SEASONAL EFFECTS ON GROWTH AND PRODUCT QUALITY IN ATLANTIC SALMON FED DIETS CONTAINING TERRESTRIAL OILS AS ASSESSED BY A LONG-TERM, ON-FARM GROWTH TRIAL

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Abstract

Seasonal changes in water temperature affect the utilisation of dietary fatty acids in Atlantic salmon (*Salmo salar L.*). Furthermore, fatty acid profiles of terrestrial oils dictate their suitability in terms of provision of metabolic energy and final product quality. An on-farm, growth trial of Atlantic salmon was conducted in Tasmania, Australia over the final year of grow-out (323 days), consisting of a 'summer phase' and a 'winter phase'. Poultry by-product oil, canola oil and tallow were fed at high dietary lipid inclusion level (80 %) to assess growth, fillet fatty acid composition and sensorial attributes. In the summer phase, the tallow diet appeared to provide added substrate for metabolic energy, potentially enhancing the deposition of n-3 LC PUFA into the fillet, despite lower final weight and a reduced apparent lipid digestibility. Subsequent winter phase results suggested all diets adequately provided metabolic energy and fillet n-3 LC PUFA concentrations were comparable. Additionally, this study highlights the importance of a well-considered experimental design and subsequent statistical interpretation, for commercial scale, on-farm feeding trials. Ultimately, this study demonstrates the importance of seasonally tailored diets for Atlantic salmon, using high terrestrial oil inclusion, under challenging Australian farming conditions.

Keywords:

Aquafeed; Oil; Quality; *Salmo salar*; Season; Water temperature; Lipid

1. Introduction

Aquaculture has been in a state of rapid growth and now produces over 50% of total available seafood (FAO, 2015), however, its further expansion is limited by the cost and the availability of suitable dietary oil sources (Tacon & Metian, 2015; Tocher, 2015; Turchini, Torstensen, & Ng, 2009). Historically, the Atlantic salmon (*Salmo salar*) aquaculture industry has relied upon the utilisation of fish oil - a rich source of omega-3 long-chain polyunsaturated fatty acids, n-3 LC PUFA. However, fish oil supply cannot expand, and its price is constantly rising (Hixson et al., 2017; Tocher, 2015; Ytrestøyl, Aas, & Åsgård, 2015). Consequently, the incorporation of alternative dietary lipids is commonplace, but these can impact the growth performance, nutritional quality and consumer acceptance of the final product (Henriques, Dick, Tocher, & Bell, 2014; Nichols, Glencross, Petrie, & Singh, 2014; Sprague, Dick, & Tocher, 2016).

Water temperature, and its effects on overall fish physiology, including fatty acid metabolism, influences how different dietary lipids are utilised by Atlantic salmon (Handeland, Berge, Bjornsson, Lie, & Stefansson, 2000; Handeland, Imsland, & Stefansson, 2008; Huguet, Norambuena, Emery, Hermon, & Turchini, 2015; Jobling & Bendiksen, 2003; Kullgren et al., 2013; Ng, Sigholt, & Bell, 2004; Norambuena, Morais, Emery, & Turchini, 2015). Water temperatures in excess of optimum (~13 ^{o}C) (Handeland et al., 2008) negatively affect the intake and digestibility of fatty acids (Bendiksen, Berg, Jobling, Arnesen, & Måsøval, 2003; Bendiksen & Jobling, 2003; Hevrøy et al., 2012; Huguet et al., 2015; Kullgren et al., 2013) and increase energetic cost, resulting in the preferential β-oxidation of certain fatty acids, affecting the fillet lipid composition (Viant et al., 2003). Southern hemisphere salmonid culture conditions are typified by mild winter conditions contrasted with summer water temperatures which regularly exceed optimum, resulting in increasingly negative impacts on the aquaculture industry (Last et al., 2011; Oliver et al., 2017). The occurrence of sub-optimal conditions is likely to increase concomitant with rising sea temperatures across temperate regions in Australia and globally (Cochrane, De Young, Soto, & Bahri, 2009; Hobday, Lyne, Thresher, Spillman, & Norman-Lopez, 2011;

58 Last et al., 2011; Oliver et al., 2017). Accordingly, seasonally tailored diets must effectively utilise
59 alternative lipid sources to provide adequate metabolic energy, whilst preserving n-3 LC PUFA,
60 particularly during sub-optimal summer conditions.

61 Owing to its comparatively low cost and widespread availability, poultry by-product oil (PbO) is a
62 frequently utilised oil source in Australian, North American and South American salmonid aquafeed
63 formulations (Bureau & Meeker, 2010; Codabaccus, Carter, Bridle, & Nichols, 2012; Hatlen et al., 2014;
64 Turchini et al., 2009). PbO typically accounts for over 50% of the dietary lipid source (Codabaccus et al.,
65 2012), without compromising digestibility or performance (Hatlen et al., 2014; Liu, Barrows, Hardy, &
66 Dong, 2004; Rosenlund, Obach, Sandberg, Standal, & Tveit, 2001; Turchini, Hermon, Cleveland, et al.,
67 2013). However, the increased utilisation of PbO by the aquafeed sector, and the pet-food industry,
68 resulted in declining availability, requiring further alternatives to be investigated (Emery, Smullen,
69 Keast, & Turchini, 2016; Emery, Smullen, & Turchini, 2014). Tallow (TAL), rendered beef and lamb fat,
70 has received recent attention, owing to its reasonable price, abundant and constant supply, and lack
71 of competition with food and traditional terrestrial feed sectors (Emery et al., 2016; Emery et al., 2014).
72 Additionally, the potential of TAL as suitable for aquafeed formulations stems from its capacity to
73 'spare' n-3 LC PUFA from catabolism, as documented previously (Emery et al., 2016; Gause &
74 Trushenski, 2013). This characteristic has been reported to be facilitated by the relatively high content
75 of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) that are reported to be
76 preferentially β-oxidised for metabolic energy, thus preserving n-3 LC PUFA from catabolism (Francis
77 & Turchini, 2017; Turchini, Francis, Senadheera, Thanuthong, & De Silva, 2011). Furthermore, TAL is
78 characterised by very low levels of omega-6 polyunsaturated fatty acids (n-6 PUFA), which, when
79 deposited into the fillet, contributes negatively to the overall final product quality for human
80 consumption (Baum et al., 2012; Kris-Etherton, Harris, & Appel, 2002; Ruxton, Reed, Simpson, &
81 Millington, 2004; Simopoulos, 2002, 2008).

82 The suitability of alternative oils is influenced by the capacity to provide metabolic energy as well as
83 market availability (Turchini, Ng, & Tocher, 2010), and canola oil (CAN) possesses these qualities (Higgs
84 et al., 2006). It is rich in MUFA, particularly 18:1n-9, making it easily digestible and a good substrate for
85 β-oxidation in Atlantic salmon (Bell et al., 2001; 2003; 2003; Higgs et al., 2006). Additionally, due to its
86 availability and consumer's acceptance, CAN is currently the primary alternative oil source in European
87 Atlantic salmon aquafeed (Bellagamba, Caprino, Mentasti, Vasconi, & Moretti, 2015; Higgs et al., 2006;
88 Karalazos, Bendiksen, Dick, & Bell, 2007; Turchini et al., 2010).

89 The vast majority of published research concerning the use of alternative oils in Atlantic salmon
90 aquaculture has been conducted in small scale laboratory trials, with limited information relevant to
91 on-farm application across a major proportion of the grow-out cycle (Mock, Francis, Drumm, et al.,
92 2019; Rosenlund, Torstensen, Stubhaug, Usman, & Sissener, 2016; Sales & Glencross, 2011;
93 Thorarensen, Kubiriza, & Imsland, 2015). Likewise, in most instances, published investigations focus
94 on the Northern Hemisphere, where thermal minima and maxima vary greatly in comparison to
95 Southern Hemisphere systems.

96 Growth performance, fatty acid composition (in particular n-3 LC PUFA level) and sensorial quality
97 (consumer preference) in Atlantic salmon remain important product quality measures. As such, the
98 modification of fillet fatty acid composition resulting from the changes in aquafeed formulations and
99 seasonal variability remain an area of research with significant commercial interest (Bell, McGhee, et
100 al., 2003; Bendiksen, Johnsen, Olsen, & Jobling, 2011; Bureau & Meeker, 2010; Higgs et al., 2006;
101 Turchini et al., 2010).

102 Therefore, the current study implemented a long-term on-farm feeding approach in Southern
103 Tasmania, Australia over the last year of a grow-out cycle with two-separate 'phases' (summer and
104 winter). The viability of three terrestrial oil sources in relation to season was assessed relative to
105 performance, sensorial quality, nutrient digestibility, fatty acid composition and metabolism in

106 Atlantic salmon fed commercial-like diets containing 20% of fish oil, and either 80% of PbO, TAL or
107 CAN as the added dietary lipid source.

108

109 ***2. Methods***

110 *2.1. Location, animals, experimental design and sampling.*

111 The trial was conducted over the last year of a grow-out period (323 days, 46 weeks) from December
112 1, 2014 to October 20, 2015 in Hideaway bay, Dover, Tasmania (Huon Tasmania, Hideaway bay site;
113 43°15′ 52.2″S 147°04′37.7″E). An initial sample of six fish were randomly selected from the trial cohort,
114 euthanized in excess anaesthetic (AQUI-S, 0.5 mL L^{-1}) and stored at −20 °C for subsequent analysis.
115 The trial was split into two separate, yet consecutive, summer and winter 'phases'. At the
116 commencement of the summer phase, 3600 Atlantic salmon (average initial weight ~600g) were
117 randomly distributed between 12 floating sea pens (5m x 5m x 5m, 300 fish per pen). Each of the pens
118 was then randomly assigned one of three dietary treatments in quadruplicate (four pens per
119 treatment; n = 4, N = 12). After the completion of the first summer phase, and upon commencement
120 of the second winter phase, the trial was reduced to triplicate replication (three pens per treatments;
121 n = 3, N = 9). This partial modification of the experimental design was due to logistical reasons and the
122 number of fish was reduced to accommodate biomass increases. Briefly, 810 Atlantic salmon (average
123 weight ~2200 g) were subsequently assigned one of nine floating sea pens (5m x 5m x 5m, 270 fish
124 per pen). Fish were maintained on the same diet in both phases, the only difference being a decrease
125 in the protein/lipid ratio in line with commercial practice to account for a lower digestible
126 protein/digestible energy requirement in larger fish and during the colder part of the year (Einen &
127 Roem, 1997).

128 Over the duration of the entire trial, encompassing both summer and winter phases, fish were fed
129 with one of the three experimental diets 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, mortalities and physio-chemical parameters including water temperature (summer mean ± SD: 15.37 ± 1.42 ^{o}C, winter mean ± SD: 11.21 ± 0.86 ^{o}C) and dissolved oxygen levels (summer mean ± SD: 7.11 ± 0.48 mg L^{-1}, winter mean ± SD: 7.85 ± 0.43 mg L^{-1}) were monitored throughout the trial and remained within acceptable limits. During the last week of the summer and winter feeding phases, ten fish were randomly selected from each pen and anaesthetised for faecal collection by hand stripping, after which samples were frozen at -20 ^{o}C for subsequent digestibility analysis. At the completion of the summer phase, all fish were anaesthetised and weighed, and 24 fish from each treatment (six fish per pen) were randomly sampled, separated and allocated into two groups: the first group (12 fish) were used for the chemical analysis of the whole body, while the second group (12 fish) were used for the chemical analysis of the fillet. Both groups of fish were subjected to biometry measurements. At the completion of the winter phase, fish were again anaesthetised and weighed, and 21 fish from each treatment (seven fish per pen) were randomly sampled, separated and allocated into three groups: the first group (nine fish) were used for the chemical analysis of whole body, the second group (six fish) for the chemical analysis of fillet, and the third group (six fish) were used for sensory analysis by means of a panel taste test. All sampled fish were immediately placed in an ice slurry, with fish allocated for chemical analysis frozen and stored at −20 °C for subsequent analysis. Fish allocated to panel taste testing were taken from the slurry to be processed by Huon Aquaculture Company, Tasmania (see section 2.5).

155 *2.2. Diets*

156 Diets were manufactured by a commercial feed producer using commercial (closed formula) salmonid
157 aquafeed formulations (Ridley Aquafeed, Australia). Two batches of pellets with identical basal
158 formulations were made, with each batch divided into three sub-batches post-extrusion and vacuum
159 coated with three different oils. The three diets within each batch were iso-proteic, iso-lipidic and iso-
160 energetic and differed only in the added oil source. Fish oil, poultry by-product oil (PbO), canola oil
161 (CAN) and tallow (TAL), were used to formulate the three diets. All diets contained 20% fish oil as a
162 percentage of the added oil from two separate batches for summer and winter phase feeds,
163 respectively and the remaining added dietary lipid was made up of either PbO, CAN or TAL, resulting
164 in three diets; PbOd, CANd and TALd. Though the formulations and type of raw materials utilised were
165 kept consistent across the two periods, different batches of raw materials were utilised due to
166 logistical reasons.

167

168 *2.3. Growth performance, chemical analyses and fatty acid analysis*

169 Standard formulae were used to assess growth, feed utilisation and biometric data, as previously
170 described (Emery et al., 2016). The chemical composition of the experimental diets, faeces and fish
171 samples including; whole body and fillet, were determined via proximate composition analysis
172 according to standard methods as previously described (Palmeri, Turchini, & De Silva, 2007). Briefly,
173 moisture was determined by drying samples in an oven at 80 °C to a constant weight. Ash was
174 determined by incinerating samples in a muffle furnace (S.E.M. SA Pty. Ltd., Australia) at 550 °C for 18
175 h. Protein content (Kjeldahl nitrogen: N × 6.25) was determined using an automated Kjeltech 2300
176 (Foss Tecator, Geneva, Switzerland), while lipid was determined by dichloromethane: methanol
177 extraction (2/1) (Folch, Lees, & Sloane Stanley, 1957) where dichloromethane was used to replace
178 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 previously described in detail in Mock et al., (2019).

2.4. Nutrient digestibility and fatty acid metabolism calculations

Evaluation of digestibility was determined following methods in Atkinson (1984), using ash 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 and Francis, 2009).

2.5. Consumer acceptance testing

Six fish from each treatment (two per cage) were further subdivided in three sub-groups and underwent standard commercial procedures of processing for 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). 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 a food frequency questionnaire (Hodge, Patterson, Brown, Ireland, & Giles, 2000), including a specific salmon questionnaire. 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), following standard procedures. The hot smoked and cold smoked salmon were prepared as previously noted and served to assessors after removal from their packages without any further treatment, the raw salmon was thawed at room temperature each morning prior to assessment.

2.6. Bioeconomic assessment of fish production

Differences in production costs factoring in the incorporation of different oils in Atlantic salmon diets were estimated using the same approach previously described in Turchini et al., (2013). The costs used for the calculations were based on costs in the Australian market over a 12 month period (July 2016 to July 2017) and expressed as $US. The average cost of raw materials, excluding oils, as well as the cost of fish oil, PbO, CAN and TAL was obtained from a commodities website. The prices ($US per tonne) used for the following calculations were as follows: fish oil: $3200; PbO: $1060; CAN: $1300; TAL: $990. Estimates of the feed formulation cost were computed and they were expressed as $US per kg for raw materials only. Subsequently, zootechnical (food conversion ratio), biometrical (fillet yield %) and chemical parameters (n-3 LC PUFA content of fish fillet) recorded were used to estimate the cost for raw materials used in the feed for the production of the following: (i) 1 kg feed; (ii) 1 kg of fish; (iii) 1kg of edible fillet and iv) 100 g of edible n-3 LC PUFA. The aforementioned calculations are based only on costs associated with raw materials used in the diets and ignores other potential differences in cost, such as handling of oils at the feed plant or possible resulting modifications of the duration of the farming process to reach a specific final bodyweight. Accordingly, this analysis should be considered purely indicative, and as such, statistical analysis has not been implemented on the resultant data.

228 *2.7. Statistical analysis*

229 All data were reported as mean ± standard error; summer phase (*n* = 4, *N* = 12), winter phase (*n* = 3,
230 *N* = 9). After confirmation of normality and homogeneity of variance, data was subjected to one-way
231 ANOVA. Where significant differences were detected, a Tukey's post-hoc test for homogenous subsets
232 was performed using IBM SPSS Statistics v24.0 (SPSS Inc., Chicago, IL, USA). Significance was accepted
233 at $P < 0.05$. Post-hoc power analysis was conducted using StatMate v2.0 (Graphpad Software Inc., CA,
234 USA).

235

236 ***3. Results***

237 *3.1. Diets*

238 The three diets in the summer phase were iso-proteic, iso-lipidic and iso-energetic. The winter phase
239 diets were iso-energetic but varied slightly in total lipid and protein (Table 1). Total fatty acid
240 concentration was similar within each phase, although individual fatty acids varied according to lipid
241 source. TALd was characterised by high levels of SFA, attributable largely to 16:0, while CANd was
242 characterised by high levels of MUFA, particularly 18:1n-9. PUFA levels were markedly lower in TALd
243 owing to relatively low levels of 18:2n-6 compared to the other diets. Additionally, low n-6 PUFA in
244 TALd contributed to a lower n-6/n-3 ratio in both phases. CANd exhibited high levels of 18:3n-3
245 relative to the other diets, contributing to higher total n-3 PUFA. Levels of n-3 LC PUFA were
246 comparable between diets, within phases, owing to similar levels of 20:5n-3 and 22:6n-3. It should be
247 noted, that, although the levels of 20:5n-3 and 22:6n-3 in the experimental diets are relatively low on
248 a global scale, they are representative of levels currently used in commercial Atlantic salmon feeds in
249 Tasmania, Australia.

250

251 *3.2. Growth, feed utilisation parameters and biometric data*

All diets were readily accepted by fish and mortalities were low in both phases (<6 %) regardless of dietary treatment. Fish reached commercial size (~5kg) by the end of the trial and gained >240 %, >120 % and > 680 %, of initial body weight (~615g) in the summer phase, winter phase and in total, respectively, with an average feed conversion ratio (FCR) of ~1.3 for all treatments across phases and an average SGR of ~0.75 and ~0.5 in the summer and winter phase, respectively (Table S1). However, final weights of fish varied between treatments and CANd fish were significantly larger than TALd fish in the summer phase (2291g vs 2154g, respectively) ($P < 0.05$). Biometric parameters showed fish were in good condition with no significant differences between treatments, withstanding a higher viscerosomatic index (VSI %) recorded for CANd fish (10.9%) compared to PbOd fish (9.26%) by the end of the winter phase despite similar FY% and DP% between treatments.

3.3. Apparent nutrient and fatty acid digestibility

High nutrient digestibility values (Apparent Digestibility Coefficient – ADC %) were achieved across treatments and phases (Table S2); however, there was a clear trend toward lower digestibility of nutrients in TALd in both the summer and winter phase, including lower lipid digestibility ($P < 0.05$). Additionally, protein digestibility was lower in TALd in the summer phase, although differences were not as pronounced. In terms of apparent fatty acid digestibility, recorded values for the TALd treatment were slightly lower in both phases, including lower ($P < 0.05$) apparent digestibility for both 14:0 and 18:1n-9. No differences in n-3 fatty acids digestibility were recorded.

3.4. Tissue proximate and fatty acid composition

Proximate composition of fillet in both phases recorded no statistically significant differences, with the exception of fillet moisture which was higher in TALd compared to CANd in the winter phase (Table 2). Fatty acid composition in fillets (Table 2) was characterised by higher ($P < 0.05$) SFA concentrations

in PbOd and TALd in both summer (2275.2 and 2611.9 mg $100g^{-1}$ fillet, respectively) and winter phases (2658.8 and 2814.6 mg $100g^{-1}$ fillet, respectively). CANd recorded higher ($P < 0.05$) levels of MUFA in both phases (5497.3 and 7102.1 mg $100g^{-1}$ fillet in summer and winter phases, respectively). Total n-6 PUFA was lower ($P < 0.05$) in TALd in both phases. TALd treatment recorded a lower n-6/n-3 ratio (1.0 and 0.9 in the summer and winter phase, respectively). PbOd recorded the highest n-6/n-3 ratio in both phases (1.4 and 1.7 in the summer and winter phase, respectively) ($P < 0.05$). Total n-3 LC PUFA, despite being higher in TALd ($P < 0.05$) after the summer phase (893.9 mg $100g^{-1}$ fillet), was similar between treatments after the winter phase; ranging from 681.6 to 718.3 mg $100g^{-1}$ fillet in PbOd and TALd, respectively. Total n-3 PUFA, was higher ($P < 0.05$) in CANd in both phases, as a result of higher 18:3n-3 concentrations.

Trends were similar across phases when fatty acid data was expressed as µmol g^{-1} fillet tissue (Table S3). Total fatty acid in fillet (µmol g^{-1} of tissue) varied slightly in the winter phase, where TALd (351.6 µmol g^{-1} of tissue) was lower ($P < 0.05$) than PbOd and CANd (394.5 and 425.0 µmol g^{-1} of tissue, respectively). SFA remained highest in TALd and lowest in CANd whilst MUFA was highest in CANd. n-3 LC PUFA was higher in TALd and lower in PbOd in the summer phase ($P < 0.05$). In the winter phase, despite a similar observable trend, results were non-significant.

3.5. Apparent in vivo fatty acid metabolism

Apparent *in vivo* fatty acid β-oxidation (Table S4) was highest for 18:1n-9 and 18:2n-6, roughly in line with dietary inclusion levels. Numerous statistically significant differences were observed, and results were noticeably different between seasons. In summer, the TALd treatment recorded a considerably higher ($P < 0.05$) total fatty β-oxidation due to high β-oxidation of SFA. CANd recorded significantly higher β-oxidation of 18:3n-3, and whilst β-oxidation of 20:5n-3 was higher in TALd compared to CANd in the summer phase ($P < 0.05$), there was virtually no apparent β-oxidation of 22:6n-3 recorded for any of the treatments. In the winter phase, all treatments recorded high β-oxidation of 18:1n-9 and

18:2n-6, and, unlike the summer phase, all treatments recorded β-oxidation of SFA, although to a lesser extent in CANd compared to the other dietary treatments. β-oxidation of 20:5n-3 was recorded to a similar extent for all treatments in the winter phase, which was lower compared to the β-oxidation recorded during the summer phase. However, β-oxidation of 22:6n-3 was higher in the winter phase compared to the summer phase, and similarly to 20:5n-3, the β-oxidation of 22:6n-3 was recorded to a similar extent for all treatments.

Apparent *in vivo* enzyme activity (fatty acid neogenesis, elongation, desaturation and chain shortening) (Table S5) recorded significant differences between treatments in the summer phase, particularly in relation to the apparent *de novo* production of 12:0, and the subsequent elongation of SFA, which was recorded in the PbOd and CANd treatments. In contrast, TALd recorded no *de novo* production, nor elongation of SFA in the summer phase. Additionally, production of 22:6n-3 was observably higher, although not significant, in PbOd and CANd in the summer phase and negligible in TALd. In contrast to the summer phase, no *de novo* production of 12:0 and no production of 22:6n-3 was recorded in any of the dietary treatments. Whole body compositional data used for the computation of the apparent *in vivo* enzyme activities, in terms of mg g^{-1} lipid are reported in Table S6 and reveal differences in the total sum of fatty acids between the summer and winter phase. Specifically, the total sum of fatty acids in whole-body of fish in the summer phase is higher than that of the winter phase fish.

3.6. Sensory analysis

There were no differences in liking score between dietary treatments for the three preparation methods (hot smoked, cold smoked and raw) (Table S7) ($P > 0.05$). Additionally, no difference ($P > 0.05$) in 'just right' scores were recorded between treatments for any of the influential attributes (fishiness, saltiness and oiliness), with fishiness and oiliness attributes scoring close to zero on 'just

right' scores. For all diet conditions the raw fish lacked saltiness as indicated by -20 on the Just About Right scale.

3.7 Bioeconomic assessment of feed related production costs

The cost of diets ($US kg^{-1}) for summer and winter phases both revealed TALd to be marginally cheaper to produce (Figure S2a and S3a, respectively). In the winter phase, this was >5 % cheaper to produce in comparison to CANd, however, in terms of cost of fish production ($US kg^{-1} of fish) PbOd was cheapest in both phases (Fig. S2b and S3b). Cost for fillet production ($US kg^{-1} and % difference in cost of edible fillet) revealed PbOd was markedly less expensive (7.76 and 5.23 % cheaper than CANd and TALd, respectively) at the conclusion of the winter phase (Fig. S3c) owing to a favourable FCR and relatively low cost of dietary ingredients. Furthermore, TALd was 11.53 % cheaper than PbOd in terms of n-3 LC PUFA production ($US $100g^{-1}$ of edible n-3 LC PUFA) in the summer phase (Fig. S2d), however, after the winter phase PbOd was marginally less expensive than TALd (Fig. S3d), despite being the most expensive after the summer phase (Fig. S2d).

3.8. Power analysis

Fish fed CANd were larger ($P < 0.05$) than fish fed TALd at the end of the summer phase (Figure 1), however, there were no statistical differences at the end of the winter phase despite observable differences in recorded weights. Scatter plots of statistical power (%) versus minimal detectable difference (MDD) (expressed as % difference in final weight) between CANd and TALd treatments for both summer and winter phases showed large differences in MDD at a commonly used statistical power of 80 % (Cohen, 1992), (7.3 and 15.5 %, for the summer (n = 4) and winter (n = 3) phase, respectively).

4. Discussion

The metabolism of fatty acids in Atlantic salmon is influenced by dietary lipid source and seasonal changes in water temperature (Jobling & Bendiksen, 2003; Kullgren et al., 2013; Ng et al., 2004). However, there remains a paucity of published information regarding these effects on large Atlantic salmon. Accordingly, the effects of dietary lipid source on the provision of metabolic energy and fatty acid deposition are discussed herein. A preliminary bioeconomic assessment and a taste quality analysis of the fillet product provides added, industry relevant, information.

Growth performance over the duration of the trial was good and in line with current commercial standards in Tasmania, Australia, with fish growing from 615 g – 5000 g. Differences in nutrient digestibility, particularly lipid, during this period may partially explain the reduced growth observed in the TALd treatment in comparison to CANd. Despite mixed results in previous research regarding the digestibility of rendered animal fats in relation to water temperature (Bureau, Gibson, & El-Mowafi, 2002; Emery et al., 2016; Turchini et al., 2009), results concerning Tasmanian salmonid grow-out conditions are confined to cooler months (Emery et al., 2016). Rising sea temperatures along Tasmania's east coast are deleterious to the Atlantic salmon aquaculture industry, with water temperature spikes to over 19 ^{o}C for prolonged periods of time (Last et al., 2011; Oliver et al., 2017), exceeding the thermal optimum for this species (Elliott & Elliott, 2010; Handeland et al., 2008). The present study indicates that during a summer grow-out phase diets containing a high level of TAL may induce negative effects on growth, owing to reduced digestibility of dietary nutrients. Thus, a potential solution may be to formulate to a given total digestible lipid, in the same way diets are formulated to equal digestible protein content. As such, diets containing high levels of TAL would be formulated with a higher total lipid content, withstanding physical limitations, in order to deliver equal concentrations of digestible nutrients. Despite a reduction in lipid digestibility, total β-oxidation of fatty acids in TALd during the summer phase was clearly higher in comparison to CANd and PbOd, owing to the high β-

oxidation of SFA. CANd and PbOd demonstrated little β-oxidation of SFA and alternatively recorded a *de novo* production of SFA, suggesting these diets were providing less SFA than physiologically required by the fish. Although *de novo* fatty acid production from sources such as amino acids and glucose are purportedly minimal in Atlantic salmon, to date, there have been few attempts to quantify it (Bou et al., 2016). Nevertheless, these results indicate that a small inclusion of dietary TAL would increase total dietary SFA and in turn reduce the need for *de novo* production of SFA, potentially providing improved metabolic performance over the summer period (Bell et al., 2002; Henderson, 1996; Kiessling & Kiessling, 1993; Torstensen, Lie, & Frøyland, 2000). However, given the aforementioned reduction in digestibility, this inclusion should be limited or compensated by a proportional increase in the overall inclusion of total dietary lipid.

Similar to the summer phase, TALd recorded a reduced digestibility of lipids during the winter phase. However, despite observable trends, final weights were not statistically different. Additionally, differences in SFA β-oxidation were not as pronounced as in the summer phase and there was no *de novo* fatty acid production recorded. In accordance with a previously recorded reduction in digestibility of SFA at lower temperatures, the digestibility of SFA in TALd in the winter phase were observably lower (Bendiksen et al., 2003; Bogevik et al., 2010). Differences in SFA β-oxidation between diets containing PbO and TAL were similar to results recorded previously during a Tasmanian winter grow-out period (Emery et al., 2016).

With regard to growth and provision of metabolic energy from dietary lipids, TALd appears to have an advantage in warmer months, in terms of provision of metabolic energy, albeit is outperformed by CANd in terms of growth. PbOd appears to provide a middle point with respect to the dietary provision of digestible fatty acids and good growth performance. As such, future work focussing on a balance of added alternate oils, dependant on season, would likely better elucidate optimal dietary oil inclusion ratios.

399 Consistent with extensive research, fillet fatty acid composition reflected the dietary lipid source
400 (Bendiksen et al., 2011; Tocher, 2015; Turchini et al., 2009). However, this study also clearly showed
401 that season impacted the utilisation of fatty acids between the treatments. A disparity between the
402 total sum of fatty acids in the whole-body of fish in the summer and winter phases was observed.
403 Specifically, a higher concentration of total fatty acids in the summer phase was observed and
404 indicates a higher proportion of non-fatty acid lipid classes. This may be related to homeoviscous
405 adaptation at higher water temperatures, however, in the absence of lipid class analysis, further
406 speculation is withheld. During the summer phase, TALd recorded significantly higher levels of n-3 LC
407 PUFA in the fillet, when reported as mg 100 g^{-1} of fillet. Little *in vivo* fatty acid bioconversion was
408 observed in the TALd treatment in the summer phase, whereas both PbOd and CANd recorded *in vivo*
409 bioconversion of n-3 LC PUFA. This indicates that the TALd diet better met the physiological
410 requirements of the fish during the sub-optimal temperatures of the summer grow-out period, and
411 likely influenced the more efficient retention of n-3 LC PUFA. Despite a similar observed trend in the
412 winter phase, differences in fillet n-3 LC PUFA were much less pronounced. Previous research
413 conducted at both 10 and 12.7 ^{o}C in freshwater and saltwater, respectively, have produced
414 significantly higher levels of n-3 LC PUFA in Atlantic salmon fillet with an increase in dietary TAL
415 inclusion (Emery et al., 2016; Emery et al., 2014). This resulted in similar concentrations of
416 nutritionally valuable n-3 LC PUFA in the final fillet product.

417

418 The n-6/n-3 ratio has been adopted as a measure of relative “healthiness” when comparing food
419 products (Calder, 2010; Kris-Etherton et al., 2002; Wall, Ross, Fitzgerald, & Stanton, 2010).Despite
420 low levels of 18:3n-3, TALd fish had low levels of n-6 PUFA, resulting in a favourable n-6/n-3 ratio
421 (0.9), Additionally, 18:1n-9, present in high amounts in TALd fish, has also been linked to health
422 benefits (Baum et al., 2012). Therefore, the present study indicates that for consumers relying on
423 fish to ‘re-balance’ their ratio of n-6 to n-3 fatty acid consumption, fish fed a high concentration of

TAL may provide superior nutritional value. This is an important finding of the current study, and is in agreement with previous research as currently the use of TAL in diets for Atlantic salmon has been limited primarily due to the perceived risks of decreased digestibility and subsequent reductions in growth (Emery et al., 2016; Gause & Trushenski, 2013). However, from a final eating quality point of view and in consideration of the very competitive price of TAL from a bio-economical point of view, TAL appears to have advantages over other oils.

Although fish consumption is relatively low in Australia, (8.7 and 10.2 kg $year^{-1}$ for males and females, respectively) (Meyer, 2016), taste and sensorial quality is a major driver (Christenson, O'Kane, Farmery, & McManus, 2017). No significant difference between treatments in either preference (like/dislike) or influential attributes were found in this study for any of the different salmon preparation types (raw, cold-smoked or hot-smoked) in either the summer or winter phase. This is in agreement with previous research finding no significant impact on taste and sensorial qualities of salmonid fillets when dietary fish oil is replaced by terrestrial oils in aquafeed (Emery et al., 2016; Hixson et al., 2017; Turchini, Hermon, Moretti, et al., 2013). The similarity in these criteria indicate taste acceptance was not related to dietary lipid source, furthermore, that a range of products may be indistinguishable amongst Australian consumers, thus providing valuable information to Atlantic salmon producers when assessing the primary dietary lipid source for aquafeed formulation.

The assessment of the viability of resources for use in aquafeeds requires also analysis of economic factors (Hardy, 2010), including the cost associated with dietary materials and feed efficiency. Seldom has such an analysis been included in published data for market sized salmonids (Turchini, Hermon, Moretti, et al., 2013). The current study revealed the higher cost of CAN had a clear impact by producing the least cost effective fish fillet (in terms of \$ kg^{-1} edible fillet) and was almost 8% more expensive than PbOd in the winter phase. The most cost-efficient fillet product (in terms of \$ kg^{-1} of edible fillet and \$ kg^{-1} of n-3 LC PUFA) after the summer phase was produced by the TALd treatment.

449 However, by the end of the winter phase, PbOd outperformed the other treatments owing to a
450 culmination of the relatively low cost of PbO, good growth efficiency, and high fillet yield. This
451 information better equips producers to adopt a holistic approach when choosing aquafeed and
452 suggests that a blend of added oils may be preferable, particularly in the summer grow out period.

453

454 The design of fish growth studies is often a balance between the physical and economic constraints of
455 increasing replication on one hand and the statistical ramifications of reducing replication on the other
456 (Thorarensen et al., 2015). As previously outlined, statistical differences in final weight were found
457 between CANd and TALd after the summer phase, however, were not recorded after the winter phase
458 despite a difference of ~300g. In light of the apparent disparity between statistically significant results
459 and economically significant results in aquaculture studies, enhanced implementation of power
460 analysis has been recommend, yet is seldom presented (Ling & Cotter, 2003; Searcy-Bernal, 1994).
461 Power analysis revealed that the summer phase (n = 4) had a vastly reduced chance of incorrectly
462 accepting a false null hypothesis (Type II error) of no difference between fish weight compared to the
463 winter phase (n = 3). Despite increasing criticism of the use of p-values (Anderson, Burnham, &
464 Thompson, 2000; Wasserstein, Schirm, & Lazar, 2019), aquaculture remains heavily reliant upon null-
465 hypothesis testing leading to a tendency of discarding non-statistically significant results (Searcy-
466 Bernal, 1994; Thorarensen et al., 2015). The widely accepted a-priori significance value (p-value) of
467 0.05, can in simple terms be defined as a 5% chance that the null-hypothesis of no difference between
468 treatment means is true. Given the decrease in statistical power due to reduction in replication from
469 quadruplicate to triplicate, as evidenced in the present study, a statistically significant result (i.e. $p <$
470 0.05) may be difficult to detect. However, given aforementioned logistic and economic constraints,
471 the vast majority of fish growth trials are conducted with triplicate replication (Thorarensen et al.,
472 2015). Therefore, it is suggested that increasing the a-priori significance value to 0.10 (i.e. accepting
473 significance at $p < 0.10$), may be useful in selected circumstances. Clearly, this would require a

474 cautious approach as the incidence of incorrectly rejecting a true null-hypothesis (Type I error) would
475 increase. However, if used in conjunction with power analysis may result in further investigation of
476 important results which would have previously been discarded. This presents two main considerations
477 when designing on-farm growth trials where statistical significance is relied upon for product
478 comparison: i) non-statistically significant results ($p > 0.05$) require further investigation where low
479 replication and a resultant decrease in statistical power prevent the detection of statistical difference
480 between means and ii) when possible, replication in fish trials should be increased in order to narrow
481 the gap between statistical and 'real-world' significance.

482

483 ***5. Conclusions***

484 The present study is one of few long-term farm based growth trials revealing the effects of different
485 dietary lipid sources on growth, fillet nutritional quality, fatty acid metabolism and taste
486 characteristics of Atlantic salmon with an emphasis on seasonal differences in lipid utilisation. From a
487 nutritional quality point of view, TALd demonstrated several benefits, including an increased
488 deposition of fillet n-3 LC PUFA in the summer phase and a lower fillet n-6/n-3 ratio. Additionally, the
489 high dietary provision of SFA in TAL provided an enhanced substrate for metabolic energy in the sub-
490 optimal conditions of the summer phase. Dietary treatments providing limited SFA, such as CANd and
491 PbOd, showed that fish needed to increase their overall SFA status via the metabolically expensive
492 process of *de novo* production (liponeogensis) during the summer phase. However, these beneficial
493 effects of increased dietary SFA supply are counterbalanced by other negative effects. Consistent with
494 previous research, TALd exhibited a reduction in lipid digestibility, which resulted in reduced overall
495 fish growth, and CANd produced better overall results and fish were significantly larger in the summer
496 phase. In the winter phase, the lack of *de novo* fatty acid production of SFA and β-oxidation of n-3 LC
497 PUFA suggested that all dietary treatments more adequately provided for the fishes physiological fatty
498 acid requirements at these environmental conditions. PbOd appeared a middle point between CANd

and TALd in terms of nutritional quality and growth. Accordingly, by the time fish were market size (~5kg), PbOd was the most cost effective owing to the low cost of dietary oil, good growth efficiency and high fillet yield. As water temperatures increase and heatwave events become more prevalent, further research is required to better elucidate cost-effective, seasonally tailored aquafeed formulations. This may require multiple dietary lipid sources in order to mitigate potential negative effects of reduced digestibility and sub-optimal growth whilst simultaneously fulfilling the resulting modified metabolic requirements of fish and maximising final product quality, including final n-3 LC PUFA content.

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Conflict of interest

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

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734 Figure 1. Final weights of Atlantic salmon fed the three experimental diets for 173 and 150 days in
735 the summer and winter phase, respectively. Statistical denotations 'n' and 'N' appear in parenthesis
736 for each phase followed by start and finish dates for respective phases. Scatter plots above summer
737 and winter phases show increasing statistical power (%) (Y-axis) vs percentage difference in final
738 weights (X-axis) between CANd and TALd in the summer and winter phase, respectively.

739

740 Figure S1. Hedonic LMS liking scale used in Compusense for the sensory analysis of liking scores for
741 Atlantic salmon prepared three different ways; hot smoked, cold smoked and raw.

742

743 Figure S2. Estimated production costs at the end of the summer phase: (a) Costs for feed
744 formulation: costs associated only with raw materials used in formulation ($ per kg of diet); (b) Costs
745 for fish production: costs associated with feed (costs for feed formulation) needed for the
746 production of 1 kg of fish ($US per kg of fish); (c) Costs for fillet production: costs associated with
747 feed (costs for feed formulation) needed for the production of 1 kg of edible fillet ($US per kg of
748 edible fillet); and (d) Costs for n-3 LC-PUFA production: costs associated with feed (costs for feed
749 formulation) needed for the production of 100 g of edible n-3 LC PUFA ($US per 100 g of edible n-3
750 LC PUFA). Values above each bar show percentage differences in aforementioned costs between the
751 dietary treatments, with PbOd equated to 100 %.

752

753 Figure S3. Estimated production costs at the end of the winter phase: (a) Costs for feed formulation:
754 costs associated only with raw materials used in formulation ($ per kg of diet); (b) Costs for fish
755 production: costs associated with feed (costs for feed formulation) needed for the production of 1 kg
756 of fish ($US per kg of fish); (c) Costs for fillet production: costs associated with feed (costs for feed
757 formulation) needed for the production of 1 kg of edible fillet ($US per kg of edible fillet); and (d)
758 Costs for n-3 LC-PUFA production: costs associated with feed (costs for feed formulation) needed for
759 the production of 100 g of edible n-3 LC PUFA ($US per 100 g of edible n-3 LC PUFA). Values above
760 each bar show percentage differences in aforementioned costs between the dietary treatments,
761 with PbOd equated to 100 %.

762

Table 1

Proximate composition and total fatty acids and fatty acid (mg g^{-1} diet) composition of experimental diets used in the Atlantic salmon growth trial for 173 and 150 days in summer and winter phases, respectively.

		Summer phase diets[a]			Winter phase diets		
	PD	PbOd(s)	CANd(s)	TALd(s)	PbOd(w)	CANd(w)	TALd(w)
Proximate composition (mg g^{-1}) (dry weight)							
Moisture	36.1	46.4	48.3	51.7	28.9	49.4	44.3
Protein	469.8	475.1	480.3	480.1	403.9	412.0	402.6
Lipid	256.4	250.5	246.2	258.5	330.5	365.6	358.2
NFE	157.4	140.6	144.3	128.7	164.6	102.0	125.0
Ash	86.2	85.5	83.2	83.8	72.2	71.0	69.9
Energy (kJ g^{-1})	23.3	23.1	23.0	23.1	25.4	25.9	25.8
Total fatty acids and fatty acid (mg g^{-1} diet) composition							
Total FA (mg g^{-1} diet)[b]	209.1	209.2	204.3	208.0	253.1	261.2	250.9
SFA[c]	62.4	58.2	31.9	82.3	78.7	44.3	104.1
14:0	5.4	5.0	3.9	7.9	4.1	2.8	7.9
16:0	40.3	38.6	18.5	46.0	54.1	26.2	60.8
18:0	11.8	10.7	6.4	22.9	18.7	12.8	31.2
Other SFA[d]	4.9	3.9	3.1	5.5	1.8	2.5	4.2
MUFA[e]	99.6	97.1	113.5	89.2	123.8	153.9	115.4
16:1n-7	11.4	10.0	4.5	9.6	12.2	4.2	10.6
18:1n-9	76.3	74.7	96.3	66.9	95.9	132.2	86.0
18:1n-7	5.3	5.4	5.9	4.6	6.2	7.5	5.2
20:1n-9	1.7	1.8	2.4	1.5	4.2	5.3	4.6
Other MUFA[f]	4.9	5.2	4.4	6.6	5.3	4.7	9.0
Total trans FA[g]	0.8	1.0	0.4	1.9	1.2	0.6	5.2
PUFA[h]	47.1	53.8	58.9	36.4	49.2	62.2	25.9
18:2n-6	22.9	29.0	27.8	14.0	29.9	34.8	9.4
20:2n-6	0.5	0.7	0.8	0.6	0.4	0.5	0.5
20:4n-6	1.0	1.2	0.9	1.1	0.9	0.6	0.7
Other n-6 PUFA[i]	1.5	1.9	1.5	2.0	1.8	1.4	1.6
n-6 PUFA[j]	25.9	32.8	31.0	17.7	33.0	37.3	12.2
18:3n-3	2.8	4.8	11.2	2.3	4.8	15.1	2.0
20:5n-3	4.8	7.5	7.0	7.4	3.9	3.7	3.9
22:5n-3	7.6	1.2	1.1	1.2	0.9	0.8	1.0
22:6n-3	1.2	5.0	4.7	5.2	5.4	5.1	5.8
n-3 PUFA[k]	4.9	19.4	26.4	16.8	15.9	24.6	13.3
Other n-3 PUFA[l]	1.1	0.9	2.4	0.7	1.0	0.0	0.6
LC PUFA[m]	19.5	18.5	18.8	18.4	13.8	12.0	14.0
n-6 LC PUFA[n]	14.8	3.5	3.0	3.4	2.9	2.5	2.7
n-3 LC PUFA[o]	18.1	14.4	15.1	14.5	10.9	9.5	11.3
n-6/n-3 ratio[p]	5.3	1.7	1.2	1.1	2.1	1.5	0.9

[a] Diets: PD = 6mm commercial diet fed previous to experimental diets, added oil 20% fish oil and 80% poultry by-product oil; PbOd = poultry by-product oil diet, added oil 20% fish oil, 80% poultry by-product oil; CANd = canola oil diet, added oil 20% fish oil, 80% canola oil; TALd = tallow diet, added 20% fish oil, 80% tallow. (s) = summer phase diet, (w) = winter phase diet.

[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 18:3n-6, 20:2n-6, 20:3n-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 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 S1
Growth, feed efficiency and biometry of Atlantic salmon fed the experimental diets for 173 days in summer phase and 150 days in winter phase.

	Summer Phase diets[a]			Winter Phase diets		
	PbOd(s)	CANd(s)	TALd(s)	PbOd(w)	CANd(w)	TALd(w)
Initial weight (g)	615 ± 12	615 ± 12	618 ± 12	2219 ± 7	2279 ± 38	2164 ± 37
Final weight (g)	2217 ± 9ab	2292 ± 36a	2154 ± 31b	5053 ± 33	5138 ± 199	4835 ± 67
Gain (g)	1602 ± 19ab	1676 ± 36a	1537 ± 31b	2834 ± 28	2858 ± 164	2670 ± 33
Gain (%)	261 ± 7.71	273 ± 8.42	249 ± 7.56	128 ± 1.12	125 ± 5.37	123 ± 1.16
Feed (% body weight day-1)	0.86 ± 0.03	0.85 ± 0.02	0.85 ± 0.02	0.65 ± 0.02	0.65 ± 0.07	0.67 ± 0.02
FCR[b]	1.31 ± 0.04	1.28 ± 0.02	1.34 ± 0.03	1.26 ± 0.04	1.27 ± 0.16	1.34 ± 0.03
SGR[c]	0.74 ± 0.01ab	0.77 ± 0.01a	0.72 ± 0.01b	0.53 ± 0.00	0.53 ± 0.02	0.53 ± 0.00
K[d]	1.62 ± 0.04	1.68 ± 0.04	1.69 ± 0.04	1.82 ± 0.10	1.77 ± 0.09	1.77 ± 0.11
DP (%)[e]	89.36 ± 0.26	89.39 ± 0.39	89.32 ± 0.24	90.41 ± 0.52	91.72 ± 2.72	89.71 ± 0.35
FY (%)[f]	67.62 ± 0.45	67.95 ± 0.67	68.94 ± 1.09	60.47 ± 0.73	58.80 ± 0.35	60.05 ± 0.67
HSI (%)[g]	1.19 ± 0.03	1.24 ± 0.04	1.21 ± 0.09	1.02 ± 0.01	1.98 ± 0.92	1.05 ± 0.08
VSI (%)[h]	10.07 ± 0.27	10.05 ± 0.32	10.06 ± 0.25	9.26 ± 0.45a	10.90 ± 0.27b	10.00 ± 0.38ab

Data are expressed as mean ± S.E.M., summer phase; *n* = 4, *N* = 12, 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. NB: each phase was analysed independently.

[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 S2

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

	Summer phase diets[a]			Winter phase diets		
	PbOd(s)	CANd(s)	TALd(s)	PbOd(w)	CANd(w)	TALd(w)
Nutrients[b]						
DM[b]	64.8 ± 0.8ab	66.4 ± 0.6a	61.6 ± 1.1b	66.3 ± 3.9	69.0 ± 1.2	61.4 ± 4.0
Protein	80.2 ± 0.6	80.0 ± 0.5	79.2 ± 1.5	75.5 ± 3.1	76.9 ± 0.2	74.1 ± 3.7
Lipid	92.4 ± 0.8a	94.5 ± 0.9a	82.5 ± 1.5b	88.8 ± 3.3ab	94.2 ± 0.5a	78.5 ± 4.0b
NFE[b]	68.7 ± 1.0	68.9 ± 1.3	69.1 ± 1.4	78.3 ± 2.3	70.8 ± 2.3	75.8 ± 1.8
Energy[c]	77.4 ± 0.7a	78.8 ± 0.4a	72.6 ± 1.3b	76.8 ± 3.8	81.0 ± 0.9	71.4 ± 4.1
Fatty acids[d]						
Total FA (mg g-1 diet)	91.6 ± 1.3	93.3 ± 1.2	89.5 ± 1.3	89.4 ± 3.4ab	95.3 ± 0.4a	76.9 ± 4.5b
12:0	90.9 ± 0.7	88.8 ± 2.1	91.4 ± 0.4	91.8 ± 2.5	94.7 ± 1.3	84.2 ± 3.4
14:0	88.8 ± 1.0a	93.0 ± 0.8a	75.1 ± 1.5b	87.6 ± 3.3a	92.5 ± 0.7a	73.3 ± 4.0b
16:0	88.2 ± 4.3	89.2 ± 1.3	91.7 ± 4.9	79.8 ± 4.1	81.0 ± 2.1	68.4 ± 4.1
18:0	70.3 ± 1.7a	81.9 ± 1.8b	100[e]	65.8 ± 6.5	60.8 ± 4.6	56.9 ± 4.3
16:1n-7	96.9 ± 0.8	96.1 ± 0.8	94.2 ± 1.3	96.4 ± 2.4	99.1 ± 0.0	88.7 ± 4.2
18:1n-9	96.0 ± 1.3a	95.8 ± 1.2a	90.1 ± 1.6b	94.7 ± 3.2ab	99.2 ± 0.1a	84.0 ± 5.1b
18:1n-7	94.7 ± 1.3ab	95.8 ± 1.6a	88.5 ± 1.9b	93.9 ± 3.1ab	98.9 ± 0.1a	82.9 ± 5.2b
20:1n-9	100	100	99.4 ± 0.6	92.9 ± 3.6	98.2 ± 0.2	82.4 ± 5.6
18:2n-6	97.5 ± 0.8	96.8 ± 0.8	94.3 ± 0.9	96.1 ± 2.6	99.4 ± 0.1	85.5 ± 5.3
20:2n-6	94.5 ± 2.1	97.1 ± 0.6	92.5 ± 2.1	93.0 ± 2.6	96.9 ± 0.1	85.9 ± 3.9
20:4n-6	97.1 ± 0.7	96.3 ± 0.6	94.3 ± 2.0	95.7 ± 2.1	98.3 ± 0.2	86.6 ± 5.4
18:3n-3	98.0 ± 0.7	97.6 ± 0.7	95.4 ± 0.7	96.7 ± 2.3	99.7 ± 0.1	88.1 ± 4.6
20:5n-3	98.8 ± 0.3	98.2 ± 0.4	97.7 ± 0.6	97.6 ± 1.6	99.4 ± 0.0	90.5 ± 4.4
22:5n-3	95.7 ± 0.7	95.7 ± 0.4	94.0 ± 1.2	96.1 ± 2.6	99.1 ± 0.1	86.5 ± 5.6
22:6n-3	97.0 ± 0.7	96.8 ± 0.5	94.3 ± 2.1	96.3 ± 2.1	98.7 ± 0.1	87.8 ± 5.1

Data are expressed as mean ± S.E.M., summer phase; n = 4, N = 12, 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. NB: each phase was analysed independently.

[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 2

Proximate (g $100g^{-1}$) and fatty acid composition (as mg $100g^{-1}$ edible fillet and % of total fatty acids in brackets and italics) of Atlantic salmon fed the three experimental diets in summer and winter phases, (173 and 150 days, respectively).

	Summer phase diets[a]			Winter phase diets		
	PbOd(s)	CANd(s)	TALd(s)	PbOd(w)	CANd(w)	TALd(w)
Proximate composition (g $100g^{-1}$ fillet)						
Moisture	64.33 ± 0.62	63.71 ± 0.42	64.74 ± 0.27	64.45 ± 0.62ab	63.65 ± 0.32a	66.12 ± 0.59b
Protein	22.77 ± 0.14	22.25 ± 0.26	22.44 ± 0.3	21.97 ± 0.52	21.86 ± 0.06	21.28 ± 0.52
Lipid	10.71 ± 0.44	11.61 ± 0.41	11.17 ± 0.13	13.22 ± 0.59	14.02 ± 0.07	12.29 ± 0.42
Ash	1.29 ± 0.06	1.29 ± 0.06	1.28 ± 0.03	0.99 ± 0.02	0.97 ± 0.01	0.95 ± 0.04
Fatty acids (mg $100g^{-1}$ fillet and % of total FA in brackets)						
20:5n-3	208.1 ± 6.8a (*2.2*)	222.5 ± 6.8ab (*2.2*)	236.4 ± 3.1b (*2.5*)	136.1 ± 3.7 (*1.2*)	147.8 ± 4.6 (*1.2*)	147.9 ± 3.8 (*1.5*)
22:5n-3	97.9 ± 4.9 (*1.0*)	99.3 ± 1.3 (*1.0*)	109.1 ± 0.7 (*1.1*)	66.9 ± 3.0 (*0.6*)	62.3 ± 1.9 (*0.5*)	72.9 ± 2.6 (*0.7*)
22:6n-3	421.2 ± 6.5a (*4.5*)	444.6 ± 11.1ab (*4.3*)	474.6 ± 5.1b (*5.0*)	395.1 ± 22.5 (*3.6*)	386.4 ± 22.8 (*3.3*)	414.8 ± 7.7 (*4.3*)
SFA[b]	2275.2 ± 99.2a (*24.1*)	1936.8 ± 50.6b (*18.7*)	2611.9 ± 54.5c (*27.2*)	2658.8 ± 151.2a (*24.1*)	1889.9 ± 80.1b (*15.6*)	2814.6 ± 79.5a (*28.7*)
MUFA	4498 ± 158.8a (*48.3*)	5497.3 ± 162.4b (*53.8*)	4631.2 ± 53.6a (*48.8*)	5762.5 ± 256.1a (*52.4*)	7102.1 ± 258.4b (*59.3*)	5227.8 ± 83.0a (*53.4*)
PUFA	2532.4 ± 73.1a (*27.0*)	2796.1 ± 72.9b (*27.2*)	2223.6 ± 17.5c (*23.3*)	2521.6 ± 100.8a (*22.5*)	2945 ± 114.3b (*24.3*)	1644.7 ± 16.1c (*16.4*)
LC-PUFA	1049 ± 23.4a (*11.2*)	1061 ± 18.7ab (*10.3*)	1119.3 ± 5.0b (*11.7*)	925.8 ± 43.2 (*8.0*)	908.1 ± 40.7 (*7.3*)	879.7 ± 12.8 (*8.6*)
Trans	33.4 ± 1.0a (*0.4*)	31.1 ± 3.0a (*0.3*)	53.6 ± 3.6b (*0.6*)	47.2 ± 2.1a (*0.4*)	28.4 ± 1.0b (*0.2*)	71.8 ± 2.1c (*0.7*)
n-6 PUFA	1456.5 ± 50.9a (*15.5*)	1495.8 ± 40.6a (*14.5*)	1089.8 ± 14.3b (*11.4*)	1561.8 ± 77.4a (*14.2*)	1693.7 ± 65.1a (*14.1*)	743.0 ± 8.7b (*7.7*)
n-6 LC PUFA	252.4 ± 7.9a (*2.7*)	223.4 ± 3.6b (*2.2*)	225.5 ± 4.9b (*2.4*)	244.2 ± 9.7a (*2.0*)	210.3 ± 9.8a (*1.6*)	161.4 ± 2.6b (*1.4*)
n-3 PUFA	1022.4 ± 20a (*10.9*)	1242.6 ± 29.8b (*12.1*)	1068.9 ± 7a (*11.2*)	907.1 ± 24.1a (*8.0*)	1204.8 ± 47.8b (*9.9*)	850.4 ± 9.6a (*8.3*)
n-3 LC PUFA	796.6 ± 16.4a (*8.5*)	837.6 ± 16.5a (*8.1*)	893.9 ± 6.5b (*9.4*)	681.6 ± 34.2 (*6.0*)	697.8 ± 31.0 (*5.7*)	718.3 ± 10.8 (*7.2*)
n-6/n-3 ratio	1.4 ± 0.0a	1.2 ± 0.0b	1.0 ± 0.0c	1.7 ± 0.1a	1.4 ± 0.0b	0.9 ± 0.0c

Data are expressed as mean ± S.E.M., summer phase; $n = 4$, $N = 12$, 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. NB: each phase was analysed independently.

[a] See Table 1 for experimental diet abbreviations.

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

Table S3

Fatty acid composition (µmol g^{-1} tissue) of fillets of Atlantic salmon fed the three experimental diets in summer and winter phases, (173 and 150 days, respectively).

	Summer phase diets[a]			Winter phase diets		
	PbOd(s)	CANd(s)	TALd(s)	PbOd(w)	CANd(w)	TALd(w)
Fatty acids (µmol g-1 of tissue)						
Total FA[b]	335.2 ± 12.0	366.0 ± 10.1	342.7 ± 4.3	394.5 ± 18.4ab	425 ± 16.1a	351.6 ± 6.6b
SFA[c]	87.2 ± 3.8a	74.0 ± 1.9b	100.2 ± 2.1c	102.2 ± 5.8a	72.2 ± 3.1b	108.3 ± 3.0a
14:0	8.7 ± 0.4a	8.5 ± 0.3b	11.4 ± 0.2c	8.2 ± 0.4a	6.8 ± 0.2b	11.3 ± 0.2c
16:0	57.6 ± 2.7a	45.9 ± 1.3b	62.2 ± 1.3a	71.5 ± 4.4a	46.4 ± 2.1b	69.6 ± 1.9a
18:0	15.6 ± 0.7a	13.7 ± 0.3a	20.3 ± 0.6b	19.0 ± 0.9a	14.5 ± 0.6b	22.7 ± 0.8c
Other SFA[d]	5.4 ± 0.1a	5.9 ± 0.1b	6.2 ± 0.1b	3.5 ± 0.1a	4.5 ± 0.1b	4.6 ± 0.2b
MUFA	160.8 ± 5.7a	195.5 ± 5.8b	165.9 ± 1.9a	204.3 ± 9.2a	250.3 ± 9.1b	185.6 ± 3.0a
16:1n-7	14.9 ± 0.7a	10.6 ± 0.2b	16.3 ± 0.2a	19.1 ± 0.9a	9.0 ± 0.4b	17.6 ± 0.4a
18:1n-9	124.4 ± 4.3a	160.4 ± 4.9b	127.1 ± 1.6a	158.6 ± 7.6a	210.8 ± 7.7b	141.9 ± 2.1a
18:1n-7	9.9 ± 0.3a	11.3 ± 0.3b	10 ± 0.2a	11.7 ± 0.7ab	13.1 ± 0.5a	10.5 ± 0.2b
20:1n-9	5.5 ± 0.2a	6.6 ± 0.2b	5.7 ± 0.1a	8.9 ± 0.0a	10.6 ± 0.4b	8.8 ± 0.1a
Other MUFA[e]	6.0 ± 0.1a	6.6 ± 0.2ab	6.8 ± 0.1b	6.0 ± 0.2	6.9 ± 0.2	6.8 ± 0.2
Total trans FA	1.2 ± 0.0a	1.1 ± 0.1a	1.9 ± 0.1b	1.7 ± 0.1a	1.0 ± 0.0b	2.5 ± 0.1c
PUFA	86.0 ± 2.5a	95.4 ± 2.5a	74.7 ± 0.6b	86.1 ± 3.4a	101.4 ± 3.9b	54.9 ± 0.5c
18:2n-6	42.2 ± 1.5a	44.6 ± 1.3a	30.3 ± 0.4b	46.7 ± 2.5a	52.5 ± 2.0a	21.1 ± 0.2b
20:2n-6	3.1 ± 0.1a	2.8 ± 0.1ab	2.4 ± 0.0b	3.3 ± 0.2a	3.2 ± 0.1a	1.9 ± 0.0b
20:4n-6	1.8 ± 0.0a	1.6 ± 0.0b	1.9 ± 0.0a	1.6 ± 0.1a	1.1 ± 0.1b	1.4 ± 0.1ab
Other n-6 PUFA[f]	3.9 ± 0.1	3.5 ± 0.1	3.5 ± 0.2	4.1 ± 0.2a	3.4 ± 0.2b	2.3 ± 0.1c
n-6 PUFA	51.0 ± 1.8a	52.5 ± 1.4a	38.1 ± 0.5b	55.6 ± 2.8a	60.3 ± 2.3a	26.7 ± 0.3b
n-6 LC PUFA	8.1 ± 0.3a	7.2 ± 0.1b	7.2 ± 0.2b	7.8 ± 0.3a	6.8 ± 0.3a	5.2 ± 0.1b
18:3n-3	8.0 ± 0.2a	14.4 ± 0.5b	6.3 ± 0.1c	7.8 ± 0.7a	18.2 ± 0.7b	3.8 ± 0.0c
20:5n-3	6.9 ± 0.2a	7.4 ± 0.2ab	7.8 ± 0.1b	4.5 ± 0.1	4.9 ± 0.2	4.9 ± 0.1
22:5n-3	3.0 ± 0.1	3.0 ± 0.0	3.3 ± 0.0	2.0 ± 0.1	1.9 ± 0.1	2.2 ± 0.1
22:6n-3	12.8 ± 0.2	13.5 ± 0.3	14.4 ± 0.2	12.0 ± 0.7	11.8 ± 0.7	12.6 ± 0.2
Other n-3 PUFA[g]	2.2 ± 0.0	2.3 ± 0.1	2.3 ± 0.1	2.9 ± 0.1a	3.2 ± 0.1ab	3.6 ± 0.1b
n-3 PUFA	32.9 ± 0.6a	40.6 ± 1b	34.1 ± 0.2a	29.3 ± 0.7a	40.0 ± 1.6b	27.1 ± 0.3a
n-3 LC PUFA	24.8 ± 0.5a	26.1 ± 0.5a	27.8 ± 0.2c	21.2 ± 1.1	21.8 ± 1.0	22.3 ± 0.3
LC PUFA	32.9 ± 0.7	33.2 ± 0.6	35.0 ± 0.1	29 ± 1.3	28.5 ± 1.3	27.5 ± 0.4
n-6/n-3 ratio	1.5 ± 0.0a	1.3 ± 0.0b	1.1 ± 0.0c	1.9 ± 0.1a	1.5 ± 0.0b	1.0 ± 0.0c

Data are expressed as mean ± S.E.M., summer phase; *n* = 4, *N* = 12, 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. NB: each phase was analysed independently.

[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 S4

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 in summer and winter phases, (173 and 150 days, respectively).

	Summer phase diets[a]			Winter phase diets		
	PbOd(s)	CANd(s)	TALd(s)	PbOd(w)	CANd(w)	TALd(w)
12:0	0.62 ± 0.62a (*10.0*)	–	13.94 ± 0.43b (*63.1*)	3.25 ± 0.8ab (*56.0*)	1.55 ± 0.36a (*49.8*)	4.99 ± 0.19b (*61.1*)
14:0	8.95 ± 8.95 (*4.7*)	–	26.91 ± 4.99 (*8.7*)	57.8 ± 10.83ab (*44.2*)	25.66 ± 7.92a (*27.2*)	82.86 ± 4.45b (*31.6*)
16:0	11.78 ± 11.78a (*0.9*)	–	744.31 ± 31.57b (*46.3*)	501.09 ± 83.55a (*32.9*)	26.19 ± 14.55b (*3.3*)	465.92 ± 19.08a (*25.9*)
18:0	–[d]	–	472.59 ± 13.06 (*65.5*)	82.72 ± 22.09a (*17.5*)	84.34 ± 7.69a (*24.8*)	328.86 ± 6.1b (*39.5*)
20:0	0.19 ± 0.19 (*1.3*)	–	0.12 ± 0.12 (*0.8*)	–	–	–
22:0	13.31 ± 0.68 (*45.9*)	–	12.49 ± 1.01 (*46.8*)	6.02 ± 0.58a (*0.6*)	20.75 ± 0.66b (*81.9*)	8.22 ± 0.3a (*18.9*)
24:0	0 ± 0	0.9 ± 0.07 (*10.1*)	–	–	3.05 ± 0.4 (*27.2*)	–
SFA[b,c]	34.84 ± 22.19a	0.9 ± 0.07a	1270.37 ± 50.57b	650.87 ± 117.76a	161.54 ± 30.87b	890.86 ± 29.91a
14:1n-5	5.11 ± 0.59a (*45.6*)	2.39 ± 0.1b (*45.6*)	15.97 ± 0.6c (*57.1*)	9.1 ± 0.68a (*65.6*)	1.69 ± 0.11b (*53.3*)	32.29 ± 1.08c (*59.3*)
16:1n-7	129.25 ± 12.61a (*38.1*)	22.48 ± 3.75b (*14.7*)	119.88 ± 5.12a (*35.3*)	180.25 ± 26.16a (*52.2*)	59.18 ± 5.58b (*47.7*)	138.91 ± 6.67a (*43.8*)
18:1n-7	27.36 ± 5.19ab (*16.6*)	34.53 ± 0.97a (*18.9*)	13.66 ± 3.48b (*9.4*)	62.01 ± 15.14ab (*39.2*)	86.23 ± 6.06a (*43.3*)	30.15 ± 3.41b (*21.7*)
18:1n-9	582.49 ± 53.1a (*25.5*)	673.04 ± 21.04a (*22.7*)	394.97 ± 43.68b (*18.6*)	1145.91 ± 198.01ab (*46.8*)	1607.9 ± 97.56a (*45.6*)	755.65 ± 48.64b (*32.7*)
20:1n-9	–	–	–	17.58 ± 11.55 (*18.2*)	24.8 ± 3.57 (*19.1*)	6.08 ± 2.18 (*5.4*)
22:1n-9	4.71 ± 0.5a (*23.5*)	0.54 ± 0.45b (*2.9*)	3.17 ± 0.22a (*18.3*)	8.26 ± 2.34a (*30.9*)	0.81 ± 0.41b (*3.1*)	7.14 ± 0.68a (*24.3*)
24:1n-9	7.93 ± 1.06 (*61.2*)	4.38 ± 1.12 (*28.6*)	5.88 ± 1.58 (*46.5*)	1.76 ± 0.93a (*16.2*)	5.62 ± 0.32b (*36.3*)	3.18 ± 0.24ab (*24.8*)
20:1n-11	17.98 ± 1.36a (*48.2*)	9.76 ± 0.26b (*27.5*)	18.94 ± 0.55a (*49.4*)	11.65 ± 2.12a (*45.6*)	1.17 ± 1.17b (*5.3*)	14.7 ± 0.7a (*50.0*)
22:1n-11	16.81 ± 0.81a (*51.6*)	16.13 ± 0.48a (*51.8*)	10.19 ± 0.39b (*35.5*)	43.63 ± 0.2a (*100*)	43.51 ± 1.14a (*100*)	52.16 ± 0.5b (*100*)
MUFA	791.64 ± 72.6	763.25 ± 17.92	582.65 ± 53.9	1480.15 ± 256.17ab	1830.91 ± 115.12a	1040.27 ± 63.86b
18:2n-6	308.71 ± 26.67a (*34.6*)	244.45 ± 7.11a (*28.3*)	106.4 ± 8.77b (*23.8*)	395.07 ± 65.95a (*51.4*)	439.44 ± 31.35a (*47.0*)	101.73 ± 9.65b (*39.8*)
20:2n-6	–	–	–	–	–	0.28 ± 0.15 (*2.3*)
22:2n-6	–	–	–	8.43 ± 0.07a (*79.5*)	–	4.86 ± 0.13b (*78.2*)
18:3n-6	0.28 ± 0.28a (*3.9*)	–	2.87 ± 0.25b (*30.2*)	–	–	1.23 ± 0.09b (*45.0*)
20:3n-6	1.86 ± 1.31a (*7.1*)	0.48 ± 0.33a (*2.2*)	7.25 ± 0.48b (*26.8*)	1.11 ± 1.11a (*8.9*)	10.67 ± 1.72b (*55.7*)	11.18 ± 0.59b (*77.2*)
20:4n-6	11.74 ± 1.14a (*34.1*)	6.97 ± 0.39b (*26.1*)	10.88 ± 0.47a (*33.4*)	10.56 ± 1.73 (*51.4*)	7.44 ± 0.82 (*49.7*)	9.53 ± 0.41 (*53.6*)
22:4n-6	1.32 ± 0.17a (*23.2*)	–	2.23 ± 0.1b (*34.3*)	1.36 ± 0.26 (*56.4*)	0.96 ± 0.08 (*64.6*)	1.05 ± 0.03 (*40.6*)
22:5n-6	2.9 ± 0.12a (*28.2*)	1.97 ± 0.36b (*20.9*)	5.11 ± 0.03c (*43.9*)	8.43 ± 0.33a (*82.7*)	10.94 ± 0.43b (*81.0*)	8.57 ± 0.2a (*74.9*)
n-6 PUFA	326.82 ± 29.01a	253.87 ± 7.46b	134.76 ± 9.75c	424.97 ± 69.13a	469.45 ± 34.11a	138.43 ± 11.14b
18:3n-3	59.58 ± 2.44a (*39.9*)	157.75 ± 5.05b (*45.0*)	22.71 ± 2.21c (*31.1*)	71.82 ± 11.97a (*57.9*)	211.29 ± 10.72b (*51.8*)	1.24 ± 1.01c (*2.2*)
18:4n-3	2.92 ± 0.16a (*72.2*)	1.66 ± 0.18b (*47.1*)	0.16 ± 0.1c (*0.5*)	–	–	–

20:4n-3	2.58 ± 0.43a (*20.9*)	0.97 ± 0.22b (*8.1*)	0.08 ± 0.08b (*0.8*)	–	–	–
20:3n-3	–	44.96 ± 0 (*100*)	–	–	–	–
22:3n-3	3.26 ± 0.26 (*38.1*)	2.5 ± 0.15 (*31.8*)	3.25 ± 0.2 (*35.7*)	11.77 ± 0.05a (*100*)	2.91 ± 0.08b	11.92 ± 0.11a (*100*)
20:5n-3	111.97 ± 11.24ab (*52.4*)	89.82 ± 4.97a (*44.4*)	121.16 ± 4.55b (*55.1*)	71.55 ± 5.36 (*77.8*)	59.51 ± 2.56 (*65.2*)	66.73 ± 2.26 (*67.8*)
22:5n-3	–	–	–	9.02 ± 3.01 (*46.8*)	5.79 ± 1.07 (*33.0*)	7.14 ± 0.2 (31.8)
22:6n-3	0.92 ± 0.92 (*0.7*)	–	3.6 ± 1.27 (*2.6*)	42.64 ± 13.42 (*35.9*)	33.52 ± 8.41 (*28.6*)	35.85 ± 3.71 (*26.7*)
n-3 PUFA	181.23 ± 12.7a	297.67 ± 6.85b	150.96 ± 6.34a	206.81 ± 33.63a	313.02 ± 22.38b	122.88 ± 5.05a
Total FA	1334.52 ± 127.52a	1315.69 ± 30.19a	2138.74 ± 115.41b	2762.8 ± 476.33	2774.93 ± 201.32	2192.44 ± 109.67

Data are expressed as mean ± S.E.M., summer phase; n = 4, N = 12, 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. NB: each phase was analysed independently.

[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 S5

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 323 days.

	Summer phase diets[a]			Winter phase diets		
	PbOd(s)	CANd(s)	TALd(s)	PbOd(w)	CANd(w)	TALd(w)
de novo fatty acid production						
12:0	85.21 ± 41.57	178.42 ± 11.44	–	–	–	–
Fatty acid elongation[b]						
12:0 to 14:0	86.67 ± 41.87	177.67 ± 11.53	–	–	–	–
14:0 to 16:0	101.37 ± 43.45	196.09 ± 11.15	–	–	4.12 ± 4.12	–
16:0 to 18:0	111.55 ± 11.05	105.29 ± 3.59	–	–	–	–
18:0 to 20:0	2.05 ± 0.70a	18.82 ± 1.56b	0.71 ± 0.37a	2.18 ± 0.52a	11.42 ± 1.23b	1.59 ± 0.05a
18:1n-9 to 20:1n-9	9.39 ± 2.92	10.50 ± 2.73	16.5 ± 2.74	–	–	–
20:1n-9 to 22:1n-9	–	0.71 ± 0.42	–	–	0.67 ± 0.67	–
20:0 to 22:0	–	22.05 ± 0.88	–	–	–	–
22:0 to 24:0	0.42 ± 0.04	–	0.49 ± 0.17	3.64 ± 0.4	–	4.04 ± 0.20
18:2n-6 to 20:2n-6	19.27 ± 2.92a	15.32 ± 2.26ab	9.46 ± 0.88b	13.93 ± 4.6a	16.43 ± 0.33a	0.47 ± 0.47b
20:2n-6 to 22:2n-6	2.72 ± 0.33a	2.67 ± 0.26a	1.63 ± 0.11b	–	1.58 ± 0.05	–
18:3n-6 to 20:3n-6	0.50 ± 0.37	1.08 ± 0.92	–	0.86 ± 0.67	–	–
18:3n-3 to 20:3n-3	–	–	–	2.18 ± 0.58a	14.22 ± 0.23b	1.96 ± 0.12a
20:4n-6 to 22:4n-6	–	0.33 ± 0.09	–	–	–	–
18:4n-3 to 20:4n-3	–	–	0.29 ± 0.16	12.56 ± 1.94a	15.88 ± 0.44ab	18.55 ± 0.24b
20:5n-3 to 22:5n-3	25.22 ± 10.32	28.18 ± 3.17	12.72 ± 1.52			
22:5n-3 to 24:5n-3	20.60 ± 9.65	21.88 ± 1.81	8.72 ± 1.04	1.51 ± 0.81	1.45 ± 0.67	1.28 ± 0.38
Fatty acid Δ-6 desaturation						
18:2n-6 to 18:3n-6	2.60 ± 1.05	4.44 ± 1.11	–	4.57 ± 1.72	7.33 ± 1.26	–
18:3n-3 to 18:4n-3	–	–	0.09 ± 0.08	9.55 ± 1.93a	15.31 ± 0.42b	31.11 ± 0.73c
24:5n-3 to 24:6n-3	15.26 ± 8.70	17.32 ± 1.80	4.36 ± 0.52	1.52 ± 0.54	1.05 ± 0.46	1.11 ± 0.16
Fatty acid Δ-9 desaturation						
20:0 to 20:1n-11	–	–	–	–	1.82 ± 1.27	–
Fatty acid chain shortening						
24:6n-3 to 22:6n-3	11.27 ± 8.55	12.6 ± 1.68	0.16 ± 0.16	–	–	–

Data are expressed as mean ± S.E.M., summer phase; n = 4, N = 12, 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. NB: each phase was analysed independently.

[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 S6

Proximate (mg 100 g^{-1}) and fatty acid composition (mg g^{-1} lipid) of Atlantic salmon whole body fed the three experimental diets in summer and winter phases, (173 and 150 days, respectively).

	Summer phase diets[a]			Winter phase diets		
	PbOd(s)	CANd(s)	TALd(s)	PbOd(w)	CANd(w)	TALd(w)
Proximate composition (mg g^{-1} of tissue)						
Moisture	594.5 ± 3.9	585.5 ± 7.6	601.6 ± 5.7	576.5 ± 0.6ab	565.4 ± 1.1b	595.9 ± 0.3a
Protein	199.1 ± 2.7	192.4 ± 5.4	195 ± 3	180.0 ± 0.3	183.2 ± 0.5	184.3 ± 0.3
Lipid	165.4 ± 6.6	166 ± 3.7	170.4 ± 3	207.9 ± 0.9ab	227.0 ± 0.7b	192.0 ± 0.5a
Ash	16.7 ± 0.7	16.4 ± 1.3	16.3 ± 0.4	14.0 ± 0.1	13.1 ± 0.7	13.9 ± 0.8
Fatty acids (mg g^{-1} lipid)						
SFA[b]	220.5 ± 3.8a	180.8 ± 3.2b	242.2 ± 7.2b	179.9 ± 12.1c	125.5 ± 3.4a	219.8 ± 5.9b
14:0	20.1 ± 0.3a	18.6 ± 0.4a	24.9 ± 0.6b	13.0 ± 1.0bd	11.0 ± 0.2b	20.9 ± 0.5a
16:0	142.2 ± 2.5a	107.5 ± 2.2b	145 ± 4.2a	124.3 ± 8.2bc	78.7 ± 2.1a	138.3 ± 3.7b
18:0	43.3 ± 0.7a	36.4 ± 0.5b	54.8 ± 2c	35.9 ± 2.5c	27.1 ± 0.9a	50.4 ± 1.5b
Other SFA[c]	14.9 ± 0.5a	18.3 ± 0.5b	17.5 ± 0.4b	6.7 ± 0.5c	8.7 ± 0.2b	10.2 ± 0.3a
MUFA	464.9 ± 6a	525.8 ± 11.1b	432.3 ± 5.6c	388.4 ± 26.8b	467.6 ± 10.8a	410.8 ± 10.4ab
16:1n-7	38.7 ± 0.8a	25.9 ± 0.7b	38.7 ± 0.6a	34.2 ± 2.4bc	16 ± 0.4a	35.9 ± 0.7c
18:1n-9	359.5 ± 6.1a	428 ± 10.1b	326.9 ± 4.3c	301.7 ± 20.4b	391.6 ± 9.3a	314.3 ± 8.1b
18:1n-7	28.3 ± 0.4a	3.00 ± 0.5b	25.5 ± 0.3c	22.8 ± 1.7ab	24.3 ± 0.6b	23.3 ± 0.6a
20:1n-9	17.1 ± 0.3a	20.7 ± 0.4b	16.9 ± 0.3a	17.8 ± 1.6b	21.4 ± 0.6b	21.4 ± 0.7b
Other MUFA[d]	21.3 ± 0.5a	21.2 ± 0.3a	24.3 ± 0.2b	12.0 ± 0.7d	14.2 ± 0.1be	15.9 ± 0.3c
Total trans FA	3.5 ± 0.3a	2.9 ± 0.3a	4.9 ± 0.1b	3.3 ± 0.2b	1.9 ± 0a	6.3 ± 0.1a
PUFA	246.1 ± 2.8a	255.3 ± 4.9a	192.8 ± 1b	170.4 ± 10.8bc	197.2 ± 2.9d	129.6 ± 3.1a
18:2n-6	118.8 ± 1a	117 ± 2.7a	75.5 ± 0.7b	90.3 ± 5.8bc	99.7 ± 1.9c	48.3 ± 1.0a
20:2n-6	9.0 ± 0.4a	8.8 ± 0.3a	7.1 ± 0.1b	6.9 ± 0.7b	6.6 ± 0.2b	4.6 ± 0.1a
20:4n-6	5.2 ± 0.2a	4.5 ± 0.1b	5.1 ± 0.1a	3.2 ± 0.1c	2.4 ± 0.0a	3.1 ± 0.1c
Other n-6 PUFA[e]	3.2 ± 0.2ab	2.5 ± 0.4a	3.7 ± 0.1b	8.2 ± 0.4b	6.5 ± 0.2a	5.8 ± 0.1a
n-6 PUFA	136.1 ± 1.2a	132.8 ± 3.1a	91.4 ± 0.8b	108.6 ± 6.9bc	115.2 ± 1.9c	61.8 ± 1.3a

n-6 LC PUFA	24.4 ± 0.7a	22.6 ± 0.7ab	21.5 ± 0.2b	16.3 ± 1.1b	13.7 ± 0.1a	12.6 ± 0.3a
18:3n-3	22.4 ± 0.9a	37.1 ± 1.3b	14.9 ± 0.2c	13.3 ± 0.9c	34 ± 0.7b	8.5 ± 0.2a
20:5n-3	18.5 ± 0.7	18.6 ± 0.3	19.8 ± 0.6	9.4 ± 0.6b	10.5 ± 0.3ab	11.9 ± 0.4a
22:5n-3	8.9 ± 0.1ab	8.5 ± 0.1a	9.3 ± 0.2b	4.6 ± 0.4b	4.3 ± 0.1b	5.8 ± 0.3a
22:6n-3	38.9 ± 1.8	36.9 ± 0.6	37.7 ± 0.6	26 ± 1.5c	24.5 ± 0.2ac	30.3 ± 1.1d
Other n-3 PUFA[f]	7.0 ± 0.3	7.2 ± 0.1	6.8 ± 0.2	6.2 ± 0.4bc	6.6 ± 0.2c	8.7 ± 0.3a
n-3 PUFA	95.7 ± 2.3a	108.4 ± 1.7b	88.5 ± 0.5c	59.5 ± 3.7c	79.9 ± 1.0b	65.3 ± 1.9c
n-3 LC PUFA	73.0 ± 1.9	70.8 ± 0.8	73.6 ± 0.6	45.8 ± 2.8ab	45.8 ± 0.3ab	54.8 ± 1.7c
LC PUFA	97.5 ± 2.2	93.4 ± 1.4	95.1 ± 0.8	62.1 ± 3.9ab	59.5 ± 0.4ab	67.4 ± 2a
n-6/n-3 ratio	1.5 ± 0.0a	1.3 ± 0.0b	1.1 ± 0.0c	1.8 ± 0.0a	1.4 ± 0.0b	0.9 ± 0.0a

Data are expressed as mean ± S.E.M., summer phase; n = 3, N = 12, 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. NB: each phase was analysed independently.

[a] See Table 1 for experimental diet abbreviations.

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

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

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

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

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

Table S7

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

	Diets		
	PbO	CAN	TAL
Preference; Like (+) or Dislike (-)			
Raw	4.11 ± 1.18	9.85 ± 2.47	6.21 ± 0.40
Cold smoked	14.63 ± 2.24	20.35 ± 1.35	13.16 ± 1.87
Hot smoked	22.6 ± 1.31	19.71 ± 3.42	19.15 ± 4.02
Influential attributes ('Just Right' scores; +/ve = too much, -/ve = too little)			
Fishiness			
Raw	-0.6 ± 2.64	0.77 ± 1.02	0.5 ± 0.97
Cold smoked	3.91 ± 0.80	-0.29 ± 3.44	3.82 ± 0.85
Hot smoked	-0.68 ± 1.71	1.49 ± 1.88	0.15 ± 2.33
Saltiness			
Raw	-20.26 ± 3.66	-18.21 ± 3.27	-21.32 ± 0.39
Cold smoked	9.85 ± 1.57	4.97 ± 1.69	6.31 ± 0.95
Hot smoked	5.73 ± 1.53	8.54 ± 0.83	5.66 ± 0.53
Oiliness			
Raw	-3.8 ± 1.78	-2.8 ± 1.12	-2.63 ± 2.18
Cold smoked	3.52 ± 0.27	2.19 ± 2.58	6.02 ± 0.69
Hot smoked	-0.45 ± 1.63	-0.52 ± 2.73	-2.74 ± 0.47

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 letter denote statistically significant difference.

[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

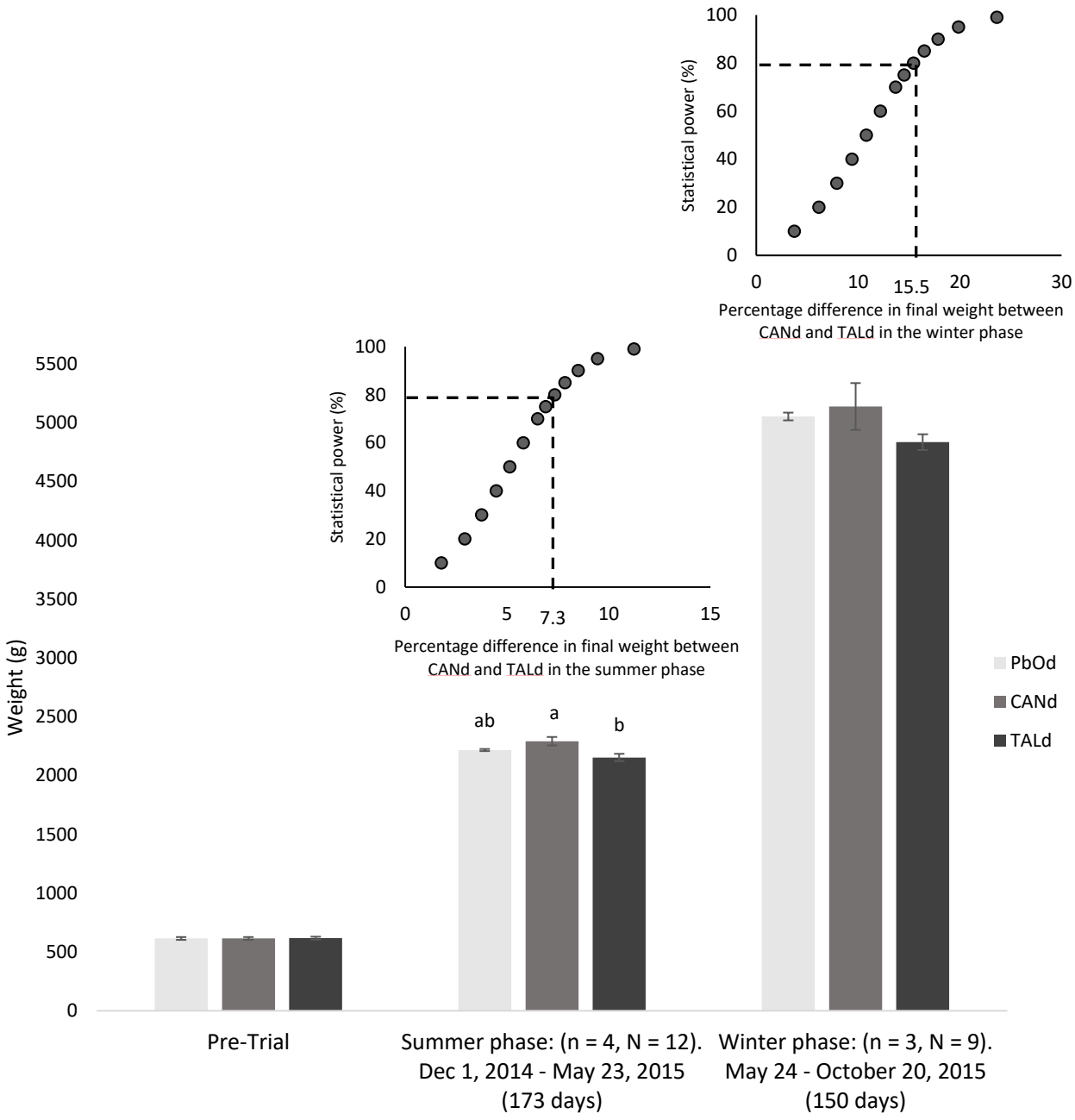
Statistical power (%)
100
80
60
40
20
0
0
10
15.5
20
30
Percentage difference in final weight between CANd and TALd in the winter phase
Statistical power (%)
100
80
60
40
20
0
0
5
7.3
10
15
Percentage difference in final weight between CANd and TALd in the summer phase
Weight (g)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
ab
a
b
PbOd
CANd
TALd
Pre-Trial
Summer phase: (n = 4, N = 12). Dec 1, 2014 - May 23, 2015 (173 days)
Winter phase: (n = 3, N = 9). May 24 - October 20, 2015 (150 days)

Figure 1

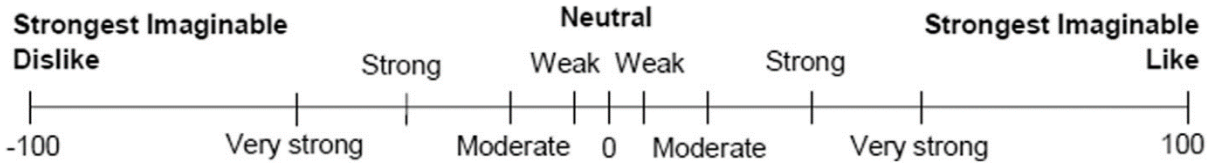
Strongest Imaginable Dislike
Neutral
Strongest Imaginable Like
Strong
Weak
Weak
Strong
-100
Very strong
Moderate
0
Moderate
Very strong
100

Figure S 1.

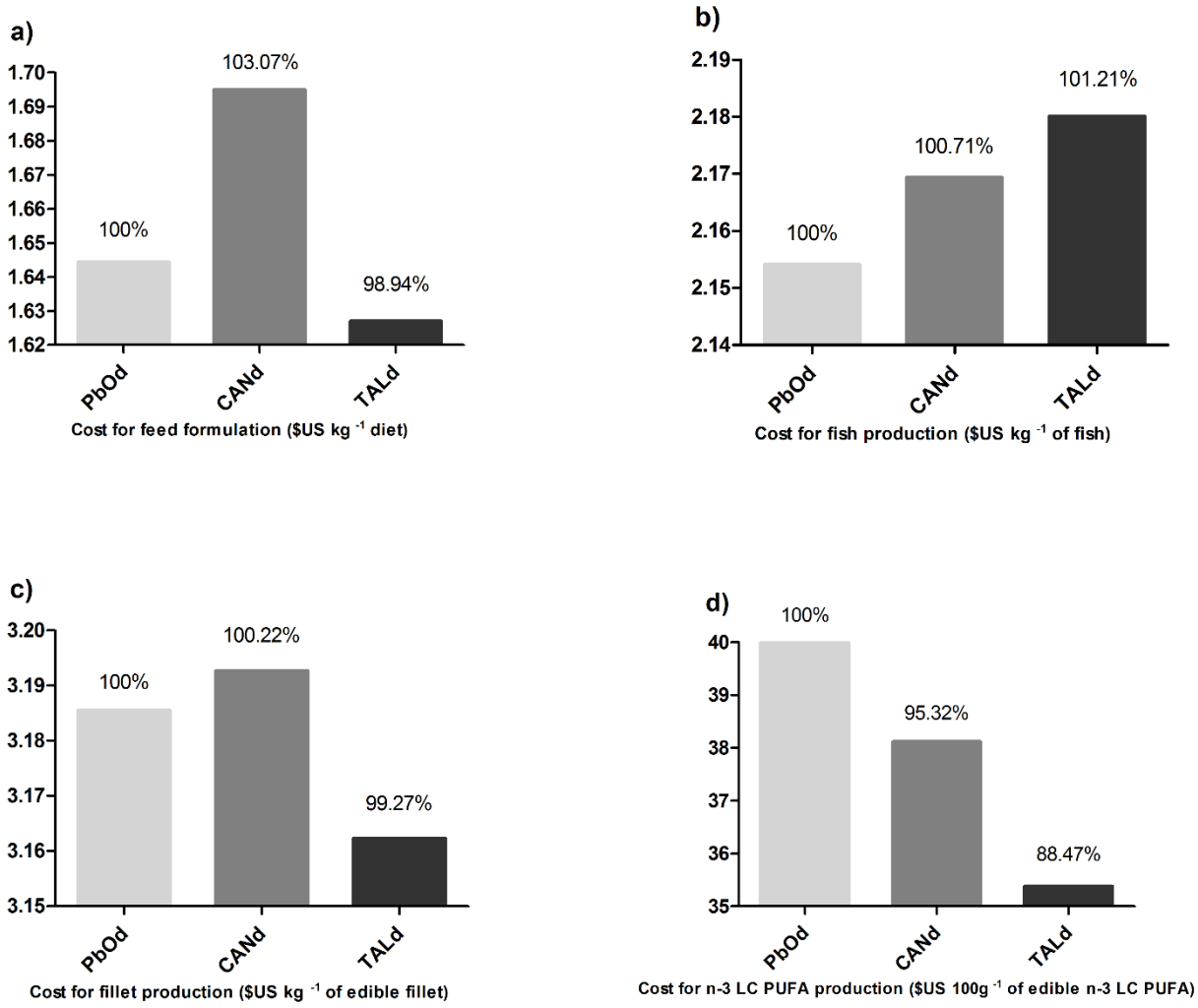
a)
1.70
1.69
1.68
1.67
1.66
1.65
1.64
1.63
1.62
103.07%
100%
98.94%
PbOd
CANd
TALd
Cost for feed formulation ($US kg -1 diet)
b)
2.19
2.18
2.17
2.16
2.15
2.14
101.21%
100.71%
100%
PbOd
CANd
TALd
Cost for fish production ($US kg -1 of fish)
c)
3.20
3.19
3.18
3.17
3.16
3.15
100.22%
100%
99.27%
PbOd
CANd
TALd
Cost for fillet production ($US kg -1 of edible fillet)
d)
40
39
38
37
36
35
100%
95.32%
88.47%
PbOd
CANd
TALd
Cost for n-3 LC PUFA production ($US 100g -1 of edible n-3 LC PUFA)

Figure S2

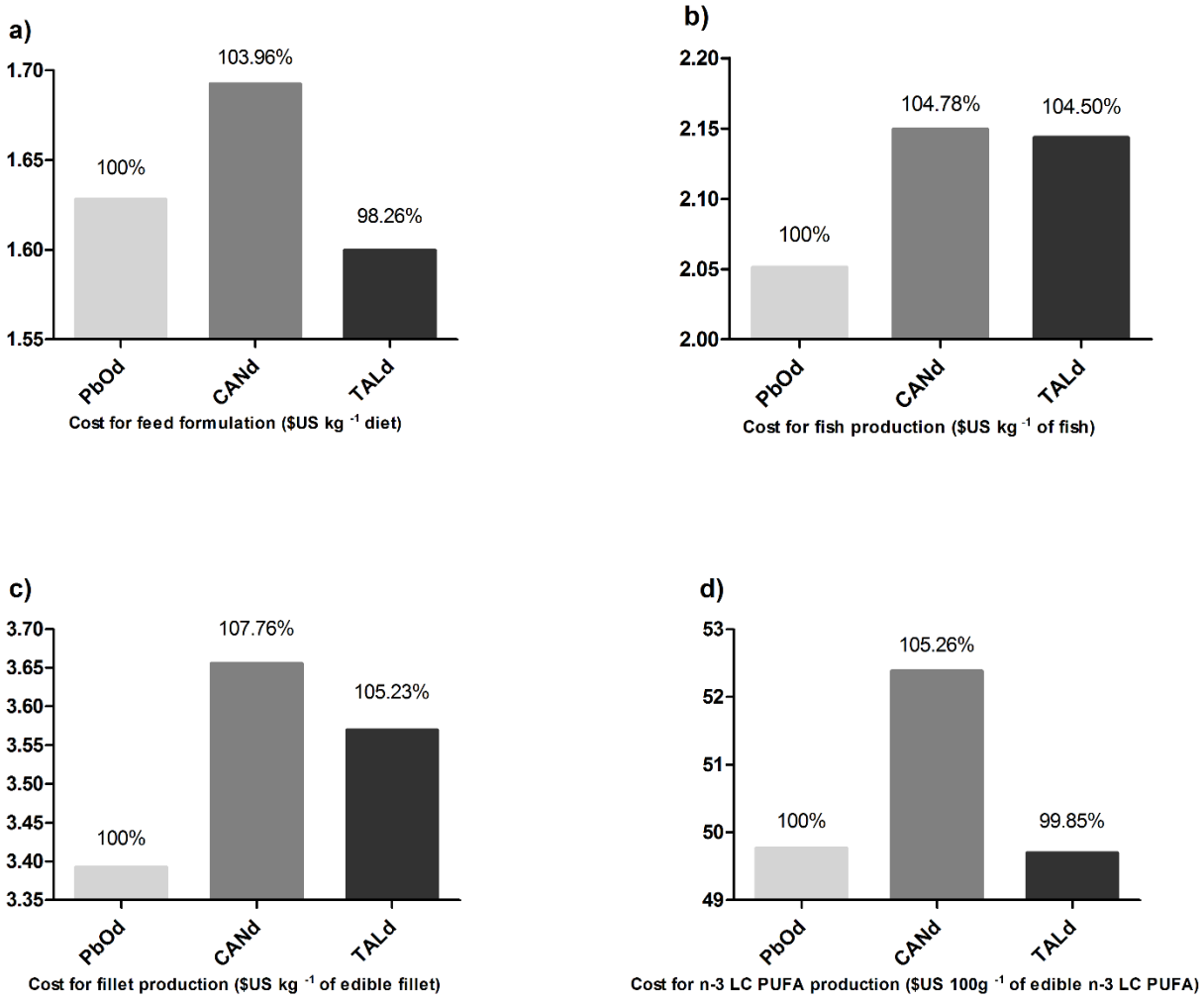
a)
1.70
1.65
1.60
1.55
103.96%
100%
98.26%
PbOd
CANd
TALd
Cost for feed formulation ($US kg -1 diet)
b)
2.20
2.15
2.10
2.05
2.00
104.78%
104.50%
100%
PbOd
CANd
TALd
Cost for fish production ($US kg -1 of fish)
c)
3.70
3.65
3.60
3.55
3.50
3.45
3.40
3.35
107.76%
105.23%
100%
PbOd
CANd
TALd
Cost for fillet production ($US kg -1 of edible fillet)
d)
53
52
51
50
49
105.26%
100%
99.85%
PbOd
CANd
TALd
Cost for n-3 LC PUFA production ($US 100g -1 of edible n-3 LC PUFA)

Figure S3