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Title: Endogenous biosynthesis of n-3 LC PUFA in Atlantic salmon

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Short title: Quantification of endogenous n-3 LC PUFA production in large, post-smolt Atlantic salmon

Keywords: Fatty acid, metabolism, omega-3, bioconversion, aquaculture, fish oil

Abstract

A more efficient utilisation of marine derived sources of dietary omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA) in cultured Atlantic salmon could, amongst other strategies, be facilitated by nutritional strategies that maximise endogenous n-3 LC PUFA synthesis. The objective of the current study was to quantify the extent of n-3 LC PUFA biosynthesis and the resultant effect on fillet nutritional quality in large, market size Atlantic salmon. Four diets were manufactured providing altered levels of dietary omega-3 substrate, namely 18:3n-3, and end-products, namely, 20:5n-3 and 22:6n-3. After 283 days of feeding, fish grew to in excess of 3000g and no differences in growth performance or biometrical parameters were recorded. An analysis of fatty acid composition and *in vivo* metabolism revealed that post-smolt Atlantic salmon have the potential to endogenously produce n-3 LC PUFA when provided with a substantial amount of dietary omega-3 substrate. Moreover, the extent of endogenous production resulted in fillet levels of n-3 LC PUFA comparable to fish fed a diet with added fish oil. Another major finding was that the presence of abundant dietary omega-3 substrate with the addition of dietary omega-3 end-product (i.e. fish oil) had a positive effect on final fillet levels of n-3 LC PUFA. This was likely the result of the preferential β-oxidation of dietary C_{18} n-3 PUFA resulting in an apparent conservation of n-3 LC PUFA from catabolism. Ultimately, this study highlights the potential for endogenous synthesis of n-3 LC PUFA to, at least partially, support a substantial reduction, in the amount of dietary fish oil in diets for market sized Atlantic salmon reared in seawater.

1. Introduction

The intense market volatility and reduced availability of traditional protein and lipid sources such as fishmeal and fish oil for use in aquafeed has led to constantly evolving dietary formulations (Bendiksen et al. 2011; Tocher 2015; Turchini et al. 2010). 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, omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA) (Sprague et al. 2016; Tocher 2015; Turchini et al. 2009). Resultantly, 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 (Emery et al. 2016; Francis & Turchini 2017; Nuez Ortin et al. 2015; Torstensen et al. 2004). 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 various aspects of fatty acid metabolism, including *in vivo* fatty acid β-oxidation and bioconversion (Hixson et al. 2017; Norambuena et al. 2015; Tocher 2003; Torstensen et al. 2000). Concomitant with an increased understanding of fatty acid metabolism within popular cultured species in recent years, dietary formulations have been manipulated in order 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 (Francis & Turchini 2017; Hixson et al. 2017; Karalazos et al. 2011; Torstensen et al. 2004). The n-3 LC PUFA biosynthetic pathway is facilitated by desaturase and elongase enzymes found in many fish and mammalian species (Monroig et al. 2010; Nakamura & Nara 2004; Tocher 2003). 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 is not uniform across species (Castro et al. 2016; Tocher 2003). Specifically, marine species of fish are purported to have a severely limited capacity to biosynthesise 22:6n-3 from 18:3n-3 dietary substrate (Monroig et al. 2011; Morais et al. 2009; Venegas-Calerón et al. 2010). It is hypothesised that the abundance of n-3 LC PUFA in marine ecosystems, which originate in lower trophic eukaryotes and prokaryotes (Kabeya et al.

2018), has rendered the n-3 LC PUFA biosynthetic pathway largely redundant (Monroig et al. 2011; Morais et al. 2009; Nakamura & Nara 2004; Tocher 2003). 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 (Bell et al. 2001; Ruyter & Thomassen 1999; Sissener et al. 2016; Turchini et al. 2013).

Atlantic salmon have a demonstrated ability to bioconvert 18:3n-3 to 22:6n-3. Resultantly, numerous metabolic responses to dietary fatty acid changes have been elucidated in this species (Bell et al. 2001; Giri et al. 2016; Monroig et al. 2010; Norambuena et al. 2015; Nordgarden et al. 2003; Nuez Ortin et al. 2015; Ruyter & Thomassen 1999; Tocher et al. 2002; Torstensen et al. 2000). 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 (Glencross et al. 2014; Hixson et al. 2017; Thanuthong et al. 2011; Thomassen et al. 2012; Turchini & Francis 2009). Oppositely, the presence of end-product (22:6n-3) may elicit a negative feedback mechanism on the pathway (Thomassen et al. 2012). 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 (Mellery et al. 2016; Tocher 2003; Torstensen et al. 2004). For instance, n-3 LC PUFA synthesis is generally more efficient in the juvenile stages, and then decreases in the seawater growth phase (Glencross 2009). Therefore, endogenous synthesis has been documented not to be able to enrich the fillet tissue with n-3 LC PUFA to the same extent as dietary added fish oil in Atlantic salmon (Kjær et al. 2016; Leaver et al. 2008; Sissener et al. 2016; Tocher et al. 2003; Torstensen et al. 2000; Turchini et al. 2011b; Xue et al. 2015). Nevertheless, there remains multiple, interrelated dietary and environmental factors which dictate the final concentration of n-3

LC PUFA that can be synthesised *de novo* (Giri et al. 2016; Hixson et al. 2017; Lewis et al. 2013; Senadheera et al. 2012a, b; Zheng et al. 2005).

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 and Atlantic salmon, in particular, are popular with consumers, in-part, due to historically high levels of n-3 LC PUFA (Christenson et al. 2017; Strobel et al. 2012; Tocher 2010). 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 (Nichols et al., 2014; Sprague et al. 2016) 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 (Francis & Turchini 2017; Tacon & Metian 2015; Tocher 2015). 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 (Giri et al. 2016; Hixson et al. 2017; Tocher 2010). 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 focussed 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 (Tocher 2010). 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 (Talbot et al. 1999). Accordingly, the current 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.

2. Materials and Methods

2.1. 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.

2.2. Animals, trial facility and sampling

Juvenile Atlantic salmon (*Salmo 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 6mm salmonid diet (Ridley Aquafeed Pty. Ltd.) prior to the commencement of the trial period. The experiment was conducted in a closed-loop, thermostatically controlled, recirculating aquaculture system containing eight (5000 L) rearing tanks. Physical and biological filtration (drum filter fitted with a 60 µm screen; Hydrotech, Vellinge, Sweden)

and UV disinfection, maintained water quality throughout the experiment. The system was maintained on a 12:12 hour light: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 levels of metabolic waste, total ammonia, phosphorus, nitrite and nitrate were monitored daily using Aquamerck test kits (Merck, Darmstadt, Germany) and 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^{-1}) and stored at -20 °C until analysis. Initially, 272 fish were distributed amongst eight tanks (34 fish per tank) and assigned one of four dietary treatments in duplicate (two tanks per treatment; n = 2, N = 8). After 14 and 28 weeks, weight checks were performed and fish removed to improve stocking densities. Fish were fed to apparent satiation twice a day at 0900 and 1600 h for the entire (40 week) grow-out period. Feed consumption and mortalities were recorded throughout the trial and remained within acceptable limits. Faeces were collected two weeks prior to final sampling for the estimation of digestibility. At the completion of the grow-out phase, all fish were euthanised in excess anaesthetic (AQUI-S, 0.05 ml/L) and weighed. Subsequently, 18 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 (10 fish per treatment; five fish per tank) were used for analysis of biometry and for chemical analysis of whole-body, the second group (eight fish per treatment; four fish per tank) were used for biometry and chemical analysis of fillet.

2.3. Experimental diets

Four experimental diets were formulated to be iso-lipidic (310 mg g^{-1}), iso-proteic (440 mg g^{-1}) and iso-energetic (26 kJ g^{-1}) (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 by using three different lipid sources; poultry by-product oil, fish oil and camelina oil, to create varied ratios of short-chain to long-chain omega-3 fatty acids and therefore four varied compositions of 'substrate' (18:3n-3) and 'end-product' (20:5n-3 and 22:6n-3) in terms of *in vivo* bioconversion of omega-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 % fish oil; and
- High substrate: High end-product (High:High), where added oil consisted of 80 % camelina oil and 20 % fish oil.

2.4. 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 (DP %), 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. (2014). The chemical composition of the experimental diets, faeces and fish samples was determined via proximate composition analysis according to standard methods, as previously described (Emery et al., 2013). 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., SA, PTY LTD Australia) at 550°C for 18 h. Protein (Kjeldahl nitrogen: N × 6.25) content was determined using an automated Kjeltech 2300 (Foss Tecator, Geneva, Switzerland) and lipid was determined by solvent extraction (2:1) (Folch et al., 1957), 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 gas chromatography as described in Norambuena et al. (2013). Resulting peaks were identified relative to known external standards, and then corrected for theoretical FID response and methyl transformation, required for GC analysis. The resulting quantitative fatty acid data were eventually reported as mg g^{-1} for the experimental diets and as μmol g-1 tissue (wet weight) for fillet tissue, as recommended by Parrish (2016).

2.5. Nutrient digestibility and metabolism calculations

Evaluation of digestibility was determined following methods presented in Atkinson (1984), 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., (2006) with further development (Turchini et al. 2007; Turchini & Francis 2009). The results of the whole-body fatty acid balance method are reported as nmol g^{-1} day^{-1} and percentage of net intake as presented in Norambuena (2015).

2.6. Statistical analysis

All data were reported as mean ± standard error; (n = 2, N = 8). After confirmation of normality and homogeneity of variance, data was 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

v24.0 (SPSS Inc., Chicago, IL, USA). Significance was accepted at $P < 0.05$, where: * = $P < 0.05$; ** = $P < 0.01$ and *** = $P < 0.001$.

3. Results

3.1. 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^{-1} 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 18:1n-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 18:3n-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 22:6n-3 concentrations. The ratio of n-6 to n-3 fatty acids varied considerably between treatments ranging from 0.8 to 4.1 in High:High and Low:Low, respectively ($P < 0.05$).

3.2. 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 ~2000g and grew to weights in excess of 3000g 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 omega-3 substrate, end-product or substrate by end-product interactions recorded with regard to any growth performance parameters.

3.3. Apparent nutrient and fatty acid digestibility

High nutrient digestibility values (Apparent Digestibility Coefficient – ADC %) were observed across treatments with only one significant difference recorded, where NFE digestibility was higher in Low:Low in comparison to High:High ($P < 0.05$) (Table 3). Fatty acid digestibility values were high with no significant differences recorded between treatments, although there was a significant effect of end-product recorded for the digestibility of 20:5n-3.

3.4. 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^{-1} 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, although differences were not significant ($P > 0.05$). Expectedly, fillet levels of 22:6n-3 were higher in High:High compared to Low:Low in terms of both µmol g^{-1} of fillet tissue and mg $100g^{-1}$ of fillet ($P < 0.05$). However, High:Low had comparable levels of 22:6n-3 compared to both Low:High and High:High ($P > 0.05$). Furthermore, High:Low had marginally higher levels of total n-3 LC PUFA compared to 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 to Low:Low ($P < 0.05$). The n-6 to 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^{-1} of fillet tissue and mg $100g^{-1}$ of fillet , however, no significant substrate by end-product interaction was recorded.

3.5. 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 day; nmol g^{-1} day^{-1} and as % of net intake) (Table 5 and Figure 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 on the basis % of net intake, both the low substrate treatments (Low:Low and Low:High) recorded higher values (~70%). Notably, 22:6n-3 was highly conserved across treatments and recorded low β-oxidation values.

Apparent *in vivo* fatty acid bioconversion (expressed as nmol of fatty acid bioconverted per gram of fish per day; nmol g^{-1} day^{-1} and as % of net intake) (Table 6 and Figure 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 higher levels of 24:6n-3 desaturation. Additionally, 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, although results for the latter were not significant. Recorded levels of 24:6n-3 chain shortening, the final step of endogenous 22:6n-3 production, was highest in High:Low, although, despite relatively minimal amounts recorded in all other treatments, the differences were not significant ($P > 0.05$).

4. 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 demonstrates a clear capacity for the endogenous synthesis of n-3 LC PUFA in post-smolt Atlantic salmon via *in vivo* fatty acid bioconversion. Moreover, when provided with abundant dietary supply of 18:3n-3, the extent of endogenous production can considerably enhance 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 (Føre et al. 2016). As reviewed by Glencross (2009) and Turchini (2009), 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 (Betancor et al. 2011; Glencross & Rutherford 2011; Ostbye et al. 2011; Ruyter et al. 2000). However, currently used levels of dietary n-3 LC PUFA in commercial aqufeed 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 (Hixson et al. 2017; Rosenlund et al. 2016). With respect to the 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. 2011; Sales & Glencross 2011; Tocher 2015; Turchini et al. 2009). However, given the demonstrated capacity of Atlantic salmon to endogenously synthesise 22:6n-3 from dietary precursors, the mirroring effect between diet and fillet fatty acid compositions may be partially obscured, especially when diets are devoid of added fish oil (Miller et al. 2008; Tocher 2015). 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 (Bell et al. 2004; Turchini et al. 2009). Therefore, the marginally higher fillet levels of n-3 LC PUFA, expressed as both μmol g^{-1} and mg 100 g^{-1} of fillet tissue recorded in fish fed the High:Low diet compared to the Low:High diet was somewhat unexpected considering the High:Low diet contained no added fish oil.

As will be discussed, these differences are a clear result of differences in lipid metabolism, namely, β-oxidation, bioconversion and deposition of 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 (Tocher 2003). In particular, monounsaturated fatty acids, including 18:1n-9 and saturated fatty acids, including 16:0, are known to be readily catabolised for this specific purpose (McKenzie et al. 1998; Sargent et al. 2003). However, due to the increasing commercial utilisation of dietary oils rich in C_{18} n-3 PUFA in aquafeed formulations (eg. 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 (Bell et al. 2010; Hixson et al. 2014; Hixson et al. 2017; Turchini et al. 2011b). Research suggests 18:3n-3 is readily catabolised in fish, including salmonids, proportional to dietary inclusion levels (Mourente et al. 2005; Stubhaug et al. 2007; Tocher et al. 2002; Turchini et al. 2011a). 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 % 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^{-1} day^{-1}). 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 (Bell et al. 2002; Budge et al. 2011; Francis et al. 2014; Stubhaug et al. 2007). However, considering the increased total amount of 18:3n-3 in the high dietary 18:3n-3 treatments, the actual amount β-oxidised was greater compared to 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 (Glencross et al. 2014; Hixson et al. 2017; Thanuthong et al. 2011; Turchini & Francis 2009; Xue et al. 2015). The findings of the present study support this and found that the Δ-6 desaturation of 18:3n-3 was significantly enhanced by the dietary addition of 18:3n-3 (both in terms of nmol g^{-1} day^{-1} and % of net intake). Additionally, 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 (Raz et al. 1997; Tocher et al. 2003).

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' (Codabaccus et al. 2012; Eroldoğan et al. 2013; Rombenso et al. 2015; Trushenski et al. 2013; Turchini et al. 2011a). Specifically, the dietary addition of saturated and monounsaturated fatty acids has been shown to enhance the retention efficiency of n-3 LC PUFA (Emery et al. 2016; Francis et al. 2014; Turchini et al. 2011a). 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 (Bell et al. 2001; Bell et al. 2003a; Bell et al. 2003b; Hixson et al. 2014; Sinclair et al. 2002). Moreover, they agree with findings in juvenile salmon advocating the potential for added dietary 18:3n-3 to spare n-3 LC PUFA from catabolism (Berge et al. 2004).

Despite no dietary lipid 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 (Codabaccus et al. 2011; Stubhaug et al. 2007; Tocher 2010). Furthermore, in accordance with previous research, the presence of dietary n-3 LC PUFA appeared to negatively affect the bioconversion of 20:5n-3 (Raz et al. 1997; Thomassen et al. 2012; Tocher 2003).

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 (Bell et al. 2003b; Bransden et al. 2003; Mourente et al. 2005; Pratoomyot et al. 2010; Torstensen et al. 2004). 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, did not inhibit n-3 LC PUFA biosynthesis via the presence of end-product desaturase inhibitors. Consequently, the addition of abundant dietary 18:3n-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.

5. Conclusion

In the absence of added fish oil, the high dietary provision of 18:3n-3 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 are highly nutritious at the time of harvest. Complimentary methods, such as real-time polymerase chain reaction 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 bioconversion pathway, and in particular the ability to differentiate from *de novo* synthesised LC-PUFA vs LC-PUFA spared from catabolic processes. Nevertheless, the information provided herein, scant for post-smolt Atlantic salmon, is essential in order to create dietary strategies that mitigate a 'low fish oil future'.

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Conflict of interest

The authors wish to declare funding and donation of materials for the growth trial from Ridley Aquafeed Ltd. (Brisbane, Australia). The authors wish to clarify that the current trial compared experimental dietary lipid compositions in aquafeed and was not comparing commercially produced products by Ridley Aquafeed Ltd.

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Figure 1; Metabolic fate of dietary 18:3n-3, 20:5n-3 and 22:6n-3 (β-oxidation, bioconversion and deposition, expressed as % 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 days. 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 different superscripts 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 (ns = not significant; * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$). See Table 1 for experimental dietary abbreviations.

Diet Formulations ?

Table 1

Proximate composition, total fatty acids and fatty acid (mg g^{-1} diet) composition of the four experimental diets.

	Diets[a]			
	Low : Low	Low : High	High : Low	High : High
Proximate composition (mg g^{-1})				
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^{-1})	25.8	26.0	25.7	26.0
Total FA (mg g^{-1} diet)[b]	272.6	270.6	247.4	253.7
SFA[c]	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[d]	2.4	3.6	3.3	4.6
MUFA[e]	143.0	132.7	115.3	108.1
16:1n-7	14.1	14.7	8.2	9.0
18:1n-9	118.9	105.6	82.7	71.5
18:1n-7	6.6	6.9	4.6	5.0
20:1n-9	1.7	2.4	14.8	16.2
Other MUFA[f]	1.7	3.0	4.9	6.3
Total trans FA[g]	1.7	1.5	0.8	0.6
PUFA[h]	44.7	49.9	75.5	83.2
18:2n-6	34.1	29.7	36.3	33.2
20:2n-6	0.3	0.3	1.8	1.9
20:4n-6	0.6	1.3	0.4	1.0
Other n-6 PUFA[i]	0.8	1.2	0.6	1.1
n-6 PUFA[j]	35.8	32.6	39.2	37.3
18:3n-3	5.3	4.7	32.3	33.5
20:5n-3	1.0	4.6	0.8	4.2
22:5n-3	0.4	1.0	0.3	0.9
22:6n-3	1.5	5.5	1.2	4.9
n-3 PUFA[k]	8.7	16.6	36.2	45.2
Other n-3 PUFA[l]	0.5	0.8	1.6	1.7
LC PUFA[m]	4.6	14.2	6.5	15.5
n-6 LC PUFA[n]	1.4	2.5	2.7	3.9
n-3 LC PUFA[o]	3.2	11.7	3.8	11.6
n-6/n-3 ratio[p]	4.1	2.0	1.1	0.8

[a] 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 : High = high substrate, high end-product diet consisting of 44% protein and 31% lipid, added oil consists of 80% camelina oil and 20% fish oil;

[b] Total FA = total fatty acids mg/g of diet.

[c] SFA = saturated fatty acids.

[d] Other SFA = sum of 12:0, 15:0, 17:0, 20:0, 22:0 & 24:0.

[e] MUFA = monounsaturated fatty acids.

[f] Other MUFA = sum of 14:1n-5, 15:1n-5, 17:1n-7, 20:1n-13, 20:1n-11, 22:1n-11, 22:1n-9 & 24:1n-9.

[g] Total trans FA = sum of 18:1n-9t, 18:1n-7t & 18:2n-6t.

[h] PUFA = polyunsaturated fatty acids.

[i] Other n-6 PUFA = sum of 18:3n-6, 20:2n-6, 20:3n-6, 22:2n-6, 22:4n-6 & 22:5n-6.

[j] n-6 PUFA = omega-6 polyunsaturated fatty acids.

[k] n-3 PUFA = omega-3 polyunsaturated fatty acids.

[l] Other n-3 PUFA = sum of 20:3n-3, 20:4n-3, 24:5n-3 & 24:6n-3.

[m] LC-PUFA = long chain polyunsaturated fatty acids.

[n] n-6 LC PUFA = omega-6 long chain polyunsaturated fatty acids.

[o] n-3 LC PUFA = omega-3 long chain polyunsaturated fatty acids.

[p] 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 days.

	Diets[a]				Effect of substrate, end-product and substrate / end-product interaction		
	Low : Low	Low : High	High : Low	High : High	Substrate	End-product	Interaction
Initial wt (g)	1116 ± 1	1126 ± 3	1118.7 ± 1	1128 ± 1	ns	ns	ns
Final wt (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[b]	0.40 ± 0.01	0.40 ± 0.00	0.44 ± 0.03	0.41 ± 0.00	ns	ns	ns
FCR[b]	1.14 ± 0.06	1.16 ± 0.01	1.18 ± 0.01	1.26 ± 0.04	ns	ns	ns
SGR[c]	0.36 ± 0.01	0.35 ± 0.00	0.35 ± 0.00	0.34 ± 0.00	ns	ns	ns
K[d]	1.6 ± 0.1	1.5 ± 0.0	1.9 ± 0.4	1.4 ± 0.1	ns	ns	ns
DP (%)[e]	87.0 ± 8.1	94.9 ± 3.2	94.8 ± 3.2	92.2 ± 6.5	ns	ns	ns
FY (%)[f]	52.2 ± 5.2	55.7 ± 0.8	57.1 ± 0.3	52.0 ± 3.5	ns	ns	ns
HSI (%)[g]	1.2 ± 0.1	1.4 ± 0.0	1.4 ± 0.1	1.3 ± 0.0	ns	ns	ns
VSI (%)[h]	9.8 ± 0.5	9.3 ± 0.3	9.2 ± 1.5	9.0 ± 0.9	ns	ns	ns

Data are expressed as mean ± S.E.M., *n* = 2, *N* = 8. P < 0.05; Treatment means analysed by one-way ANOVA with Tukey's post-hoc test of multiple comparisons. Effect of substrate, end-product and substrate / end-product interaction analysed by two-way ANOVA (ns = not significant) * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

[b]Feed ration (% bw / day)

[c] FCR = food conversion ratio.

[d] SGR = specific growth rate.

[e] K = condition factor.

[f] DP (%) = dress-out percentage.

[g] FY (%) = fillet yield percentage.

[h] HSI (%) = hepatosomatic index.

[i] VSI (%) = viscerosomatic index.

Table 3

Nutrient and fatty acids digestibility (apparent digestibility coefficient - ADC %) of the four experimental diets in Atlantic salmon.

	Diets[a]				Effect of substrate, end-product and substrate / end-product interaction		
	Low : Low	Low : High	High : Low	High : High	Substrate	End-product	Interaction
Nutrients[b]							
DM[b]	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[b]	81.9 ± 0.1a	76.6 ± 0.1ab	78.4 ± 1.6ab	73.7 ± 2.1b	ns	ns	ns
Energy[c]	94.1 ± 0.3	92.8 ± 0.4	93.3 ± 0.2	92.2 ± 1.2	ns	ns	ns
Total FA[d]	96.8 ± 0.6	96.5 ± 0.1	97.2 ± 0.4	96.1 ± 0.9	ns	ns	ns
Fatty acids							
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	*
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	*	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

Data are expressed as mean ± S.E.M., n = 2, N = 8. $P < 0.05$; Treatment means analysed by one-way ANOVA with Tukey's post-hoc test of multiple comparisons. Effect of substrate, end-product and substrate / end-product interaction analysed by two-way ANOVA (ns = not significant) * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

[a] See Table 1 for experimental diet abbreviations.

[b] Nutrients: DM, dry matter; NFA, nitrogen-free extract.

[c] Calculated on the basis of 23.6, 39.5 and 17.2 kJ g^{-1} of protein, fat and carbohydrate, respectively.

[d] Total FA = total fatty acids

[e] Value of 100 = fatty acid not detected in faeces.

Table 4

Proximate (mg g^{-1} of tissue) and fatty acid composition (µmol g^{-1} tissue) of fillets of Atlantic salmon fed the four experimental diets for 283 days.

					Effect of substrate, end-product and substrate / end-product interaction		
	Low : Low	Low : High	High : Low	High : High	Substrate	End-product	Interaction
Proximate composition (mg g-1 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
Fatty acids (µmol g-1 of tissue)							
Total FA[b]	277.1 ± 52.4	221.9 ± 10.0	296.6 ± 54.7	321.4 ± 11.5	ns	ns	ns
SFA[c]	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[d]	1.6 ± 0.3	1.6 ± 0.0	2.3 ± 0.4	2.8 ± 0.1	*	ns	ns
MUFA	161.5 ± 32.3	120.9 ± 6.7	153.4 ± 28.0	155 ± 4.1	ns	ns	ns
16:1n-7	14.5 ± 3.3	11.9 ± 0.2	11.6 ± 2.3	13.4 ± 0.7	ns	ns	ns
18:1n-9	129.2 ± 25.8	94.2 ± 5.5	111.1 ± 20.0	107.9 ± 2.3	ns	ns	ns
18:1n-7	8.7 ± 1.7	7.3 ± 0.5	7.1 ± 1.3	7.9 ± 0.2	ns	ns	ns
20:1n-9	6.0 ± 0.9a	5.0 ± 0.5a	13.9 ± 2.2b	17.0 ± 0.1b	**	ns	ns
Other MUFA[e]	3.2 ± 0.6ab	2.4 ± 0.0a	9.7 ± 2.2b	8.8 ± 1.0ab	**	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	*	ns	ns
18:2n-6	27.7 ± 5.0	23.3 ± 1.3	36.7 ± 7.8	39.2 ± 1.7	ns	ns	ns
20:2n-6	1.9 ± 0.2ab	1.7 ± 0.2a	2.9 ± 0.6ab	3.6 ± 0b	*	ns	ns

20:4n-6	2.8 ± 0.5	2.0 ± 0.1	1.9 ± 0.2	2.0 ± 0.1	ns	ns	ns
Other n-6 PUFA[f]	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
18:3n-3	2.2 ± 0.4a	2.3 ± 0.1a	17.3 ± 4.4b	24.2 ± 2.2b	**	ns	ns
20:5n-3	1.5 ± 0.3a	2.2 ± 0.0a	3.1 ± 0.5ab	4.3 ± 0.3b	**	*	ns
22:5n-3	0.8 ± 0.1a	1.3 ± 0.1ab	1.5 ± 0.0b	2.1 ± 0.0c	**	**	ns
22:6n-3	9.0 ± 1.0a	12.3 ± 0.7ab	10.4 ± 0.7ab	14.1 ± 0.1b	ns	**	ns
Other n-3 PUFA[g]	0.7 ± 0.1a	0.9 ± 0.0a	3.0 ± 0.5b	4.7 ± 0.1b	***	*	*
n-3 PUFA	14.1 ± 1.9a	19 ± 0.9ab	35.2 ± 6.1bc	49.4 ± 2.4c	**	ns	ns
n-3 LC PUFA	11.8 ± 1.5a	16.7 ± 0.8a	17.9 ± 1.6a	25.1 ± 0.2b	**	**	ns
LC PUFA	20.6 ± 3.0a	23.0 ± 1.4ab	25.9 ± 2.7ab	33.7 ± 0.3b	*	ns	ns

Data are expressed as mean ± S.E.M., *n* = 2, *N* = 8. P < 0.05; Treatment means analysed by one-way ANOVA with Tukey's post-hoc test of multiple comparisons. Effect of substrate, end-product and substrate / end-product interaction analysed by two-way ANOVA (ns = not significant) * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

[b] Total FA = total fatty acids µg/g of tissue

[c] See table 2 for fatty acid classes and abbreviations.

[d] Other SFA = sum of 12:0, 15:0, 17:0, 20:0, 21:0, 22:0 & 24:0.

[e] Other MUFA = sum of 14:1n-5, 15:1n-5, 17:1n-7, 20:1n-11, 22:1n-11 & 24:1n-9.

[f] Other n-6 PUFA = sum of 18:3n-6, 20:3n-6, 22:2n-6, 22:4n-6, 22:5n-6.

[g] Other n-3 PUFA = sum of 18:4n-3, 20:4n-3, 22:3n-3, 24:5n3 & 24:6n-3.

Table 5

Fillet fatty acid composition (as mg 100 g^{-1} of edible product) of Atlantic salmon fillet fed the four experimental diets for 283 days.

	Diets[a]				Effect of substrate, end-product and substrate / end-product interaction		
mg 100 g^{-1} of fillet	Low : Low	Low : High	High : Low	High : High	Substrate	End-product	Interaction
20:5n-3	44.2 ± 7.9a	67.1 ± 1.1a	93.0 ± 13.8ab	130.5 ± 7.8b	**	*	ns
22:5n-3	27.2 ± 4.6a	42.8 ± 2.8a	48.7 ± 0.7a	69.8 ± 0.6b	**	**	ns
22:6n-3	294.5 ± 31.7a	404.9 ± 24.5ab	340.8 ± 23.4ab	462.1 ± 4.4b	ns	**	ns
SFA[b]	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	*	ns	ns
LC-PUFA	656.4 ± 95.3a	738.7 ± 44.3a	821.9 ± 83.6ab	1072.5 ± 10.3b	*	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 ± 60.1a	607.2 ± 29ab	1056.8 ± 175.6bc	1482 ± 68.8c	**	*	ns
n-3 LC PUFA	384.5 ± 48.8a	543.2 ± 27.7a	575.1 ± 52.1a	806.0 ± 8.0b	**	**	ns
n-6/n-3 ratio	2.4 ± 0.1c	1.4 ± 0.0a	1.2 ± 0.0ab	0.9 ± 0.0b	***	***	**

Data are expressed as mean ± S.E.M., *n* = 2, *N* = 8. P < 0.05; Treatment means analysed by one-way ANOVA with Tukey's post-hoc test of multiple comparisons. Effect of substrate, end-product and substrate / end-product interaction analysed by two-way ANOVA (ns = not significant) * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

[b] See table 2 for fatty acid classes and abbreviations.

Table 6

The apparent *in vivo* fatty acid β-oxidation (nmol g^{-1} day^{-1}) in Atlantic salmon fed the four experimental diets for 283 days.

	Diets[a]				Effect of substrate, end-product and substrate / end-product interaction		
	Low : Low	Low : High	High : Low	High : High	Substrate	End-product	Interaction
12:0	2.7 ± 0.0a	2.8 ± 0.1a	1.2 ± 0.0b	1.9 ± 0.1b	***	*	ns
14:0	34.1 ± 0.5a	60.7 ± 0.4b	18.9 ± 1.0c	49.3 ± 3.5d	**	***	ns
16:0	526.5 ± 2.9a	537.8 ± 2.7a	293.8 ± 2.2b	367.5 ± 23.4b	***	*	ns
18:0	121.3 ± 2.3a	130.4 ± 0.4a	42.4 ± 5.7b	85.7 ± 6.4b	***	**	*
20:0	–[d]	5.0 ± 0.0	–	–	–	–	–
22:0	1.0 ± 0a	2.0 ± 0.0b	3.1 ± 0.2c	4.6 ± 0.2d	***	**	ns
24:0	1.3 ± 0.1a	2.0 ± 0.1bc	2.3 ± 0.1c	5.3 ± 0.2b	***	***	**
SFA[b,c]	686.9 ± 0.3a	740.7 ± 2.7a	361.8 ± 7.1b	514.3 ± 33.8b	***	**	*
14:1n-5	5.9 ± 0.1a	5.3 ± 0.1b	3.2 ± 0.0c	2.8 ± 0.1c	***	**	ns
16:1n-7	99.6 ± 0.6a	109.0 ± 1.3a	54.7 ± 2.5b	71.0 ± 6.5b	***	*	ns
18:1n-7	26.5 ± 1.0	30.2 ± 1.0	21.5 ± 0.2	28.0 ± 3.5	ns	ns	ns
18:1n-9	556.0 ± 38.3	551.6 ± 11.4	382.5 ± 12.7	371.6 ± 56.3	**	ns	ns
20:1n-9	–	–	69.3 ± 5.9	85.0 ± 13.1	–	–	–
22:1n-9	–	–	15.8 ± 1.3a	25.3 ± 1.7b	–	–	–
24:1n-9	–	1.2 ± 0.2a	1.9 ± 0.5ab	4.0 ± 0.6a	**	*	ns
20:1n-11	–	3.5 ± 0.1	–	–	–	–	–
22:1n-11	3.6 ± 0.1a	6.0 ± 0.2b	3.0 ± 0.0a	5.8 ± 0.5b	ns	**	ns
MUFA	691.6 ± 40.1	706.7 ± 14.1	551.9 ± 18.2	593.6 ± 82.5	ns	ns	ns
18:2n-6	179.2 ± 8.6	170.8 ± 0.2	203.1 ± 12.2	197.8 ± 22.1	ns	ns	ns
20:2n-6	–	–	1.0 ± 1.0	1.9 ± 1.9	–	–	–
22:2n-6	–	–	0.4 ± 0.1	0.5 ± 0.2	–	–	–
20:4n-6	–	1.7 ± 0.3	–	2.4 ± 0.8	–	–	–
22:4n-6	–	0.4 ± 0a	0.1 ± 0.1a	0.6 ± 0.1b	ns	**	ns
22:5n-6	–	1.5 ± 0.1a	0.1 ± 0.1b	1.7 ± 0.2a	ns	***	ns
n-6 PUFA	179.2 ± 8.6	174.4 ± 0.1	204.7 ± 13.1	204.8 ± 23.6	ns	ns	ns

18:3n-3	50.6 ± 2.6a	41.9 ± 0.1a	251.6 ± 0.2b	273.5 ± 16.6b	***	ns	ns
18:4n-3	0.9 ± 0.9	1.3 ± 0.0	–	–	–	–	–
20:4n-3	0.3 ± 0.3	1.2 ± 0.1	–	–	–	–	–
20:3n-3	0.1 ± 0.1	–	3.0 ± 0.8	1.0 ± 1.0	*	ns	ns
22:3n-3	–	–	2.6 ± 0.1	2.8 ± 0.2	–	–	–
20:5n-3	5.7 ± 5.7	42.3 ± 0.1	–	28.0 ± 1.7	*	***	ns
22:5n-3	1.8 ± 1.8	4.3 ± 0.1	–	–	–	–	–
22:6n-3	0.2 ± 0.2	3.2 ± 1.0	–	0.2 ± 0.2	*	*	ns
n-3 PUFA	59.4 ± 11.3a	94.2 ± 1.5a	257.2 ± 1.1b	305.5 ± 15.8b	***	*	ns
Total FA	2549.7 ± 75.2	2700.3 ± 33.7	2394.7 ± 72.1	2731.8 ± 278	ns	ns	ns

Data are expressed as mean ± S.E.M., *n* = 2, *N* = 8. P < 0.05; Treatment means analysed by one-way ANOVA with Tukey's post-hoc test of multiple comparisons. Effect of substrate, end-product and substrate / end-product interaction analysed by two-way ANOVA (ns = not significant) * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

[b] See table 2 for fatty acid classes and abbreviations.

[c] Fatty acids not recording any β-oxidation are not reported in this table.

[d] β-oxidation not detected.

Table 7

The apparent *in vivo* fatty acid bioconversion (nmol g^{-1} day^{-1}) in Atlantic salmon fed the four experimental diets for 283 days.

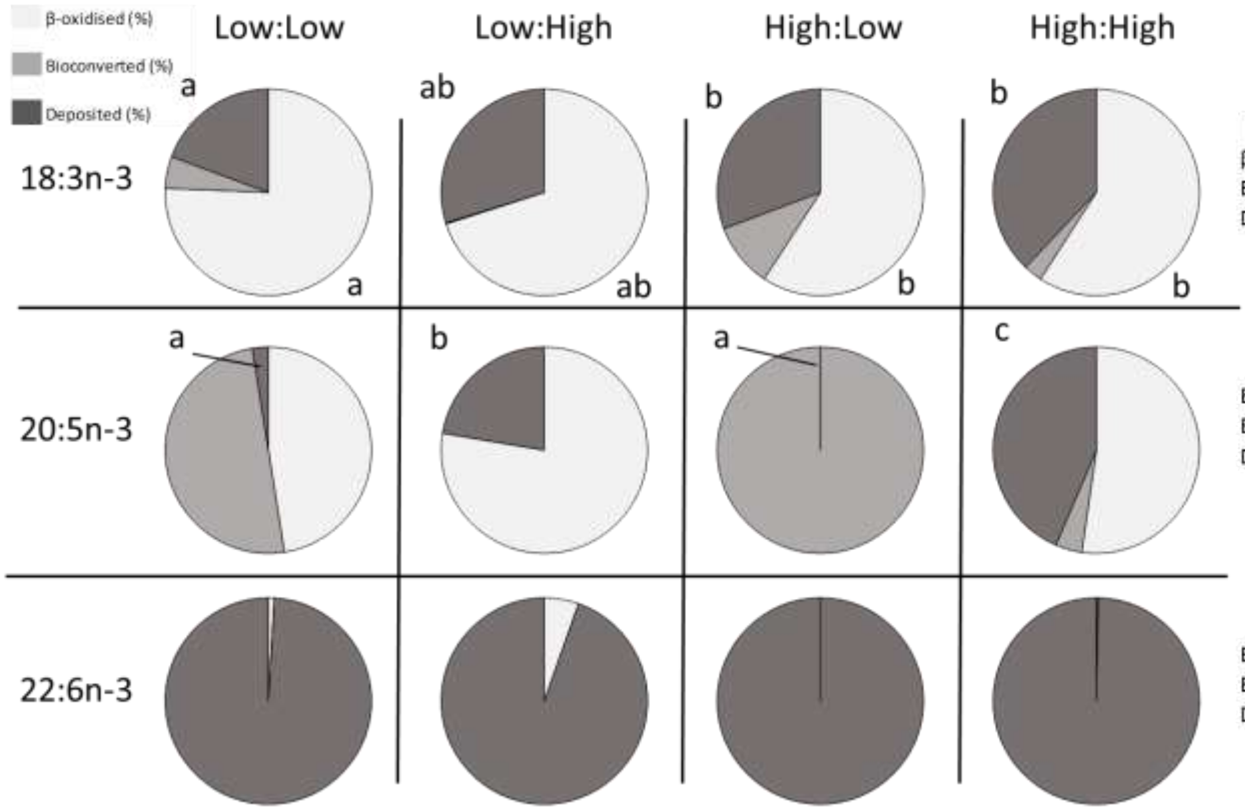
	Diets[a]				Effect of substrate, end-product and substrate / end-product interaction		
	Low : Low	Low : High	High : Low	High : High	Substrate	End-product	Interaction
Fatty acid elongation[b]							
18:0 to 20:0	1.28 ± 0.06a	–[c]	31.56 ± 5.72b	9.83 ± 1.27a	**	*	*
18:1n-9 to 20:1n-9	20.15 ± 4.38	10.68 ± 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 ± 1.87a	10.82 ± 0.80a	0.26 ± 0.26b	0.55 ± 0.55b	**	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 ± 5.31a	9.84 ± 0.77b	21.19 ± 0.77b	8.71 ± 1.10b	*	**	*
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 ± 4.23a	–	43.64 ± 6.44b	13.48 ± 0.33a	**	*	*
20:5n-3 to 22:5n-3	8.10 ± 8.10a	–	24.82 ± 5.51b	2.22 ± 1.02a	ns	*	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	*	ns
Fatty acid Δ-9 desaturation							
20:0 to 20:1n-11	3.67 ± 0.13a	–	43.24 ± 5.13b	26.37 ± 0.45c	***	*	ns
Fatty acid Δ-6 desaturation							
18:2n-6 to 18:3n-6	52.74 ± 4.75a	13.28 ± 0.69b	36.79 ± 1.12c	15.26 ± 1.31bc	ns	***	*
24:4n-6 to 24:5n-6	1.73 ± 0.11	–	0.03 ± 0.03	–	–	–	–
18:3n-3 to 18:4n-3	3.31 ± 3.31a	–	43.02 ± 6.52b	13.16 ± 0.33a	**	*	*
24:5n-3 to 24:6n-3	8.59 ± 8.45	0.38 ± 0.04	20.40 ± 4.16	1.52 ± 0.91	ns	*	ns
Fatty acid Δ-5 desaturation							
20:3n-6 to 20:4n-6	15.91 ± 2.61a	–	5.07 ± 0.41b	–	–	–	–
20:4n-3 to 20:5n-3	4.00 ± 4.00a	–	31.50 ± 6.16b	–	–	–	–
Fatty acid chain shortening							
24:5n-6 to 22:5n-6	1.73 ± 0.11a	–	0.03 ± 0.03b	–	–	–	–
24:6n-3 to 22:6n-3	8.45 ± 8.45	–	19.89 ± 4.26	1.02 ± 1.02	ns	*	ns

Data are expressed as mean ± S.E.M., *n* = 2, *N* = 8. P < 0.05; Treatment means analysed by one-way ANOVA with Tukey's post-hoc test of multiple comparisons. Effect of substrate, end-product and substrate / end-product interaction analysed by two-way ANOVA (ns = not significant) * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

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

[c] Not detected



	Substrate	End-product	Interaction
β-oxidation	**	ns	ns
Bioconversion	ns	ns	ns
Deposition	**	*	ns

Beta-oxidation	ns	ns	ns
Bioconversion	ns	*	ns
Deposition	**	***	**

Beta-oxidation	*	ns	ns
Bioconversion	–	–	–
Deposition	ns	ns	ns

Figure 1