1 g/kg) as a digestibility marker[24]. On days 108–111 (freshwater phase), faecal samples from all tanks were collected from faecal settlement collectors (Guelph system) attached to the tanks between 11.00–17.00 and 19.00–09.00 hours[24,25]. At the end of the experiment (seawater phase), fish were stripped for collection of faeces[26]. Faecal samples were freeze-dried before chemical analysis. Apparent digestibility was calculated using the standard formula: apparent digestibility (%) = 100 − (100 (Y_{diet}/Y_{faeces}) × (FA_{faeces}/FA_{diet})), where *Y* is the percentage of yttrium oxide and FA is the percentage of particular FA[27].

Chemical analysis

Standard methods were used to determine DM (freeze dry to constant weight followed by drying at 135°C for 2 h)[28]; total lipid[29], nitrogen (Kjeldahl using Se catalyst; crude protein was calculated as N × 6·25), energy (bomb calorimeter; Gallenkamp Autobomb, Loughborough, Leics, UK, calibrated with benzoic acid) and ash were measured by combustion at 600°C for 2 h[28]. Apart from DM, freeze-dried samples were used for chemical analyses and corrected for DM.

Lipid extraction and isolation

Whole carcass and faecal samples were freeze-dried and extracted overnight using a modified Bligh & Dyer protocol[29]. This involved a single-phase extraction using $CHCl_3$–MeOH–H_2O (1:2:0·8, by vol.), followed by phase separation to yield a total lipid extract.

An aliquot of the total lipid extract was transmethylated in MeOH–$CHCl_3$–HCl (10:1:1, by vol.) for 2 h at 100°C. After addition of Milli-Q water (1 ml), the mixture was extracted with hexane–chloroform (4:1, v/v) to obtain FA methyl esters. Samples with an internal injection standard (19 : 0 FA methyl esters) added were analysed by GC using an Agilent Technologies 7890B GC (Palo Alto, CA, USA) equipped with an Equity™-1 fused silica capillary column (15 m × 0·1 mm internal diameter and 0·1 μm film thickness), a flame ionisation detector, a split/splitless injector and an Agilent Technologies 7683 B Series autosampler. Helium was used as the carrier gas. Samples were injected in splitless mode at an oven temperature of 120°C. After injection, oven temperature was raised to 270°C at 10°C/min and finally to 310°C at 5°C/min. Peaks were quantified with Agilent Technologies ChemStation software. GC results are typically subject to an error of up to ±5 % of individual component area.

Individual components were identified by mass spectral data and by comparing retention time data with authentic and laboratory standards. GC-MS analyses were performed on a Finnigan ThermoQuest GCQ GC-MS fitted with an on-column injector and using ThermoQuest Xcalibur software (Austin, TX, USA). The GC was equipped with an HP-5 cross-linked methyl silicone-fused silica capillary column (50 m × 0·32 mm internal diameter). Helium was used as the carrier gas, with operating conditions as described previously[30].

Fatty acid mass balance

A whole-body FAMB was performed on the *n*-3 biosynthetic pathway to compare individual FA appearance or disappearance and the accretion of individual *n*-3 PUFA as described previously[17].

Statistical analysis

Values are reported as means with their standard errors. Normality and homogeneity of variance were confirmed, and the percentage data were arcsine-transformed before analysis. Comparison between treatments for FA, growth performance, osmolality, *K* and mass balance means was done by one-way ANOVA, followed by multiple comparison using Tukey–Kramer's honestly significant difference wherever applicable. Significance was accepted at $P \leq 0{\cdot}05$. Statistical analysis was performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL, USA).

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Results

Parr–smolt transformation

There were no significant differences ($P \geq 0{\cdot}05$) in blood plasma osmolality of fish fed on all diets 7 d post-transfer to seawater. Mean *K* for all groups was similar during the short-day period, but shortly after switching to continuous light, RO and EO fish displayed higher *K* compared with FO fish before seawater transfer ($P < 0{\cdot}05$; Fig. 1). After seawater transfer, elevated *K* was observed particularly for RO fish. The pattern was similar for all groups and was typical for smolting fish, with a steady drop in *K* after onset of continuous light, which was carried on for 28 d in seawater before increasing thereafter.

Growth

Growth (final weight, weight gain and specific growth rate) and efficiency were higher ($P < 0{\cdot}05$) for RO fish compared with EO fish in freshwater, whereas FO fish did not differ significantly ($P \geq 0{\cdot}05$) in performance and efficiency from the other two treatments. Feed consumption and survival were not significantly different ($P \geq 0{\cdot}05$) between treatments (Table 2).

Growth (final weight, weight gain and specific growth rate) was higher for RO fish compared with fish fed on the EO and FO diets in seawater, with no difference ($P \geq 0{\cdot}05$) in growth performance for EO and FO fish (Table 2). Survival was higher in RO fish compared with FO and EO fish.

Chemical composition

During the freshwater phase, feeds had no significant effect on carcass DM (pooled mean 326·1 (SEM 2·5) g/kg), crude protein (159·5 (SEM 1·1) g/kg, w/w), total lipid (124·4 (SEM 3·4) g/kg, w/w) or ash (26·3 (SEM 0·5) g/kg, w/w). During the seawater phase, feeds had no significant effect on carcass crude protein

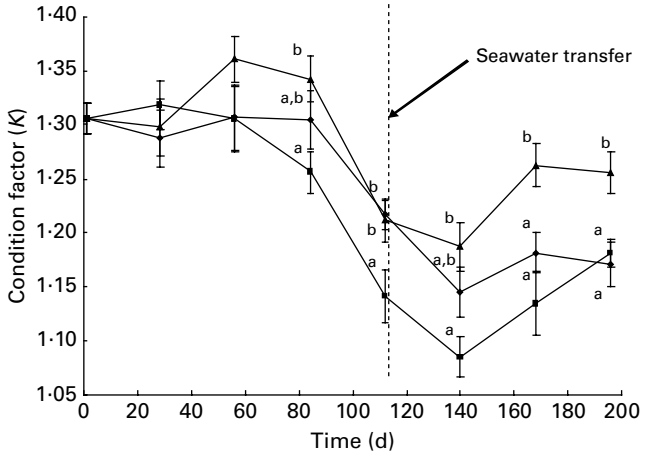

Fig. 1. Condition factor (*K*) of Atlantic salmon (*Salmo salar* L.) fed on different oil sources from parr to smolt. Values are means representing *K* of Atlantic salmon fed diets containing *Echium* oil (—◆—), fish oil (—■—) and rapeseed oil (—▲—), with standard errors represented by vertical bars (*n* 16). [a,b] Mean values with unlike letters at each time interval were significantly different in *K* between diets ($P < 0{\cdot}05$).

(pooled mean 146·6 (SEM 1·0) g/kg, w/w), total lipid (120·2 (SEM 2·7) g/kg, w/w) or ash (25·4 (SEM 0·4) g/kg, w/w). Significant differences for carcass DM ($P < 0{\cdot}05$) were obtained (EO 314·4 (SEM 3·3); FO 320·8 (SEM 4·6) and RO 328·0 (SEM 3·6)), with EO fish having a lower DM than RO fish.

FO fish had higher whole-carcass EPA and DHA content than either EO or RO fish in freshwater. EO fish had significantly higher ($P < 0{\cdot}01$) SDA, α-linolenic acid (ALA; 18 : 3*n*-3) and eicosatetraenoic acid (ETA; 20 : 4*n*-3) compared with fish fed on the other two diets (Table 3). Total *n*-3 for EO fish was comparable with FO fish, and total PUFA was higher for EO fish compared with FO fish, whereas for RO fish, both total *n*-3 and total PUFA were lowest ($P < 0{\cdot}01$). The *n*-3:*n*-6 ratios for FO, EO and RO fish were in the order FO > EO > RO.

FO fish had higher whole-carcass EPA and DHA content than either EO or RO fish in seawater. EO fish had significantly higher ($P < 0{\cdot}01$) ALA, SDA and ETA compared with fish fed on the other two diets (Table 4). There was significantly higher ($P < 0{\cdot}05$) EPA and docosapentaenoic acid (DPA; 22 : 5*n*-3) in EO fish compared with RO fish. Total *n*-3 and total PUFA were higher ($P < 0{\cdot}01$) for EO fish compared with FO and RO fish. The *n*-3:*n*-6 ratios for FO, EO and RO fish were in the order FO > EO > RO. Total FA was significantly higher ($P < 0{\cdot}05$) in RO fish compared with EO and FO fish.

Fatty acid mass balance

Biosynthesis of ETA and EPA was higher ($P < 0{\cdot}05$) for EO fish compared with both RO and FO fish and with no difference in the biosynthesis of DPA among all groups in freshwater (Table 5). DHA showed a positive mass balance, which was not different between EO and RO fish. The main negative mass balance (NMB) in EO fish was for ALA, which represented 69 % of the net intake (Table 5) followed by SDA (42 %). Total elongated/desaturated *n*-3 LC-PUFA products (ETA + EPA + DPA + DHA = 1249 μmol/fish) represented 15 % of the combined NMB for ALA and SDA in EO fish (8414 μmol/fish). There was a NMB of 2327 μmol/fish for ALA in RO fish, which represented 63 % of net intake (Table 5). A NMB of 1096 μmol/fish (47 %) for ALA was obtained in RO fish as a result of elongation and desaturation, out of which SDA biosynthesis represented 42 % (464 μmol/fish). Total elongated/desaturated *n*-3 LC-PUFA products (ETA + EPA + DPA + DHA) in RO fish were 632 μmol/fish. In FO fish, the main NMB was for EPA, with a disappearance of about 66 % of net intake (4264 μmol/fish), mainly due to β-oxidation as only 228 μmol/fish were used for the biosynthesis of DPA.

Higher biosynthesis of ETA ($P < 0{\cdot}05$) occurred in EO fish compared with RO and FO fish in seawater. There were no significant differences ($P > 0{\cdot}05$) in EPA, ETA and DHA between EO and RO fish, but negative values of EPA, ETA and DHA were obtained for RO fish (Table 5). In contrast, there was a positive mass balance of all *n*-3 LC-PUFA along the *n*-3 pathway for EO fish (Table 5).

There was a NMB of 12 836 and 3472 μmol/fish for ALA and SDA, respectively, for EO fish in seawater accounting

Table 2. Growth and efficiencies of Atlantic salmon (*Salmo salar* L.) fed the *Echium* oil (EO), fish oil (FO) and rapeseed oil (RO) diets
(Mean values with their standard errors, *n* 3, 4 and 4, respectively)

		Initial weight (g)		Final weight (g)		Weight gain (g)		SGR (%/d)		FC (g)		FER (g/g)		Survival (%)	
	Diet*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Freshwater	EO	26·1	0·5	96·3^{a}	0·9	70·2^{a}	0·9	1·2^{a}	0·0	73·5	1·1	1·0^{a}	0·0	93·8	1·4
	FO	25·7	0·3	103·5^{a,b}	2·7	77·9^{a,b}	2·8	1·2^{a,b}	0·0	76·8	1·5	1·0^{a,b}	0·0	94·7	0·8
	RO	26·4	0·2	109·4^{b}	3·0	83·1^{b}	3·0	1·3^{b}	0·0	79·1	1·6	1·1^{b}	0·0	95·5	0·0
Seawater	EO	96·3^{a}	0·9	204·4^{a}	2·4	108·1^{a}	1·8	0·9^{a}	0·0	134·6^{a}	4·3	0·8	0·0	81·3^{a}	1·2
	FO	103·5^{a,b}	4·3	223·0^{a}	11·0	119·5^{a}	6·7	0·9^{a}	0·0	143·2^{a,b}	2·0	0·8	0·0	81·9^{a}	2·1
	RO	109·4^{b}	3·0	252·8^{b}	8·7	143·4^{b}	5·9	1·0^{b}	0·0	159·7^{b}	5·3	0·9	0·0	92·7^{b}	2·6

SGR, specific growth rate; FC, feed consumption; FER, feed efficiency ratio.
^{a,b} Mean values within a column with unlike superscript letters belonging to either freshwater or seawater were significantly different ($P<0·05$).
* See Table 1 for diet definitions.

for 73 and 51 % of net intakes. Total elongated/desaturated products of *n*-3 LC-PUFA (ETA + EPA + DPA + DHA) were 696 μmol/fish for EO fish representing only 4 % of combined NMB for ALA and SDA. There was a NMB of 74 % (5468 μmol/fish) for ALA net intake from RO fish, with only 13 % (689 μmol/fish) elongated/desaturated along the pathway largely as SDA (633 μmol/fish). There was no positive mass balance of FA for FO fish in seawater, and the main NMB was for EPA (79 % net intake) followed by DHA (52 % net intake).

Higher accretion of Δ5 desaturated and elongated FA ($P<0·05$) in EO fish compared with RO fish was observed in freshwater. The accretion of elongated and desaturated FA could not be computed for RO and FO fish in seawater as a result of high NMB along the *n*-3 pathway.

Table 3. Fatty acid (FA) content (mg/g) of whole carcasses of Atlantic salmon (*Salmo salar* L.) parr fed the *Echium* oil (EO), fish oil (FO) and rapeseed oil (RO) diets*
(Mean values with their standard errors)

	Initial		EO		FO		RO		
FA	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*f*
14 : 0	11·3^{b}	0·3	1·9^{a}	0·2	9·6^{b}	0·8	1·9^{a}	0·2	100·5
16 : 0	38·8^{b}	0·6	24·7^{a}	0·6	40·4^{b}	1·5	22·4^{a}	1·1	68·8
18 : 0	11·4^{a,b}	0·0	13·0^{b}	0·0	11·7^{b}	0·5	9·4^{a}	0·4	18·1
Other SFA†	5·1^{b}	0·1	1·3^{a}	0·2	4·8^{b}	0·3	1·7^{a}	0·2	59·1
Total SFA	66·5^{b}	1·2	40·9^{a}	1·1	66·5^{b}	2·6	35·5^{a}	1·5	81·7
16 : 1*n*-7*c*	21·6^{b}	0·3	5·8^{a}	0·3	21·1^{b}	0·7	5·7^{a}	0·5	295·2
18 : 1*n*-9*c*	41·8^{a}	1·4	44·3^{a}	1·0	35·6^{a}	1·0	107·7^{b}	5·1	111·7
18 : 1*n*-7*c*	11·1^{b}	0·2	4·7^{a}	0·1	10·8^{b}	0·4	9·7^{b}	0·4	88·4
20 : 1*n*-9*c*	7·8^{b}	0·7	3·6^{a}	0·2	4·1^{a}	0·2	4·7^{a}	0·2	27·5
Other MUFA‡	7·9^{c}	0·3	2·7^{a}	0·2	5·4^{b}	0·2	3·3^{a}	0·3	46·2
Total MUFA	90·2^{b}	2·3	61·0^{a}	1·5	77·0^{a,b}	1·8	131·1^{c}	5·8	68·8
18 : 3*n*-3 ALA	0·5^{a}	0·3	24·7^{c}	1·1	2·3^{a}	0·3	9·9^{b}	0·6	169·3
18 : 4*n*-3 SDA	3·4^{a}	0·4	18·5^{b}	1·0	5·1^{a}	0·2	4·2^{a}	0·3	120·3
20 : 4*n*-3 ETA	2·6^{b}	0·3	3·1^{b}	0·2	1·2^{a}	0·3	1·1^{a}	0·1	27·6
20 : 5*n*-3 EPA	12·6^{b}	1·5	5·9^{a}	0·4	21·8^{c}	1·0	4·5^{a}	0·3	151·5
22 : 5*n*-3 DPA	5·4^{b}	0·5	2·8^{a}	0·2	8·9^{c}	0·4	1·7^{a}	0·3	120·7
22 : 6*n*-3 DHA	17·8^{b}	1·2	8·6^{a}	0·7	20·4^{b}	0·8	7·4^{a}	0·5	78·5
Other *n*-3§	1·0^{b}	0·0	0·0^{a}	0·0	1·5^{c}	0·1	0·1^{a}	0·1	212·7
Total *n*-3	43·5^{b}	4·3	63·6^{c}	2·9	61·2^{c}	2·6	28·9^{a}	1·4	46·9
18 : 2*n*-6 LA	7·7^{a}	0·9	31·4^{b}	1·1	11·1^{a}	0·5	31·1^{b}	1·2	114·5
18 : 3*n*-6 GLA	0·0^{a}	0·0	11·2^{c}	0·5	0·0^{a}	0·0	3·0^{b}	0·2	227·3
20 : 3*n*-6	0·0^{a}	0·0	3·5^{c}	0·1	0·1^{a}	0·1	2·3^{b}	0·1	122·3
20 : 4*n*-6 ARA	1·6^{b}	0·1	0·9^{a}	0·1	2·2^{c}	0·1	1·2^{a,b}	0·1	28·7
Other *n*-6‖	0·9	0·1	0·2	0·1	0·4	0·2	0·8	0·3	1·2
Total *n*-6	10·3^{a}	0·9	47·1^{c}	1·7	13·9^{a}	0·6	38·5^{b}	1·6	121·1
Other PUFA¶	1·7^{b}	0·2	0·2^{a}	0·1	2·6^{c}	0·1	0·2^{a}	0·1	98·2
Total PUFA	55·5^{a}	5·4	110·9^{c}	4·5	77·7^{b}	3·1	67·5^{a,b}	2·3	39·7
n-3:*n*-6	4·2^{c}	0·0	1·3^{b}	0·0	4·4^{c}	0·2	0·8^{a}	0·0	273·5
Total FA	212·2	3·3	212·8	6·4	221·2	4·6	234·1	8·9	1·1

f, Mean sum of squares; ALA, α-linolenic acid; SDA, stearidonic acid; ETA, eicosatetraenoic acid; DPA, docosapentaenoic acid; LA, linoleic acid; GLA, γ-linolenic acid; ARA, arachidonic acid.
^{a,b,c} Mean values across the row with unlike superscript letters were significantly different as determined by Tukey–Kramer's honestly significant difference (df = 3, $P<0·01$).
* See Table 1 for diet definitions.
† Includes 15 : 0, 17 : 0, 20 : 0, 22 : 0 and 24 : 0.
‡ Includes 16 : 1*n*-9, 16 : 1*n*-5, 18 : 1*n*-5, 20 : 1*n*-7, 22 : 1*n*-9, 22 : 1*n*-11 and 24 : 1*n*-9.
§ Includes 21 : 5*n*-3 and 24 : 6*n*-3.
‖ Includes 20 : 2*n*-6, 22 : 4*n*-6 and 24 : 5*n*-6.
¶ Includes 16 : 2*n*-4, 16 : 3*n*-4 and 18 : 2*n*-9.

Table 4. Fatty acid (FA) content (mg/g) of whole carcasses of Atlantic salmon (*Salmo salar* L.) smolt fed the *Echium* oil (EO), fish oil (FO) and rapeseed oil (RO) diets†
(Mean values with their standard errors)

	EO		FO		RO		
FA	Mean	SEM	Mean	SEM	Mean	SEM	*f*
14 : 0	1·1[a]	0·1	6·4[b]	0·7	1·3[a]	0·1	64·9
16 : 0	22·1[a]	0·1	33·6[b]	0·9	19·1[a]	0·5	99·0
18 : 0	12·5[b]	0·4	9·2[a]	0·3	8·6[a]	0·2	45·5
Other SFA‡	0·9[a]	0·1	4·0[c]	0·3	2·2[b]	0·1	73·7
Total SFA	36·8[b]	1·3	53·3[c]	1·5	31·2[a]	0·8	80·4
16 : 1*n*-7*c*	2·8[a]	0·2	16·9[b]	0·4	3·2[a]	0·2	776·8
18 : 1*n*-9*c*	45·5[b]	1·3	33·4[a]	1·5	123·4[c]	2·0	870·2
18 : 1*n*-7*c*	3·6[a]	0·1	9·6[b]	0·3	10·0[b]	0·2	252·5
20 : 1*n*-9*c*	2·8[a]	0·1	5·1[b]	0·5	4·6[b]	0·2	17·6
Other MUFA§	2·0[a]	0·2	6·5[b]	0·6	1·5[a]	0·2	55·8
Total MUFA	56·7[a]	1·7	71·4[b]	3·0	142·6[c]	2·4	420·5
18 : 3*n*-3 ALA	31·1[c]	1·4	2·5[a]	0·3	10·4[b]	0·2	248·7
18 : 4*n*-3 SDA	22·6[b]	1·4	4·3[a]	0·2	4·1[a]	0·1	146·3
20 : 4*n*-3 ETA	3·0[b]	0·2	0·8[a]	0·2	0·6[a]	0·1	70·4
20 : 5*n*-3 EPA	4·5[b]	0·1	21·5[c]	1·0	2·6[a]	0·1	405·6*
22 : 5*n*-3 DPA	1·7[b]	0·2	7·9[c]	0·6	0·6[a]	0·2	136·2
22 : 6*n*-3 DHA	5·1[a]	0·2	17·9[b]	0·5	4·1[a]	0·2	575·5
Other *n*-3‖	0·0[a]	0·0	1·2[b]	0·2	0·0[a]	0·0	78·1
Total *n*-3	68·0[c]	3·3	56·1[b]	2·5	22·5[a]	0·5	105·2
18 : 2*n*-6 LA	38·2[b]	1·4	10·3[a]	0·3	39·8[b]	0·5	266·0
18 : 3*n*-6 GLA	13·9[c]	0·7	0·1[a]	0·1	3·8[b]	0·1	256·8
20 : 3*n*-6	3·7[c]	0·2	0·0[a]	0·0	2·8[b]	0·1	159·1
20 : 4*n*-6 ARA	0·4[a]	0·1	2·1[c]	0·1	1·1[b]	0·1	46·3
Other *n*-6¶	0·5[a]	0·1	0·4[a]	0·1	1·1[b]	0·2	8·1
Total *n*-6	56·7[c]	2·4	12·9[a]	0·6	48·7[b]	0·6	188·3
Other PUFA**	0·0[a]	0·0	2·0[b]	0·1	0·0[a]	0·0	364·4
Total PUFA	124·7[b]	5·7	71·0[a]	3·1	71·1[a]	0·8	65·6
n-3:*n*-6	1·2[b]	0·0	4·4[c]	0·1	0·5[a]	0·0	788·5
Total FA	218·2[a]	8·1	195·7[a]	6·5	245·0[b]	3·7	14·2*

f, Mean sum of squares; ALA, α-linolenic acid; SDA, stearidonic acid; ETA, eicosatetraenoic acid; DPA, docosapentaenoic acid; LA, linoleic acid; GLA, γ-linolenic acid; ARA, arachidonic acid;
[a,b,c] Mean values across the row with unlike superscript letters were significantly different as determined by Tukey–Kramer's honestly significant difference (df = 3, $P<0·01$, $^*P<0·05$).
† See Table 1 for diet definitions.
‡ Includes 15 : 0, 17 : 0, 20 : 0, 22 : 0 and 24 : 0.
§ Includes 16 : 1*n*-9, 16 : 1*n*-5, 18 : 1*n*-5, 20 : 1*n*-7, 22 : 1*n*-9, 22 : 1*n*-11 and 24 : 1*n*-9.
‖ Includes 21 : 5*n*-3 and 24 : 6*n*-3.
¶ Includes 20 : 2*n*-6, 22 : 4*n*-6 and 24 : 5*n*-6.
** Includes 16 : 2*n*-4, 16 : 3*n*-4 and 18 : 2*n*-9.

Discussion

Growth and parr–smolt transformation

The transfer and growth of fish in seawater were key elements in the present study, and both the *K* (Fig. 1) and osmolality values for all groups were indicative of smolted fish. Feeding Atlantic salmon a diet of 100 % RO had a positive impact on growth over the duration of the experiment. Alterations in lipid metabolism are regarded as an integral part of the parr–smolt transformation, and a VO diet might be better suited for Atlantic salmon to adapt to seawater since its FA composition more closely resembles those of fish from the wild[31]. The importance of dietary VO during smoltification might be multiple: higher osmoregulatory capacity[32,33], increased growth[34] and acting as a protection barrier against translocation of pathogens[35]. In agreement with previous findings on the beneficial effects of VO on fish undergoing smoltification, RO fish showed higher growth and survival, indicating that RO fish were better prepared for transition in seawater than EO and FO fish. To the best of our knowledge, in all previous studies involving complete substitution of FO by VO in Atlantic salmon undergoing parr–smolt transformation, fishmeal contributed some *n*-3 LC-PUFA to the diet, with EPA and DHA composition ranging from 1 to 4 % of total FA[32–34]. In the present study, defatted fishmeal was used, and only trace amounts of *n*-3 LC-PUFA were present in the VO diets, with EPA and DHA composition ranging from 0·2 to 0·5 % of total FA (0·3–0·9 g/kg; Table 1). An important finding of the present study in relation to parr–smolt transformation was that fish fed exclusively on the VO diets (EO or RO) successfully smolted. This finding could prove to be important for feed formulation in the context of FO substitution in Atlantic salmon in freshwater and also extends to fishmeal replacement in the freshwater life cycle of Atlantic salmon.

n-3 Fatty acid metabolism – freshwater phase

Our prime objective was to test whether feeding Atlantic salmon a diet rich in SDA from parr to smolt would result in

Table 5. Fatty acid mass balance (μmol/fish) for whole carcasses of Atlantic salmon (*Salmo salar* L.) fed the *Echium* oil (EO), fish oil (FO) and rapeseed oil (RO) diets*
(Mean values with their standard errors, *n* 6 and *n* 9 for FO in freshwater and seawater, respectively, *n* 8 and *n* 12 for EO and RO in freshwater and seawater, respectively)

		ALA (18 : 3*n*-3)		SDA (18 : 4*n*-3)		ETA (20 : 4*n*-3)		EPA (20 : 5*n*-3)		DPA (22 : 5*n*-3)		DHA (22 : 6*n*-3)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Freshwater	Net intake												
	EO	9813·0^{c}	144·5	3792·4^{c}	55·8	13·7^{b}	0·2	54·7^{a}	0·8	0·0^{a}	0·0	72·8^{a}	1·1
	FO	498·8^{a}	10·0	1089·8^{b}	21·8	272·5^{c}	5·4	6504·1^{b}	129·8	705·5^{b}	14·1	2067·9^{b}	41·3
	RO	3697·4^{b}	75·8	34·6^{a}	0·7	0·0^{a}	0·0	217·9^{a}	4·5	0·0^{a}	0·0	130·5^{a}	2·7
	Accumulation												
	EO	3006·2^{c}	199·6	2185·4^{b}	116·4	282·8^{b}	26·0	369·4^{a}	55·5	263·4^{a}	20·3	474·7^{a}	73·2
	FO	280·2^{a}	48·2	555·3^{a}	46·0	69·8^{a}	56·3	2240·2^{b}	200·2	933·1^{b}	64·4	1783·8^{b}	155·0
	RO	1370·7^{b}	74·0	498·9^{a}	46·8	72·2^{a}	15·0	247·7^{a}	46·3	200·3^{a}	18·6	459·4^{a}	80·9
	Appearance/disappearance												
	EO	−6806·8^{a}	151·7	−1607·0^{a}	110·7	269·1^{c}	25·0	314·7^{c}	55·6	263·4^{a}	20·3	401·8^{b}	72·9
	FO	−218·7^{c}	45·3	−534·5^{b}	29·4	−202·7^{a}	51·7	−4264·0^{a}	119·0	227·6^{a}	66·2	−284·1^{a}	143·7
	RO	−2326·8^{b}	120·9	464·4^{c}	47·5	72·2^{b}	15·0	29·7^{b}	50·6	200·3^{a}	18·0	328·9^{b}	82·9
Seawater	Net intake												
	EO	17 514·7^{c}	599·6	6808·5^{c}	233·1	0·0^{a}	0·0	104·0^{a}	3·6	0·0^{a}	0·0	140·1^{a}	4·8
	FO	907·4^{a}	7·4	1973·5^{b}	16·0	470·5^{b}	3·8	11 725·3^{c}	95·2	1276·4^{b}	10·4	3579·4^{c}	29·1
	RO	7398·0^{b}	243·5	69·8^{a}	2·3	0·0^{a}	0·0	446·1^{b}	14·7	0·0^{a}	0·0	266·8^{b}	8·8
	Accumulation												
	EO	4678·9^{c}	518·3	3336·1^{b}	538·2	337·2^{b}	39·4	330·9^{a}	42·3	112·6^{a}	30·2	159·7^{a}	79·5
	FO	356·1^{a}	107·5	455·6^{a}	97·4	63·9^{a}	50·6	2485·5^{b}	449·5	725·3^{b}	250·0	1705·1^{b}	348·2
	RO	1930·4^{b}	180·8	702·4^{a}	96·4	56·0^{a}	28·9	182·4^{a}	79·5	−72·5^{a}	35·9	208·3^{a}	89·7
	Appearance/disappearance												
	EO	−12 835·9^{a}	676·1	−3472·3^{a}	573·8	337·2^{c}	39·4	226·9^{c}	39·6	112·6^{b}	30·2	19·6^{b}	75·5
	FO	−551·3^{c}	101·6	−1517·8^{b}	86·9	−406·6^{a}	47·2	−9239·8^{a}	382·0	−551·1^{a}	241·6	−1874·3^{a}	320·5
	RO	−5467·6^{b}	104·8	632·6^{c}	94·2	56·0^{b}	28·9	−263·7^{b}	68·1	−72·5^{b}	35·9	−58·5^{b}	85·7

ALA, α-linolenic acid; SDA, stearidonic acid; ETA, eicosatetraenoic acid; DPA, docosapentaenoic acid.
[a,b,c] Mean values within a column with unlike superscript letters belonging to either freshwater or seawater were significantly different ($P<0·05$).
* See Table 1 for diet definitions.

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higher biosynthesis of *n*-3 LC-PUFA. In freshwater, EO fish had higher ETA compared with fish fed the other diets (Table 3). Atlantic salmon parr towards the end of the freshwater period were at an important phase in their life cycle, preparing for the transfer to seawater. This critical period for the fish is accompanied by an increase in desaturation and elongation activities along both the *n*-3 and *n*-6 pathways for the production of LC-PUFA[32,33]. Therefore, if provided with enough substrate (ALA), fish can meet their EPA and DHA requirements as observed in RO fish, and the presence of SDA in the EO diet, which is a precursor for EPA and allows bypassing of the initial Δ6 desaturase enzyme[13,16], has enabled higher biosynthetic activity along the *n*-3 LC-PUFA pathway resulting in an increase in ETA. In a previous study on Atlantic salmon parr fed an EO diet, comparable levels of EPA and DHA in muscle were obtained for FO fish[16]. In the present study, we did not obtain a similar result. However, in the present study, the *K* value was higher because the diet contained 200 g/kg total lipids compared with 129 g/kg in the previous study. Therefore, due to the lower dietary lipid level, there was a reduction in TAG proportion relative to polar lipids in the white muscle, which might explain the retention of DHA[16]. Furthermore, the previous study was conducted over a short duration (42 d), which coincided with the critical parr–smolt transformation period characterised by an increase in desaturation and elongation activities.

A point of focus was the biosynthetic activity along the *n*-3 pathway, and the FAMB approach was used to verify our hypothesis. For EO fish, ETA and EPA biosynthesis was higher compared with RO fish due to the presence of high SDA (9·2 % of total FA) in the EO diet. In contrast, for RO fish, ALA had to be desaturated to SDA, adding an extra step along the pathway at the cost of 42 % of total elongation and desaturation products. As a result, there was a twofold increase in total *n*-3 LC-PUFA biosynthesis (1249 *v.* 631 μmol/fish) in EO fish. There was no difference in DHA biosynthesis between EO and RO fish, hereby underlying the importance of DHA in fish undergoing smoltification and life in seawater. Previous research has demonstrated that in Atlantic salmon undergoing parr–smolt transformation, there was an increase in DHA in gill and liver polar lipids in fish fed on VO diets[32,33]. In other studies[36,37], there were preferential deposition and retention of DHA in muscle lipids irrespective of the concentration in the diet, which was attributed to the specificity of fatty acyl transferase enzymes towards incorporation of DHA into flesh TAG and polar lipids.

FO fish in freshwater showed some degree of biosynthetic activity especially for DPA, and while we do not neglect the fact that EPA and DHA could have been produced, it might have been masked by the high dietary presence of these FA. This masking is regarded as a shortcoming associated with the FAMB approach[17,19,38]. Consequently, when computing the last step of the method, it was not possible to detect any increment of FA as a result of Δ5 and Δ6 desaturases, and elongase enzymes except for the conversion of EPA to DPA (Fig. 2) with a positive mass balance obtained for DPA. Other limitations may occur; the computation of the FAMB proceeds only in the direction of its specific pathway,

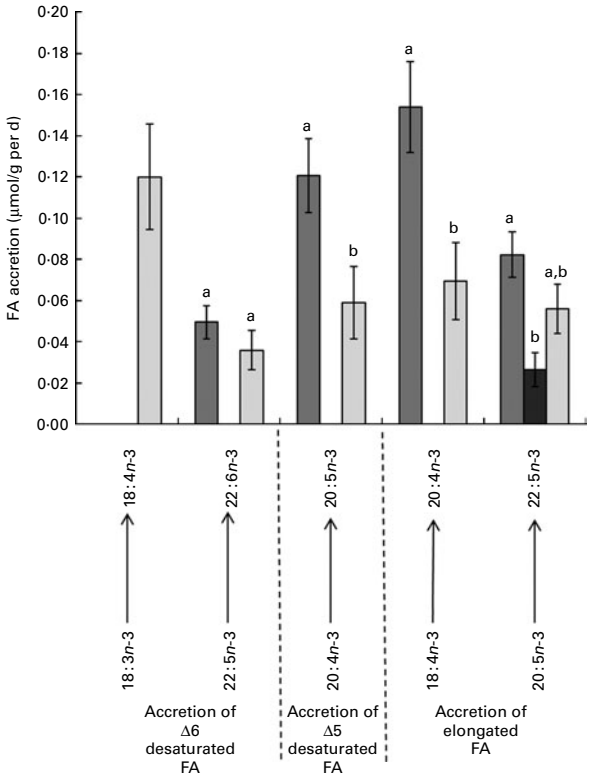

Fig. 2. Individual appearance (accretion) of Δ5 and Δ6 desaturated and elongated fatty acids (FA) in the whole carcass of Atlantic salmon (*Salmo salar* L.) parr fed on different oil sources in freshwater. Values are means, with standard errors represented by vertical bars (*n* 6 for fish oil (■) and *n* 8 for *Echium* oil (■) and rapeseed oil (□)). [a,b] Mean values with unlike letters were significantly different in FA accretion between the diets ($P<0·05$).

therefore it does not take into account possible FA chain shortening such as retroconversion of DHA to DPA or the β-oxidation of FA previously elongated and desaturated[17,19]. The production of eicosanoids, resolvins and protectins from their precursor's arachidonic acid, EPA and DHA is also not quantified. However, the production of these functional metabolites is minimal, probably having little impact on the total FAMB[17,19]. In rats, the production of eicosanoids, as measured by their urinary excretion, does not exceed 1 μg/d[39]. Most of these metabolites are generally involved in inflammatory processes and are either potent pro- or anti-inflammatory at nanomolar concentrations, and their production is measured in minute amounts (ng/mg of protein) in tissues[40,41]. Arguably, the FAMB might be more limited with respect to production of these functional metabolites in cases of infected or injured fish. Similar limitations also occur with other methods employing labelled FA to assess FA metabolism in fish[19]. As the use of the FAMB approach expands, including via incorporation of measurement of metabolites present at low abundance, the method may be further fine-tuned.

Similarly, for EO fish, the high presence of SDA in the diet might have masked its desaturation from ALA, therefore any accretion of Δ6 desaturated SDA could not be obtained (Fig. 2). Hence, when assessing the biosynthetic activity along the *n*-3 pathway, this method might be best suited

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when comparing between oils with ALA as the main precursor and very low amounts of other *n*-3 PUFA. In freshwater, ALA was the main FA that is β-oxidised in RO fish (33% of net intake), while in EO fish, 69 and 42% of ALA and SDA net intake showed a NMB. The NMB for 42% of SDA equated to 1607 μmol/fish, which was greater than the total elongated/desaturated products (1249 μmol/fish). Therefore, theoretically 78% of SDA NMB was biosynthesised along the *n*-3 pathway, and dietary ALA did not contribute to any *n*-3 LC-PUFA biosynthesis in EO fish. Since some ALA might have been elongated to SDA and then further metabolised, as mentioned earlier, the high dietary amount of SDA might have masked this step.

In FO fish, 66% of EPA net intake showed a NMB mainly through β-oxidation. It has been well documented that excess dietary EPA is readily β-oxidised[19,38,42]. Moreover, it has been shown that at around seawater transfer, there is an increase in β-oxidation capacity in the liver and muscle of Atlantic salmon[42,43], which would explain the apparent β-oxidation of EPA. The FAMB approach was developed to determine enzymatic activity[17]; however, since the enzyme activity is usually measured over a limited incubation time, it was proposed to report either any FA accretion as an indication of enzymatic activity[19] or apparent enzyme activity[38]. In the present study, the accretion of certain FA could not be computed due to the masking effect of the FA present in high amounts in the diet. However, in the freshwater phase, we confirmed that in EO fish, there was higher *n*-3 LC-PUFA production as a result of higher accretion of desaturated and elongated FA (Fig. 2).

n-3 Fatty acid metabolism – seawater phase

The same scenario as in freshwater was observed in seawater for whole-carcass FA content, with fish fed on the FO diet having higher amounts of EPA and DHA (Table 4). However, between the VO diets, the presence of SDA in EO fish in seawater resulted in greater *n*-3 LC-PUFA biosynthesis since higher ETA, EPA and DPA were accumulated. This observation was confirmed through the FAMB (Table 5), where positive values were obtained at all levels of desaturation/elongation along the *n*-3 pathway leading to a net gain of 696 μmol/fish in total *n*-3 LC-PUFA for EO fish compared with a net loss (−439 μmol/fish) for RO fish. Yet again, the presence of SDA in the EO diet resulted in these differences because of the extra step involved in producing SDA in RO fish at the expense of 633 μmol/fish (Table 5). In a similar study[13], it has been found that an EO diet promoted an increase in elongase and Δ5 desaturase gene expression in Atlantic salmon smolt when compared with fish fed a FO diet, and that the increase in activity led to higher EPA in the liver compared with fish fed a RO diet.

It was evident that the biosynthetic activity along the *n*-3 pathway was negligible in seawater for all three diets (Table 5). This observation has been previously documented, whereby marine fish cannot convert dietary ALA from VO sources to EPA and DHA at a physiologically significant rate[44,45] due to the evolutionary consequence of a natural diet rich in *n*-3 LC-PUFA[46]. *Ex vivo* approaches were used to assess *n*-3 biosynthetic capacity of Atlantic salmon in isolated hepatocytes in previous studies[32,33,46] and showed lower hepatic desaturation of ALA to *n*-3 LC-PUFA in Atlantic salmon post-smolts compared with parr. Our *in vivo* approach has also shown different biosynthetic capacity for Atlantic salmon in fresh and seawater. Due to the low *n*-3 biosynthetic activity at the cellular level of key tissues of Atlantic salmon in seawater and the NMB of substantial amounts of FA through β-oxidation, any significant accretion of *n*-3 LC-PUFA could not be detected when examined at the whole organism level in the present study. We have analysed the whole carcass, therefore it will also be important to examine the individual tissues (muscle and liver) in future studies.

In seawater fish, the FAMB was characterised by high NMB of specific substrates mainly due to β-oxidation: ALA for RO fish (74% net intake), ALA and SDA for EO fish (73 and 51% net intake), EPA and DHA for FO fish (79 and 52% net intake). While there is a preferential order of FA for β-oxidation, this is subservient to β-oxidation of excess FA[19]. ALA has been shown to be readily β-oxidised[47,48] in Atlantic salmon, while SDA in EO fish and EPA in FO fish were probably supplied surplus to requirements from their respective diets. In general, immediately after seawater transfer, the feed intake of Atlantic salmon smolt is reduced due to stress, and this period is accompanied by a reduction in condition (*K*) as fish use their lipid stores as an energy source. Therefore, it is very likely that fish were initially using their lipid reserves in seawater, which also contributed to the large NMB in FA.

The use of FO can be regarded as a rather wasteful practice due to the β-oxidation of substantial amounts of EPA and DHA; however, high amounts were still accumulated and resulted in the observed three- to fivefold difference compared with fish fed on the VO diets. It should be stressed that from a human health perspective, the SDA-enriched oil might be a more suitable substitute due to the improved *n*-3:*n*-6 ratio, high levels of total *n*-3 (mostly as ALA and SDA) and total PUFA. Furthermore, high intake of SDA, from genetically modified soyabean oil, increased the *n*-3 index and lowered the risk of cardiac events in humans[49]. EO in the present study was used as a model oil due to its high SDA naturally, but the use of EO is currently uneconomic as a substitute for FO[3]. Nevertheless, plant genomics research is underway to increase the synthesis of SDA in commercially viable oil seed plants and also to further improve the *n*-3:*n*-6 ratio[50]. In the near future, such plant oils, containing EPA and DHA[2,3], might be commercially available and could be suitable for FO substitution in the diet of Atlantic salmon[51,52].

Conclusion

Several major findings were drawn from the present study. First, complete substitution of FO with both EO and RO in the diets led to successful parr–smolt transformation without any additional input of *n*-3 LC-PUFA. Second, in freshwater, both RO and EO fish were able to biosynthesise *n*-3 LC-PUFA to meet their requirements, and the presence

of SDA in the EO diet resulted in higher *n*-3 LC-PUFA biosynthesis. Third, in seawater, *n*-3 LC-PUFA biosynthetic activity was non-existent at the whole-body level for RO and FO fish, whereas some *n*-3 LC-PUFA biosynthesis occurred in EO fish probably as a result of the long feeding history on SDA, but to a lesser extent than in freshwater fish. In addition, the FAMB approach has been a useful tool to assess FA metabolism at the whole-body level in the present study, although further research is required to fine-tune the method. Finally, although an EO diet increased the *n*-3 LC-PUFA biosynthesis, EPA and DHA contents in both fresh and seawater fish were still lower compared with in those fed the FO diet. However, due to higher ALA, SDA and total *n*-3 PUFA obtained in fish, oil enriched with SDA in aquafeeds would be more beneficial from a consumer perspective compared with conventional VO.

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References

1. FAO (2007) Fisheries Technical Paper no. 500: Food and Agricultural Organisation of the United Nation. *State of World Aquaculture 2006*. Rome: FAO.
2. Naylor RL, Goldburg RJ, Primavera J, *et al.* (2000) Effect of aquaculture on world fish supplies. *Nature* **405**, 1017–1024.
3. Miller MR, Nichols PD & Carter CG (2008) *n*-3 Oil sources for use in aquaculture – alternatives to the unsustainable harvest of wild fish. *Nutr Res Rev* **21**, 85–96.
4. Naylor RL, Hardy RW, Bureau DP, *et al.* (2009) Feeding aquaculture in an era of finite resources. *Proc Natl Acad Sci U S A* **106**, 15103–15110.
5. Pickova J & Morkore T (2007) Alternate oils in fish feeds. *Eur J Lipid Sci Technol* **109**, 56–263.
6. Jacobs MN, Covaci A & Schepens P (2002) Investigation of selected persistent organic pollutants in farmed Atlantic salmon (*Salmo salar*), salmon aquaculture feed, and fish oil components of the feed. *Environ Sci Technol* **36**, 2797–2805.
7. Bell JG, McGhee F, Dick JR, *et al.* (2005) Dioxin and dioxin-like polychlorinated biphenyls (PCBs) in Scottish farmed salmon (*Salmo salar*): effects of replacement of dietary marine fish oil with vegetable oils. *Aquaculture* **243**, 305–314.
8. Turchini GM, Torstensen BE & Ng WK (2009) Fish oil replacement in finfish nutrition. *Rev Aquac* **1**, 10–57.
9. Bell JG, Henderson RJ, Tocher DR, *et al.* (2002) Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. *J Nutr* **132**, 222–230.
10. Bransden MP, Carter CG & Nichols PD (2003) Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. *Comp Biochem Physiol B Biochem Mol Biol* **135**, 611–625.
11. Torstensen BE, Froyland L & Lie O (2004) Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein composition and lipogenic enzyme activities. *Aquac Nutr* **10**, 175–192.
12. Bell JG, Tocher DR, Henderson RJ, *et al.* (2003) Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *J Nutr* **133**, 2793–2801.
13. Miller MR, Bridle AR, Nichols PD, *et al.* (2008) Increased elongase and desaturase gene expression with stearidonic acid enriched diet did not enhance long-chain omega 3 content of seawater Atlantic salmon (*Salmo salar* L.). *J Nutr* **138**, 2179–2185.
14. Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* **56**, 365–379.
15. Ruxton CH, Reed SC, Simpson MJ, *et al.* (2004) The health benefits of omega-3 PUFA: a review of the evidence. *J Hum Nutr Diet* **17**, 449–459.
16. Miller MR, Nichols PD & Carter CG (2007) Replacement of dietary fish oil for Atlantic salmon parr (*Salmo salar* L.) with a stearidonic acid containing oil has no effect on omega-3 long-chain polyunsaturated fatty acid concentrations. *Comp Biochem Physiol B Biochem Mol Biol* **146**, 197–206.
17. Turchini GM, Francis DS & De Silva SS (2007) A whole body, *in vivo*, fatty acid balance method to quantify PUFA metabolism (desaturation, elongation and beta-oxidation). *Lipids* **42**, 1065–1071.
18. Turchini GM, Francis DS & De Silva SS (2006) Fatty acid metabolism in the freshwater fish Murray cod (*Maccullochella peelii peelii*) deduced by the whole-body fatty acid balance method. *Comp Biochem Physiol B Biochem Mol Biol* **144**, 110–118.
19. Turchini GM & Francis DS (2009) Fatty acid metabolism (desaturation, elongation and β-oxidation) in rainbow trout fed fish oil or linseed oil-based diets. *Br J Nutr* **102**, 69–81.
20. Carter CG, Bransden MP, Lewis TE, *et al.* (2003) Potential of thraustochytrids to partially replace fish oil in Atlantic salmon feeds. *Mar Biotechnol* **5**, 480–492.
21. Wedemeyer GA (1996) *Physiology of Fish in Intensive Culture Systems*, pp. 61–93. New York: Chapman & Hall.
22. McCormick SD, Moriyama S & Björnsson BT (2000) Low temperature limits the photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. *Am J Physiol Regul Integr Comp Physiol* **278**, 1352–1361.
23. Arnesen AM, Johnsen HK, Mortensen A, *et al.* (1998) Acclimation of Atlantic salmon (*Salmo salar* L.) smolts to 'cold' sea water following direct transfer from fresh water. *Aquaculture* **168**, 351–367.

British Journal of Nutrition

24. Carter CG, Lewis TE & Nichols PD (2003) Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon (*Salmo salar* L.) diets. *Aquaculture* **225**, 341–351.
25. Ward DA, Carter CG & Townsend AT (2005) The use of yttrium oxide and the effect of faecal collection timing for determining the apparent digestibility of minerals and trace elements in Atlantic salmon (*Salmo salar* L.) feeds. *Aquac Nutr* **11**, 49–59.
26. Percival SB, Lee PS & Carter CG (2001) Validation of a technique for determining apparent digestibility in large (up to 5 kg) Atlantic salmon (*Salmo salar* L.) in seacages. *Aquaculture* **201**, 315–327.
27. Maynard LA & Loosli JK (1969) *Animal Nutrition*, 6th ed. New York: McGraw-Hill.
28. AOAC (1995) *Official Methods of Analysis*, 16th ed. Washington, DC: AOAC International.
29. Bligh E & Dyer WJ (1959) Rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
30. Miller MR, Nichols PD, Barnes J, *et al.* (2006) Regiospecificity profiles of storage and membrane lipids from the gill and muscle tissue of Atlantic salmon (*Salmo salar* L.) grown at elevated temperature. *Lipids* **41**, 865–876.
31. Bell JG, Ghioni C & Sargent JR (1994) Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon (*Salmo salar*): a comparison with commercial diets. *Aquaculture* **128**, 301–313.
32. Bell JG, Tocher DR, Farndale BM, *et al.* (1997) The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr–smolt transformation. *Lipids* **32**, 515–525.
33. Tocher DR, Bell JG, Dick JR, *et al.* (2000) Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr–smolt transformation and the effects of dietary linseed and rapeseed oils. *Fish Physiol Biochem* **23**, 59–73.
34. Bendiksen EA, Arnessen AM & Jobling M (2003) Effects of dietary fatty acid profile and fat content on smolting and seawater performance in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **225**, 149–163.
35. Jutfelt F, Olsen RE, Björnsson BT, *et al.* (2007) Parr–smolt transformation and dietary vegetable lipids affect intestinal nutrient uptake, barrier function and plasma cortisol levels in Atlantic salmon. *Aquaculture* **273**, 298–311.
36. Bell JG, McEvoy J, Tocher DR, *et al.* (2001) Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J Nutr* **131**, 1535–1543.
37. Bell JG, Henderson RJ, Tocher DR, *et al.* (2002) Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects tissue fatty acid compositions and hepatic fatty acid metabolism. *J Nutr* **132**, 222–230.
38. Francis DS, Peters DJ & Turchini GM (2009) Apparent *in vivo* Δ-6 desaturase activity, efficiency, and affinity are affected by total dietary C18 PUFA in the freshwater fish Murray cod. *J Agric Food Chem* **57**, 4381–4390.
39. Hansen HS & Jensen B (1983) Urinary prostaglandin E_2 and vasopression excretion in essential fatty acid deficient rats. *Lipids* **18**, 682–690.
40. Marcheselli VL, Hong S, Lukiw W, *et al.* (2003) Novel docosanoids inhibit brain ischemia–reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *J Biol Chem* **278**, 43807–43817.
41. Hudert CA, Weylandt KH, Lu Y, *et al.* (2006) Transgenic mice rich in endogeneous omega-3 fatty acids are protected from colitis. *Proc Natl Acad Sci U S A* **103**, 11276–11281.
42. Stubhaug I, Lie O & Torstensen BE (2007) Fatty acid productive value and β-oxidation capacity in Atlantic salmon (*Salmo salar* L.) fed on different lipid sources along the whole growth period. *Aquac Nutr* **13**, 145–155.
43. Stubhaug I, Lie O & Torstensen BE (2006) β-Oxidation capacity in liver increases during parr–smolt transformation of Atlantic salmon (*Salmo salar* L.) fed vegetable and fish oil. *J Fish Biol* **69**, 504–517.
44. Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Rev Fish Sci* **11**, 107–184.
45. Sargent JR, Tocher DR & Bell JG (2002) The lipids. In *Fish Nutrition*, pp. 181–257 [JE Halver and RW Hardy, editors]. San Diego, CA: Academic Press, Elsevier.
46. Tocher D, Bell J, Dick J, *et al.* (1997) Fatty acyl desaturation in isolated hepatocytes from Atlantic salmon (*Salmo salar*): stimulation by dietary borage oil containing γ-linolenic acid. *Lipids* **32**, 1237–1247.
47. Torstensen BE & Stubhaug I (2004) Beta-oxidation of 18 : 3*n*-3 in Atlantic salmon (*Salmo salar* L.) hepatocytes treated with different fatty acids. *Lipids* **39**, 1–8.
48. Stubhaug I, Froyland L & Torstensen BE (2005) β-Oxidation capacity of red and white muscle and liver in Atlantic salmon (*Salmo salar* L.) – effects of increasing dietary levels of rapeseed oil and olive oil to replace capelin oil. *Lipids* **40**, 39–47.
49. Harris W, Lemke S, Hansen S, *et al.* (2008) Stearidonic acid-enriched soybean oil increased the omega-3 index, an emerging cardiovascular risk marker. *Lipids* **43**, 805–811.
50. Ursin VM (2003) Modification of plant lipids for human health: development of functional land-based omega-3 fatty acids. *J Nutr* **133**, 4271–4274.
51. Graham IA, Cirpus P, Rein D, *et al.* (2004) The use of very long chain polyunsaturated fatty acids to ameliorate metabolic syndrome: transgenic plants as an alternative sustainable source to fish oils. *Nutr Bull* **29**, 228–233.
52. Venegas-Caleron M, Sayanova O & Napier JA (2010) An alternative to fish oils: metabolic engineering of oil-seed crops to produce omega-3 long chain PUFA. *Prog Lipid Res* **49**, 108–119.

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