Endogenous biosynthesis of *n*-3 long-chain PUFA in Atlantic salmon

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Abstract

A more efficient utilisation of marine-derived sources of dietary *n*-3 long-chain PUFA (*n*-3 LC PUFA) in cultured Atlantic salmon (*Salmo salar* L.) could be achieved by nutritional strategies that maximise endogenous *n*-3 LC PUFA synthesis. The objective of the present study was to quantify the extent of *n*-3 LC PUFA biosynthesis and the resultant effect on fillet nutritional quality in large fish. Four diets were manufactured, providing altered levels of dietary *n*-3 substrate, namely, $18:3n$-3, and end products, namely, $20:5n$-3 and $22:6n$-3. After 283 d of feeding, fish grew in excess of 3000 g and no differences in growth performance or biometrical parameters were recorded. An analysis of fatty acid composition and *in vivo* metabolism revealed that endogenous production of *n*-3 LC PUFA in fish fed a diet containing no added fish oil resulted in fillet levels of *n*-3 LC PUFA comparable with fish fed a diet with added fish oil. However, this result was not consistent among all treatments. Another major finding of this study was the presence of abundant dietary *n*-3 substrate, with the addition of dietary *n*-3 end product (i.e. fish oil) served to increase final fillet levels of *n*-3 LC PUFA. Specifically, preferential *β*-oxidation of dietary C_{18} *n*-3 PUFA resulted in conservation of *n*-3 LC PUFA from catabolism. Ultimately, this study highlights the potential for endogenous synthesis of *n*-3 LC PUFA to, partially, support a substantial reduction in the amount of dietary fish oil in diets for Atlantic salmon reared in seawater.

Key words: Fatty acids: Metabolism: *n*-3 Fatty acids: Bioconversion: Aquaculture: Fish oil

The intense market volatility and reduced availability of traditional protein and lipid sources such as fishmeal and fish oil for use in aquafeed have led to constantly evolving dietary formulations[1–3]. The aquaculture industry has been active in adopting plant and animal protein and lipid sources for the incorporation into aquafeed; however, most of these ingredients contain none or considerably lower levels of the health beneficial fatty acids, namely, *n*-3 long-chain ($C \geq 20$) PUFA (*n*-3 LC PUFA)[2,4,5]. Consequently, maximising the deposition efficiency of increasingly limited dietary *n*-3 LC PUFA into the final edible product will prove integral to ensure the ongoing viability of the aquaculture sector[6–9]. The possible solutions available to address this challenge are based on the knowledge that changes to the fatty acid composition of the aquafeed influence not only the final fatty acid composition of fish fillets but also the various aspects of fatty acid metabolism, including *in vivo* fatty acid *β*-oxidation and bioconversion[10–13]. Concomitant with an increased understanding of fatty acid metabolism within popular cultured species in recent years, dietary formulations have been manipulated to promote the sparing of

n-3 LC PUFA from catabolism as well as stimulate endogenous production via the *n*-3 LC PUFA biosynthetic pathway[7,9,10,14]. The *n*-3 LC PUFA biosynthetic pathway is facilitated by desaturase and elongase enzymes found in many fish and mammalian species[12,15,16]. However, the activity and efficiency of these enzymes in converting shorter (C_{18}), less unsaturated fatty acids into longer (C_{20-22}), more unsaturated fatty acids are not uniform across species[12,17]. Specifically, carnivorous marine species of fish are purported to have a severely limited capacity to biosynthesise $22:6n$-3 from $18:3n$-3 dietary substrate[18–20]. It is hypothesised that the abundance of *n*-3 LC PUFA in marine ecosystems, which originate in lower trophic microbial and invertebrate organisms[21], has rendered the *n*-3 LC PUFA biosynthetic pathway largely redundant[12,16,18,19]. Conversely, freshwater and anadromous fish have adapted to a relative paucity of dietary available *n*-3 LC PUFA and exhibit a much higher capacity to biosynthesise physiologically required *n*-3 LC PUFA from dietary $18:3n$-3[22–25].

Atlantic salmon (*Salmo salar*) have a demonstrated, albeit often limited, ability to bioconvert $18:3n$-3 to $22:6n$-3[9,26,27].

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Consequently, numerous metabolic responses to dietary fatty acid changes have been elucidated in this species[8,11,13,15,22,23,28–30]. It has been shown that endogenous production of 22 : 6n-3 in Atlantic salmon is heavily influenced by the dietary ratio of shorter-chain to long-chain n-3 PUFA. Specifically, an increase in substrate (18 : 3n-3) availability appears to enhance the activity of the Δ-6 desaturase enzyme necessary for the first and one of the last steps of the n-3 PUFA bioconversion pathway[10,31–34]. Conversely, the presence of end product (22 : 6n-3) may elicit a negative feedback mechanism on the pathway[33]. Furthermore, the extent of endogenous synthesis of 22 : 6n-3 in Atlantic salmon may only occur to an extent that satisfies a minimum physiological requirement, which itself, is dictated by changing environmental conditions and life history stage[9,12,35]. For instance, n-3 LC PUFA synthesis is generally more efficient in the juvenile stages and then decreases in the seawater growth phase[36]. Therefore, endogenous synthesis has been documented to be unable to enrich the fillet tissue with n-3 LC PUFA to the same extent as dietary added fish oil in Atlantic salmon[13,24,37–41]. Nevertheless, there remains multiple, interrelated dietary and environmental factors that dictate the final concentration of n-3 LC PUFA that can be synthesised de novo[10,28,42–45].

Currently, commercial aquafeed formulations for Atlantic salmon contain a physiological excess of n-3 LC PUFA to ensure a high level of deposition of these fatty acids into the fillet tissue. Atlantic salmon, in particular, are popular with consumers, in part, due to historically high levels of n-3 LC PUFA[46–48]. However, this is becoming increasingly marginal due to mounting pressure from both within and outside the aquaculture industry for n-3 LC PUFA-rich oil sources. Levels of n-3 LC PUFA in farmed Atlantic salmon have followed a declining trend[4] and are approaching the minimal levels needed to satisfy both, the physiological needs of the animal and consumer expectation. Therefore, novel approaches to maximise the deposition efficiency of nutritionally valuable fatty acids in this popular table fish are increasingly sought after[2,7,49]. In the future, a more efficient utilisation of n-3 LC PUFA in cultured salmonids may be reliant on dietary strategies that maximise n-3 PUFA bioconversion[10,28,46]. Hence, further research that elucidates the potential to exploit fatty acid metabolism, namely, n-3 LC PUFA, bioconversion in large Atlantic salmon is warranted.

To date, the majority of laboratory-based fish trials have focused on smaller sub-market-sized fish due to logistical constraints. This limits commercial relevance as physiological fatty acid requirements, and by extension in vivo fatty acid metabolism, change in response to ontogenic development[46]. Furthermore, trials conducted on farm may compromise the accuracy of feed and fatty acid uptake measurements due to a typically higher food conversion ratio and difficulties quantifying uneaten feed[50]. Accordingly, the present experiment was conducted in a laboratory set-up with fish grown up to a marketable size. The trial utilised three oil sources, namely, fish oil, poultry by-product oil and camelina oil to create four experimental diets with varying levels of shorter-chain and long-chain n-3 PUFA. The objective, therefore, was to provide altered substrate (18 : 3n-3) and end product (20 : 5n-3 and 22 : 6n-3)

ratios along the n-3 LC PUFA bioconversion pathway and subsequently quantify the extent of n-3 LC PUFA biosynthesis, towards enhancing current knowledge, and industry relevance, regarding the potential for endogenous production of n-3 LC PUFA in market-sized Atlantic salmon.

Methods

Ethics statement

All animals and procedures in this experiment were approved by the Deakin University Animal Welfare Committee (B25-2015). All possible steps to minimise animal suffering and provide an enriched environment were taken.

Animals, trial facility and sampling

Juvenile Atlantic salmon (S. salar) were sourced from a commercial producer (Mountain Fresh Trout and Salmon Farm, Harrietville, VIC, Australia) and transported to the Deakin Aquaculture Futures Facility (Deakin University, Warrnambool Campus, VIC, Australia). Juvenile fish were acclimatised to the facility in freshwater before gradually being exposed to saltwater. Smoltification of fish was confirmed by plasma chloride analysis. Following smoltification, fish were acclimatised to the experimental conditions and maintained on a commercial 6 mm salmonid diet (Ridley Aquafeed Pty Ltd) before the commencement of the trial period. The experiment was conducted in a closed-loop, thermostatically controlled, recirculating aquaculture system containing eight (5000 litres) rearing tanks. Physical and biological filtration (drum filter fitted with a 60 μm screen; Hydrotech) and UV disinfection maintained water quality throughout the experiment. The system was maintained on a 12 h light–12 h dark cycle and temperature was kept at 15·0 ± 0·5°C for the duration of the experiment. Dissolved oxygen was maintained at optimal conditions, and the levels of metabolic waste, total ammonia, P, nitrite and nitrate were monitored daily using Aquamerck Test kits (Merck), which remained within acceptable limits throughout the trial. Immediately preceding the trial, an initial sample of six fish were euthanised in excess anaesthetic (AQUI-S, 0·05 ml/l) and stored at –20°C until analysis. Initially, 272 fish were distributed amongst eight tanks (thirty-four fish per tank) and assigned one of four dietary treatments in duplicate (two tanks per treatment; n 2, N 8). After 98 and 196 d, weight checks were performed and fish removed to improve stocking densities. Fish were fed to apparent satiation twice a day at 09.00 and 16.00 h for the entire (283 d) grow-out period. Feed consumption and mortalities (<2 %) were recorded throughout the trial and remained within acceptable limits. Faeces were collected 2 weeks before final sampling for digestibility estimation. At the completion of the grow-out phase, all fish were euthanised in excess anaesthetic (AQUI-S, 0·05 ml/l) and weighed. Subsequently, eighteen fish per treatment (nine fish per tank) were selected and stored at –20°C until analysed. The sampled fish were separated into two groups: the first group (ten fish per treatment; five fish pooled per tank) was used for analysis of biometry and for chemical analysis of whole body, the second group (eight fish

per treatment; four fish pooled per tank) was used for biometry and chemical analysis of fillet.

Experimental diets

Four experimental diets were formulated to be iso-lipidic (310 mg/g), iso-proteic (440 mg/g) and iso-energetic (26 kJ/g) (Table 1). For manufacturing the four experimental diets, a 9 mm extruded pellet for Atlantic salmon was produced by a commercial feed producer (Ridley Aquafeed Pty Ltd) and removed from the production process at the feed mill post-extrusion, before oil coating and delivered to Deakin University for subsequent vacuum oil coating of the experimental oils. A small-scale, laboratory-based, vacuum-coater equipped with a vacuum pump and rotation mechanism was used to add the dietary lipid. The four experimental diets were achieved using three different lipid sources, poultry by-product oil, fish oil and camelina oil, to create varied ratios of short-chain and long-chain *n*-3 fatty acids and therefore four varied compositions of 'substrate' (18 : 3*n*-3) and 'end product' (20 : 5*n*-3 and 22 : 6*n*-3) in terms of *in vivo* bioconversion of *n*-3 fatty acids via the desaturation, elongation enzymatic pathway. The experimental oil blends were added to the 'uncoated' pellets and resulted in the following four diets:

- Low substrate:Low end product (Low:Low), where added oil consisted of 100 % poultry by-product oil.
- Low substrate:High end product (Low:High), where added oil consisted of 80 % poultry by-product oil and 20 % fish oil.
- High substrate:Low end product (High:Low), where added oil consisted of 80 % camelina oil and 20 % poultry by-product oil
- High substrate:High end product (High:High), where added oil consisted of 80 % camelina oil and 20 % fish oil.

Growth performance, chemical analyses and fatty acid analysis

Standard formulae were used to assess growth, feed utilisation and biometrical data. These included initial and final average weight, weight gain (g), weight gain %, feed conversion ratio (FCR), specific growth rate (SGR), Fulton's condition factor (*K*), dress-out percentage, fillet yield percentage (FY%), hepatosomatic index (HSI%) and viscera-somatic index (VSI %). Calculations for these common formulae are presented in detail in Francis *et al.*[51]. The chemical composition of the experimental diets, faeces and fish samples was determined via proximate composition analysis according to standard methods, as previously described by Emery *et al.*[52]. Briefly, moisture was determined by drying samples in an oven at 80°C to a constant weight, while ash was determined by incinerating samples in a muffle furnace (S.E.M. (S.A.) Pty Ltd) at 550°C for 18 h. Protein (Kjeldahl N: N × 6·25) content was determined using an automated Kjeltech 2300 (Foss Tecator) and lipid was determined by solvent extraction[53], where dichloromethane was used to replace chloroform for safety considerations. Following lipid extraction, fatty acids were esterified into methyl esters using an acid-catalysed

methylation method and then analysed by GC as described in Norambuena *et al.*[54]. Resulting peaks were identified relative to known external standards and then corrected for theoretical flame ionisation detector response and methyl transformation required for GC analysis. The resulting quantitative fatty acid data were eventually reported as mg/g for the experimental diets and as μmol/g tissue (wet weight) for fillet tissue, as recommended by Parrish[55].

Apparent nutrient digestibility and metabolism calculations

Apparent digestibility was determined following methods presented in Atkinson *et al.*[56], using ash instead of acid-insoluble ash as the inert marker. The calculation of apparent *in vivo* fatty acid metabolism was performed using the whole-body fatty acid balance method, as initially proposed and described by Turchini *et al.*[57] with further development[34,58]. The results of the whole-body fatty acid balance method are reported as nmol/g per d and percentage of net intake as presented in Norambuena *et al.*[11].

Statistical analysis

All data were reported as means with their standard errors (*n* 2, *N* 8). After confirmation of normality and homogeneity of variance, data were subjected to two-way ANOVA to assess the effects of dietary substrate, dietary end product and dietary substrate by end product interactions. A Tukey's *post hoc* test determined statistical significance between homogenous subsets and was performed where statistical significant differences were identified. The analysis was performed using IBM SPSS Statistics version 24.0 (SPSS Inc.). Significance was accepted at $P < 0.05$, where * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

Before the experiment, based on recent research with a similar study design and similar sized fish[59], a between-group, within-treatment standard deviation of 197·4 was expected for an outcome of importance, specifically, final fish weight. Power analysis revealed that the minimum detectable difference in final weight between treatment groups given the sample size in the present study and assuming a statistical power of 80 % was 262 g. This was considered acceptable for the present study, considering the expectedly large final weights of the fish. Power analysis was performed using GraphPad StatMate version 2.0 (Graphpad Software Inc.).

Results

Diets

The four experimental diets were iso-energetic and proximate compositions were similar (Table 1). Total fatty acid concentration ranged from 247·4 to 272·6 mg/g diet in High:Low and Low:Low, respectively. Levels of individual fatty acids varied according to lipid source. Accordingly, Low:Low and Low:High were characterised by higher levels of both SFA and MUFA due to typically high 18 : 1*n*-9 concentrations in poultry by-product oil. Total PUFA levels were higher in High:Low and High:High; however, *n*-6 PUFA was relatively consistent

between dietary treatments. Levels of *n*-3 PUFA were markedly higher in High:Low and High:High, owing to high levels of 18 : 3*n*-3. Diet *n*-3 LC PUFA levels were predictably dictated by fish oil inclusion and were higher in Low:High and High:High owing to higher 22 : 6*n*-3 concentrations. The ratio of *n*-6:*n*-3 fatty acids varied considerably between treatments, ranging from 0·8 to 4·1 in High:High and Low:Low, respectively ($P < 0.05$).

Growth, feed utilisation parameters and biometric data

Mortality rates were low during the trial and unrelated to diet. Diets were generally well accepted by fish. However, some maturation of fish occurred in the latter stages of the trial, which led to poorer than expected growth and food conversion. Fish gained approximately 2000 g and grew to weights in excess of 3000 g, with no statistical differences evident between treatments. FCR ranged from 1·14 to 1·26 in Low:Low and High:High, respectively (Table 2) ($P > 0.05$). Overall, there were no significant differences in either growth or biometry measures between dietary treatments, including SGR, FCR, feed ration % (relative to body mass), *K*, FY% HSI% and VSI%. There were no significant effects of dietary *n*-3 substrate, end product or substrate by end product interactions recorded with regard to any growth performance parameters.

Table 1. Proximate composition, total fatty acids (FA) and FA (mg/g diet) composition of the four experimental diets

	Diets*			
	Low:Low	Low:High	High:Low	High:High
Proximate composition (mg/g)				
Moisture	39·8	39·0	38·6	45·7
Protein	430·6	432·8	440·7	446·4
Lipid	320·2	328·3	311·8	322·4
NFE	176·4	165·6	173·4	155·8
Ash	71·8	72·5	72·8	74·3
Energy (kJ/g)	25·8	26·0	25·7	26·0
Total FA (mg/g diet)†	272·6	270·6	247·4	253·7
SFA	83·2	86·4	55·8	61·8
14 : 0	3·3	5·8	2·0	4·4
16 : 0	59·8	59·6	38·3	40·1
18 : 0	17·7	17·5	12·2	12·7
Other SFA‡	2·4	3·6	3·3	4·6
MUFA	143·0	132·7	115·3	108·1
16 : 1*n*-7	14·1	14·7	8·2	9·0
18 : 1*n*-9	118·9	105·6	82·7	71·5
18 : 1*n*-7	6·6	6·9	4·6	5·0
20 : 1*n*-9	1·7	2·4	14·8	16·2
Other MUFA§	1·7	3·0	4·9	6·3
Total *trans*-FA‖	1·7	1·5	0·8	0·6
PUFA	44·7	49·9	75·5	83·2
18 : 2*n*-6	34·1	29·7	36·3	33·2
20 : 2*n*-6	0·3	0·3	1·8	1·9
20 : 4*n*-6	0·6	1·3	0·4	1·0
Other *n*-6 PUFA¶	0·8	1·2	0·6	1·1
n-6 PUFA	35·8	32·6	39·2	37·3
18 : 3*n*-3	5·3	4·7	32·3	33·5
20 : 5*n*-3	1·0	4·6	0·8	4·2
22 : 5*n*-3	0·4	1·0	0·3	0·9
22 : 6*n*-3	1·5	5·5	1·2	4·9
n-3 PUFA	8·7	16·6	36·2	45·2
Other *n*-3 PUFA**	0·5	0·8	1·6	1·7
LC PUFA	4·6	14·2	6·5	15·5
n-6 LC PUFA	1·4	2·5	2·7	3·9
n-3 LC PUFA	3·2	11·7	3·8	11·6
n-6:*n*-3 ratio††	4·1	2·0	1·1	0·8

NFE, N-free extract; LC, long chain.
* Diets: Low:Low = low substrate, low end product diet consisting of 43 % protein and 32 % lipid, added oil consists of 100 % poultry by-product oil; Low:High = low substrate, high end product diet consisting of 43 % protein and 33 % lipid, added oil consists of 80 % poultry by-product oil and 20 % fish oil; High:Low = high substrate, low end product diet consisting of 44 % protein and 31 % lipid, added oil consists of 80 % camelina oil and 20 % poultry by-product oil; High:High = high substrate, high end product diet consisting of 45 % protein and 32 % lipid, added oil consists of 80 % camelina oil and 20 % fish oil.
† Total FA = total FA mg/g of diet.
‡ Other SFA = sum of 12 : 0, 15 : 0, 17 : 0, 20 : 0, 22 : 0 and 24 : 0.
§ Other MUFA = sum of 14 : 1*n*-5, 15 : 1*n*-5, 17 : 1*n*-7, 20 : 1*n*-13, 20 : 1*n*-11, 22 : 1*n*-11, 22 : 1*n*-9 and 24 : 1*n*-9.
‖ Total *trans*-FA = sum of 18 : 1*n*-9*t*, 18 : 1*n*-7*t* and 18 : 2*n*-6*t*.
¶ Other *n*-6 PUFA = sum of 18 : 3*n*-6, 20 : 2*n*-6, 20 : 3*n*-6, 22 : 2*n*-6, 22 : 4*n*-6 and 22 : 5*n*-6.
** Other *n*-3 PUFA = sum of 20 : 3*n*-3, 20 : 4*n*-3, 24 : 5*n*-3 and 24 : 6*n*-3.
†† *n*-6:*n*-3 ratio = ratio between *n*-6 PUFA and *n*-3 PUFA.

Table 2. Growth, feed efficiency and biometry of Atlantic salmon fed the four experimental diets for 283 d* (Mean values with their standard errors, n 2, N 8)

| | Diets† | | | | | | | | Effect of substrate, end product and substrate/end product interaction (P) | | |
| | Low:Low | | Low:High | | High:Low | | High:High | | | | |
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Substrate	End product	Interaction
Initial weight (g)	1116	1	1126	3	1119	1	1128	1	NS	NS	NS
Final weight (g)	3283	171	3227	14	3308	237	3049	110	NS	NS	NS
Gain (g)	2167	171	2102	17	2190	236	1921	109	NS	NS	NS
Gain (%)	194.1	15.5	186.7	1.9	195.7	20.9	170.3	9.5	NS	NS	NS
Feed ration (% bw/d)	0.40	0.01	0.40	0.00	0.44	0.03	0.41	0.00	NS	NS	NS
FCR	1.14	0.06	1.16	0.01	1.18	0.01	1.26	0.04	NS	NS	NS
SGR	0.36	0.01	0.35	0.00	0.35	0.00	0.34	0.00	NS	NS	NS
K‡	1.6	0.1	1.5	0.0	1.9	0.4	1.4	0.1	NS	NS	NS
DP (%)	90.2	0.5	90.7	0.3	90.8	1.5	91.0	0.9	NS	NS	NS
FY (%)	52.2	5.2	55.7	0.8	57.1	0.3	52.0	3.5	NS	NS	NS
HSI (%)	1.2	0.1	1.4	0.0	1.4	0.1	1.3	0.0	NS	NS	NS
VSI (%)	9.8	0.5	9.3	0.3	9.2	1.5	9.0	0.9	NS	NS	NS

FCR, food conversion ratio; SGR, specific growth rate; DP, dress-out percentage; FY, fillet percentage; HSI, hepatosomatic index; VSI, viscera-somatic index.
* Treatment means were analysed by one-way ANOVA with Tukey's post hoc test of multiple comparisons. Effects of substrate, end product and substrate/end product interaction were analysed by two-way ANOVA.
† See Table 1 for experimental diet abbreviations.
‡ K = condition factor.

Apparent nutrient and fatty acid digestibility

High nutrient digestibility values (apparent digestibility coefficient %) were observed across treatments, with only one significant difference recorded, where N-free extract digestibility was higher in Low:Low in comparison with High:High ($P < 0.05$) (Table 3). Fatty acid digestibility values were high with no significant differences recorded between treatments, though there was a significant effect of end product recorded for the digestibility of 20:5n-3.

Tissue proximate and fillet fatty acid composition

No significant differences in fillet proximate composition were evident (Table 4), even if fillet lipid concentrations varied between treatments, ranging from 75·1 to 104·7 mg/g tissue in Low:High and High:High, respectively ($P > 0.05$). Owing to differences in fillet lipid, total fatty acid concentration was lowest in Low:High and highest in High:High, though differences were not significant ($P > 0.05$). Expectedly, fillet levels of 22:6n-3 were higher in High:High compared with Low:Low in terms of both µmol/g of fillet tissue and mg/100 g of fillet ($P < 0.05$). However, High:Low had comparable levels of 22:6n-3 compared with both Low:High and High:High ($P > 0.05$). Furthermore, High:Low had marginally higher levels of total n-3 LC PUFA compared with Low:High, despite the latter treatment diet containing no added fish oil ($P > 0.05$). As expected, High:High had higher levels of n-3 LC PUFA compared with Low:Low ($P < 0.05$). The n-6:n-3 PUFA ratios varied across treatments and followed dietary trends, ranging from 1·0 to 2·7 in High:High and Low:Low, respectively ($P < 0.05$). Across treatments, there was a clear effect of substrate and end product recorded for 20:5n-3, 22:5n-3 and total n-3 LC PUFA in terms of both µmol/g of fillet tissue and mg/100 g of fillet; however, no significant substrate by end product interaction was recorded.

Apparent in vivo fatty acid metabolism

Apparent in vivo fatty acid β-oxidation (expressed as nmol of fatty acid β-oxidised, per gram of fish per d; nmol/g per d and as percentage of net intake) (Table 6 and Fig. 1, respectively) as calculated by the whole-body fatty acid balance method was highest in 16:0 and 18:1n-9 across all treatments, relative to high dietary inclusion levels. Accordingly, there was a significant effect of substrate and end product concentration on the β-oxidation of SFA ($P < 0.001$ and $P < 0.01$, respectively). MUFA was β-oxidised to a similar extent as SFA where treatments demonstrated a similar trend; however, no significant differences were recorded between treatments ($P > 0.05$). Substrate concentration had a clear effect of the β-oxidation of 18:3n-3 ($P < 0.001$), where the High:Low and High:High treatments recorded significantly higher β-oxidation of 18:3n-3 ($P < 0.05$). However, in terms of β-oxidation calculated based the percentage of net intake, both the low substrate treatments (Low:Low and Low:High) recorded higher values (approximately 70 %). Notably, 22:6n-3 was highly conserved across treatments and recorded low β-oxidation values.

Table 3. Nutrient and fatty acids (FA) digestibility (apparent digestibility coefficient, %) of the four experimental diets in Atlantic salmon*
(Mean values with their standard errors, n 2, N 8)

| | \multicolumn{8}{c}{Diets†} | | | | | | | Effect of substrate, end product and substrate/end product interaction (P) | | |
| | Low:Low | | Low:High | | High:Low | | High:High | | | | |
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Substrate	End product	Interaction
Nutrients											
DM	86·2	0·2	84·6	0·3	85·0	0·2	83·9	1·1	NS	NS	NS
Protein	94·8	0·0	93·3	0·4	93·5	0·3	93·3	0·8	NS	NS	NS
Lipid	79·9	3·2	82·3	2·3	84·7	0·3	81·7	3·4	NS	NS	NS
NFE	81·9[a]	0·1	76·6[a,b]	0·1	78·4[a,b]	1·6	73·7[b]	2·1	NS	NS	NS
Energy‡	94·1	0·3	92·8	0·4	93·3	0·2	92·2	1·2	NS	NS	NS
Total FA	96·8	0·6	96·5	0·1	97·2	0·4	96·1	0·9	NS	NS	NS
FA											
14:0	96·5	0·3	95·7	0·1	96·0	0·3	95·3	0·5	NS	NS	NS
16:0	94·7	0·2	93·8	0·2	94·6	0·3	94·2	0·3	NS	NS	NS
18:0	92·8	0·1	91·8	0·3	92·4	0·3	92·6	0·1	NS	NS	<0·05
16:1n-7	98·8	0·5	98·7	0·2	98·9	0·2	98·0	0·8	NS	NS	NS
18:1n-9	97·5	0·9	97·6	0·1	97·9	0·4	96·3	1·4	NS	NS	NS
18:1n-7	97·3	0·9	97·3	0·1	97·5	0·4	95·8	1·5	NS	NS	NS
20:1n-9	95·9	0·8	96·0	0·1	96·6	0·6	94·4	1·9	NS	NS	NS
18:2n-6	98·5	0·7	98·7	0·1	98·7	0·3	97·7	0·8	NS	NS	NS
20:2n-6	95·8	0·9	95·4	0·5	97·4	0·5	95·5	1·6	NS	NS	NS
20:4n-6	97·8	0·7	98·8	0·2	96·5	1·3	98·3	0·1	NS	NS	NS
18:3n-3	98·8	0·6	98·9	0·1	98·9	0·2	98·0	0·7	NS	NS	NS
20:5n-3	98·8	0·3	99·5	0·0	98·2	0·4	99·1	0·1	NS	<0·05	NS
22:5n-3	96·9	0·0	98·6	0·0	96·2	1·1	97·7	0·3	NS	NS	NS
22:6n-3	97·0	1·0	98·7	0·2	95·0	1·8	97·7	0·1	NS	NS	NS

NFE, N-free extract.
[a,b] Mean values within a row with unlike superscript letters were significantly different (P < 0·05).
* Treatment means were analysed by one-way ANOVA with Tukey's *post hoc* test of multiple comparisons. Effects of substrate, end product and substrate/end product interaction were analysed by two-way ANOVA.
† See Table 1 for experimental diet abbreviations.
‡ Calculated on the basis of 23·6, 39·5 and 17·2 kJ/g of protein, fat and carbohydrate, respectively.
§ Value of 100 = FA not detected in faeces.

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Table 4. Proximate (mg/g of tissue) and fatty acid (FA) composition (µmol/g tissue) of fillets of Atlantic salmon fed the four experimental diets for 283 d*
(Mean values with their standard errors, *n* 2, *N* 8)

| | Diets† | | | | | | | | Effect of substrate, end product and substrate/end product interaction (*P*) | | |
| | Low:Low | | Low:High | | High:Low | | High:High | | | | |
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Substrate	End product	Interaction
Proximate composition (mg/g of tissue)											
Moisture	692·4	13·9	699·3	5·8	683·8	18·2	677·9	6·6	NS	NS	NS
Protein	207·9	1·9	214·0	2·3	206·5	1·3	205·5	5·2	NS	NS	NS
Lipid	92·1	17·3	75·1	3·7	97·9	16·6	104·7	3·1	NS	NS	NS
Ash	8·1	0·5	8·7	0·1	9·4	0·1	8·7	1·1	NS	NS	NS
FA (µmol/g of tissue)											
Total FA (µg/g of tissue)	277·1	52·4	221·9	10·0	296·6	54·7	321·4	11·5	NS	NS	NS
SFA‡	61·5	10·8	50·2	0·6	59·6	10·9	66·1	3·0	NS	NS	NS
14:0	3·8	0·7	4·0	0·0	3·4	0·7	5·4	0·3	NS	NS	NS
16:0	43·1	7·5	34·3	0·2	41·0	7·8	44·7	2·4	NS	NS	NS
18:0	13·1	2·2	10·2	0·4	12·8	2·0	13·2	0·2	NS	NS	NS
Other SFA§	1·6	0·3	1·6	0·0	2·3	0·4	2·8	0·1	<0·05	NS	NS
MUFA	161·5	32·3	120·9	6·7	153·4	28·0	155	4·1	NS	NS	NS
16:1*n*-7	14·5	3·3	11·9	0·2	11·6	2·3	13·4	0·7	NS	NS	NS
18:1*n*-9	129·2	25·8	94·2	5·5	111·1	20·0	107·9	2·3	NS	NS	NS
18:1*n*-7	8·7	1·7	7·3	0·5	7·1	0·9	7·9	0·2	NS	NS	NS
20:1*n*-9	6·0^a	0·9	5·0^a	0·5	13·9^b	2·2	17·0^b	0·1	<0·01	NS	NS
Other MUFA‖	3·2^{a,b}	0·6	2·4^a	0·0	9·7^b	2·2	8·8^{a,b}	1·0	<0·01	NS	NS
Total *trans*-FA	1·2	0·2	0·8	0·0	0·9	0·2	0·9	0·0	NS	NS	NS
PUFA	52·8	9·1	49·9	2·7	82·6	15·6	99·3	4·3	<0·05	NS	NS
18:2*n*-6	27·7	5·0	23·3	1·3	36·7	7·8	39·2	1·7	NS	NS	NS
20:2*n*-6	1·9^{a,b}	0·2	1·7^a	0·2	2·9^{a,b}	0·6	3·6^b	0·0	<0·05	NS	NS
20:4*n*-6	2·8	0·5	2·0	0·1	1·9	0·2	2·0	0·1	NS	NS	NS
Other *n*-6 PUFA¶	6·1	1·3	3·4	0·3	5·7	0·8	4·4	0·0	NS	NS	NS
n-6 PUFA	38·5	7·0	30·3	1·8	47·1	9·4	49·2	1·8	NS	NS	NS
n-6 LC PUFA	8·8	1·5	6·3	0·5	8·0	1·0	8·6	0·1	NS	NS	NS
18:3*n*-3	2·2^a	0·4	2·3^a	0·1	17·3^b	4·4	24·2^b	2·2	<0·01	NS	NS
20:5*n*-3	1·5^a	0·3	2·2^a	0·0	3·1^{a,b}	0·5	4·3^b	0·3	<0·01	<0·05	NS
22:5*n*-3	0·8^a	0·1	1·3^{a,b}	0·1	1·5^b	0·0	2·1^c	0·0	<0·01	<0·01	NS
22:6*n*-3	9·0^a	1·0	12·3^{a,b}	0·7	10·4^{a,b}	0·7	14·1^b	0·1	NS	<0·01	NS
Other *n*-3 PUFA††	0·7^a	0·1	0·9^a	0·0	3·0^b	0·5	4·7^b	0·1	<0·001	<0·05	<0·05
n-3 PUFA	14·1^a	1·9	19·0^{a,b}	0·9	35·2^{b,c}	6·1	49·4^c	2·4	<0·01	NS	NS
n-3 LC PUFA	11·8^a	1·5	16·7^a	0·8	17·9^a	1·6	25·1^b	0·2	<0·01	<0·01	NS
LC PUFA	20·6^a	3·0	23·0^{a,b}	1·4	25·9^{a,b}	2·7	33·7^b	0·3	<0·05	NS	NS

LC, long chain.

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different (*P* < 0·05).

* Treatment means were analysed by one-way ANOVA with Tukey's *post hoc* test of multiple comparisons. Effects of substrate, end product and substrate/end product interaction were analysed by two-way ANOVA.

† See Table 1 for experimental diet abbreviations.

‡ See Table 2 for FA classes and abbreviations.

§ Other SFA = sum of 12:0, 15:0, 17:0, 20:0, 21:0, 22:0 and 24:0.

‖ Other MUFA = sum of 14:1*n*-5, 15:1*n*-5, 17:1*n*-7, 20:1*n*-11, 22:1*n*-11 and 24:1*n*-9.

¶ Other *n*-6 PUFA = sum of 18:3*n*-6, 20:3*n*-6, 22:2*n*-6, 22:4*n*-6, 22:5*n*-6.

†† Other *n*-3 PUFA = sum of 18:4*n*-3, 20:4*n*-3, 22:3*n*-3, 24:5*n*3 and 24:6*n*-3.

Table 5. Fillet fatty acid composition (as mg/100 g of edible product) of Atlantic salmon fillet fed the four experimental diets for 283 d*
(Mean values with their standard errors, n 2, N 8)

| | Diets† | | | | | | | | Effect of substrate, end product and substrate/end product interaction (P) | | |
| | Low:Low | | Low:High | | High:Low | | High:High | | | | |
mg/100 g of fillet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Substrate	End product	Interaction
20 : 5n-3	44·2[a]	7·9	67·1[a]	1·1	93·0[a,b]	13·8	130·5[b]	7·8	<0·01	<0·05	NS
22 : 5n-3	27·2[a]	4·6	42·8[a]	2·8	48·7[a]	0·7	69·8[b]	0·6	<0·01	<0·01	NS
22 : 6n-3	294·5[a]	31·7	404·9[a,b]	24·5	340·8[a,b]	23·4	462·1[b]	4·4	NS	<0·01	NS
SFA‡	1607·2	282·1	1307·9	16·2	1561·2	283·6	1725·3	78·4	NS	NS	NS
MUFA	4548	906·1	3404·8	189·4	4376·3	797·9	4423·2	118·2	NS	NS	NS
PUFA	1557·5	265·3	1490·3	81·3	2408·6	444·2	2906·6	120·8	<0·05	NS	NS
LC-PUFA	656·4[a]	95·3	738·7[a]	44·3	821·9[a,b]	83·6	1072·5[b]	10·3	<0·05	NS	NS
Trans	33·1	6·6	22·7	0·9	25·2	6·4	24·1	0·4	NS	NS	NS
n-6 PUFA	1074·3	196·2	841·8	50·1	1324·6	263·5	1377·0	50·8	NS	NS	NS
n-6 LC PUFA	271·9	46·6	195·4	16·6	246·9	31·5	266·5	2·3	NS	NS	NS
n-3 PUFA	446·3[a]	60·1	607·2[a,b]	29	1056·8[b,c]	175·6	1482[c]	68·8	<0·01	<0·05	NS
n-3 LC PUFA	384·5[a]	48·8	543·2[a]	27·7	575·1[a]	52·1	806·0[b]	8·0	<0·01	<0·01	NS
n-6:n-3 ratio	2·4[c]	0·1	1·4[a]	0·0	1·2[a,b]	0·0	0·9[b]	0·0	<0·001	<0·001	<0·01

LC, long chain.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).
* Treatment means were analysed by one-way ANOVA with Tukey's *post hoc* test of multiple comparisons. Effects of substrate, end product and substrate/end product interaction were analysed by two-way ANOVA.
† See Table 1 for experimental diet abbreviations.
‡ See Table 2 for fatty acid classes and abbreviations.
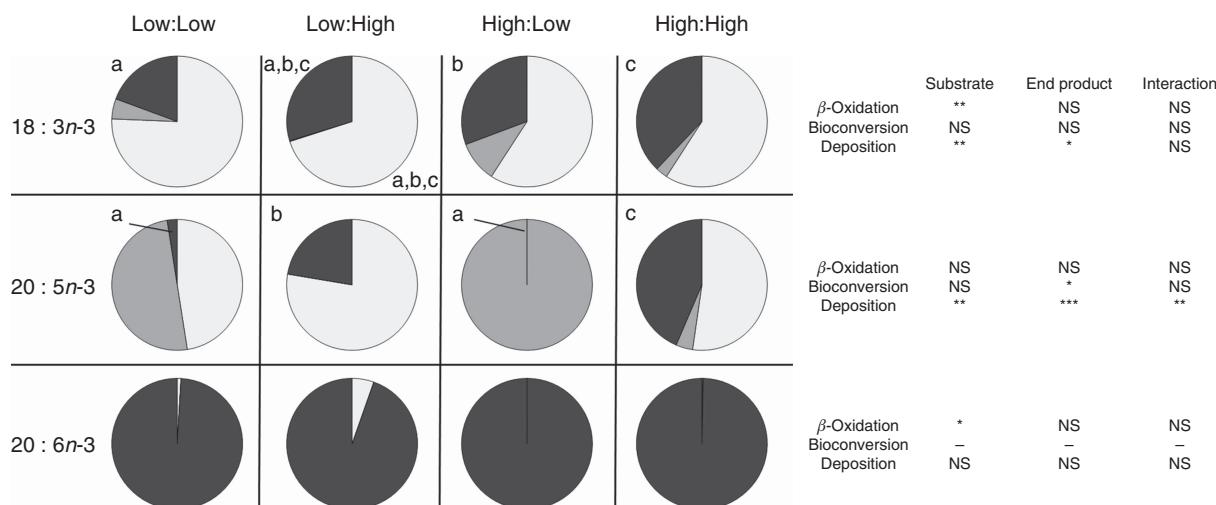
Fig. 1. Metabolic fate of dietary $18:3n$-3, $20:5n$-3 and $22:6n$-3 (β-oxidation, bioconversion and deposition, expressed as percentage of net intake) in post-smolt Atlantic salmon fed experimental diets with altered dietary substrate ($18:3n$-3) and end product ($20:5n$-3 and $22:6n$-3) ratios for 283 d. [a,b,c] Values in the same row (either $18:3n$-3, $20:5n$-3 or $22:6n$-3) and for the same category (β-oxidation, bioconversion and deposition) with unlike letters are significantly different ($P<0.05$) and P values relative to the two-way ANOVA comparing substrate, end product and substrate by end product interactions are reported on the right (* $P<0.05$, ** $P<0.01$, *** $P<0.001$). See Table 1 for experimental dietary abbreviations. ☐, β-Oxidised (%); ▨, bioconverted (%); ■, deposited (%).

Apparent *in vivo* fatty acid bioconversion (expressed as nmol of fatty acid bioconverted per gram of fish per d; nmol/g per d and as percentage of net intake) (Table 7 and Fig. 1, respectively) highlighted the elongation of $18:0$ in three of the four treatments (Low:Low, High:Low and High:High), with the highest values recorded in the High:Low treatment ($P<0.05$). There was a significant effect of end product concentration on the Δ-6 desaturation of $18:2n$-6 ($P<0.001$) with Low:Low recording the highest level of activity ($P<0.05$). Elongation of $18:3n$-6 was noted in all treatments; however, this was significantly higher in Low:Low ($P<0.05$). Likewise, Δ-5 desaturation of $20:3n$-6 was highest in Low:Low ($P<0.05$), whilst Δ-5 desaturation of $20:4n$-3 was highest in High:Low, with negligible activity recorded in the other treatments ($P<0.05$). The High:Low treatment recorded the highest level of Δ-6 desaturation of $18:3n$-3 ($P<0.05$) and numerically, albeit not statistically, higher levels of $24:6n$-3 desaturation. In addition, there was a significant effect of substrate, end product and a substrate by end product interaction recorded for the Δ-6 desaturation of $18:3n$-3. The High:Low treatment recorded significantly higher levels of elongation of $20:5n$-3 ($P<0.05$) and also higher elongation of $22:5n$-3, though the results for the latter were not significant. Recorded levels of $24:6n$-3 chain shortening, the final step of endogenous $22:6n$-3 production, were highest in High:Low; despite relatively minimal amounts recorded in all other treatments, the differences were not significant ($P>0.05$).

Discussion

While previous research has highlighted the capacity for endogenous *n*-3 LC PUFA production in salmonid species, the extent of this metabolic activity has, up until now, not been well quantified in post-smolt Atlantic salmon. In this respect, the present study demonstrated that the amount of apparent endogenous production of *n*-3 LC PUFA was variable between treatment groups of fish fed diets containing altered levels of dietary *n*-3 substrate, namely, $18:3n$-3, and end products, namely, $20:5n$-3 and $22:6n$-3. Despite this it was demonstrated that when provided a diet containing relatively high levels $18:3n$-3 and low levels of $20:5n$-3 and $22:6n$-3, post-smolt Atlantic salmon possesses a capacity for the endogenous synthesis of *n*-3 LC PUFA via *in vivo* fatty acid bioconversion. Moreover, when provided with abundant dietary supply of $18:3n$-3, the extent of endogenous production has the potential to enhance total fillet concentrations of *n*-3 LC PUFA, complementing, and independent of, dietary *n*-3 LC PUFA provision. Furthermore, the present study highlights the positive effect of high dietary substrate inclusion ($18:3n$-3), concomitant with the supply of dietary fish oil, on the final concentration of fillet *n*-3 LC PUFA, attributable to the provision of a suitable substrate for β-oxidation, thus, sparing *n*-3 LC PUFA from catabolism. These important findings are discussed in further detail herein.

The effect of various lipid sources used in aquafeed on the nutritional value of the fish understandably garners substantial research attention. Nevertheless, growth performance remains a key indicator of the suitability of aquafeed formulations for the use in commercial aquaculture operations[60]. As reviewed by Glencross[36] and Turchini *et al.*[5], the substitution of fish oil with terrestrial-based oils has been widely reported to have minimal effect on the growth of salmonids, even at high inclusion levels. Moreover, it has been reported that an excessive inclusion level of LC-PUFA may elicit detrimental effects on fish performance in several species[61–64]. However, currently used levels of dietary *n*-3 LC PUFA in commercial aquafeed are unlikely to elicit any negative effects on growth performance, given the low levels of marine-sourced oils presently utilised. Recent research advocates dietary *n*-3 LC PUFA at an inclusion level in excess of 2·7 % of fatty acids is necessary for optimal growth in post-smolt Atlantic salmon[10,59]. With respect to the

Table 6. Apparent *in vivo* fatty acid β-oxidation (nmol/g per d) in Atlantic salmon fed the four experimental diets for 283 d*
(Mean values with their standard errors, *n* 2, *N* 8)

| | Diets† | | | | | | | | Effect of substrate, end product and substrate/end product interaction (*P*) | | |
| | Low:Low | | Low:High | | High:Low | | High:High | | | | |
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Substrate	End product	Interaction
12:0	2.7a	0.0	2.8a	0.1	1.2b	0.0	1.9b	0.1	<0.001	<0.05	NS
14:0	34.1a	0.5	60.7b	0.4	18.9c	1.0	49.3d	3.5	<0.01	<0.001	NS
16:0	526.5a	2.9	537.8a	2.7	293.8b	2.2	367.5b	23.4	<0.001	<0.05	NS
18:0	121.3a	2.3	130.4a	0.4	42.4b	5.7	85.7b	6.4	<0.001	<0.01	<0.05
20:0	−‡		5.0	0.0	−		−		−	−	−
22:0	1.0a	0	2.0b	0.0	3.1c	0.2	4.6d	0.2	<0.001	<0.01	NS
24:0	1.3a	0.1	2.0b,c	0.1	2.3c	0.1	5.3b	0.2	<0.001	<0.001	<0.01
SFA§‖	686.9a	0.3	740.7a	2.7	361.8b	7.1	514.3b	33.8	<0.001	<0.01	<0.05
14:1*n*-5	5.9a	0.1	5.3b	0.1	3.2c	0.0	2.8c	0.1	<0.001	<0.01	NS
16:1*n*-7	99.6a	0.6	109.0a	1.3	54.7b	2.5	71.0b	6.5	<0.001	<0.05	NS
18:1*n*-7	26.5	1.0	30.2	1.0	21.5	0.2	28.0	3.5	NS	NS	NS
18:1*n*-9	556.0	38.3	551.6	11.4	382.5	12.7	371.6	56.3	<0.01	NS	NS
20:1*n*-9	−		−		69.3	5.9	85.0	13.1	−	−	−
22:1*n*-9	−		−		15.8a	1.3	25.3b	1.7	−	−	−
24:1*n*-9	−		1.2a	0.2	1.9a,b	0.5	4.0a	0.6	<0.01	<0.05	NS
20:1*n*-11	−		3.5	0.1	−		−		−	−	−
22:1*n*-11	3.6a	0.1	6.0b	0.2	3.0a	0.0	5.8b	0.5	NS	<0.01	NS
MUFA	691.6	40.1	706.7	14.1	551.9	18.2	593.6	82.5	NS	NS	NS
18:2*n*-6	179.2	8.6	170.8	0.2	203.1	12.2	197.8	22.1	NS	NS	NS
20:2*n*-6	−		−		1.0	1.0	1.9	1.9	−	−	−
22:2*n*-6	−		−		0.4	0.1	0.5	0.2	−	−	−
20:4*n*-6	−		1.7	0.3	−		2.4	0.8	−	−	−
22:4*n*-6	−		0.4a	0.0	0.1a	0.1	0.6b	0.1	NS	<0.01	NS
22:5*n*-6	−		1.5a	0.1	0.1b	0.1	1.7a	0.2	NS	<0.001	NS
n-6 PUFA	179.2	8.6	174.4	0.1	204.7	13.1	204.8	23.6	NS	NS	NS
18:3*n*-3	50.6a	2.6	41.9a	0.1	251.6b	0.2	273.5b	16.6	<0.001	NS	NS
18:4*n*-3	0.9	0.9	1.3	0.0	−		−		−	−	−
20:4*n*-3	0.3	0.3	1.2	0.1	−		−		−	−	−
20:3*n*-3	0.1	0.1	−		3.0	0.8	1.0	1.0	<0.05	NS	NS
22:3*n*-3	−		−		2.6	0.1	2.8	0.2	−	−	−
20:5*n*-3	5.7	5.7	42.3	0.1	−		28.0	1.7	<0.05	<0.001	NS
22:5*n*-3	1.8	1.8	4.3	0.1	−		−		−	−	−
22:6*n*-3	0.2	0.2	3.2	1.0	−		0.2	0.2	<0.05	<0.05	NS
n-3 PUFA	59.4a	11.3	94.2a	1.5	257.2b	1.1	305.5b	15.8	<0.001	<0.05	NS
Total FA	2549.7	75.2	2700.3	33.7	2394.7	72.1	2731.8	278	NS	NS	NS

a,b,c,d Mean values within a row with unlike superscript letters were significantly different (*P* < 0.05).
* Treatment means were analysed by one-way ANOVA with Tukey's *post hoc* test of multiple comparisons. Effects of substrate, end product and substrate/end product interaction were analysed by two-way ANOVA.
† See Table 1 for experimental diet abbreviations.
‡ β-Oxidation not detected.
§ See Table 2 for fatty acid classes and abbreviations.
‖ Fatty acids not recording any β-oxidation are not reported in this table.

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Table 7. Apparent *in vivo* fatty acid bioconversion (nmol/g per d) in Atlantic salmon fed the four experimental diets for 283 d*
(Mean values with their standard errors, *n* 2, *N* 8)

	Low:Low Mean	SEM	Low:High Mean	SEM	High:Low Mean	SEM	High:High Mean	SEM	Substrate	End product	Interaction
Fatty acid elongation‡											
18:0 to 20:0	1.28^a	0.06	—§		31.56^b	5.72	9.83^a	1.27	<0.01	<0.05	<0.05
18:1n-9 to 20:1n-9	20.15	4.38	10.68	2.25	—		—		—	—	—
20:1n-9 to 22:1n-9	2.06	0.60	0.25	0.24	—		—		—	—	—
22:1n-9 to 24:1n-9	0.27	0.07	—		—		—		—	—	—
18:2n-6 to 20:2n-6	10.31^a	1.87	10.82^a	0.80	0.26^b	0.26	0.55^b	0.55	<0.01	NS	NS
20:2n-6 to 22:2n-6	0.85	0.13	0.92	0.07	—		—		—	—	—
18:3n-6 to 20:3n-6	39.18^a	5.31	9.84^b	0.77	21.19^b	0.77	8.71^b	1.10	<0.05	<0.01	<0.05
20:4n-6 to 22:4n-6	2.31	0.11	—		0.08	0.08	—		—	—	—
22:4n-6 to 24:4n-6	1.73	0.11	—		0.03	0.03	—		—	—	—
18:3n-3 to 20:3n-3	0.02	0.02	0.10	0.10	—		0.50	0.50	NS	NS	NS
18:4n-3 to 20:4n-3	4.23^a	4.23	—		43.64^b	6.44	13.48^a	0.33	<0.01	<0.05	<0.05
20:5n-3 to 22:5n-3	8.10^a	8.10	—		24.82^b	5.51	2.22^a	1.02	NS	<0.05	NS
22:5n-3 to 24:5n-3	8.58	8.53	0.36	0.09	20.76	4.19	1.85	0.80	NS	<0.05	NS
Fatty acid Δ-9 desaturation											
20:0 to 20:1n-11	3.67^a	0.13	—		43.24^b	5.13	26.37^c	0.45	<0.001	<0.05	NS
Fatty acid Δ-6 desaturation											
18:2n-6 to 18:3n-6	52.74^a	4.75	13.28^b	0.69	36.79^c	1.12	15.26^{b,c}	1.31	NS	<0.001	<0.05
24:4n-6 to 24:5n-6	1.73	0.11	—		0.03	0.03	—		—	—	—
18:3n-3 to 18:4n-3	3.31^a	3.31	—		43.02^b	6.52	13.16^a	0.33	<0.01	<0.05	<0.05
24:5n-3 to 24:6n-3	8.59	8.45	0.38	0.04	20.40	4.16	1.52	0.91	NS	<0.05	NS
Fatty acid Δ-5 desaturation											
20:3n-6 to 20:4n-6	15.91^a	2.61	—		5.07^b	0.41	—		—	—	—
20:4n-3 to 20:5n-3	4.00^a	4.00	—		31.50^b	6.16	—		—	—	—
Fatty acid chain shortening											
24:5n-6 to 22:5n-6	1.73^a	0.11	—		0.03^b	0.03	—		—	—	—
24:6n-3 to 22:6n-3	8.45	8.45	—		19.89	4.26	1.02	1.02	NS	<0.05	NS

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0.5$).

* Treatment means were analysed by one-way ANOVA with Tukey's *post hoc* test of multiple comparisons. Effects of substrate, end product and substrate/end product interaction were analysed by two-way ANOVA.

† See Table 1 for experimental diet abbreviations.

‡ Fatty acids not recording any bioconversion (elongation or desaturation) are not reported in this table.

§ Not detected.

present study, there was no difference between major growth parameters between any of the treatments, despite no added fish oil in two of the four dietary treatments; hence, it cannot support this minimum recommended requirement for dietary n-3 LC PUFA in terms of growth performance.

Despite apparent contention regarding growth performance, it is widely accepted that the amount of n-3 LC PUFA in the fillet is a reflection of the dietary inclusion level, as reviewed by Bendiksen et al.[1], Tocher[2], Turchini et al.[5] and Sales & Glencross[65]. However, given the demonstrated capacity of Atlantic salmon to endogenously synthesise $22:6n$-3 from dietary precursors[59,66], the mirroring effect between diet and fillet fatty acid compositions may be partially obscured, especially when diets are devoid of added fish oil[2,67]. Despite this, the biosynthesis of n-3 LC PUFA from $18:3n$-3 in salmonids is recognised as a mechanism to satiate the fundamental physiological minimal requirements of n-3 LC PUFA and is generally insufficient to enrich fillets with n-3 LC PUFA to the same extent as dietary added fish oil and in line with consumer expectations[5,68]. Therefore, the numerically higher fillet levels of n-3 LC PUFA, expressed as both µmol/g and mg/100 g of fillet tissue recorded in fish fed the High:Low diet compared with the Low:High diet, were somewhat unexpected considering the High:Low diet contained no added fish oil. Although it should be noted that similar results were not observed between fish fed the High:Low diet and fish fed the High:High diet. Nevertheless, as it will be discussed, these results highlight differences in lipid metabolism, namely, β-oxidation, bioconversion and deposition of dietary fatty acids, specifically, $18:3n$-3, $20:5n$-3 and $22:6n$-3.

It is well understood that dietary fatty acids provide the vast majority of metabolic energy for salmonids[12]. In particular, MUFA, including $18:1n$-9, and SFA, including $16:0$, are known to be readily catabolised for this specific purpose[69,70]. However, due to the increasing commercial utilisation of dietary oils rich in C_{18} n-3 PUFA in aquafeed formulations (e.g. canola oil, linseed oil and potentially in the future, camelina oil) $18:3n$-3 is increasingly relied upon by farmed salmon as a dietary energy substrate[10,40,71,72]. Research suggests $18:3n$-3 is readily catabolised in fish, including salmonids, proportional to dietary inclusion levels[30,73–75]. With respect to the present study, dietary n-3 PUFA substrate level had a significant effect on the β-oxidation of $18:3n$-3 ($P < 0.001$). Specifically, an increase in dietary $18:3n$-3 led to a proportional decrease in β-oxidation of $18:3n$-3 (in terms of percentage of net intake). However, considering the enhanced dietary supply of $18:3n$-3 in the high-substrate treatments, quantitatively more $18:3n$-3 was β-oxidised in both high dietary $18:3n$-3 treatments (in terms of nmol/g per d). Furthermore, the deposition of $18:3n$-3 proportionally increased with an increased dietary supply. This is in contrast to the notion of decreasing deposition efficiency of n-3 PUFA with increased dietary supply[51,73,76,77]. However, considering the increased total amount of $18:3n$-3 in the high dietary $18:3n$-3 treatments, the actual amount of β-oxidised was greater than the low dietary $18:3n$-3 treatments.

In addition to providing a suitable substrate for β-oxidation in salmonids, the first step of n-3 PUFA bioconversion has been shown to be primarily modulated by the availability of C_{18} n-3 PUFA by providing a substrate for Δ-6 desaturase activity[10,31,32,34,41]. The findings of the present study support this, and it was found that the Δ-6 desaturation of $18:3n$-3 was significantly enhanced by the dietary addition of $18:3n$-3 (in terms of both nmol/g per d and percentage of net intake). In addition, and in accordance with previous research, the absence of added dietary n-3 LC PUFA appeared to further enhance bioconversion activity relative to the high dietary n-3 LC PUFA treatments owing to the absence of a previously identified negative feedback mechanism acting upon the n-3 LC PUFA bioconversion pathway in both mice and Atlantic salmon[39,78].

Further to $18:3n$-3 bioconversion, there were observable differences between treatments in relation to the metabolism of $20:5n$-3. Previous research has suggested n-3 LC PUFA can be preserved from catabolism by careful manipulation of the dietary fatty acid composition termed 'n-3 LC PUFA sparing'[74,79–82]. Specifically, the dietary addition of saturated and MUFA has been shown to enhance the retention efficiency of n-3 LC PUFA[6,51,74]. With respect to the present study, diet had a clear effect on the deposition of $20:5n$-3. The Low:High treatment appeared to favour the catabolism of longer, more unsaturated fatty acids, namely, $20:5n$-3. In contrast, the High:High treatment had a lesser reliance on n-3 LC PUFA β-oxidation and instead appeared to preferentially β-oxidise $18:3n$-3. These findings support previous research advocating the suitability of $18:3n$-3 as a dietary energy substrate[22,72,83–85]. Moreover, they agree with the findings in juvenile salmon advocating the potential for added dietary $18:3n$-3 to spare n-3 LC PUFA from catabolism[86].

Despite a very limited dietary provision of n-3 LC PUFA in the High:Low treatment, virtually all endogenously synthesised $20:5n$-3 was further bioconverted, culminating in a recorded chain-shortening of $24:6n$-3 – the final step in n-3 LC PUFA biosynthesis – to produce $22:6n$-3. In accordance with previous research, it therefore appears that a production of $22:6n$-3 was favoured over $20:5n$-3 deposition, which appears to be present predominately as an intermediate step toward the de novo biosynthesis of $22:6n$-3[46,73,87]. Furthermore, in accordance with previous research that identified a negative feedback mechanism on the n-3 PUFA bioconversion pathway, the presence of dietary n-3 LC PUFA appeared to negatively affect the bioconversion of $20:5n$-3[12,33,78].

In general, dietary $22:6n$-3 is well conserved from catabolism in Atlantic salmon, resulting in similar fillet levels to those provided by the diet[9,75,84,88,89]. The present study largely confirms this and high retention of $22:6n$-3 was recorded in all treatments. However, a significant effect of dietary substrate level ($18:3n$-3) on the β-oxidation of $22:6n$-3 was recorded. Specifically, the β-oxidation of $22:6n$-3 was reduced in both high dietary substrate ($18:3n$-3) treatments. Resultantly, a high dietary $18:3n$-3 content provided (i) a suitable and highly available β-oxidation substrate that, consequently, enabled the sparing of n-3 LC PUFA from β-oxidation, (ii) an enhanced substrate for bioconversion and (iii) in contrast to high end product treatments, it did not inhibit n-3 LC PUFA biosynthesis which, as mentioned, can elicit a negative feedback mechanism on the n-3 PUFA bioconversion pathway[12,78]. Consequently,

the addition of abundant dietary 18:3*n*-3 in diets devoid of added fish oil had a positive effect on the final *n*-3 LC PUFA level in the fillet of post-smolt Atlantic salmon.

In the absence of added fish oil, the high dietary provision of 18:3*n*-3 potentially provided multiplicative benefits in terms of facilitating the deposition of *n*-3 LC PUFA in to the fillet tissue by favouring the *β*-oxidation of dietary C_{18} *n*-3 PUFA resulting in an apparent conservation of *n*-3 LC PUFA from catabolism and by providing sufficient substrate for *n*-3 LC PUFA biosynthesis. These findings demonstrate the capacity for endogenous *n*-3 LC PUFA synthesis in post-smolt Atlantic salmon to ameliorate the potential negative consequences on fillet nutritional quality often attributed to a severe reduction or removal of dietary provided fish oil. This provides a commercially relevant platform for the further development of tailored diets with respect to production stage to ensure Atlantic salmon is highly nutritious at the time of harvest. Complementary methods, such as real-time PCR and stable isotope analysis, are suggested in future trials on post-smolt Atlantic salmon. This will enable a better quantification of the possible modulation of known genes associated with *n*-3 LC PUFA bioconversion processes and a more accurate trace of individual fatty acids along the *n*-3 LC PUFA bio-conversion pathway, and in particular the ability to differentiate from *de novo* synthesised LC-PUFA *v*. LC-PUFA spared from catabolic processes. Nevertheless, the information provided herein, scant for post-smolt Atlantic salmon, is essential to create dietary strategies that mitigate a 'low fish oil future'.

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