The impact of dietary protein: lipid ratio on growth performance, fatty acid metabolism, product quality and waste output in Atlantic salmon (Salmo salar)

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Keywords: *Atlantic salmon, protein:lipid ratio, fatty acid utilisation, digestibility, production cost, nitrogen waste*

Accepted refereed manuscript of:

Mock TS, Francis DS, Jago MK, Glencross BD, Smullen RP, Keast RSJ & Turchini GM (2019) The impact of dietary protein: lipid ratio on growth performance, fatty acid metabolism, product quality and waste output in Atlantic salmon (Salmo salar). *Aquaculture*, 501, pp. 191-201.

DOI: https://doi.org/10.1016/j.aquaculture.2018.11.012

Highlights:

“As part of the submission process, authors are required to provide 3 or 4 highlights, each one sentence long. Beyond stating key discoveries, these highlights must explicitly establish why the work is novel and why it has an application to aquaculture. It is not sufficient to state that the species is one that is farmed.”

- Lipid utilisation, including, fillet fatty acid composition and fatty acid metabolism, remained virtually unchanged despite an altered dietary protein : lipid ratio, this information is scarce for market-sized Atlantic salmon.

- A reduction in protein : lipid ratio resulted in lower nitrogenous waste output emanating from undigested protein, a highly relevant finding given the enhanced scrutiny on farms to limit environmental degradation.

- An assessment of the cost of dietary formulation and subsequent fish production clearly showed differences in growth parameters equate to considerable economic differences – an analysis of this kind is seldom provided in published literature.

Abstract

A common strategy for aquafeed manufacturers has been the utilisation of relatively large amounts of terrestrial oil sources to produce diets with a high energy content. The provision of high fat diets promotes the utilisation of energy from lipid, thus increasing the amount of dietary protein used for tissue synthesis. However, in recent years the cost of dietary lipid has risen, at the same time, farming operations are under increasing pressure to limit environmental degradation associated with nitrogenous waste effluent. Currently there is limited information available regarding the environmental and cost effects of an altered dietary protein : lipid ratio in diets for large Atlantic salmon reared in saltwater, presenting a potential impediment to nutritional based solutions. Accordingly the present study compared two iso-energetic diets with varied protein : lipid ratios by an assessment of growth, fatty acid utilisation, nutritional quality, nitrogenous waste output and bio-economic considerations. The trial, conducted over the final 150 days of an on-farm grow-out period found minimal differences in growth, fatty acid utilisation and fillet quality. A decreased dietary protein : lipid ratio showed a more efficient protein utilisation both in terms of digestibility and assimilation into fish and, therefore, nitrogenous waste output was reduced. However, due to small differences in feed utilisation, the cost of fish production was higher.

Keywords: protein, lipid, environment, Atlantic salmon, aquafeed

Statement of relevance

Atlantic salmon aquaculture is subject to growing scrutiny to limit the potential for environmental degradation whilst efficiently utilising increasingly expensive dietary lipids. To assess the efficacy of a decreased dietary protein : lipid ratio in aquafeed for market-sized Atlantic salmon, the current trial evaluated commercially relevant production parameters including growth, fillet quality (including, fatty acid composition and taste attributes), nitrogenous waste output as well as a preliminary calculation of the cost of fish production. Despite minimal effects on growth and fillet quality, a

reduction in the dietary protein : lipid ratio was shown to decrease the amount of undigested nitrogen and increase the amount of nitrogen assimilated into fish. However, an increased cost of fish production was incurred. This study provides relevant information for future nutritional-based solutions which aim to enhance the environmental and economic sustainability of the Atlantic salmon aquaculture industry.

1. Introduction

The central objective for aquafeed manufacturers is to achieve least-cost formulations able to maintain optimal fish performance. This can be realised via optimising the utilisation efficiency of dietary nutrients whilst simultaneously reducing the inclusion of increasingly expensive marine-derived ingredients. The resultant dietary formulations inevitably involve a series of 'trade-offs' between the cost of added macronutrients, adequate provision of nutrients for both anabolism (growth and tissue synthesis) and catabolism (metabolic energy), nutritional and organoleptic quality of the final product and limiting the negative impacts on the surrounding aquatic environment (Bendiksen et al. 2011; Bureau 2004; Tocher 2015; Turchini et al. 2010).

A common strategy for aquafeed manufacturers has been the utilisation of relatively large concentrations of terrestrial oil sources to produce diets with a high energy content. Traditionally referred to as 'protein sparing' (Einen & Roem 1997; Francis & Turchini 2017; Karalazos et al. 2011b; Kaushik & de Oliva Teles 1985), the provision of high fat diets promotes the utilisation of energy from lipid, thus increasing the amount of dietary protein available for tissue synthesis. The protein sparing concept has been particularly popular in salmonid aquaculture, given the innate ability of this species to efficiently use large amounts of dietary lipids as an energy source. Thus, coupled with the historically lower price of dietary lipid in comparison to protein sources, high energy formulations are widely favoured in Atlantic salmon aquafeed (Bendiksen et al. 2011; Einen & Roem 1997; Pratoomyot et al. 2010; Turchini et al. 2010). However, various lipid sources are now as, if not more, expensive than protein sources, in particular those rich in omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA) such as fish oil. This is a function of stagnant supply of marine derived oils and increasing demand from aquaculture, agriculture and nutraceutical sectors (Francis & Turchini 2017; Tacon & Metian 2008; Turchini et al. 2010; Turchini 2013). At the same time, the well-documented health benefits of n-3 LC PUFA consumption have influenced consumer expectation of farmed fish to provide

a dependable source of edible n-3 LC PUFA (Christenson et al. 2017; Tur et al. 2012; Turchini et al. 2011). The Atlantic salmon aquaculture industry sits at the centre of this paradox given their reputation as reliable source of edible n-3 LC PUFA whilst itself consuming a relatively high proportion of globally available fish oil. Given the increased value placed on dietary sources of n-3 LC PUFA, attempts have been made to retro-engineer the protein sparing concept, in order to conserve n-3 LC PUFA from catabolism in Atlantic salmon via the provision of high protein diets (Francis & Turchini 2017). It is known that dietary n-3 LC PUFA, including 20:5n-3 and 22:6n-3 are readily β-oxidised for metabolic energy when in excess of physiological requirements. Hence, it has been hypothesised that in contrast to the protein sparing concept, an increase in the protein : lipid ratio would increase the utilisation of dietary protein for catabolic processes and thus favour the retention of dietary fatty acids, in particular, n-3 LC PUFA. However, to date results have been inconclusive and further investigation has been suggested (Francis & Turchini 2017). Despite this, an increase in the dietary protein : lipid ratio has been shown to improve the food conversion ratio and thermal growth coefficient in farm reared Atlantic salmon (Weihe et al. 2018).

Importantly, however, any variation of the dietary protein : lipid ratio in aquafeed would not only affect the growth and nutritional quality of Atlantic salmon, but a sub-optimal digestible protein : digestible lipid ratio would decrease nitrogen retention efficiency. This would stimulate the catabolism of protein for energy, resulting in an increase of dissolved nitrogenous waste, predominantly, ammonia (Crab et al. 2007; Hardy & Gatlin 2002; Karalazos et al. 2011a; Kaushik & Cowey 1991). Poor dietary protein retention causes an increased output of undigested nitrogen entering the surrounding aquatic environment, eliciting potentially deleterious effects on water quality, including eutrophication, particularly in close proximity to the farming operation (Amirkolaie 2011; Crab et al. 2007; Rabalais 2002; Wu 1995). Meanwhile, aquaculture operations are subject to enhanced scrutiny to limit nitrogenous waste effluent, and as a result, effective nutritional strategies are being sought

(*Australian Government* 2015; Cho & Bureau 2001; Crab et al. 2007; Hardy & Gatlin 2002). Various approaches have been implemented by aquaculture operations to address this, including; reducing uneaten feed and tailoring the digestible protein : digestible lipid ratio to limit the amount of protein which is undigested or catabolised for metabolic energy (Bureau 2004; Cho et al. 1994; Cho & Bureau 1997; Crab et al. 2007). Specifically, a decrease in the dietary protein : lipid ratio has been shown to significantly reduce nitrogenous waste output in intensive aquaculture systems due to an increase in nitrogen retention efficiency (Crab et al. 2007; Einen & Roem 1997; Hardy & Gatlin 2002; Kaushik & Médale 1994; Kaushik 1998).

Despite the clear importance of this topic, published information quantifying the effect of an altered dietary protein : lipid ratio in grow-out diets for Atlantic salmon on fish nutritional quality, fatty acid metabolism and protein utilisation, remains sparse, especially in relation to the farming conditions of the southern hemisphere. Furthermore, the extent of possible n-3 LC PUFA sparing remains unclear. Thus, the adoption of modified dietary formulations which limit the negative environmental impact of Atlantic salmon aquaculture may be impeded by a lack of available research data. Therefore, this study aimed to compare two commercial-like, iso-energetic diets, both containing the same raw materials and dietary oil blend (80% poultry by-product oil and 20% fish oil), but with varied protein : lipid ratios; 45:33 and 35:36, respectively. These diets were tested on-farm, in a real-word/commercial environment over a five month grow-out period, involving an assessment of industry relevant production performance indicators, such as: nutrient digestibility, fillet fatty composition and utilisation, taste evaluation and an evaluation of undigested protein output. Furthermore, the potential disparity between feed related production costs between the two diets was assessed through a preliminary bio-economic analysis.

2. Materials and methods

2.1. Location, animals, experimental design and sampling.

The current trial was conducted from May 24 to October 20, 2015 (150 days) in a commercial salmon farm 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 for the trial, an initial sample of 6 fish was randomly selected from the trial cohort, euthanized in excess anaesthetic (AQUI-S, 0.5 ml L^{-1}) and stored at −20 °C until subsequent analysis. Five hundred and forty Atlantic salmon (average initial weight ~2250g) were assigned one of six floating sea pens (5m x 5m x 5m, 270 fish per pen) (n = 2, N = 6). Feeding of the two 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, mortalities and environmental parameters were monitored throughout the trial and remained within acceptable limits, including water temperature (mean ± SD: 11.21 ± 0.86 ^{o}C) and dissolved oxygen (mean ± SD: 7.85 ± 0.43 mg L^{-1}). In the final week of feeding, 10 fish were selected for faecal collection by hand stripping and samples were used for an estimation of digestibility. At the completion of the feeding trial, all fish were anaesthetised and weighed and 21 fish from each treatment (seven fish per pen) were randomly selected and separated into 3 groups: the first group (nine fish) were used for the chemical analysis of whole body, the second group (six fish) were used for the chemical analysis of fillet and the third group (six fish) were used for sensory analysis by means of a panel taste test. These separated fish were immediately placed in an ice slurry, following this, the fish used for chemical analysis were frozen to −20 °C and stored until subsequent analysis. Fish allocated to panel taste testing were taken from the slurry to be processed by Huon Aquaculture Company, Tasmania (as described below).

2.2. Diets

Diets were manufactured by a commercial feed producer using closed formula Atlantic salmon aquafeed formulations (Ridley Aquafeed, Australia). Two separate batches of 9 mm pellets differing in their protein : lipid ratio were extruded and vacuum coated with lipid, using identical raw materials varying only in their respective inclusion levels. The dietary lipid source used was identical for the two diets, consisting of a blend of 20% fish oil and 80% poultry by-product oil. The 40:33 treatment was formulated with 40% protein and 33% lipid, while the 35:36 treatment was formulated with 35% protein and 36% lipid. Both diets were formulated to be iso-energetic.

2.3. Growth performance, chemical analysis and fatty acid analysis

Standard formulae were used to assess growth, feed utilisation and biometric data, all reported previously in detail (Francis et al. 2014). These included initial and final average weight, total feed consumption, total and % gain in weight, specific growth rate (SGR), feed conversion ratio (FCR), feed ration % (relative to body mass), dress-out percentage (DP %), fillet yield percentage (FY %), hepatosomatic index (HSI %), viscera-somatic index (VSI %), condition factor (K), net protein utilisation (NPU %), protein growth ratio (PGR) and fat deposition rate (FDR). The chemical composition of the experimental diets, faeces and fish samples were determined via proximate composition analysis according to standard methods (Norambuena et al. 2013). 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) technique of Folch et. al. (1957), additionally, dichloromethane was used to replace chloroform for safety reasons. 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 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 Food Frequency Questionnaire (FFQ) developed by Cancer Council Victoria (Hodge et al. 2000), including a specific salmon questionnaire which determined 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 their packages 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 then given the same samples again, but were 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. Further, participants were given the opportunity to comment on each sample if they chose, or if there were additional factors that had influenced their liking.

2.6. Bio-economic assessment

Differences in production costs due to the varied protein : lipid ratio present in Atlantic salmon diets in this study were estimated following a series of bioeconomic calculations, as previously presented in Turchini et al. (2013b). 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 and PbO was obtained from a commodities website and a commercial feed production company (Ridley Aquafeed Ltd). The prices used for the calculations were as follows: fish oil: $3200; PbO: $1060 (all prices expressed as $US ton^{-1}). With this information, estimates of the feed formulation cost were possible, expressed as $US kg^{-1} for raw materials only. Subsequently, zootechnical (FCR) and biometrical parameters (FY %) 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 and (iii) 1kg of edible fillet. Additionally, these costs were also expressed as percentage difference between the two experimental diets. The calculations were 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 and all costs associated with possible differences in grow-out time. Accordingly, this analysis should be considered purely indicative, and as such, statistical analysis has not been implemented on the resultant data.

2.7. Protein utilisation, assimilation and nitrogenous waste output

The amount of feed and protein required to produce one ton of fish, as well as the amount of undigested nitrogenous waste subsequently produced was calculated for the two dietary treatments. Calculations were based on parameters already described, including; FCR, dietary protein content and ADC % of protein in the diet. Additionally, undigested protein was converted to undigested nitrogen by a conversion factor of 6.25. Hence, it was possible to calculate the amount of: i) feed required ii) protein required, iii) digested protein and iv) undigested nitrogen for each of the two dietary treatments (in terms of kg ton^{-1} of fish produced). It should be noted that the present study makes no

attempt to quantify the total amount of nitrogenous waste produced by the fish which includes nitrogen excretion from the gills in the form of ammonia and additional nitrogen loss from the surface of the fish (eg scale loss).

Nitrogen assimilation, in terms of % assimilation, g $fish^{-1}$ per fish and kg ton^{-1} of fish was calculated using a mass balance approach, whereby, initial and final weights of fish, feed intake, nitrogen content of diets and initial and final nitrogen content of whole-body fish were used to calculate the percentage of nitrogen assimilated into fish fed the dietary treatments. From this it was possible to calculate the amount of nitrogen assimilated in terms of both g $fish^{-1}$ and kg ton^{-1} of fish.

2.8. Statistical analysis

All data, were reported as mean ± standard error; (n = 2, N = 6). After confirmation of normality and homogeneity of variance, data was subjected to an independent samples T-test. Significance was accepted at $P < 0.05$, and P-values were reported as; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. All statistical analyses were performed using IBM SPSS Statistics v24.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Treatment diets

Total energy content was similar between the two treatment diets (~25.5 KJ g^{-1}). (Table 1). Major fatty acid classes including; SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA were comparable between diets. n-3 LC PUFA levels (mg g^{-1} diet) were similar (10.9 and 11.1 mg g^{-1} diet for 40:33 and 35:36, respectively). Additionally, the n-6:n-3 ratio was 2:1 in both diets owing to high dietary 18:2n-6 and relatively low 18:3n-3.

3.2. Growth, feed utilisation parameter and biometrical data

Each diet was readily accepted by fish and mortality rates were low and unrelated to treatment. Fish in both treatments more than doubled in size (2250g - 5100g), gaining over 2800g (Table 2). Overall, growth, feed utilisation and biometrical parameters were similar and there were no statistically significant differences between treatments. However, FCR was slightly lower in the 40:33 treatment (1.26) compared to the 35:36 treatment (1.38). Additionally, net protein utilisation (NPU %) was slightly higher in 35:36 compared to 40:33 (32.42 and 37.12 %, respectively), although differences were not significant ($P > 0.05$).

3.3. Apparent nutrient and fatty acid digestibility

Nutrient and fatty acid digestibility values (ADC %) were high across treatments (Table 3), and although not significantly different ($P > 0.05$), apparent protein digestibility was higher in 35:36 (80.1 and 75.5% for 35:36 and 40:33, respectively). Individual fatty acid apparent digestibility results were similar between treatments ($P > 0.05$), however, there was a general trend of lower digestibility of SFA compared to unsaturated fatty acids.

3.4. Tissue proximate and fatty acid composition

Fillet proximate composition (g $100g^{-1}$ fillet) (Table 4) was similar between treatments with no significant differences were recorded ($P > 0.05$). Fillet fatty acid composition, both in terms of µmol g^{-1} tissue (Table 4) and g $100g^{-1}$ of edible fillet (Table 5) reflected the make-up of dietary lipid profile ($P > 0.05$), including, n-3 LC PUFA levels, which were identical between treatments (21.2 µmol g^{-1} fillet tissue). Fillet n-6:n-3 ratios (in terms of g $100g^{-1}$ of edible fillet) were lower than the dietary ratios, ranging from 1.7 to 1.8 in 40:33 and 35:36, respectively.

3.6. Apparent in vivo fatty acid metabolism

Overall, total apparent *in vivo* fatty acid β-oxidation (expressed as nmol of fatty acid β-oxidation per gram of fish per day; nmol g^{-1} day^{-1}) (Table 6) was similar between treatments ($P > 0.05$). There were few differences between treatments in terms of individual fatty acid β-oxidation, with both treatments heavily utilising 18:1n-9 for catabolism. Higher β-oxidation of 22:6n-3 was recorded in the 40:33 treatment, (in terms of both nmol g^{-1} day^{-1} and % of total intake) however, differences were not significant ($P > 0.05$) (35.9 and 29.0 % of intake β-oxidised in 40:33 and 35:36, respectively). Apparent *in vivo* enzymatic activity, desaturation, elongation or chain shortening, (expressed as nmol of fatty acid per gram of fish per day; nmol g^{-1} day^{-1}) (Table 7) was low and similar between treatments, with the exception of the Δ-6 desaturation of 18:3n-3, which was higher in 35:36 compared to 40:33 (20.0 and 9.6 nmol g^{-1} day^{-1}, respectively) ($P < 0.05$). Despite some recorded Δ-6 desaturation of 24:5n-3, no *de novo* production of 22:6n-3 was recorded in either of the treatments.

3.6. Consumer preferences

There were no differences in liking score between the dietary treatments across all three preparation methods; cold-smoked, hot-smoked and raw (Table 8) ($P > 0.05$). However, the 35:36 treatment scored slightly higher than 40:33 for the cold smoked fillet (18.7 ± 0.5 and 14.6 ± 2.2, respectively), conversely, the 40:33 treatment scored slightly higher for the hot smoked fillet than 35:36 (22.6 ± 1.3 and 18.5 ± 5.4, respectively). No differences were recorded between treatments for any influential attributes (fishiness, saltiness and oiliness) ($P > 0.05$). Moreover, both fishiness and oiliness both recorded scores close to zero on the 'Just About Right' scale. For both dietary treatments, the raw fish lacked saltiness as indicated by scores of -20.3 ± 3.7 and -18.3 ± 1.3 for 40:33 and 35:36, respectively.

3.7. Bioeconomic assessment of fish production

Results of estimated feed-related production costs for a) feed, b) whole fish and c) fillet are presented in Figure 1 and expressed as i) $US ton^{-1} and ii) % difference in cost between treatments. Costs of feed production for the two experimental treatment diets were similar and differed by < 1 cent kg^{-1} (Figure 1ai). However, cost of fish production showed 40:33 to be more cost effective than 35:36 (2.05 and 2.23 $US kg^{-1} of fish, respectively) (Figure 1bi). Subsequently, 40:33 remained the more cost effective diet in terms of $US kg^{-1} of fillet (Figure 1ci). When expressed as % difference in cost, feed formulation costs differed by < 1 %, whilst the cost of fish and fillet production was 8.9 and 8.6 % cheaper in 40:33, respectively (Figure 1bii and Figure 1cii).

3.8. Feed, protein usage and nitrogenous waste output from feed

The amount of a) feed, b) protein, c) digested protein and c) undigested nitrogen (kg ton^{-1} of fish produced) are presented in Figure 2. The amount of feed required to produce one ton of fish differed slightly between treatments, although not significantly ($P > 0.05$) (Figure 2a). The amount of protein required to produce one ton of fish was similar between treatments (~500 kg ton^{-1} fish) (Figure 2b). Additionally, there was no difference in the amount of digested protein between treatments ($P > 0.05$) (Figure 2c). Nitrogenous waste from undigested protein was significantly different between treatments (20.0 and 15.8 kg ton^{-1} fish) for the 40:33 and 35:36 diets, respectively ($P = 0.009$). Nitrogen assimilation, was higher in the 35:36 treatment (in terms of % assimilated, g fish^{-1} and kg ton^{-1} fish), however, results were not significantly different ($P > 0.05$).

4. Discussion

This study has clearly demonstrated that a variation in the dietary protein : lipid ratio has no significant effect on growth performance, fatty acid metabolism or final product quality. However, a reduction in the dietary protein : lipid ratio reduced the amount of undigested protein resulting in a reduction in

nitrogenous waste. However, the bio-economical analysis revealed that due to small, yet important, differences in growth parameters, an increased cost of fish production was incurred.

Previous research has led to the widespread implementation of nutritional strategies aiming to spare protein for growth (Karalazos et al. 2011a; Weihe et al. 2018), minimise nitrogenous waste (Bendiksen et al. 2011; Bureau 2004) and efficiently incorporate health promoting fatty acids into the fillet tissue (Francis & Turchini 2017). Previous research suggests an increased dietary protein : lipid ratio may affect the n-3 LC PUFA sparing capacity and the growth performance of Atlantic salmon (Francis & Turchini 2017; Weihe et al. 2018). On the other hand, reductions in nitrogenous waste outputs are achievable through a decreased dietary protein : lipid ratio. However, to date there remains a paucity of published information specifically relating to market sized Atlantic salmon reared in seawater. The results discussed herein aim to provide added commercial relevance to existing available information.

The current feeding trial lasted 150 days and fish grew in-line with commercial expectations, where they more than doubled in size to a final weight in excess of 5000 g. Despite a disparity in FCR between the two treatments, no statistical differences in growth or biometrical parameters were observed. Considering the deposition of protein is responsible for the majority of weight gain in fish (Sveier et al. 2000), the numerically higher net protein utilisation % in the 35:36 treatment may have compensated for the slight reduction in food conversion efficiency. Although not pronounced in the present study, previous research has supported an increase in protein utilisation efficiency in Atlantic salmon fed diets with a reduced protein : lipid ratio (Einen & Roem 1997; Francis & Turchini 2017; Hardy & Gatlin 2002; Karalazos et al. 2011a). Ultimately, these findings support previous work that suggest growth is not negatively affected when the protein : lipid ratio is reduced in diets for Atlantic salmon (Azevedo et al. 2004; Bendiksen et al. 2003a; Einen & Roem 1997; Karalazos et al. 2011b; Solberg 2004).

A reduction in the dietary protein : lipid ratio, concomitant with a high dietary lipid concentration (39%) has previously been found to increase lipid content and result in obesity in large Atlantic salmon (Refstie et al. 2001). However, in the present study, fillet proximate composition was highly comparable between treatments, suggesting that, under near optimal growing conditions (Handeland et al. 2008; Kullgren et al. 2013) an alteration in the dietary protein : lipid ratio within the limits tested in the current experiment has little effect on fillet composition. Consistent with extensive research, fatty acid composition of the fillet mirrored dietary fatty acid inclusion (Bell et al. 2001a; Bell et al. 2004; Emery et al. 2014; Emery et al. 2016; Turchini et al. 2009; Turchini et al. 2013a). Accordingly, high levels of MUFA, namely 18:1n-9, were present in the fillet tissue owing to the high dietary inclusion of poultry by-product oil. In addition to high fillet levels, *in vivo* fatty acid β-oxidation results demonstrated that 18:1n-9 was heavily β-oxidised in both treatments, consistent with previous literature demonstrating the suitability of 18:1n-9 as a good source of metabolic energy (Bell et al. 2003; Torstensen et al. 2000; Turchini et al. 2009).

The protein sparing effect has been well described in numerous fish species and supports the use of high energy diets in aquafeed to conserve protein for growth, whilst utilising lipid for metabolic energy (Einen & Roem 1997; Hemre & Sandnes 1999; Karalazos et al. 2011b; Kaushik & de Oliva Teles 1985). However, market volatility and escalating scarcity of dietary lipids rich in n-3 LC PUFA (i.e. fish oil), has led to efforts to conserve valuable n-3 LC PUFA via manipulation of the dietary protein : lipid ratio. Specifically, it was hypothesised that a higher protein : lipid ratio could preserve n-3 LC PUFA from catabolism and resultantly increase retention (Francis & Turchini 2017). Consistent with previous research, the digestibility of polyunsaturated fatty acids, including 20:5n-3 and 22:6n-3 was high in both treatments (Turchini et al. 2009). Additionally, similar and relatively low, concentrations of 22:6n-3 were β-oxidised and almost identical amounts were present in the edible fillet. This suggests, in accordance with prior research in juvenile salmon, that the alteration in dietary protein : lipid ratio in

the current study has little effect on the deposition of nutritionally valuable n-3 LC PUFA, provided dietary supply is surplus to physiological requirement (Francis & Turchini 2017). A greater alteration of the dietary protein : lipid ratio may have enhanced the potential of the n-3 LC PUFA sparing effect and is, therefore, a warranted pathway for future research.

Sub-optimal dietary formulations elicit measurable metabolic effects, potentially resulting in deleterious outcomes for the health and nutritional quality of farmed fish (Sargent et al. 1999). An analysis of *in vivo* fatty acid bioconversion, as recorded by the whole-body fatty acid balance method, was used in the current study to elucidate metabolic responses to diets with altered protein : lipid ratios. Atlantic salmon have a recorded capacity for endogenous n-3 and n-6 PUFA synthesis, furthermore, the actual extent of *de novo* production is heavily modulated by dietary fatty acid provision (Bell et al. 2001b; Giri et al. 2016; Martinez-Rubio et al. 2013; Tocher 2003; Turchini & Francis 2009). With respect to the present study, Δ-6 desaturation of 18:3n-3 and 18:2n-6 was recorded in both treatments as was the endogenous production of 20:3n-6 and 20:4n-3. Despite a higher dietary provision of 18:2n-6 relative to 18:3n-3, greater levels of bioconversion were recorded for n-3 PUFA in comparison to n-6 PUFA, supporting previous research showing that Δ-6 desaturation of 18:3n-3 is not limited by presence of 18:2n-6 (Emery et al. 2013; Vagner & Santigosa 2011). Despite the observed n-3 and n-6 PUFA synthesis, no endogenous production of either 20:4n-6 or 20:5n-3 was recorded. It appears, therefore, that Δ-5 desaturase activity may have been supressed by a negative feedback mechanism owing to the dietary provision of 22:6n-3, as previously shown in Atlantic salmon (Jordal et al. 2005; Tocher et al. 2003; Zheng et al. 2005). Additionally, evidence suggests that the synthesis of 20:4n-6 is correlated with immune responses at sub-optimal water temperatures (Norambuena et al. 2015). Therefore, the near optimal water temperature experienced during the current trial (Handeland et al. 2008; Stehfest et al. 2017) were not expected to elicit endogenous 20:4n-6 synthesis in response to temperature stress. Ultimately,

the dietary provision of fish oil in both treatments appears to have been sufficient to satiate physiological requirements under the current experimental conditions. Furthermore, the differences in *in vivo* bioconversion resulting from an alteration to the dietary protein : lipid ratio had little effect on the final fatty acid composition of the fillet.

As well as fillet nutritional quality, taste is a major determinant of seafood consumption (Christenson et al. 2017). Previous research suggests taste and sensorial attributes of Atlantic salmon products are influenced by the dietary lipid level and fatty acid composition of aquafeed (Waagbø et al. 1993). The present study, therefore, investigated whether an alteration to the dietary protein : lipid ratio incurred any effect on overall liking or sensorial attributes when the fillet was prepared as three commercially available products, namely, hot smoked, cold smoked and raw. Dietary lipid level is a significant predictor of taste preference in both smoked and raw salmonid products (Einen & Skrede 1998; Johansson et al. 2000). Despite a higher dietary lipid level in the 35:36 treatment (albeit a marginal increase), there were no significant differences in either preference (like or dislike) or sensorial attributes, including: fishiness, saltiness or oiliness. Hence, it appears that the overall liking and sensory attributes of salmon products are unaffected by an alteration of the protein : lipid ratio in diets for Atlantic salmon.

From an economic perspective, market forces inevitably influence dietary formulations in aquafeed, however, analysis of costs are seldom considered in published literature (Turchini et al. 2013b). The present study includes a preliminary assessment of feed and fish production costs associated with the ingredients used in the treatment diets. As stated, this analysis should be considered indicative only as it omits associated costs such as transport and handling of raw dietary ingredients. As expected, the cost of ingredients used in the treatment diets were very similar and could be considered negligible for the present study. However, due to the lower FCR in the 40:33 treatment, the cost of

fish production was appreciably lower. Due to similar FY % in both treatments, differences in cost of fillet production still reflect the aforementioned differences in FCR. The present bioeconomic analysis highlights the potential for considerable economic repercussions, despite no statistical difference in FCR between the treatments. Thorarensen (2015) describes the difficultly a large number of fish growth studies have in detecting a statistical significance in growth parameters due to experimental design constraints. In light of this, enhanced scrutiny of even small differences in growth parameters is suggested to better understand practically significant effect sizes which may relate to large differences in on-farm production costs for commonly farmed aquaculture species. Regardless of statistical interpretation, aquaculture operations are heavily reliant on the cost-effectiveness of aquafeed (Liu et al. 2016; Turchini et al. 2013b). Therefore, results from the present study indicate that, in a commercial sense, alterations to dietary formulations such as a reduction in the dietary protein : lipid ratio should unequivocally apply cost-benefit analyses based on pre-production research.

Environmental degradation, resultant from nitrogenous waste output from aquaculture operations, has stimulated the development of feed related mitigation measures (Bureau 2004; Cho & Bureau 1997; Crab et al. 2007; Hardy & Gatlin 2002; Kaushik & Cowey 1991). It is well established, that when protein, or more specifically, amino acids, are catabolised for metabolic energy rather than utilised for tissue synthesis, nitrogen is excreted via the gills, primarily, in the form of ammonia (Crab et al. 2007; Hardy & Gatlin 2002; Kaushik & Cowey 1991). Decreasing the digestible protein : digestible energy ratio of the aquafeed allows for the utilisation of dietary lipids for the majority of metabolic energy requirements, and as a result, sparing protein for growth (Francis & Turchini 2017; Grisdale-Helland et al. 2008; Karalazos et al. 2011a; Kaushik & Cowey 1991). Similarly, high protein digestibility is crucial in limiting excess nitrogen effluent. Nitrogen is the primary factor responsible for the eutrophication of temperate coastal marine environments (Howarth & Marino 2006), leading to, among other

outcomes, toxic phytoplankton blooms, reduced water clarity, elevated pH and the depletion of dissolved oxygen in the water column, as reviewed by Smith (1999). Both protein and lipid digestibility are purported to be largely unaffected by variations in the dietary protein : lipid ratio and remain highly digestible, providing they are kept within practical limits (Bendiksen et al. 2003b; Einen & Roem 1997; Francis & Turchini 2017; Solberg 2004). However, in the present study a 5 % higher protein digestibility was recorded in the 35:36 treatment. This resulted in significant differences in terms of undigested nitrogenous waste effluent. Despite an improved FCR in the 40:33 treatment, the reduction in dietary protein digestibility resulted in similar total amounts of digested protein between treatments (in terms of kg ton^{-1} fish produced). Thus, given the higher level of dietary protein in the 40:33 diet, the amount of undigested protein, was significantly higher (in terms of kg ton^{-1} fish produced). However, it should be noted that the results presented in this study focus on the amount of nitrogenous waste entering the aquatic environment from undigested protein only. In fact, a precise, reliable method to quantify nitrogenous waste as a result of catabolised protein is not available (Bureau 2004; Houlihan et al. 1993). In consideration of this, a parallel approach was implemented to assess the differences in nitrogen assimilation between treatments through simple mass balance calculations. Although not significantly different, results were complimentary to the analysis of undigested protein, in that, the 35:36 treatment recorded higher nitrogen assimilation compared to the 40:33 treatment. To the best of the authors' knowledge, published literature examining the effect of varying the protein : lipid ratio of iso-energetic diets fed to seawater reared Atlantic salmon on the digestibility of protein and subsequent nitrogenous waste, has been confined to juvenile fish (Dessen et al. 2017). Given the reduced digestible protein : digestible energy ratio in the 35:36 diet, the aforementioned results were expected. Importantly, however, there was minimal effect on lipid utilisation between the treatments, as evidenced by comparable: i) fillet fatty acid composition, ii) fatty acid β-oxidation iii) taste quality of the final fillet product and, iv) growth and biometrical parameters.

5. Conclusion

The present study suggests that a reduction in the protein : lipid ratio in aquafeed formulations for market-sized Atlantic salmon elicits minimal effects on lipid and fatty acid utilisation and ultimately found no reduction in fillet nutritional quality including levels of n-3 LC PUFA. Additionally, taste quality was not compromised. Importantly, a significant reduction in undigested nitrogenous waste was observed when the dietary protein : lipid ratio was decreased. However, whereas overall performance was unaffected, there was a slight increase in FCR, which was reflected in the bioeconomic analysis by an increased cost of fish and fillet production. Therefore, the current study shows that the cost of fish production and the ability to decrease the potential for environmental degradation are in antagonism.

6.0 References

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Table 1

Proximate fatty acid composition (mg g^{-1} diet) of experimental diets

	Diets[a]	
	40:33	35:36
Proximate composition (mg g^{-1})		
Moisture	28.9	43.6
Protein	403.9	357.0
Lipid	330.5	367.7
Protein : lipid ratio	1.2	1.0
NFE	164.6	162.7
Ash	72.2	69.0
Energy (KJ g^{-1})	25.4	25.7
Total FA (mg g^{-1} diet)[b]	253.1	267.7
SFA[c]	78.7	81.8
14:0	4.1	4.3
16:0	54.1	56.1
18:0	18.7	19.6
Other SFA[d]	1.8	1.9
MUFA[e]	123.8	132.1
16:1n-7	12.2	12.5
18:1n-9	95.9	103.4
18:1n-7	6.2	6.5
20:1n-9	4.2	4.4
Other MUFA[f]	5.3	5.3
Total trans FA[g]	1.2	1.2
PUFA[h]	49.2	52.4
18:2n-6	29.9	32.5
20:2n-6	0.4	0.5
20:4n-6	0.9	1.0
Other n-6 PUFA[i]	1.8	1.4
n-6 PUFA[j]	33.0	35.4
n-6 LC PUFA[n]	2.9	2.6
18:3n-3	4.8	5.6
18:4n-3	0.2	0.2
20:4n-3	0.0	0.0
20:5n-3	3.9	3.8
22:5n-3	0.9	0.9
22:6n-3	5.4	5.6
Other n-3 PUFA[l]	1.0	0.7
n-3 PUFA[k]	15.9	16.7
n-3 LC PUFA[o]	10.9	11.1
LC PUFA[m]	13.8	13.7
n-6:n-3 ratio[p]	2.1	2.1

[a] Diets: 40:33 = poultry by-product oil and fish oil diet consisting of 40% protein and 35% lipid, added oil consists of 20% fish oil, 80% poultry by-product oil; 35:36 = poultry by-product oil and fish oil diet consisting of 35% protein and 35% lipid, added oil consists of 20% fish oil, 80% poultry by-product 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 & 24:0.
[e] MUFA = monounsaturated fatty acids.
[f] Other MUFA = sum of 14:1n-5, 15:1n-5, 17:1n-7, 20:1n-13, 20:1n-11, 22:1n-11, 22:1n-9 & 24:1n-9.
[g] Total trans FA = sum of 18:1n-9t, 18:1n-7t & 18:2n-6t.
[h] PUFA = polyunsaturated fatty acids.
[i] Other n-6 PUFA = sum of 18:3n-6, 20:2n-6, 20:3n-6, 22:2n-6, 22:4n-6 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, 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 two experimental diets for 150 days.

	Diets[a]			
	40:33	35:36	P-value	
Initial wt (g)	2219 ± 7	2290 ± 21		
Final wt (g)	5053 ± 33	5096 ± 83	ns	0.995
Gain (g)	2834 ± 28	2806 ± 63	ns	0.913
Gain (%)	127.7 ± 1.1	122.5 ± 1.7	ns	0.991
Feed ration[b]	0.65 ± 0.02	0.69 ± 0.02	ns	0.244
FCR[c]	1.26 ± 0.04	1.38 ± 0.05	ns	0.130
SGR[d]	0.53 ± 0.00	0.54 ± 0.01	ns	0.187
K[e]	1.82 ± 0.10	1.79 ± 0.05	ns	0.799
DP (%)[f]	90.41 ± 0.52	90.84 ± 0.9	ns	0.700
FY (%)[g]	60.47 ± 0.73	60.66 ± 0.57	ns	0.849
HSI (%)[h]	1.02 ± 0.01	1.09 ± 0.10	ns	0.538
VSI (%)[i]	9.26 ± 0.45	9.77 ± 0.21	ns	0.360
NPU (%)[j]	32.42 ± 1.05	37.12 ± 2.07	ns	0.113
PGR[k]	0.48 ± 0.01	0.51 ± 0.02	ns	0.259
FDR[l]	0.70 ± 0.04	0.72 ± 0.02	ns	0.730

Data are expressed as mean ± S.E.M., *n* = 2, *N* = 6. P < 0.05; an independent T-test: ns = not significant (P > 0.05); * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

[b] Feed ration (% BW day^{-1})

[c] FCR = food conversion ratio.

[d] SGR = specific growth rate.

[e] K = condition factor

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

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

[h] HSI (%) = hepatosomatic index.

[i] VSI (%) = viscerosomatic index.

[j] NPU (%) = net protein utilisation

[k] PGR = protein growth rate

[l] FDR = fat deposition rate

Table 3

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

	Diets[a]			
	40:33	35:36	P-value	
Nutrients[b]				
DM[b]	66.3 ± 3.9	67.1 ± 2.1	ns	0.860
Protein	75.5 ± 3.1	80.1 ± 1.8	ns	0.260
Lipid	88.8 ± 3.3	90.7 ± 1.2	ns	0.604
Total fatty acids[d]	89.4 ± 4.2	90.2 ± 1.6	ns	0.840
NFE[b]	78.3 ± 2.3	83.2 ± 1.2	ns	0.134
Energy[c]	76.8 ± 3.8	79.5 ± 1.9	ns	0.556
Fatty acids				
12:0	91.8 ± 3.1	92.5 ± 1.1	ns	0.791
14:0	87.6 ± 4.1	89.2 ± 1.6	ns	0.665
16:0	79.8 ± 5.0	81.1 ± 1.7	ns	0.783
18:0	65.8 ± 8.0	64.8 ± 1.8	ns	0.888
16:1n-7	96.4 ± 2.9	97.1 ± 1.2	ns	0.795
18:1n-9	94.7 ± 3.9	95.5 ± 1.7	ns	0.818
18:1n-7	93.9 ± 3.8	94.9 ± 1.7	ns	0.801
20:1n-9	92.9 ± 4.4	93.7 ± 1.9	ns	0.846
18:2n-6	96.1 ± 3.2	96.5 ± 1.6	ns	0.891
20:2n-6	93.0 ± 3.1	92.5 ± 2.4	ns	0.897
20:4n-6	95.7 ± 2.6	96.7 ± 1.3	ns	0.690
18:3n-3	96.7 ± 2.8	97.3 ± 1.3	ns	0.836
20:5n-3	97.6 ± 2.0	97.6 ± 1.2	ns	0.986
22:5n-3	96.1 ± 3.1	96.3 ± 1.6	ns	0.951
22:6n-3	96.3 ± 2.6	96.1 ± 1.5	ns	0.927

Data are expressed as mean ± S.E.M., n = 2, N = 6. P < 0.05; an independent T-test: ns = not significant (P > 0.05); * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

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

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

[d] Total FA = total fatty acids.

[e] Fatty acid not detected in feed.

[f] 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]			
	40:33	35:36	P-value	
Proximate composition (mg g^{-1} of tissue)				
Moisture	644.5 ± 6.2	635.8 ± 9.3	ns	0.480
Protein	219.7 ± 5.2	219.8 ± 3.3	ns	0.991
Lipid	132.2 ± 5.9	134.1 ± 6.9	ns	0.845
Ash	9.9 ± 0.2	9.7 ± 0.2	ns	0.614
Fatty acids (μmol g^{-1} of tissue)				
Total FA[b]	394.5 ± 18.4	396.0 ± 33.1	ns	0.971
SFA[c]	102.2 ± 5.8	101.1 ± 9.2	ns	0.926
14:0	8.2 ± 0.4	7.8 ± 0.7	ns	0.639
16:0	71.5 ± 4.4	71.2 ± 6.6	ns	0.974
18:0	19.0 ± 0.9	18.7 ± 1.6	ns	0.904
Other SFA[d]	3.5 ± 0.1	3.4 ± 0.3	ns	0.654
MUFA	204.3 ± 9.2	206 ± 16.9	ns	0.936
16:1n-7	19.1 ± 0.9	19.4 ± 1.7	ns	0.901
18:1n-9	158.6 ± 7.6	160.3 ± 12.9	ns	0.913
18:1n-7	11.7 ± 0.7	11.8 ± 1.0	ns	0.930
20:1n-9	8.9 ± 0.0	8.5 ± 0.8	ns	0.680
Other MUFA[e]	6.0 ± 0.2	5.9 ± 0.6	ns	0.838
Total trans FA	1.7 ± 0.1	1.7 ± 0.2	ns	0.959
PUFA	86.1 ± 3.4	87 ± 6.8	ns	0.910
18:2n-6	46.7 ± 2.5	48.5 ± 3.9	ns	0.713
20:2n-6	3.3 ± 0.2	3.3 ± 0.3	ns	0.932
20:4n-6	1.6 ± 0.1	1.6 ± 0.1	ns	0.976
Other n-6 PUFA[f]	4.1 ± 0.2	4.1 ± 0.4	ns	0.978
n-6 PUFA	55.6 ± 2.8	57.5 ± 4.7	ns	0.756
n-6 LC PUFA	7.8 ± 0.3	7.8 ± 0.7	ns	0.928
18:3n-3	7.8 ± 0.7	7.0 ± 0.5	ns	0.405
18:4n-3	0.3 ± 0.0	0.3 ± 0.0	ns	0.209
20:4n-3	1.5 ± 0.2	1.7 ± 0.2	ns	0.522
20:5n-3	4.5 ± 0.1	4.4 ± 0.4	ns	0.767
22:5n-3	2.0 ± 0.1	2.1 ± 0.2	ns	0.867
22:6n-3	12.0 ± 0.7	12.1 ± 0.7	ns	0.979
Other n-3 PUFA[g]	2.9 ± 0.1	3.0 ± 0.2	ns	0.837
n-3 PUFA	29.3 ± 0.7	28.4 ± 2.1	ns	0.717
n-3 LC PUFA	21.2 ± 1.1	21.2 ± 1.5	ns	0.997
LC PUFA	29.0 ± 1.3	29.0 ± 2.2	ns	0.981
n-6:n-3 ratio	1.9 ± 0.1	2.0 ± 0.0	ns	0.184

Data are expressed as mean ± S.E.M., $n = 2$, $N = 6$. $P < 0.05$; an independent T-test: ns = not significant ($P > 0.05$); * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

[a] See Table 1 for experimental diet abbreviations.

[b] Total FA = total fatty acids μg g^{-1} of tissue

[c] See table 1 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 22:3n-3, 24:5n3 & 24:6n-3.

Table 5

Fillet fatty acid composition (as mg 100 g^{-1} of edible fillet) of Atlantic salmon fillet fed the two experimental diets for 150 days

	Diets[a]			
mg 100 g^{-1} of fillet	40:33	35:36	P-value	
20:5n-3	136.1 ± 3.7	131.7 ± 13.5	ns	0.767
22:5n-3	66.9 ± 3.0	68.1 ± 6.3	ns	0.867
22:6n-3	395.1 ± 22.5	396.0 ± 23.8	ns	0.979
SFA[b]	2658.8 ± 151.2	2631.0 ± 239.4	ns	0.926
MUFA	5762.5 ± 256.1	5806.6 ± 476.7	ns	0.939
PUFA	2521.6 ± 100.8	2547.6 ± 199.2	ns	0.913
LC-PUFA	925.8 ± 43.2	924.0 ± 68.5	ns	0.983
trans	47.2 ± 2.1	47.5 ± 4.4	ns	0.958
n-6 PUFA	1561.8 ± 77.4	1612.0 ± 131.1	ns	0.758
n-6 LC PUFA	244.2 ± 9.7	241.9 ± 21.1	ns	0.925
n-3 PUFA	907.1 ± 24.1	883.4 ± 64.1	ns	0.747
n-3 LC PUFA	681.6 ± 34.2	682.1 ± 48.8	ns	0.994
n-6:n-3 ratio	1.7 ± 0.1	1.8 ± 0.0	ns	0.176

Data are expressed as mean ± S.E.M., *n* = 2, *N* = 6. P < 0.05; an independent T-test: ns = not significant (P > 0.05); * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

[b] See table 1 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 two experimental diets for 150 days.

	Diets[a]			
	40:33	35:36	P-value	
12:0	3.3 ± 0.8 (*56.1*)	4.2 ± 0.2 (*69.0*)	ns	0.310
14:0	57.8 ± 10.8 (*44.2*)	65.7 ± 5.2 (*47.3*)	ns	0.546
16:0	501.1 ± 83.6 (*33.0*)	536.0 ± 44.1 (*33.0*)	ns	0.731
18:0	82.7 ± 22.1 (*17.5*)	87.8 ± 10.7 (*17.2*)	ns	0.846
22:0	6.0 ± 0.6 (*0.6*)	5.7 ± 0.5 (*0.0*)	ns	0.676
SFA[b,c]	650.9 ± 117.8	699.4 ± 60.5	ns	0.733
14:1n-5	9.1 ± 0.7 (*65.6*)	9.7 ± 0.4 (*66.5*)	ns	0.509
16:1n-7	180.3 ± 26.2 (*52.2*)	159.7 ± 12.0 (*43.8*)	ns	0.515
18:1n-7	62.0 ± 15.1 (*39.2*)	60.9 ± 7.2 (*35.4*)	ns	0.949
18:1n-9	1145.9 ± 198.0 (*46.8*)	1215 ± 86.6 (*44.7*)	ns	0.765
20:1n-9	17.6 ± 11.6 (*18.2*)	17.9 ± 6.1 (*17.0*)	ns	0.984
22:1n-9	8.3 ± 2.3 (*30.9*)	4.8 ± 1.3 (*18.1*)	ns	0.267
24:1n-9	1.8 ± 0.9 (*16.2*)	3.0 ± 0.3 (*26.5*)	ns	0.268
20:1n-11	11.7 ± 2.1 (*45.6*)	9.7 ± 1.335.9	ns	0.486
22:1n-11	43.6 ± 0.2 (*100*)	43.9 ± 0.0 (*100*)	ns	0.277
MUFA	1480.2 ± 256.2	1524.6 ± 115.0	ns	0.882
18:2n-6	395.1 ± 66.0 (*51.5*)	347.8 ± 30.5 (*40.4*)	ns	0.551
22:2n-6	8.4 ± 0.1 (*79.5*)	–[d]		
20:3n-6	1.1 ± 1.1 (*8.9*)	0.8 ± 0.8 (*5.7*)	ns	0.841
20:4n-6	10.6 ± 1.7 (*51.4*)	11.9 ± 0.8 (*48.7*)	ns	0.515
22:4n-6	1.4 ± 0.3 (*56.4*)	0.8 ± 0.1 (*29.2*)	ns	0.104
22:5n-6	8.4 ± 0.3 (*81.7*)	8.0 ± 0.2 (*76.3*)	ns	0.275
n-6 PUFA	425.0 ± 69.1	369.3 ± 31.8	ns	0.505
18:3n-3	71.8 ± 12.0 (*57.9*)	84.0 ± 5.4 (*56.1*)	ns	0.406
22:3n-3	11.8 ± 0.1 (*100*)	12.0 ± 0.0 (*100*)	*	**0.010**
20:5n-3	71.6 ± 5.4 (*77.9*)	66.5 ± 3.8 (*71.0*)	ns	0.479
22:5n-3	9.0 ± 3.0 (*46.8*)	5.0 ± 1.9 (*24.2*)	ns	0.319
22:6n-3	42.6 ± 13.4 (*35.9*)	37.0 ± 4.3 (*29.0*)	ns	0.711
n-3 PUFA	206.8 ± 33.6	204.5 ± 14.4	ns	0.953
Total FA	2762.8 ± 476.3	2797.7 ± 221.4	ns	0.950

Data are expressed as mean ± S.E.M., n = 2, *N* = 6. P < 0.05; an independent T-test: ns = not significant (P > 0.05); * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

[b] See table 1 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 two experimental diets for 150 days.

	Diets[a]			
	40:33	35:36	P-value	
Fatty acid elongation[b]				
18:0 to 20:0	2.2 ± 0.5	1.6 ± 0.2	ns	0.397
22:0 to 24:0	3.6 ± 0.4	4.3 ± 0.4	ns	0.300
18:2n-6 to 20:2n-6	13.9 ± 4.6	20.0 ± 2.3	ns	0.301
20:2n-6 to 22:2n-6	–[c]	2.2 ± 0.1	ns	
18:3n-6 to 20:3n-6	0.9 ± 0.7	1.9 ± 1.0	ns	0.448
18:3n-3 to 20:3n-3	2.2 ± 0.6	–		
18:4n-3 to 20:4n-3	12.6 ± 1.9	17.1 ± 1.2	ns	0.117
22:5n-3 to 24:5n-3	1.5 ± 0.8	3.0 ± 0.4	ns	0.178
Fatty acid Δ-6 desaturation				
18:2n-6 to 18:3n-6	4.6 ± 1.7	8.6 ± 2.2	ns	0.226
18:3n-3 to 18:4n-3	9.6 ± 1.9	20.0 ± 1.6	*	**0.014**
24:5n-3 to 24:6n-3	1.5 ± 0.5	1.7 ± 0.1	ns	0.785

Data are expressed as mean ± S.E.M., *n* = 2, *N* = 6. P < 0.05; an independent T-test: ns = not significant (P > 0.05); * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

[a] See Table 1 for experimental diet abbreviations.

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

[c] Not detected

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 two dietary treatments.

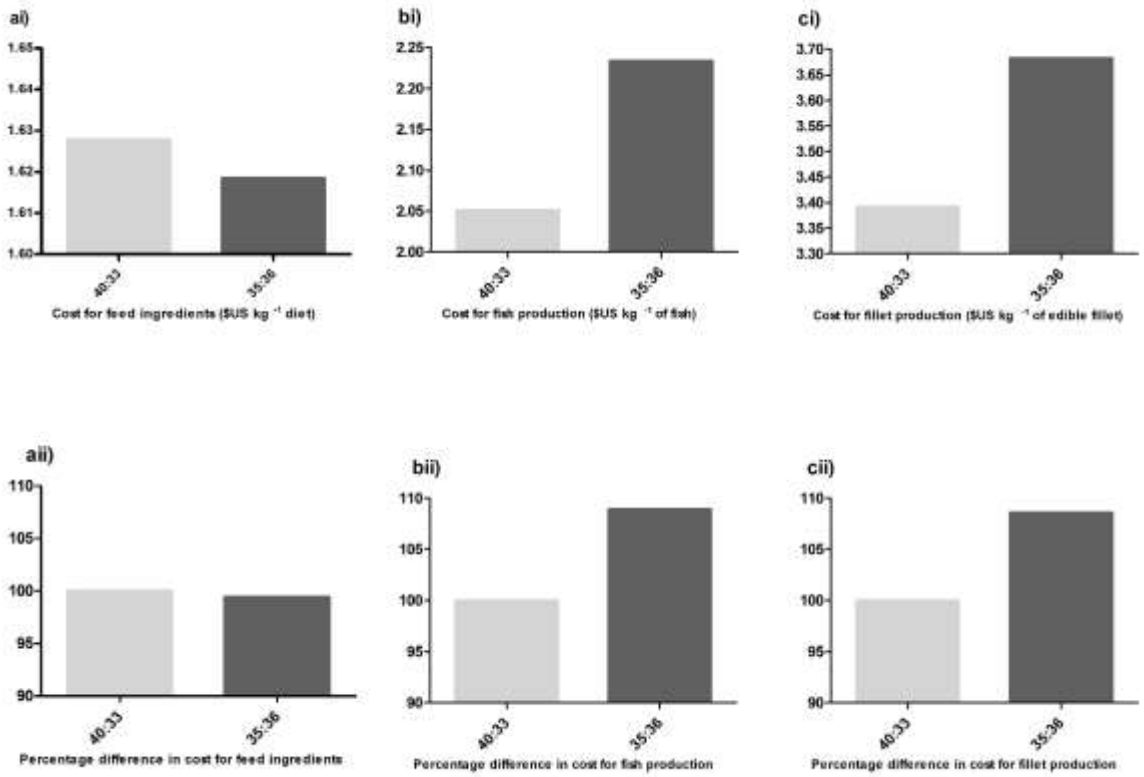
	Diets[a]			
	40:33	35:36	P-value	
Preference; Like (+) or Dislike (-)[b]				
Raw	4.1 ± 1.2	3.5 ± 1.7	ns	0.797
Cold smoked	14.6 ± 2.2	18.7 ± 0.5	ns	0.220
Hot smoked	22.6 ± 1.3	18.5 ± 5.4	ns	0.535
Influential attributes[c]				
Fishiness[c]				
Raw	-0.6 ± 2.6	0.1 ± 0.3	ns	0.816
Cold smoked	3.9 ± 0.8	3.2 ± 1.9	ns	0.759
Hot smoked	-0.7 ± 1.7	1.1 ± 0.7	ns	0.435
Saltiness[c]				
Raw	-20.3 ± 3.7	-18.3 ± 1.3	ns	0.670
Cold smoked	9.8 ± 1.6	9.1 ± 0.4	ns	0.694
Hot smoked	5.7 ± 1.5	6.3 ± 0.5	ns	0.755
Oiliness[c]				
Raw	-3.8 ± 1.8	-3.5 ± 0.1	ns	0.902
Cold smoked	3.5 ± 0.3	4.4 ± 1.6	ns	0.640
Hot smoked	-0.5 ± 1.6	-1.2 ± 2	ns	0.810

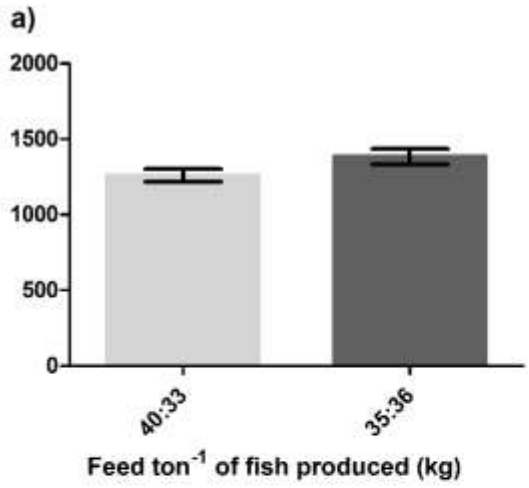
Data are expressed as mean ± S.E.M., $n = 2$, $N = 6$. $P < 0.05$; an independent T-test: ns = not significant ($P > 0.05$); * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

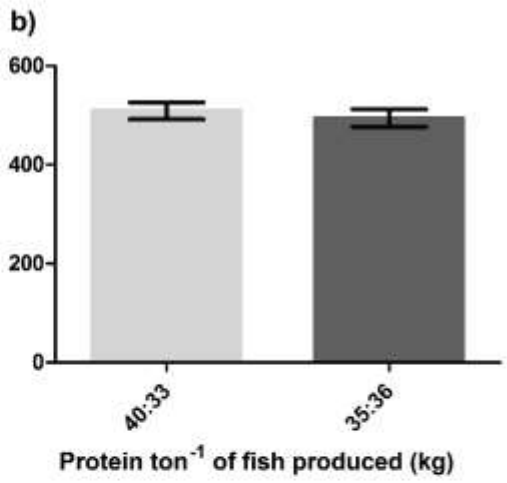
[a] See Table 1 for experimental diet abbreviations.

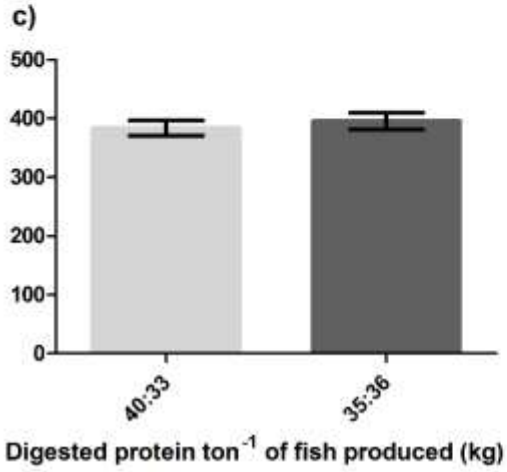
[b] Salmon preferences were assessed using hedonic LMS scales.

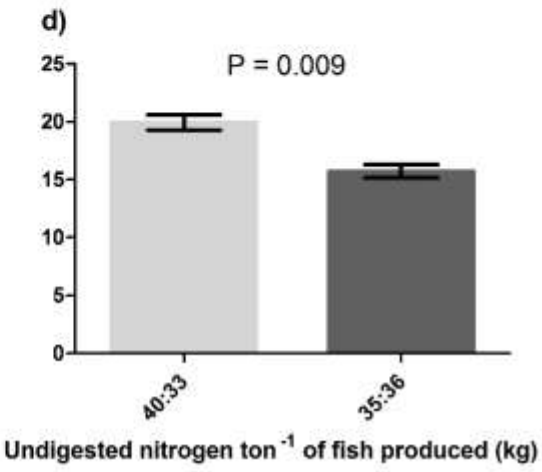
[c] Attributes consumers determined had greatest influence over preference


ai)
1.65
1.64
1.63
1.62
1.61
1.60
40:33
35:36
Cost for feed ingredients ($US kg -1 diet)
bi)
2.25
2.20
2.15
2.10
2.05
2.00
40:33
35:36
Cost for fish production ($US kg -1 of fish)
ci)
3.70
3.65
3.60
3.55
3.50
3.45
3.40
3.35
3.30
40:33
35:36
Cost for fillet production ($US kg -1 of edible fillet)
aii)
110
105
100
95
90
40:33
35:36
Percentage difference in cost for feed ingredients
bii)
110
105
100
95
90
40:33
35:36
Percentage difference in cost for fish production
cii)
110
105
100
95
90
40:33
35:36
Percentage difference in cost for fillet production

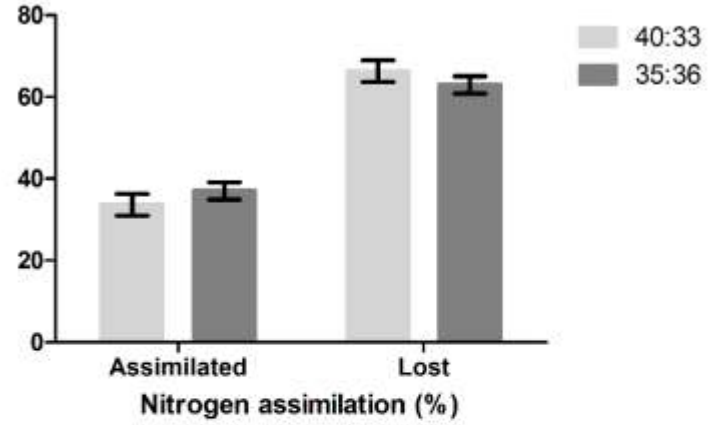

a)
2000
1500
1000
500
0
40:33
35:36
Feed ton-1 of fish produced (kg)


b)
600
400
200
0
40:33
35:36
Protein ton-1 of fish produced (kg)

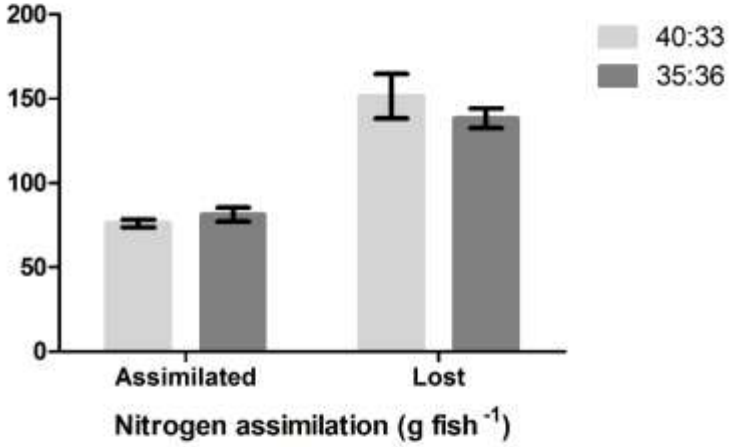

c)
500
400
300
200
100
0
40:33
35:36
Digested protein ton-1 of fish produced (kg)


d)
P = 0.009
25
20
15
10
5
0
40:33
35:36
Undigested nitrogen ton-1 of fish produced (kg)

a)


80
60
40
20
0
Assimilated
Lost
40:33
35:36
Nitrogen assimilation (%)

b)


200
150
100
50
0
Assimilated
Lost
40:33
35:36
Nitrogen assimilation (g fish -1)

c)

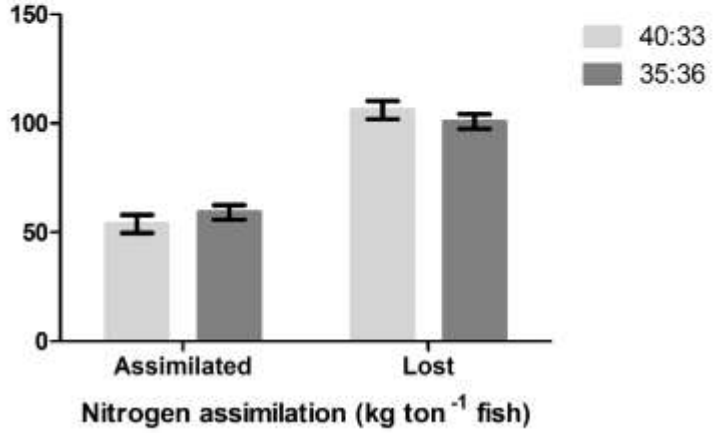

150
100
50
0
Assimilated
Lost
40:33
35:36
Nitrogen assimilation (kg ton -1 fish)

Figure 1; Preliminary assessment of feed related production costs associated with the ingredients used to formulate two commercial-like diets for large Atlantic salmon, one containing 40 % protein and 33 % lipid (40:33) and the other containing 35 % protein and 36 % lipid (35:36), including ai) Cost of feed ingredients in $US kg^{-1} of diet, bi) cost of fish production in $US kg^{-1} of fish based on feed ingredients and food conversion ratio for the respective diets, ci) cost of fillet production in $US kg^{-1} of fillet based on cost of diet ingredients, food conversion ratio and fillet yield for the respective diets. aii), bii) and cii) represent percentage differences between the two treatments for the cost of feed ingredients, fish production and fillet production, respectively.

Figure 2; Feed, protein usage and nitrogenous waste from undigested protein; associated with two commercial-like diets for large Atlantic salmon, one containing 40 % protein and 33 % lipid (40:33) and the other containing 35 % protein and 36 % lipid (35:36), including a) feed ton^{-1} fish produced (kg) based on FCR, b) protein used ton^{-1} fish produced (kg), based on FCR and protein content of the treatment diets, c) retained protein ton^{-1} of fish produced (kg), based on FCR, protein content of diet and ADC % of protein for the two treatment diets and d) undigested nitrogen ton^{-1} fish produced (kg), based on FCR, protein content of diet, ADC % of protein and converting undigested protein to nitrogen for the two treatment diets.

Figure 3; Nitrogen assimilation or 'loss' based on mass balance calculations in large Atlantic salmon associated with two commercial-like diets, one containing 40 % protein and 33 % lipid (40:33) and the other containing 35 % protein and 36 % lipid (35:36), including a) Nitrogen assimilation / loss (%), based on initial and final fish weights, protein intake and protein content of the diets and whole-body of fish from respective treatments, b) Nitrogen assimilation / loss (g $fish^{-1}$), based on total protein intake and % assimilation and c) Nitrogen assimilation / loss (kg ton^{-1} fish), based on % assimilated, protein was converted to nitrogen by dividing by 6.25.