Altered levels of shorter vs long-chain omega-3 fatty acids in commercial diets for market-sized Atlantic salmon reared in seawater – effects on fatty acid composition, metabolism and product quality.

Authors: Thomas S. Mock[1*], David S. Francis[1], Matthew K. Jago[1], Brett D. Glencross[2], Richard P. Smullen[3], Russell S.J. Keast[4], Giovanni M. Turchini[1]

[1]School of Life and Environmental Sciences, Deakin University, Geelong, Australia

75 Pigdons Road, Waurn Ponds, Victoria 3216

[2] Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland

[3] Ridley Aqua-Feed Pty Ltd, Deception Bay, Queensland, Australia

[4]Centre for Advanced Sensory Science, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia

*Corresponding author email: mock@deakin.edu.au

Accepted refereed manuscript of:
Mock TS, Francis DS, Jago MK, Glencross BD, Smullen RP, Keast RSJ & Turchini GM (2019) Altered levels of shorter vs long-chain omega-3 fatty acids in commercial diets for market-sized Atlantic salmon reared in seawater – Effects on fatty acid composition, metabolism and product quality. *Aquaculture*, 499, pp. 167-177.
DOI: https://doi.org/10.1016/j.aquaculture.2018.09.020

Abstract

There is a growing trend of 'replacing' long-chain omega-3 polyunsaturated fatty acid (n-3 LC PUFA) rich oils with C_{18} shorter-chain omega-3 polyunsaturated fatty acid rich oils in Atlantic salmon aquafeed formulations. n-3 LC PUFA, including 20:5n-3 and 22:6n-3, play contrasting physiological roles and are metabolised differently in comparison to C_{18} PUFA. Accordingly, the present study recorded the effect of replacing n-3 LC PUFA rich dietary fish oil with C_{18} n-3 PUFA rich camelina oil at two inclusion levels in commercial-like diets fed to market-sized Atlantic salmon. This assessment was achieved by an analysis of industry relevant production parameters including growth performance, fatty acid composition and metabolism, nutrient digestibility and consumer preference (taste and sensorial analysis of fillet). The trial was conducted over the final 150 days of an on-farm grow-out period in seawater. The dietary replacement of n-3 LC PUFA with C_{18} n-3 PUFA resulted in a significant decrease in fillet n-3 LC PUFA and a poorer growth performance. However, in the absence of fish oil, the inclusion of camelina oil at high levels (40 %) contributed to an improved n-6/n-3 ratio and partially ameliorated low dietary n-3 LC PUFA by providing added substrate for endogenous n-3 LC PUFA synthesis in comparison to a 20% camelina oil inclusion. Furthermore, taste quality was largely unaffected by the dietary addition of camelina oil. Finally, this study provides evidence to suggest that a more complete reporting of the relative contribution of shorter-chain C_{18} n-3 PUFA and n-3 LC PUFA in fish and seafood products would benefit consumers from a nutritional quality standpoint.

Keywords: fatty acid, metabolism, *in vivo,* camelina oil, omega-3, consumer, Atlantic salmon

1.0 Introduction

The global population is increasingly reliant upon aquaculture to supply edible omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA), despite a series of step-wise reductions in their dietary provision in commercial aquafeed formulations (Sprague et al. 2016; Subasinghe et al. 2009; Tocher 2015). The stable supply of n-3 LC PUFA rich oils, primarily fish oil, can no longer be guaranteed due to stagnant production and increased competition originating outside of aquaculture, including nutraceutical and agricultural industries (Subasinghe et al. 2009; Tocher 2015; Turchini et al. 2010). Concomitant with the volatility of fish oil supply, the use of terrestrial oilseed crops rich in C_{18}, shorter-chain, n-3 PUFA as a dietary lipid source in aquafeed has increased (Bell et al. 2001; Bell et al. 2004; Turchini et al. 2010). n-3 LC PUFA, including 20:5n-3 and 22:6n-3, play contrasting physiological roles and are metabolised differently in fish and humans in comparison to C_{18} PUFA (Fard et al. 2018; Turchini et al. 2011b). Therefore, the growing trend of 'replacing' n-3 LC PUFA with shorter-chain n-3 PUFA increases the pertinence of investigating the resultant metabolic and product quality impacts on cultured fish species.

The Atlantic salmon (*Salmo salar*) aquaculture industry is a heavy consumer of marine derived n-3 LC PUFA and has addressed unstable fish oil supply via the incorporation of various terrestrial oil sources into aquafeed formulations (Turchini et al. 2009). Camelina (*Camelina sativa*) oil is one such oil which has been afforded recent attention, and similar to linseed/flaxseed oil, and to a lesser extent to canola oil, is characterised by high levels of 18:3n-3 (Hixson et al. 2014a; Hixson et al. 2014b). 18:3n-3 is considered an essential fatty acid as it cannot be synthesised by animals or humans and is the precursor of longer-chain, more unsaturated fatty acids 20:5n-3 and 22:6n-3 (n-3 LC-PUFA), which reportedly possesses numerous health benefits, in-part due to anti-inflammatory and cardio-protective properties (Baum et al. 2012; Nestel et al. 2015; Turchini et al. 2011b). Whilst the main dietary source of n-3 LC PUFA is fish and seafood, 18:3n-3 can be consumed from a wide variety of terrestrial dietary sources (Abedi & Sahari 2014; Garg et al. 2006; Simopoulos 2000). It is known that

in the absence of added n-3 LC PUFA, several fish species, including Atlantic salmon, have the capacity to endogenously produce 20:5n-3 and 22:6n-3 when provided 18:3n-3 as a substrate for *in vivo* bioconversion (Nakamura & Nara 2004; Tocher 2003). However, the extent of endogenous n-3 LC-PUFA production is limited and dependent on: i) both substrate (18:3n-3) and end-product (22:6n-3) availability; ii) on the physiological requirement for 22:6n-3, which itself is influenced by a number of factors including developmental stage and water temperature (Tocher et al. 2003; Torstensen et al. 2004; Turchini & Francis 2009; Turchini et al. 2011b); and iii) on other external factors, such as, amongst others, dietary co-enzyme and co-factor availability (Giri et al. 2016; Lewis et al. 2013; Senadheera et al. 2012b, a) and the presence of promoters or inhibitors (Pickova et al. 2010). Nevertheless, the endogenous production of 22:6n-3 from 18:3n-3 is not considered sufficient to substantially enrich the fillet with 22:6n-3 to the same extent as dietary fish oil (Cleveland et al. 2012; Francis et al. 2007; Tocher et al. 2003; Turchini & Francis 2009).

Owing to the extensively reported health benefits of n-3 LC PUFA (Garg et al. 2006; Kris-Etherton et al. 2002; Ruxton et al. 2004; Valfré et al. 2003), health agencies have long iterated the importance of fish consumption (Meyer 2016; NHFA 2008). However, an enhanced holistic approach regarding the 'healthy' consumption of fatty acids advocates the consumption of a diet which also minimises the n-6 to n-3 fatty acid ratio (Calder 2010; Nestel et al. 2015; Simopoulos 2008; Turchini et al. 2011b). The balance of these fatty acids has become a relative measure of the health promoting benefit of oil sources due to reported anti-inflammatory properties and resultant risk reduction of cardiovascular disease achieved by diets with a reduced n-6/n-3 ratio (Abedi & Sahari 2014; Harris et al. 2009; Simopoulos 2000, 2002, 2008; Turchini et al. 2011b). Despite the aforementioned functional differences between 18:3n-3 and 22:6n-3, the reported n-6/n-3 ratio in fish often does not distinguish between long and short-chain n-3 fatty acids and as a result has the potential to mislead consumers, who fail to make this technical distinction (Turchini et al. 2011b). This re-iterates the importance of distinguishing the effects of dietary lipid sources of C_{18}, shorter-, and C_{20-22}, long- chain fatty acids in aquafeed on the overall health characteristics of Atlantic salmon.

Notably, the adoption of dietary formulations in aquaculture clearly depends on more than the 'healthiness' of the fillet product. Indeed, overall fish performance, including growth remains a primary consideration of producers (Lysfjord et al. 2004; Rosenlund et al. 2016; Thorarensen et al. 2015). To date, few long-term growth trials have been conducted with salmon in seawater fed diets containing low n-3 LC PUFA concentrations, although admittedly, its dietary addition appears essential for optimal growth (Bell et al. 2010; Rosenlund et al. 2016). The present study compared three commercial-like diets containing either added n-3 LC PUFA, from fish oil, or added shorter-chain n-3 PUFA (at two levels), from camelina oil to assess growth performance, fillet fatty acid composition, nutrient and fatty acid digestibility, *in vivo* fatty acid metabolism, and sensorial characteristics in market-sized Atlantic salmon.

2.0 Materials and Methods

2.1 Location, animals, experimental design and sampling.

The present experiment was conducted on a commercial farm, from May 24 to October 20, 2015 (150 days) in Hideaway bay, Dover, Tasmania (Huon Tasmania, Hideaway bay site; 43°15' 52.2"S 147°04'37.7"E). Immediately preceding the allocation of fish into trial pens an initial sample of 6 fish were randomly taken from the trial cohort, euthanized in excess anaesthetic (AQUI-S, 0.5 ml L^{-1}) and stored at −20 °C until subsequent analysis. A total of 2430 Atlantic salmon (average initial weight ~2200g) were assigned to one of nine floating sea pens (5m x 5m x 5m, 270 fish per pen) (n = 3, N = 9). Feeding of the three experimental diets to trial pens was achieved by using a Sterner feeder fitted with a 40 L hopper and spinning feed spreading mechanism that dispersed feed over ~80% of the cage surface. Fish were fed twice per day to satiation by an automated AQ1 feed system, with the first feeding programmed for 15 minutes before sunrise and the second feeding 15 minutes after sunset. A 0.5 m diameter, 0.5 m deep cone was positioned at a depth of 4 m to channel uneaten feed past an infrared sensor which detected uneaten pellets and automatically turned the feeder off. All feeding

sessions were overseen by an observer to ensure the operation of all automated systems were correct and consistent. Feed consumption and mortalities were monitored throughout the trial and physio-chemical parameters were recorded, including water temperature (mean ± SD: 11.21 ± 0.86 ^{o}C) and dissolved oxygen (mean ± SD: 7.85 ± 0.43 mg L^{-1}). During the last week of feeding, 10 fish were randomly selected for faecal collection by hand stripping and samples were used for subsequent nutrient digestibility assessment. At the completion of the feeding trial, all fish were anaesthetised and weighed, and 21 fish from each treatment (seven fish per pen/replicate unit) were randomly selected and separated. These fish were allocated into 3 groups: the first group (nine fish) were used for the chemical analysis of whole body samples, the second group (six fish) were used for biometrical measurements and for the chemical analysis of fillet samples and the third group (six fish) were used for sensory analysis by means of a panel taste test. Sampled fish were immediately placed in an ice slurry, where fish used for chemical analysis were then frozen to −20 °C and stored until subsequent analysis, while fish allocated to panel taste testing were immediately processed by Huon Aquaculture Company, Tasmania, as described below.

2.2 Experimental Diets

The experimental diets were manufactured by a commercial feed producer using a closed formula Atlantic salmon aquafeed formulations (Ridley Aquafeed, Australia). A single batch of 9mm pellets with an identical basal formulation was made, then divided into three separate sub-batches post extrusion and vacuum coated with different oils. The three diets were isoproteic, isolipidic and isoenergetic and differed only in the added oil source. All diet manufacturing followed normal commercial pellet production procedures at a commercial mill (Ridley Aquafeed, Narangba, QLD, Australia).

Three lipid sources, tallow, fish oil and camelina oil, were used to obtain the three experimental diets. The FO20 diet was formulated with 20% fish oil and 80% tallow as the added dietary lipid. FO20 was

therefore the diet providing dietary omega-3 fatty acids in the form of n-3 LC PUFA. The CAM20 diet also contained 80% tallow, however, fish oil was substituted by camelina oil resulting in dietary lipid composed of 20% camelina oil and 80% tallow. The third treatment diet was made up of 60% tallow and 40% camelina oil as the added dietary lipid (CAM40). The CAM20 and CAM40 were therefore the two diets providing dietary omega-3 fatty acids in the form of the shorter, C_{18} n-3 PUFA, at two different levels.

2.3 Growth performance, chemical analysis and fatty acid analysis

Standard formulae were used to assess growth, feed utilisation and biometrical data, as previously described (Emery et al. 2016). The chemical composition of the experimental diets, faeces and fish samples were determined via proximate composition analysis according to standard methods as previously described (Palmeri et al. 2007). Briefly, moisture was determined by drying samples in an oven at 80 °C to a constant weight. 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). Lipid was determined by dichloromethane: methanol extraction (2/1) according to 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. Briefly, a known aliquot of C23:0 was added to each sample as an internal standard (Sigma-Aldrich, Inc., St. Louis, MO, USA). Fatty acid methyl esters were isolated and identified using an Agilent Technologies GC 7890A (Agilent Technologies, Santa Clara, California, USA) equipped with a BPX70 capillary column (120 m, 0.25 mm internal diameter, 0.25-µm film thickness; SGE Analytical Science, Ringwood, Victoria, Australia), a flame ionisation detector (FID), an Agilent Technologies 7693 autosampler injector, and a split injection system (split ratio 50/1). Fatty acids were identified relative to known external standards, and resulting peaks were corrected by the theoretical

relative FID response factors and for methyl transformation, and then quantified relative to the internal standard.

2.4. Nutrient digestibility and fatty acid metabolism calculations

Evaluation of digestibility was determined following methods in Atkinson (1984), the only difference being ash was used instead of acid insoluble ash. 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., (2007) with further development (Turchini et al. 2008; Turchini & Francis 2009).

2.5. Consumer acceptance testing

Six fish from each treatment (two per cage) were further subdivided in three sub-groups and were subject to standard commercial processing procedures, resulting in three different preparations: hot smoked, cold smoked and fresh fillet.

Methods for consumer acceptance testing were based on methods previously described in Emery et al., (2016). In the present study, a total of 35 regular salmon consumers (20 female, 15 male; age 37 ± 5) were recruited from locations adjacent to the Deakin University, Melbourne campus, Australia. All participants completed a validated version of the Food Frequency Questionnaire (FFQ) developed by Cancer Council Victoria (Hodge et al. 2000), which included a specific salmon questionnaire to determine that they consumed salmon or salmon products at least once every two weeks. This study was conducted according to the institutional review board regulations of Deakin University (DUREC 2013-156). The experimental protocol was also registered under the Australian New Zealand Clinical Trials Registry (ACTRN12613000701729). All participants gave written informed consent and were paid to participate. Participants attended a single lab session which included training for using the

hedonic Labelled Magnitude Scale (hedonic LMS) (Lim 2011) (Figure S1) and completion of a like / dislike questionnaire prior to rating their liking of different salmon products using the hedonic LMS). Procedures were conducted in partitioned sensory booths in the Centre for Advanced Sensory Science using Compusense Cloud Software as part of the Compusense Academic Consortium (Compusense Inc., Ontario, Canada). The hot smoked and cold smoked salmon were prepared as previously noted and served to assessors after removal from packaging without any further treatment, the raw salmon was thawed at room temperature each morning prior to assessment. Each participant was first given approximately 15 g of each sample to rate their liking using the hedonic LMS. After a one minute break, participants were given the same samples again, but were then asked to rate the intensity of fishy, salty and oily attributes using a Just About Right scale. In this case a positive value indicated a sample was too high in the attribute and a negative score indicated a sample was lacking the attribute. Thereby, for the influential attributes, a score close to zero indicated the sample was 'just about right'. These attributes were chosen after bench-top testing determined they may influence liking. Finally, participants were given the opportunity to comment on each sample or whether there were additional factors that had influenced their liking.

2.6. Statistical analysis

All data were reported as mean ± standard error; (n = 3, N = 9). After confirmation of normality and homogeneity of variance, data was subjected to one-way ANOVA, where significant differences were detected, a Tukey's post-hoc test for homogenous subsets was performed using IBM SPSS Statistics v24.0 (SPSS Inc., Chicago, IL, USA). Significance was accepted at $P < 0.05$.

3.0 Results

3.1 Diets

Proximate composition was similar between the three treatment diets, ~420 mg g^{-1} diet and ~350 mg g $diet^{-1}$ for protein and lipid, respectively (Table 1). n-6 PUFA varied across treatments and was highest in CAM40, attributable to high levels of 18:2n-6. Likewise, n-3 PUFA concentrations varied between treatments and were highest in CAM40, owing to high levels of 18:3n-3, and lowest in FO20. n-3 LC PUFA was highest in FO20 owing to elevated concentrations of 20:5n-3 and 22:6n-3. n-6/n-3 ratios were similar in FO20 and CAM40 (0.9 and 1.0, respectively) and markedly higher in CAM20 (1.9).

3.2. Growth, feed utilisation parameters and biometrical data

All diets were readily accepted and mortality rates were low across treatments (<6%, over the entire duration of the trial, and unrelated to treatment). Fish doubled in size (2200g – 4500g), with an FCR ranging between 1.34 and 1.63 in FO20 and CAM20 treatments, respectively (Table 2). Both SGR and weight gain % were significantly higher in the FO20 treatment ($P < 0.05$) and fish were observably larger, however, final weights were not statistically different. There were no significant differences in biometry measures between the treatments.

3.3. Apparent digestibility

High nutrient and fatty acid digestibility values (Apparent Digestibility Coefficient – ADC %) were observed across treatments, with only few statistically significant differences recorded (Table 3). Protein digestibility values ranged from 74.1 to 80.2 % in FO20 and CAM20, respectively and lipid digestibility values ranged from 78.5 to 84.8 % in FO20 and CAM40, respectively. Digestibility values recorded for 18:3n-3 ranged from 88.1 to 96.6 % in FO20 and CAM20, respectively and digestibility values for 22:6n-3 ranged from 87.8 and 93.6 % for FO20 and CAM20, respectively.

3.4. Tissue proximate and fatty acid composition

Proximate composition of fillet was similar between treatments and no significant differences were recorded ($P > 0.05$) (Table 4). Fillet fatty acid composition, in terms of g $100g^{-1}$ of fillet (Table 5), recorded numerous statistically significant differences, including higher SFA in FO20 (2814.6 mg $100g^{-1}$ fillet). n-6 PUFA differed significantly between all treatments ranging from 743.0 to 1276.6 mg $100g^{-1}$ of fillet in FO20 and CAM40, respectively. n-3 LC PUFA also varied between treatments ranging from 458.5 to 718.3 mg $100g^{-1}$ fillet in CAM20 and FO20, respectively, owing largely to differences in 22:6n-3 ($P < 0.05$). The n-6/n-3 ratio varied substantially between treatments, ranging from 0.9 to 1.6 in FO20 and CAM20, respectively. Trends were similar when expressed as µmol g^{-1} fillet and revealed significant differences in 18:3n-3 content which ranged from 3.8 to 14.0 µmol g^{-1} in FO20 and CAM40, respectively ($P < 0.05$) (Table 4).

3.6. Apparent *in vivo* fatty acid metabolism

Apparent *in vivo* fatty acid β-oxidation and apparent *in vivo* fatty acid bioconversion, as calculated by the whole-body fatty acid balance method, are presented in tables 6 and 7. Fatty acid β-oxidation (expressed as nmol of fatty acid β-oxidised per gram of fish per day; nmol g^{-1} day^{-1}) revealed 18:1n-9 was heavily β-oxidised in all treatments. High β-oxidation of 16:0 was recorded in FO20 and CAM20, however, almost no β-oxidation of SFA was recorded for CAM40. Significantly more EPA and DHA was β-oxidised in fish fed FO20 and was in line with dietary inclusion levels. β-oxidation of 18:3n-3 also followed dietary inclusion levels and was significantly higher in fish fed CAM40 and minimal in FO20. Apparent *in vivo* enzyme activity for fatty acid elongation, fatty acid desaturation and fatty acid chain shortening, (expressed as nmol of product per gram of fish per day; nmol g^{-1} day^{-1}) varied considerably between treatments and numerous statistically significant differences were observed. In general, the highest enzymatic activity was observed in CAM40 including *de novo* fatty acid production of 12:0. Fatty acid elongation of 12:0, 14:0 and 16:0 were also high in CAM40 and were not recorded in the

other treatments. n-3 PUFA bioconversion was highest in CAM40, including Δ-5 desaturation of 20:4n-3 to biosynthesise 20:5n-3, and chain shortening of 24:6n-3 for 22:6n-3 production.

3.6. Consumer preferences

No significant difference in liking score between dietary treatments for the three preparation methods, (hot smoked, cold smoked and raw), was observed (Table 8). FO20 scored the highest liking score for the raw preparation method whilst CAM20 and CAM40 scored highest in liking scores for the hot smoked and cold smoked preparation methods, respectively. Overall, 'just right' scores were similar between treatments for the influential attributes (fishiness, saltiness and oiliness) and trends observed appeared to be related to preparation method and not treatment. One significant difference was recorded; CAM20 scored significantly higher than FO20 for the saltiness attribute for the cold smoked preparation method (11.04 and 6.31 on the 'just right scale', respectively) ($P < 0.05$).

4.0 Discussion

The objective of the present study was to quantify the effects of replacing n-3 LC PUFA with shorter-chain C_{18} n-3 PUFA in diets for Atlantic salmon in response to the growing trend of substituting fish oil with terrestrial alternatives containing C_{18} n-3 PUFA in aquafeed formulations. It is known that, due to functional differences, n-3 LC PUFA and C_{18} n-3 PUFA are not metabolised equally and this could potentially be affecting both fish performance and product quality in this species (Bell et al. 2004; Betancor et al. 2017; Hixson et al. 2014a; Turchini et al. 2011b; Xue et al. 2015). Accordingly, industry relevant information including an analysis of growth performance, fillet nutritional quality, *in vivo* fatty acid metabolism and an evaluation of consumer taste preference were addressed in this experiment and are discussed herein.

It is widely reported that the replacement of fish oil with vegetable and animal by-product oils in diets for salmonids has little effect on overall growth performance, even when included as 100 % of the dietary lipid source (Bell et al. 2010; Bransden et al. 2003; Hixson et al. 2014a; Karalazos et al. 2007; Menoyo et al. 2005; Torstensen et al. 2000). Despite this, a minimum dietary requirement for 20:5n-3 and 22:6n-3 for optimum growth in seawater for large Atlantic salmon has been suggested (>2.7 % of fatty acids) (Rosenlund et al. 2016). In the current trial the dietary treatments containing no added fish oil recorded ~20% lower total weight gain compared to the FO20 treatment. Therefore, this study supports a minimum dietary provision of fish oil in diets for market-sized Atlantic salmon reared in on-farm conditions in seawater to maintain optimum growth performance. Alternatively, considering no other detrimental effects were observed, if the cost saving resulting from the exclusion of fish oil from feed formulation could be greater than the reduced revenues originating from the retarded fish growth, specific production decisions could be implemented accordingly. This would require a deeper and specific bio-economical assessment, which was not an objective of the present study, but may be warranted in future investigations.

In line with extensive research, fillet fatty acid composition reflected dietary inclusion levels (Bell et al. 2001; Bell et al. 2002; Bell et al. 2004; Emery et al. 2016; Francis & Turchini 2017; Friesen et al. 2008; Glencross et al. 2014; Hixson et al. 2014b; Jobling & Bendiksen 2003; Karalazos et al. 2007; Robin et al. 2003; Rosenlund et al. 2001; Tocher et al. 2003; Torstensen et al. 2000; Turchini et al. 2013). As expected, fillet levels of n-3 LC PUFA were significantly higher in FO20, compared to the no fish oil treatments, and confirm that in order for farmed Atlantic salmon to maintain their reputation as a good source of n-3 LC PUFA, the dietary inclusion of n-3 LC PUFA in aquafeed is required (Henriques et al. 2014; Sargent et al. 2003; Tur et al. 2012). Despite higher total 22:6n-3 concentrations in the fillet of FO20 fish, the deposition efficiency was noticeably lower, as evidenced by the significantly higher β-oxidation of both 20:5n-3 and 22:6n-3. Contrastingly, the β-oxidation of 20:5n-3 and 22:6n-3 was minimal in both CAM20 and CAM40 (both as total fatty acid β- oxidation and as % relative to net intake), supporting previous research which suggests an increased retention

of n-3 LC PUFA when dietary provision is limited (Codabaccus et al. 2012; Francis et al. 2014; Pratoomyot et al. 2010; Turchini et al. 2011a).

Despite similar dietary levels of n-3 LC PUFA, CAM40 recorded higher levels of n-3 LC PUFA in the fillet compared to CAM20. A possible explanation may relate to the capacity of numerous freshwater and anadromous fish, including Atlantic salmon, to endogenously produce n-3 LC PUFA via the desaturation and elongation of 18:3n-3 (Nakamura & Nara 2004; Tocher 2003). 18:3n-3 rich terrestrial oils are more readily available for the inclusion into aquafeed formulations compared to 22:6n-3 rich fish oil (Turchini et al. 2010). Hence, previous research has focussed on the dietary manipulation of shorter vs long-chain n-3 PUFA ratios in an attempt to 'augment' the endogenous synthesis of n-3 LC PUFA in commercially important finfish species such as Atlantic salmon (Betancor et al. 2014; Francis et al. 2007; Olsen & Ringø 1992; Ruyter & Thomassen 1999; Tocher et al. 2002; Torstensen et al. 2004; Turchini & Francis 2009). Marine environments contain an abundance of dietary sources of 22:6n-3 and accordingly endogenous production of 22:6n-3 in marine fish is limited. In contrast, dietary sources of 22:6n-3 in freshwater environments are relatively scarce, where resultantly, the endogenous production of 22:6n-3 is believed to be an evolutionary adaptation by freshwater and anadromous fish to meet physiological requirements (Tocher 2003). However, research suggests that the activity of the Δ-6 desaturase enzyme in salmonids, which catalyses the first, and one of the last, steps of the n-3 bioconversion pathway, is modulated primarily by the provision of n-3 substrate and that the negative feedback modulation by dietary provision of LC PUFA may have a limited influence (Glencross et al. 2014; Hixson et al. 2017; Thanuthong et al. 2011; Thomassen et al. 2012; Turchini & Francis 2009). With respect to the present study, CAM40 contained threefold higher dietary concentrations of 18:3n-3 compared to CAM20, and although not significant, recorded a higher Δ-6 desaturation of 18:3n-3. Subsequently, CAM40 recorded a Δ-5 desaturation of 20:4n-3, an elongation of 20:5n-3 and ultimately an endogenous production of 22:6n-3, although final amounts were limited. CAM20 on the other hand recorded no production of n-3 LC PUFA beyond 20:4n-3. Therefore, in accordance with previous

research, the higher dietary provision of 18:3n-3 in CAM40 explains the higher enzymatic activity in the n-3 PUFA bioconversion pathway, which may have contributed to higher fillet levels of n-3 LC PUFA (Thanuthong et al. 2011; Thomassen et al. 2012; Turchini & Francis 2009). As mentioned, higher levels of n-3 LC PUFA were recorded in the fillet tissue of CAM40 fish compared to fish fed the CAM20 diet, despite similar amounts provided in the diet and limited recorded endogenous production of 22:6n-3. The inability to detect large amounts of endogenous 22:6n-3 production in CAM40 fish using the whole-body fatty acid balance method may be a result of limitations when this method is applied to on-farm situations. These limitations may arise from a less efficient incorporation of dietary supplied fatty acids into the fish, owing to a typically higher FCR on-farm compared to laboratory-based trials. Concomitantly, inaccuracies associated with feed delivery and consumption measurements can result in a considerable over or under-estimation of feed inputs. Resultantly, dietary fatty acid availability is overestimated and, therefore, contributes to an underestimation of final whole-body fatty acid balance. Nevertheless, the apparent limited biosynthesis of 22:6n-3 in CAM40 likely indicates there are other factors responsible for the higher fillet 22:6n-3 in CAM40 fish. The most obvious explanation relates to the marginally higher dietary level of n-3 LC PUFA in CAM40 treatment diets as well as the increased retention of 22:6n-3, as evidenced by lower a β-oxidation of this fatty acid, both in terms of μmol g^{-1} day^{-1} and % of net intake. Withstanding the aforementioned limitations, this study suggests that if fish oil is replaced by camelina oil, a relatively high (~40%) dietary addition is favourable to enhance the n-3 LC PUFA content of the fillet. Furthermore, relative to a 20% inclusion of camelina oil, a reduction in the n-6/n-3 ratio was achieved, leading to net gains in potential health benefits (Harris et al. 2009; Simopoulos 2008). However, due to the multiple explanatory factors presented herein, further investigation is required to better quantify the effects on endogenous production of n-3 LC PUFA in large Atlantic salmon fed diets containing very low n-3 LC PUFA and varied levels of 18:3n-3 as a substrate for *in vivo* bioconversion.

As highlighted, dietary provision of 18:3n-3 influences the extent of n-3 PUFA bioconversion in salmonids, where more specifically, *in vivo* enzymatic activity appears proportional to 18:3n-3 substrate availability (Glencross et al. 2014; Hixson et al. 2017; Thanuthong et al. 2011). However, despite a minimal dietary provision, >60 % of 18:3n-3 was either desaturated or elongated in the FO20 dietary treatment. Despite no dietary provision of 18:4n-3, endogenous synthesis of 20:4n-3 was significantly higher in the FO20 treatment, however, no production of 20:5n-3 was recorded. It appears, in accordance with previous research, that the dietary provision of n-3 LC PUFA in the FO20 diet elicited a partial negative feedback on Δ-5 desaturase enzyme activity, yet did not inhibit Δ-6 desaturase enzyme activity (Glencross et al. 2014; Hixson et al. 2017; Thanuthong et al. 2011; Thomassen et al. 2012). However, considering the low dietary provision of 18:3n-3 there are likely other factors contributing to the high bioconversion rate. The high dietary 20:4n-6 in the FO20 diet relative to the other experimental diets has the potential to increase Δ-6 desaturation of 18:3n-3, as suggested by previous research in juvenile Atlantic salmon (Norambuena et al. 2015). Additionally, the Δ-6 desaturase enzyme has a higher affinity with n-3, as opposed to n-6 substrates, namely 18:2n-6 (Vagner & Santigosa 2011). Thus, the relatively low dietary concentration of 18:2n-6 in FO20 compared to the other treatment diets may have resulted in a preferential desaturation of 18:3n-3.

Atlantic salmon contain a 'healthy' n-6/n-3 ratio, which in turn is a direct consequence of balanced aquafeed formulations (Bendiksen et al. 2011; Leaver et al. 2011; Strobel et al. 2012; Turchini et al. 2010). The present study highlights the different utilisation of 18:3n-3 and 22:6n-3 in Atlantic salmon, in turn, affecting the nutritional value of the final product. The dietary n-6/n-3 ratios in FO20 and CAM40 were similar (0.9 and 1.0, respectively), however, in FO20 this was comprised primarily of long-chain n-3 PUFA, whereas CAM40 consisted of mostly C_{18} n-3 PUFA. In accordance with previous research, 18:3n-3 was heavily β-oxidised and this was reflected in fillet n-6/n-3 ratios which recorded values of 0.9 and 1.4 for FO20 and CAM40, respectively (in terms of mg $100g^{-1}$ of fillet) (Bell

et al. 2001; Bell et al. 2003a; Bell et al. 2003b; Sinclair et al. 2002). Therefore, dietary n-6/n-3 dietary ratios in CAM40 were not reflected in the fillet.

From a human nutritional viewpoint, an unbalanced dietary n-6/n-3 ratio continues to be used as a measure of the overall 'health' of the final product, owing the a purported decrease in occurrence of inflammatory conditions such as cardiovascular disease (Baum et al. 2012; Calder 2010; Harris et al. 2009; Simopoulos 2008; Valfré et al. 2003). However, the relative contribution of 18:3n-3 and 22:6n-3 to total n-3 PUFA is often omitted (Turchini et al. 2011b). To date, there is scant information available specifically linking 18:3n-3 to health benefits in humans, conversely, the health benefits of 22:6n-3 have been extensively reported, leading to a potential misinterpretation of the actual health benefit of the product (Fard et al. 2018; Turchini et al. 2011b). Furthermore, dependant on marketing strategy and resultant labelling practice, nutritional information can often be confined to total 'omega-3' or 'omega-3 polyunsaturated fatty acids' (Turchini et al. 2011b). In the present study, fish fed the CAM40 diet contained higher (albeit not statistically) n-3 PUFA in the fillet compared to FO20, largely due to high 18:3n-3, however, fish fed the FO20 diet had significantly higher n-3 LC PUFA. The aforementioned results exemplify the need to consider the relative contribution of 18:3n-3 and 22:6n-3 when assessing the nutritive value of both aquafeed and the final fish product. Accordingly, it is suggested that products advertising levels of 'omega-3' contain a more complete set of nutritional information, specifically, elucidating the relative contribution of individual fatty acids, especially, shorter-chain C_{18} n-3 PUFA and long-chain n-3 PUFA.

In addition to the nutritional value, taste and sensorial quality is also a major driver of fish consumption (Christenson et al. 2017). The present study indicated that consumer preference; like (+) or dislike (-) and influential attribute scores from raw, cold and hot smoked fillets was largely independent of the dietary treatment. The possible exception was CAM20, which scored a higher positive result for saltiness compared to FO20 when prepared raw (i.e. was considered too salty). These results are largely confirmatory with Hixson et. al., (2017), who found that consumers were unable to distinguish between fish fed a diet containing camelina oil and fish fed a fish oil control

diet, providing confidence that the dietary inclusion of camelina oil, in the absence of fish oil has no measurable effect on major taste attributes in market-sized Atlantic salmon.

In summary, the replacement of n-3 LC PUFA with C_{18} n-3 PUFA in diets for Atlantic salmon resulted in a significant decrease in fillet n-3 LC PUFA and a reduction in growth performance. However, in the absence of fish oil, the inclusion of camelina oil at high levels (40 %) contributed to an improved n-6/n-3 ratio and may partially ameliorate low dietary n-3 LC PUFA by providing added substrate for endogenous n-3 LC PUFA synthesis in comparison to 20% camelina oil inclusion. Furthermore, taste quality is largely unaffected by the dietary addition of camelina oil. This study provides evidence to suggest that a more complete reporting of the relative contribution of shorter-chain C_{18} n-3 PUFA and n-3 LC PUFA in fish and seafood products would benefit consumers.

Acknowledgements

The authors gratefully acknowledge the assistance and support from Ridley Aquafeed (Brisbane, Australia) and Huon Aquaculture Ltd (Dover, Tasmania). The authors also wish to thank Dr. Karen Hermon for technical assistance and Adrian Steenholdt for management of the feeding trials for their valuable contribution.

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 different lipid sources in aquafeed and was not directly comparing commercially produced products by Ridley Aquafeed Ltd.

References

Abedi, E & Sahari, MA 2014, 'Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties', *Food Science & Nutrition*, vol. 2, no. 5, pp. 443-63.

Atkinson, J, Hilton, J & Slinger, S 1984, 'Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*)', *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 41, no. 9, pp. 1384-6.

Baum, SJ, Kris-Etherton, PM, Willett, WC, Lichtenstein, AH, Rudel, LL, Maki, KC, Whelan, J, Ramsden, CE & Block, RC 2012, 'Fatty acids in cardiovascular health and disease: A comprehensive update', *Journal of Clinical Lipidology*, vol. 6, no. 3, pp. 216-34.

Bell, JG, McEvoy, J, Tocher, DR, McGhee, F, Campbell, PJ & Sargent, JR 2001, 'Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism', *Journal of Nutrition*, vol. 131, no. 5, pp. 1535-43.

Bell, JG, Henderson, RJ, Tocher, DR, McGhee, F, Dick, JR, Porter, A, Smullen, RP & Sargent, JR 2002, 'Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism', *Journal of Nutrition*, vol. 132, no. 2, pp. 222-30.

Bell, JG, McGhee, F, Campbell, PJ & Sargent, JR 2003a, 'Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil “wash out”', *Aquaculture*, vol. 218, no. 1–4, pp. 515-28.

Bell, JG, Tocher, DR, Henderson, RJ, Dick, JR & Crampton, VO 2003b, 'Altered fatty acid compositions in atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet', *The Journal of nutrition*, vol. 133, no. 9, pp. 2793-801.

Bell, JG, Henderson, RJ, Tocher, DR & Sargent, JR 2004, 'Replacement of dietary fish oil with increasing levels of linseed oil: Modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet', *Lipids*, vol. 39, no. 3, pp. 223-32.

Bell, JG, Pratoomyot, J, Strachan, F, Henderson, RJ, Fontanillas, R, Hebard, A, Guy, DR, Hunter, D & Tocher, DR 2010, 'Growth, flesh adiposity and fatty acid composition of Atlantic salmon (*Salmo salar*) families with contrasting flesh adiposity: Effects of replacement of dietary fish oil with vegetable oils', *Aquaculture*, vol. 306, no. 1-4, pp. 225-32.

Bendiksen, EÅ, Johnsen, CA, Olsen, HJ & Jobling, M 2011, 'Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar* L.)', *Aquaculture*, vol. 314, no. 1-4, pp. 132-9.

Betancor, MB, Howarth, FJE, Glencross, BD & Tocher, DR 2014, 'Influence of dietary docosahexaenoic acid in combination with other long-chain polyunsaturated fatty acids on expression of biosynthesis genes and phospholipid fatty acid compositions in tissues of post-smolt Atlantic salmon (Salmo salar)', *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, vol. 172, pp. 74-89.

Betancor, MB, Li, K, Sprague, M, Bardal, T, Sayanova, O, Usher, S, Han, L, Måsøval, K, Torrissen, O & Napier, JA 2017, 'An oil containing EPA and DHA from transgenic Camelina sativa to replace marine fish oil in feeds for Atlantic salmon (Salmo salar L.): Effects on intestinal transcriptome, histology, tissue fatty acid profiles and plasma biochemistry', *Plos One*, vol. 12, no. 4, p. e0175415.

Bransden, MP, Carter, CG & Nichols, PD 2003, 'Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (Salmo salar L.): effect on growth performance, tissue fatty acid composition and disease resistance', *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 135, no. 4, pp. 611-25.

Calder, PC 2010, 'Omega-3 fatty acids and inflammatory processes', *Nutrients*, vol. 2, no. 3, pp. 355-74.

Christenson, JK, O'Kane, GM, Farmery, AK & McManus, A 2017, 'The barriers and drivers of seafood consumption in Australia: A narrative literature review', *International Journal of Consumer Studies*, vol. 41, no. 3, pp. 299-311.

Cleveland, BJ, Francis, DS & Turchini, GM 2012, 'Echium oil provides no benefit over linseed oil for (n-3) long-chain PUFA biosynthesis in rainbow trout', *The Journal of nutrition*, vol. 142, no. 8, pp. 1449-55.

Codabaccus, BM, Carter, CG, Bridle, AR & Nichols, PD 2012, 'The "n-3 LC-PUFA sparing effect" of modified dietary n-3 LC-PUFA content and DHA to EPA ratio in Atlantic salmon smolt', *Aquaculture*, vol. 356, pp. 135-40.

Emery, JA, Smullen, RP, Keast, RSJ & Turchini, GM 2016, 'Viability of tallow inclusion in Atlantic salmon diet, as assessed by an on-farm grow out trial', *Aquaculture*, vol. 451, pp. 289-97.

Fard, GS, Wang, F, Sinclair, AJ, Elliot, G & Turchini, GM 2018, 'How does high DHA fish oil affect health? A systematic review of evidence. ', *Critical Reviews in Food Science and Nutrition*, vol. 0, no. 0, pp. 1-44.

Folch, J, Lees, M & Sloane-Stanley, G 1957, 'A simple method for the isolation and purification of total lipids from animal tissues', *J biol Chem*, vol. 226, no. 1, pp. 497-509.

Francis, DS, Turchini, GM, Jones, PL & De Silva, SS 2007, 'Dietary lipid source modulates in vivo fatty acid metabolism in the freshwater fish, Murray cod (Maccullochella peelii peelii)', *Journal of Agricultural and Food Chemistry*, vol. 55, no. 4, pp. 1582-91.

Francis, DS, Thanuthong, T, Senadheera, SPSD, Paolucci, M, Coccia, E, De Silva, SS & Turchini, GM 2014, 'n-3 LC-PUFA deposition efficiency and appetite-regulating hormones are modulated by the dietary lipid source during rainbow trout grow-out and finishing periods', *Fish Physiology and Biochemistry*, vol. 40, no. 2, pp. 577-93.

Francis, DS & Turchini, GM 2017, 'Retro-engineering the protein sparing effect to preserve n-3 LC-PUFA from catabolism and optimise fish oil utilisation: A preliminary case study on juvenile Atlantic salmon', *Aquaculture*, vol. 468, Part 1, pp. 184-92.

Friesen, EN, Ikonomou, MG, Higgs, DA, Ang, KP & Dubetz, C 2008, 'Use of Terrestrial Based Lipids in Aquaculture Feeds and the Effects on Flesh Organohalogen and Fatty Acid Concentrations in Farmed Atlantic Salmon', *Environmental Science & Technology*, vol. 42, no. 10, pp. 3519-23.

Garg, ML, Wood, LG, Singh, H & Moughan, PJ 2006, 'Means of Delivering Recommended Levels of Long Chain n-3 Polyunsaturated Fatty Acids in Human Diets', *Journal of Food Science*, vol. 71, no. 5, pp. R66-R71.

Giri, SS, Graham, J, Hamid, NKA, Donald, JA & Turchini, GM 2016, 'Dietary micronutrients and in vivo n−3 LC-PUFA biosynthesis in Atlantic salmon', *Aquaculture*, vol. 452, no. Supplement C, pp. 416-25.

Glencross, B, Tocher, D, Matthew, C & Gordon Bell, J 2014, 'Interactions between dietary docosahexaenoic acid and other long-chain polyunsaturated fatty acids on performance and fatty acid retention in post-smolt Atlantic salmon (Salmo salar)', *Fish Physiology and Biochemistry*, vol. 40, no. 4, pp. 1213-27.

Harris, WS, Mozaffarian, D, Rimm, E, Kris-Etherton, P, Rudel, LL, Appel, LJ, Engler, MM, Engler, MB & Sacks, F 2009, 'Omega-6 Fatty Acids and Risk for Cardiovascular Disease', *A Science Advisory From the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention*, vol. 119, no. 6, pp. 902-7.

Henriques, J, Dick, JR, Tocher, DR & Bell, JG 2014, 'Nutritional quality of salmon products available from major retailers in the UK: content and composition of n-3 long-chain PUFA', *British Journal of Nutrition*, vol. 112, no. 06, pp. 964-75.

Hixson, SM, Parrish, CC & Anderson, DM 2014a, 'Full substitution of fish oil with camelina (Camelina sativa) oil, with partial substitution of fish meal with camelina meal, in diets for farmed Atlantic salmon (*Salmo salar*) and its effect on tissue lipids and sensory quality', *Food Chemistry*, vol. 157, no. 0, pp. 51-61.

Hixson, SM, Parrish, CC & Anderson, DM 2014b, 'Changes in tissue lipid and fatty acid composition of farmed rainbow trout in response to dietary camelina oil as a replacement of fish oil', *Lipids*, vol. 49, no. 1, pp. 97-111.

Hixson, SM, Parrish, CC, Xue, X, Wells, JS, Collins, SA, Anderson, DM & Rise, ML 2017, 'Growth performance, tissue composition, and gene expression responses in Atlantic salmon (Salmo salar) fed varying levels of different lipid sources', *Aquaculture*, vol. 467, pp. 76-88.

Hodge, A, Patterson, AJ, Brown, WJ, Ireland, P & Giles, G 2000, 'The Anti Cancer Council of Victoria FFQ: relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation', *Australian and New Zealand journal of public health*, vol. 24, no. 6, pp. 576-83.

Jobling, M & Bendiksen, EÅ 2003, 'Dietary lipids and temperature interact to influence tissue fatty acid compositions of Atlantic salmon, *Salmo salar* L., parr', *Aquaculture Research*, vol. 34, no. 15, pp. 1423-41.

Karalazos, V, Bendiksen, EÅ, Dick, JR & Bell, JG 2007, 'Effects of dietary protein, and fat level and rapeseed oil on growth and tissue fatty acid composition and metabolism in Atlantic salmon (*Salmo salar* L.) reared at low water temperatures', *Aquaculture Nutrition*, vol. 13, no. 4, pp. 256-65.

Kris-Etherton, PM, Harris, WS & Appel, LJ 2002, 'Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease', *Circulation*, vol. 106, no. 21, pp. 2747-57.

Leaver, MJ, Taggart, JB, Villeneuve, L, Bron, JE, Guy, DR, Bishop, SC, Houston, RD, Matika, O & Tocher, DR 2011, 'Heritability and mechanisms of n−3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon', *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, vol. 6, no. 1, pp. 62-9.

Lewis, MJ, Hamid, NKA, Alhazzaa, R, Hermon, K, Donald, JA, Sinclair, AJ & Turchini, GM 2013, 'Targeted dietary micronutrient fortification modulates n− 3 LC-PUFA pathway activity in rainbow trout (Oncorhynchus mykiss)', *Aquaculture*, vol. 412, pp. 215-22.

Lim, J 2011, 'Hedonic scaling: A review of methods and theory', *Food quality and preference*, vol. 22, no. 8, pp. 733-47.

Lysfjord, G, Jobling, M & Solberg, C 2004, 'Atlantic salmon, Salmo salar L., smolt production strategy affects body composition and early seawater growth', *Aquaculture*, vol. 237, no. 1, pp. 191-205.

Menoyo, D, Lopez-Bote, CJ, Obach, A & Bautista, JM 2005, 'Effect of dietary fish oil substitution with linseed oil on the performance, tissue fatty acid profile, metabolism, and oxidative stability of Atlantic salmon', *Journal of Animal Science*, vol. 83, no. 12, pp. 2853-62.

Meyer, BJ 2016, 'Australians are not Meeting the Recommended Intakes for Omega-3 Long Chain Polyunsaturated Fatty Acids: Results of an Analysis from the 2011–2012 National Nutrition and Physical Activity Survey', *Nutrients*, vol. 8, no. 3, p. 111.

Nakamura, MT & Nara, TY 2004, 'Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases', *Nutrition*, vol. 24.

Nestel, P, Clifton, P, Colquhoun, D, Noakes, M, Mori, TA, Sullivan, D & Thomas, B 2015, 'Indications for Omega-3 Long Chain Polyunsaturated Fatty Acid in the Prevention and Treatment of Cardiovascular Disease', *Heart, Lung and Circulation*, vol. 24, no. 8, pp. 769-79.

NHFA 2008, *National Heart Foundation of Australia. Position Statement. Fish, fish oil, n-3 polyunsaturated fatty acids and cardiovascular health.*

Norambuena, F, Morais, S, Emery, JA & Turchini, GM 2015, 'Arachidonic Acid and Eicosapentaenoic Acid Metabolism in Juvenile Atlantic Salmon as Affected by Water Temperature', *Plos One*, vol. 10, no. 11, p. e0143622.

Olsen, R & Ringø, E 1992, 'Lipids of Arctic charr, Salvelinus alpinus (L.) II. Influence of dietary fatty acids on the elongation and desaturation of linoleic and linolenic acid', *Fish Physiology and Biochemistry*, vol. 9, no. 5-6, pp. 393-9.

Palmeri, G, Turchini, GM & De Silva, SS 2007, 'Lipid characterisation and distribution in the fillet of the farmed Australian native fish, Murray cod (*Maccullochella peelii peelii*)', *Food Chemistry*, vol. 102, no. 3, pp. 796-807.

Pickova, J, Sampels, S & Berntssen, MH 2010, '11 Minor Components in Fish Oil and Alternative Oils with Potential Physiological Effect', *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*, p. 351.

Pratoomyot, J, Bendiksen, EA, Bell, JG & Tocher, DR 2010, 'Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (Salmo salar L.)', *Aquaculture*, vol. 305, no. 1-4, pp. 124-32.

Robin, JH, Regost, C, Arzel, J & Kaushik, SJ 2003, 'Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis', *Aquaculture*, vol. 225, no. 1–4, pp. 283-93.

Rosenlund, G, Obach, A, Sandberg, M, Standal, H & Tveit, K 2001, 'Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L.)', *Aquaculture Research*, vol. 32, no. s1, pp. 323-8.

Rosenlund, G, Torstensen, BE, Stubhaug, I, Usman, N & Sissener, NH 2016, 'Atlantic salmon require long-chain n-3 fatty acids for optimal growth throughout the seawater period', *Journal of nutritional science*, vol. 5.

Ruxton, C, Reed, SC, Simpson, M & Millington, K 2004, 'The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence', *Journal of Human Nutrition and Dietetics*, vol. 17, no. 5, pp. 449-59.

Ruyter, B & Thomassen, MS 1999, 'Metabolism of n-3 and n-6 fatty acids in Atlantic salmon liver: Stimulation by essential fatty acid deficiency', *Lipids*, vol. 34, no. 11, pp. 1167-76.

Sargent, JR, Tocher, DR & Bell, JG 2003, '4 - The Lipids', in JEHW Hardy (ed.), *Fish Nutrition (Third Edition)*, Academic Press, San Diego, pp. 181-257.

Senadheera, SD, Turchini, GM, Thanuthong, T & Francis, DS 2012a, 'Effects of dietary vitamin B6 supplementation on fillet fatty acid composition and fatty acid metabolism of rainbow trout fed vegetable oil based diets', *Journal of Agricultural and Food Chemistry*, vol. 60, no. 9, pp. 2343-53.

Senadheera, SD, Turchini, GM, Thanuthong, T & Francis, DS 2012b, 'Effects of dietary iron supplementation on growth performance, fatty acid composition and fatty acid metabolism in rainbow trout (Oncorhynchus mykiss) fed vegetable oil based diets', *Aquaculture*, vol. 342, pp. 80-8.

Simopoulos, A 2000, 'Human requirement for N-3 polyunsaturated fatty acids', *Poultry science*, vol. 79, no. 7, pp. 961-70.

Simopoulos, A 2002, 'The importance of the ratio of omega-6/omega-3 essential fatty acids', *Biomedicine & pharmacotherapy*, vol. 56, no. 8, pp. 365-79.

Simopoulos, A 2008, 'The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases', *Experimental biology and medicine*, vol. 233, no. 6, pp. 674-88.

Sinclair, AJ, Attar-Bashi, NM & Li, D 2002, 'What is the role of α-linolenic acid for mammals?', *Lipids*, vol. 37, no. 12, pp. 1113-23.

Sprague, M, Dick, JR & Tocher, DR 2016, 'Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015', *Scientific Reports*, vol. 6, p. 21892.

Strobel, C, Jahreis, G & Kuhnt, K 2012, 'Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products', *Lipids in health and disease*, vol. 11, no. 1, p. 144.

Subasinghe, R, Soto, D & Jia, J 2009, 'Global aquaculture and its role in sustainable development', *Reviews in Aquaculture*, vol. 1, no. 1, pp. 2-9.

Thanuthong, T, Francis, DS, Manickam, E, Senadheera, SD, Cameron-Smith, D & Turchini, GM 2011, 'Fish oil replacement in rainbow trout diets and total dietary PUFA content: II) Effects on fatty acid metabolism and in vivo fatty acid bioconversion', *Aquaculture*, vol. 322–323, no. 0, pp. 99-108.

Thomassen, MS, Rein, D, Berge, GM, Ostbye, T-K & Ruyter, B 2012, 'High dietary EPA does not inhibit Delta 5 and Delta 6 desaturases in Atlantic salmon (Salmo salar L.) fed rapeseed oil diets', *Aquaculture*, vol. 360, pp. 78-85.

Thorarensen, H, Kubiriza, GK & Imsland, AK 2015, 'Experimental design and statistical analyses of fish growth studies', *Aquaculture*, vol. 448, pp. 483-90.

Tocher, D, Fonseca-Madrigal, J, Bell, JG, Dick, J, Henderson, RJ & Sargent, J 2002, 'Effects of diets containing linseed oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon (Salmo salar)', *Fish Physiology and Biochemistry*, vol. 26, no. 2, pp. 157-70.

Tocher, DR 2003, 'Metabolism and functions of lipids and fatty acids in teleost fish', *Reviews in Fisheries Science*, vol. 11, no. 2, pp. 107-84.

Tocher, DR, Bell, JG, Dick, JR & Crampton, VO 2003, 'Effects of dietary vegetable oil on Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid compositions', *Lipids*, vol. 38, no. 7, pp. 723-32.

Tocher, DR 2015, 'Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective', *Aquaculture*, vol. 449, pp. 94-107.

Torstensen, BE, Lie, Ø & Frøyland, L 2000, 'Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)—effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources', *Lipids*, vol. 35, no. 6, pp. 653-64.

Torstensen, BE, Frøyland, L & Lie, Ø 2004, 'Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon (Salmo salar L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities', *Aquaculture Nutrition*, vol. 10, no. 3, pp. 175-92.

Tur, J, Bibiloni, M, Sureda, A & Pons, A 2012, 'Dietary sources of omega 3 fatty acids: public health risks and benefits', *British Journal of Nutrition*, vol. 107, no. S2, pp. S23-S52.

Turchini, G, Francis, D & De Silva, S 2007, 'A Whole Body, In Vivo, Fatty Acid Balance Method to Quantify PUFA Metabolism (Desaturation, Elongation and Beta-oxidation)', *Lipids*, vol. 42, no. 11, pp. 1065-71.

Turchini, G, Francis, D, Senadheera, S, Thanuthong, T & De Silva, S 2011a, 'Fish oil replacement with different vegetable oils in Murray cod: evidence of an “omega-3 sparing effect” by other dietary fatty acids', *Aquaculture*, vol. 315, no. 3, pp. 250-9.

Turchini, G, Hermon, K, Cleveland, B, Emery, J, Rankin, T & Francis, D 2013, 'Seven fish oil substitutes over a rainbow trout grow-out cycle: I) Effects on performance and fatty acid metabolism', *Aquaculture Nutrition*, vol. 19, no. s1, pp. 82-94.

Turchini, GM, Francis, DS & De Silva, SS 2008, 'A Whole Body, In Vivo, Fatty Acid Balance Method to Quantify PUFA Metabolism (Desaturation, Elongation and Beta-oxidation)', *Lipids*, vol. 43, no. 10, pp. 977-.

Turchini, GM & Francis, DS 2009, 'Fatty acid metabolism (desaturation, elongation and β-oxidation) in rainbow trout fed fish oil- or linseed oil-based diets', *British Journal of Nutrition*, vol. 102, no. 01, pp. 69-81.

Turchini, GM, Torstensen, BE & Ng, W-K 2009, 'Fish oil replacement in finfish nutrition', *Reviews in Aquaculture*, vol. 1, no. 1, pp. 10-57.

Turchini, GM, Ng, W-K & Tocher, DR 2010, *Fish oil replacement and alternative lipid sources in aquaculture feeds*, CRC Press.

Turchini, GM, Nichols, PD, Barrow, C & Sinclair, AJ 2011b, 'Jumping on the Omega-3 Bandwagon: Distinguishing the Role of Long-Chain and Short-Chain Omega-3 Fatty Acids', *Critical Reviews in Food Science and Nutrition*, vol. 52, no. 9, pp. 795-803.

Vagner, M & Santigosa, E 2011, 'Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: A review', *Aquaculture*, vol. 315, no. 1-2, pp. 131-43.

Valfré, F, Caprino, F & Turchini, GM 2003, 'The Health Benefit of Seafood', *Veterinary Research Communications*, vol. 27, no. 1, pp. 507-12.

Xue, X, Hixson, SM, Hori, TS, Booman, M, Parrish, CC, Anderson, DM & Rise, ML 2015, 'Atlantic salmon (Salmo salar) liver transcriptome response to diets containing Camelina sativa products', *Comparative Biochemistry and Physiology D-Genomics & Proteomics*, vol. 14, pp. 1-15.

Figure 1. Hedonic LMS liking scale used in Compusense for the sensory analysis of liking scores for Atlantic salmon prepared three different ways: hot smoked, cold smoked and raw.

Table 1

Proximate and fatty acid (mg g^{-1} diet) composition of experimental diets used in the Atlantic salmon growth trial for 150 days.

	Diets[a]		
	FO20	CAM20	CAM40
Proximate composition			
Moisture	44.3	46.0	34.9
Protein	402.6	434.2	438.8
Lipid	358.2	324.2	354.4
NFE	125.0	124.4	98.8
Ash	69.9	71.2	73.2
Energy (KJ g^{-1})	25.8	25.2	26.1
Fatty acids composition			
Total FA[b]	250.9	246.0	253.0
SFA[c]	104.1	105.9	84.1
14:0	7.9	5.8	4.9
16:0	60.8	57.4	48.3
18:0	31.2	38.4	26.8
Other SFA[d]	4.2	4.3	4.1
MUFA[e]	115.4	104.8	110.8
16:1n-7	10.6	7.3	6.8
18:1n-9	86.0	86.2	82.1
18:1n-7	5.2	4.2	4.0
20:1n-9	4.6	3.5	11.1
Other MUFA[f]	9.0	3.7	6.8
Total trans FA[g]	5.2	3.9	4.1
PUFA[h]	25.9	30.9	53.8
18:2n-6	9.4	17.7	23.7
18:3n-6	0.1	0.1	0.2
20:2n-6	0.5	0.7	1.4
20:3n-6	0.6	0.8	0.6
20:4n-6	0.7	0.5	0.3
Other n-6 PUFA[i]	0.9	0.5	0.6
n-6 PUFA[j]	12.2	20.2	26.8
n-6 LC PUFA[n]	2.7	2.4	3.1
18:3n-3	2.0	7.5	23.4
20:5n-3	3.9	1.0	1.0
22:5n-3	1.0	0.3	0.3
22:6n-3	5.8	1.6	1.6
Other n-3 PUFA[l]	0.6	0.3	0.7
n-3 PUFA[k]	13.3	10.7	27.0
n-3 LC PUFA[o]	11.3	3.2	3.6
LC PUFA[m]	14.0	5.6	6.6
n-6/n-3 ratio[p]	0.9	1.9	1.0

[a] Diets: FO20 = tallow and fish oil diet, added oil 20% fish oil, 80% tallow; CAM20 = tallow and camelina oil diet, added oil 80% tallow, 20% camelina oil; CAM40 = tallow and camelina oil diet, added 60% tallow, 40% camelina oil.

[b] Total FA = total fatty acids mg g^{-1} of diet.
[c] SFA = saturated fatty acids.
[d] Other SFA = sum of 12:0, 15:0, 17:0, 20:0, 22:0 and 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 and 24:1n-9.
[g] Total trans FA = sum of 18:1n-9t, 18:1n-7t and 18:2n-6t.
[h] PUFA = polyunsaturated fatty acids.
[i] Other n-6 PUFA = sum of 20:2n-6, 22:2n-6, 22:4n-6 and 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 18:4n-3, 20:3n-3, 20:4n-3, 24:5n-3 and 24:6n-3.
[m] LC-PUFA = long chain (>20C) 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 three experimental diets for 150 days.

	Diets[a]		
	FO20	CAM20	CAM40
Initial wt (g)	2164 ± 37	2272 ± 34	2285 ± 2
Final wt (g)	4835 ± 67	4552 ± 39	4638 ± 49
Feed (% body weight day^{-1})	0.67 ± 0.01	0.72 ± 0.01	0.62 ± 0.06
Gain (g)	2670 ± 33a	2280 ± 30b	2353 ± 50ab
Gain (%)	123 ± 1a	100 ± 2b	103 ± 2b
FCR[b]	1.34 ± 0.03	1.63 ± 0.05	1.37 ± 0.13
SGR[c]	0.53 ± 0.0a	0.48 ± 0.01b	0.48 ± 0.01b
K[d]	1.77 ± 0.11	1.66 ± 0.12	1.72 ± 0.07
DP (%)[e]	89.71 ± 0.35	89.46 ± 0.29	89.26 ± 0.95
FY (%)[f]	60.05 ± 0.67	59.02 ± 0.7	60.68 ± 0.98
HSI (%)[g]	1.05 ± 0.08	1.03 ± 0.04	0.96 ± 0.02
VSI (%)[h]	10.00 ± 0.38	10.21 ± 0.26	9.68 ± 0.35

Data are expressed as mean ± S.E.M., *n* = 3, *N* = 9. $P < 0.05$; one-way ANOVA with Tukey's post-hoc test of multiple comparisons.

[a] See Table 1 for experimental diet abbreviations.

[b] FCR = food conversion ratio.

[c] SGR = specific growth rate.

[d] K = condition factor

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

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

[g] HSI (%) = hepatosomatic index.

[h] VSI (%) = viscerosomatic index.

Table 3

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

	Diets		
	FO20	CAM20	CAM40
Nutrients[b]			
DM[b]	61.4 ± 4.0	67.2 ± 1.1	67.0 ± 1.5
Protein	74.1 ± 3.7	80.2 ± 0.7	78.2 ± 2.1
Lipid	78.5 ± 4.0	81.0 ± 1.0	84.8 ± 1.4
NFE[b]	75.8 ± 1.8a	71.0 ± 1.4a	63.6 ± 1.7b
Energy[c]	71.4 ± 4.1	75.9 ± 1.1	76.3 ± 1.6
Fatty acids[d]			
Total FA (mg g^{-1} diet)	76.9 ± 4.5	80.4 ± 1.1	83.3 ± 1.7
12:0	84.2 ± 3.4	85.3 ± 0.3	86.0 ± 1.4
14:0	73.3 ± 4.0	71.9 ± 1.1	72.8 ± 1.4
16:0	68.4 ± 4.1	69.2 ± 1.6	69.0 ± 1.1
18:0	56.9 ± 4.3	57.7 ± 3.1	53.3 ± 1.1
16:1n-7	88.7 ± 4.2	94.6 ± 0.3	94.2 ± 1.8
18:1n-9	84.0 ± 5.1	91.1 ± 0.4	91.6 ± 2.3
18:1n-7	82.9 ± 5.2	89.6 ± 0.4	90.0 ± 2.3
20:1n-9	82.4 ± 5.6	93.3 ± 0.8	92.7 ± 2.4
18:2n-6	85.5 ± 5.3	94.7 ± 0.5	94.5 ± 2.2
20:2n-6	85.9 ± 3.9	93.0 ± 0.6	93.0 ± 2.3
20:4n-6	86.6 ± 5.4	93.9 ± 0.6	90.6 ± 1.5
18:3n-3	88.1 ± 4.6	96.6 ± 0.6	95.9 ± 2.0
20:5n-3	90.5 ± 4.4	96.5 ± 0.3	95.2 ± 1.3
22:5n-3	86.5 ± 5.6	93.5 ± 0.7	89.9 ± 2.2
22:6n-3	87.8 ± 5.1	93.6 ± 0.7	92.3 ± 1.4

Data are expressed as mean ± S.E.M., $n = 3$, $N = 9$. $P < 0.05$; one-way ANOVA with Tukey's post-hoc test of multiple comparisons, different letters denote statistically significant difference.

[a] See Table 1 for experimental diet abbreviations.

[b] Nutrients: DM, dry matter; NFE, 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 three experimental diets for 150 days.

	Diets[a]		
	FO20	CAM20	CAM40
Proximate composition (mg g^{-1} of tissue)			
Moisture	661.2 ± 5.9	657.9 ± 5.8	649.7 ± 8
Protein	212.8 ± 5.2	219.3 ± 2.1	219.4 ± 3.5
Lipid	122.9 ± 4.2	110.2 ± 7.4	121.6 ± 6.8
Ash	9.5 ± 0.4	9.5 ± 0.3	10.1 ± 0.4
Fatty acids (µmol g^{-1} of tissue)			
Total FA[b]	351.6 ± 6.6	313.8 ± 17.9	341.9 ± 9.5
SFA[c]	108.3 ± 3a	87.8 ± 5.3b	87.5 ± 2.1b
14:0	11.3 ± 0.2a	7.5 ± 0.5b	7.5 ± 0.3b
16:0	69.6 ± 1.9a	57.9 ± 3.4b	58.2 ± 1.4b
18:0	22.7 ± 0.8a	19.0 ± 1.2ab	18.0 ± 0.4b
Other SFA[d]	4.6 ± 0.2a	3.4 ± 0.2b	3.8 ± 0.2ab
MUFA	185.6 ± 3	164.1 ± 10.2	175.7 ± 5.2
16:1n-7	17.6 ± 0.4a	14.3 ± 0.9b	14.5 ± 0.7b
18:1n-9	141.9 ± 2.1	129.3 ± 7.9	133.1 ± 3.6
18:1n-7	10.5 ± 0.2a	8.4 ± 0.5b	8.7 ± 0.4b
20:1n-9	8.8 ± 0.1ab	6.7 ± 0.5a	10.7 ± 0.7b
Other MUFA[e]	6.8 ± 0.2ab	5.4 ± 0.3a	8.8 ± 1b
Total trans FA	2.5 ± 0.1a	1.6 ± 0.2b	1.5 ± 0b
PUFA	54.9 ± 0.5a	60.0 ± 2.2a	77.1 ± 2.4b
18:2n-6	21.1 ± 0.2a	30.2 ± 1.3b	37.3 ± 1.2c
20:2n-6	1.9 ± 0a	2.2 ± 0.2a	2.9 ± 0.1b
20:4n-6	1.4 ± 0.1	1.4 ± 0	1.2 ± 0.1
Other n-6 PUFA[f]	2.3 ± 0.1a	3.8 ± 0.2b	4.0 ± 0.3b
n-6 PUFA	26.7 ± 0.3a	37.6 ± 1.6b	45.4 ± 1.4b
n-6 LC PUFA	5.2 ± 0.1a	6.2 ± 0.3b	6.7 ± 0.2b
18:3n-3	3.8 ± 0a	6.7 ± 0.4a	14.0 ± 1.3b
20:5n-3	4.9 ± 0.1a	3.7 ± 0.1b	4.2 ± 0.4ab
22:5n-3	2.2 ± 0.1a	1.5 ± 0.1b	1.7 ± 0.1b
22:6n-3	12.6 ± 0.2a	7.9 ± 0.1b	8.3 ± 0.4b
Other n-3 PUFA[g]	3.6 ± 0.1a	1.8 ± 0.1b	2.6 ± 0.1c
n-3 PUFA	27.1 ± 0.3a	21.5 ± 0.5b	30.7 ± 1.5a
n-3 LC PUFA	22.3 ± 0.3a	14.3 ± 0.1b	16.2 ± 0.6c
LC PUFA	27.5 ± 0.4a	20.5 ± 0.4b	23.0 ± 0.8c
n-6/n-3 ratio	1.0 ± 0.0a	1.7 ± 0.0b	1.5 ± 0.1c

Data are expressed as mean ± S.E.M., *n* = 3, *N* = 9. P < 0.05; one-way ANOVA with Tukey's post-hoc test of multiple comparisons.

[a] See Table 1 for experimental diet abbreviations.

[b] Total FA = total fatty acids µmol g^{-1} 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 and % of total fatty acids in brackets and italics) of Atlantic salmon fillet fed the three experimental diets for 150 days.

	Diets[a]		
mg 100 g^{-1} of fillet	FO20	CAM20	CAM40
20:5n-3	147.9 ± 3.8a (*1.5*)	110.6 ± 3.8b (*1.2*)	127.3 ± 11.0ab (*1.4*)
22:5n-3	72.9 ± 2.6a (*0.8*)	48.4 ± 2.4b (*0.6*)	55.2 ± 2.7b (*0.6*)
22:6n-3	414.8 ± 7.7a (*4.2*)	259.9 ± 3.7b (*3.0*)	272.0 ± 14.4b (*2.9*)
SFA[b]	2814.6 ± 79.5a (*28.8*)	2291.3 ± 138.5b (*26.3*)	2282.4 ± 53.9b (*23.9*)
MUFA	5227.8 ± 83.0 (*53.5*)	4627.7 ± 286.7 (*53.0*)	4977.6 ± 146.5 (*52.1*)
PUFA	1644.7 ± 16.1a (*16.9*)	1756.6 ± 62.5a (*20.2*)	2242.1 ± 68.5b (*23.5*)
LC-PUFA	879.7 ± 12.8a (*9.0*)	652.8 ± 11.7b (*7.5*)	729.0 ± 24.0c (*7.6*)
trans	71.8 ± 2.1a (*0.7*)	45.6 ± 5.6b (*0.5*)	40.9 ± 0.6b (*0.4*)
n-6 PUFA	743.0 ± 8.7a (*7.6*)	1056.3 ± 46.5b (*12.1*)	1276.6 ± 38.7c (*13.4*)
n-6 LC PUFA	161.4 ± 2.6a (*1.7*)	194.2 ± 8.0b (*2.2*)	209.4 ± 7.4b (*2.2*)
n-3 PUFA	850.4 ± 9.6a (*8.7*)	660.6 ± 15.0b (*7.6*)	924.0 ± 41.3a (*9.7*)
n-3 LC PUFA	718.3 ± 10.8a (*7.4*)	458.6 ± 3.9b (*5.3*)	519.6 ± 18.2c (*5.4*)
n-6/n-3 ratio	0.9 ± 0.0a	1.6 ± 0.0b	1.4 ± 0.1c

Data are expressed as mean ± S.E.M., *n* = 3, *N* = 9. P < 0.05; one-way ANOVA with Tukey's post-hoc test of multiple comparisons.

[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} and % of total intake in brackets and italics) in Atlantic salmon fed the three experimental diets for 150 days.

	Diets[a]		
	FO20	CAM20	CAM40
12:0	5.2 ± 0.2a (*64.5*)	6.2 ± 0.2a (*74.7*)	3.7 ± 0.5b (*69.5*)
14:0	104.5 ± 5.7a (*40.3*)	92.3 ± 2.2a (*46.8*)	55.6 ± 9.7b (*39.3*)
16:0	661.3 ± 29.8a (*37.2*)	725.7 ± 14.8a (*41.4*)	380.3 ± 76.1b (*30.6*)
18:0	289.3 ± 9.6a (*35.2*)	434.2 ± 4.0b (*41.0*)	133.4 ± 27.7c (*21.4*)
22:0	8.7 ± 0.3a (*25.6*)	16.8 ± 0.2b (*87.0*)	22.9 ± 1.5c (*90.5*)
SFAb,c	1068.9 ± 45.3a	1275.3 ± 19.7a	596.0 ± 115.3b
14:1n-5	31.6 ± 1.5a (*58.7*)	14.7 ± 0.8b (*59.4*)	23.3 ± 3.2a (*64.4*)
16:1n-7	135.7 ± 8.7a (*43.3*)	87.5 ± 4.9b (*39.1*)	50.1 ± 17.3a (*27.8*)
18:1n-7	31.4 ± 4.1 (*22.9*)	47.8 ± 3.8 (*40.9*)	28.5 ± 9.3 (*29.8*)
18:1n-9	765.8 ± 60.1ab (*33.5*)	1190.7 ± 41.3a (*49.8*)	744.6 ± 181.1b (*38.4*)
20:1n-9	6.9 ± 2.6a (*6.2*)	21.7 ± 1.6a (*24.9*)	98.1 ± 20.9b (*41.3*)
22:1n-9	11.8 ± 0.9ab (*40.7*)	1.3 ± 0.7a (*8.3*)	19.1 ± 4.5b (*35.7*)
24:1n-9	7.3 ± 0.3a (*57.8*)	1.7 ± 0.4b (*37.2*)	4.5 ± 0.8c (*45.6*)
20:1n-11	16.5 ± 0.9 (*56.7*)	–[d]	–
22:1n-11	51.7 ± 0.7a (*100*)	13.1 ± 0.0b (*100*)	14.2 ± 0.5b (*100*)
MUFA	1058.6 ± 79.7	1378.4 ± 52.5	982.3 ± 237.0
18:2n-6	100 ± 10.9a (*39.6*)	250.9 ± 17.4b (*50.8*)	219.7 ± 56.3ab (*38.9*)
20:2n-6	0.4 ± 0.3 (*3.3*)	3.1 ± 0.7 (*18.1*)	6.0 ± 3.2 (*18.4*)
22:2n-6	4.7 ± 0.2a (*76.9*)	8.1 ± 0.6b (*83.2*)	3.9 ± 0.8a (*60.8*)
18:3n-6	0.4 ± 0.1 (*16.3*)	–	–
20:3n-6	11.1 ± 0.7a (*77.2*)	1.5 ± 0.3b (*10.5*)	4.4 ± 1.2b (*24.6*)
20:4n-6	9.3 ± 0.6a (*52.8*)	2.8 ± 1.3b (*22.4*)	0.2 ± 0.1b (*3.2*)
22:4n-6	1.1 ± 0.1a (*41.3*)	0.7 ± 0.1b (*44.1*)	–
22:5n-6	8.5 ± 0.3a (*73.4*)	4.9 ± 0.1b (*94.7*)	3.0 ± 0.3c (*97.3*)
n-6 PUFA	135.5 ± 12.9	272.2 ± 18.8	237.3 ± 61.7
18:3n-3	1.2 ± 1.2a (*2.1*)	153.8 ± 3.8b (*72.6*)	348.4 ± 56.1c (*63.2*)
22:3n-3	11.8 ± 0.2a (*100*)	5.9 ± 0b (*100*)	6.8 ± 0.5b (*94.3*)
20:5n-3	64.5 ± 3.2a (*66.4*)	13.5 ± 3.1b (*53.0*)	1.4 ± 1.4c (*5.6*)
22:5n-3	6.8 ± 0.1a (*30.5*)	3.3 ± 0.6b (*46.1*)	–
24:5n-3	–	0.6 ± 0.3 (*100*)	0.3 ± 0.3 (*100*)
24:6n-3	–	–	0.1 ± 0.1 (*100*)
22:6n-3	33.8 ± 3.9a (*25.5*)	7.1 ± 4.2b (*19.4*)	3.7 ± 3.5b (*10.5*)
n-3 PUFA	118.1 ± 6.9a	184.3 ± 9.1a	360.7 ± 60.5b
Total FA	2381.1 ± 144.1	3110.2 ± 99.9	2176.2 ± 473.6

Data are expressed as mean ± S.E.M., winter phase; *n* = 3, *N* = 9. P < 0.05; one-way ANOVA with Tukey's post-hoc test of multiple comparisons, different letters denote statistically significant difference.

[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 (elongation, desaturation or chain shortening) (nmol g^{-1} day^{-1}) in Atlantic salmon fed the three experimental diets for 150 days.

	Diets[a]		
	FO20	CAM20	CAM40
Fatty acid elongation[b]			
18:0 to 20:0	1.6 ± 0.0a (0.2)	12.0 ± 2.2b (1.1)	44.0 ± 3.2c (7.3)
22:0 to 24:0	4.0 ± 0.2a (57.5)	0.1 ± 0.1b (0.5)	–
18:2n-6 to 20:2n-6	0.4 ± 0.4 (0.2)	–	0.1 ± 0.1 (2.4)
20:4n-6 to 22:4n-6	–[c]	–	0.1 ± 0.1 (1.6)
18:3n-3 to 20:3n-3	2.0 ± 0.2a	2.6 ± 0.5ab	5.7 ± 1.2b
18:4n-3 to 20:4n-3	18.6 ± 0.2a	1.7 ± 0.2b	5.2 ± 2.2b
20:5n-3 to 22:5n-3	–	–	3.7 ± 2.5
22:5n-3 to 24:5n-3	1.3 ± 0.4	0.1 ± 0.1	2.4 ± 2.4
Fatty acid Δ-5 desaturation			
20:4n-3 to 20:5n-3	–	–	3.6 ± 2.0
Fatty acid Δ-6 desaturation			
18:2n-6 to 18:3n-6	–	10.6 ± 1.2	13.2 ± 1.5
18:3n-3 to 18:4n-3	31.1 ± 0.7a (60.8)	8.2 ± 0.3b (5.1)	12.3 ± 2.4b (3.5)
24:5n-3 to 24:6n-3	1.1 ± 0.2	–	2.2 ± 2.1
Fatty acid Δ-9 desaturation			
20:0 to 20:1n-11	–	10.2 ± 1.9a	37.1 ± 3.0b
Fatty acid chain shortening			
24:6n-3 to 22:6n-3	–	–	2.0 ± 2.0

Data are expressed as mean ± S.E.M., $n = 3$, $N = 9$. $P < 0.05$; one-way ANOVA with Tukey's post-hoc test of multiple comparisons, different letters denote statistically significant difference.

[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

Table 8

Consumer preference of salmon products (raw salmon, cold smoked and hot smoked fillet) and major influential attributes (fishiness, saltiness and oiliness) from the three dietary treatments.

	Diets[a]		
	FO20	CAM20	CAM40
Preference; Like (+) or Dislike (-)[b]			
Raw	6.21 ± 0.4	1.77 ± 1.94	2.68 ± 0.19
Cold smoked	13.16 ± 1.87	11.71 ± 3.19	17.52 ± 4.68
Hot smoked	19.15 ± 4.02	19.61 ± 1.55	18.95 ± 2.66
Influential attributes[c]			
Fishiness[c]			
Raw	0.5 ± 0.97	1.13 ± 0.74	-1.21 ± 3.48
Cold smoked	3.82 ± 0.85	3.94 ± 1.06	2.52 ± 0.69
Hot smoked	0.15 ± 2.33	-1.69 ± 0.39	-0.48 ± 2.76
Saltiness[c]			
Raw	-21.32 ± 0.39	-20.12 ± 1.54	-20.08 ± 3.06
Cold smoked	6.31 ± 0.95a	11.04 ± 0.8b	7.17 ± 0.36ab
Hot smoked	5.66 ± 0.53	6.84 ± 0.55	6.39 ± 3.45
Oiliness[c]			
Raw	-2.63 ± 2.18	-4.02 ± 0.71	-4.2 ± 0.62
Cold smoked	6.02 ± 0.69	3.03 ± 4.23	5.27 ± 2.3
Hot smoked	-2.74 ± 0.47	-3.75 ± 1.4	-4.27 ± 1.89

Data are expressed as mean ± S.E.M., $n = 3$, $N = 9$. $P < 0.05$; one-way ANOVA with Tukey's post-hoc test of multiple comparisons.

[a] See Table 1 for experimental diet abbreviations.

[b] Salmon preferences were assessed using hedonic LMS scales.

[c] Attributes consumers determined had greatest influence over preference

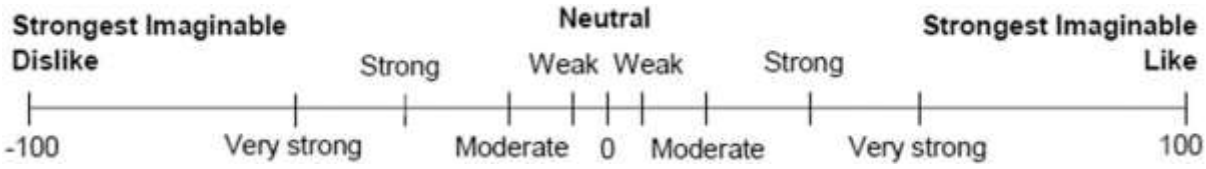
Strongest Imaginable
Dislike
Neutral
Strongest Imaginable
Like
Strong
Weak
Weak
Strong
-100
Very strong
Moderate
0
Moderate
Very strong
100

Figure 1.