Effects of dietary protein and fat level and rapeseed oil on growth and tissue fatty acid composition and metabolism in Atlantic salmon (*Salmo salar* L.) reared at low water temperatures

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Running title: Protein/fat level and rapeseed oil in Atlantic salmon diets

Abstract

A 12 week feeding trial was conducted to elucidate the interactive effects of dietary fat and protein contents and oil source on growth, fatty acid composition, protein retention efficiency (PRE) and β-oxidation activity of muscle and liver in Atlantic salmon (*Salmo salar* L.) at low water temperatures (4.2 ^{o}C). Triplicate groups of Atlantic salmon (initial weight 1168 g) were fed six isoenergetic diets formulated to provide either 390 g kg^{-1} protein and 320 g kg^{-1} fat (high protein (HP) diets) or 340 g kg^{-1} protein and 360 g kg^{-1} fat (low protein (LP) diets); within each dietary protein/fat level crude RO comprised 0, 30 or 60% (R0, R30, R60, respectively) of the added oil. After 12 weeks the overall growth and FCR were very good for all treatments (TGC; 4.76 (±0.23), FCR; 0.85 (±0.02)). Significant effects were shown due to oil source on SGR and TGC only. The liver and muscle FA compositions were highly affected by the graded inclusion of RO. The PRE was significantly affected by the dietary protein level, while no significant effects were shown in total β-oxidation capacity of liver and muscle. The results of this study suggest that more sustainable, lower protein diets with moderate RO inclusion can be used in Atlantic salmon culture at low water temperatures with no negative effects on growth and feed conversion, no major detrimental effects on lipid and fatty acid metabolism and a positive effect on protein sparing.

KEYWORDS: Rapeseed oil; Dietary protein / lipid ratio; Polyunsaturated fatty acids (PUFA); β-Oxidation; Protein sparing effect; Atlantic salmon

Introduction

Traditionally, marine fish meal (FM) and fish oil (FO) have been the major protein and oil sources in aquafeeds, especially for carnivorous species such as Atlantic salmon, mainly due to their high nutritional value (Sargent & Tacon, 1999). The aquaculture sector is at present the biggest consumer of FM and FO, consuming in 2002 about 2.685 mmt of FM and 0.666 mmt of FO, that represents 42.1 and 78.7% of the total global FM and FO production, respectively (Tacon, 2004). Moreover, the largest proportion of the total FM and FO used in aquafeeds in 2002, 26.9 and 52.4%, respectively, was consumed by salmonids (Tacon, 2004).

Clearly, there is a strong dependence on FM and FO for the salmon industry. This could be risky and even harmful to the viability, growth and profitability of the sector, as it has been estimated that the resources of wild feed grade fisheries will remain static (Pike & Barlow, 2003), while the demand for these commodities by the aquaculture feed industry will grow significantly in the next decade (Sargent & Tacon, 1999; Tidwell & Allan, 2002; Tacon, 2004). Moreover, other issues arise, that make the use of FM and FO for aquafeeds problematic; for instance, FM and FO can contain organic pollutants (e.g. dioxins, PCBs, PBDEs), that are deposited in the fish and, thereby, may limit their inclusion (SCAN, 2000; SCF, 2001; Jacobs *et al.*, 2002a; Jacobs *et al.*, 2002b; Bell *et al.*, 2005). Hence, there is a growing, pressing need for sustainable alternatives to FM and FO and for the reduction of the dependence of FM and FO for fish feeds.

Vegetable oils (VO) represent sustainable alternatives to FO. However, the replacement of FO with VO can be challenging, as VO lack the n-3 highly unsaturated fatty acids (HUFA) which are abundant in FO; the n-3 HUFA, especially eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic acids (22:6n-3; DHA), are

essential for optimal growth and development in salmon (Sargent *et al.*, 2002). In addition, the fatty acid (FA) composition of the fish tissues reflects the FA composition of the diets; hence, replacement of FO with VO results in reductions of EPA, DHA and the n-3/n-6 FA ratio, with a direct effect on the nutritional quality of the end product (Bell *et al.*, 2001; Rosenlund *et al.*, 2001; Bell *et al.*, 2003a). This is important for the human consumer as EPA and DHA and the n-3/n-6 FA ratio have been associated with numerous beneficial effects on human health and any reduction in farmed fish would be undesirable (De Deckere *et al.*, 1998; Horrocks & Yeo, 1999; Simopoulos, 1999; Hunter & Roberts, 2000; ISSFAL, 2000; Simopoulos, 2003).

RO is considered to be a good sustainable substitute for FO. It has been used successfully in a number of previous studies with salmon (Bell *et al.*, 2001; Torstensen *et al.*, 2004a; Torstensen *et al.*, 2004b). Moreover, it has a high availability (FAO, 2005), as it is the third largest production of VO in the world, after soy and palm oil (U. S. Department for Agriculture, 2005).

Currently, salmon diets contain high proportions of protein, most of it provided by FM. However, it is crucial for the aquafeed industry to optimise the use of feed protein and to improve the protein utilisation in the salmon diets. This would allow less dependence on FM, reduce the cost of the feed and also reduce the environmental impact through waste output from salmon culture(Halver & Hardy, 2002). Salmon can utilize lipids efficiently, therefore the use of high lipid diets in salmon allows protein sparing (Froyland *et al.*, 1998; Hillestad *et al.*, 1998; Bendiksen *et al.*, 2003) and subsequently improved growth.

Numerous studies have investigated the replacement of FO with RO, and/or other VO in diets of salmonids (Bell *et al.*, 2001; Rosenlund *et al.*, 2001; Tocher *et al.*, 2001; Bell *et al.*, 2002; Bell *et al.*, 2003b; Bell *et al.*, 2003a; Bendiksen *et al.*,

100 2003; Ng *et al.*, 2004; Tocher *et al.*, 2004; Torstensen *et al.*, 2004a; Torstensen *et al.*,
101 2004b; Fonseca-Madrigal *et al.*, 2005) and also the use of low protein / high lipid
102 diets (Einen & Roem, 1997; Bendiksen *et al.*, 2003; Azevedo *et al.*, 2004; Solberg,
103 2004). However, most of these studies were focusing either on the oil source or on the
104 dietary protein/fat level. In addition, very few were conducted at the very low
105 temperatures used in the present study that are common in sites located in high
106 latitudes. It is known that temperature plays a significant role in FA metabolism and,
107 in general, in fish nutrition, physiology and growth (Torstensen *et al.*, 2000;
108 Bendiksen & Jobling, 2003; Bendiksen *et al.*, 2003; Guderley, 2004; Ng *et al.*, 2004;
109 Tocher *et al.*, 2004). In particular, the important role of n-3 HUFAs in low
110 temperature adaptation has been highlighted (Hochachka & Somero, 2002).
111 The aim of this trial was to elucidate the interactive effects of dietary fat and
112 protein contents and oil source on growth, whole body proximate composition, fatty
113 acid composition and β-oxidation activity of liver and muscle in Atlantic salmon at
114 low water temperatures.

115 **Materials and methods**

116 *Fish and facilities*

117 Atlantic salmon (*Salmo salar*) of the NLA strain (03G) with overall mean
118 weight of 1168g were randomly distributed into 18 sea cages of 125 m^3 (5x5x5m)
119 with 137 fish in each cage. Prior to the trial the fish were stocked in two trial cages
120 and acclimatised for six weeks. The fish were subjected to artificial light (LD24:0)
121 from the middle of December at decreasing ambient temperature (range; 4-6oC).
122 During this holding period the fish were fed commercial pelleted feed (BioOptimal
123 CPK, 9mm, BioMar AS, Norway) in accordance with the manufacturer's

recommendations. During the experimental period (February –April 2004) the fish were subjected to artificial light (LD24:0), provided from sub-merged light (one 400W bulb shared by four cages). The temperature varied from 2.8 °C to 7.3 °C with an average temperature of 4.2 ± 0.8 °C. Salinity was 34.0 ± 0.8 g L^{-1}. Fish were bulk weighed at the start of the trial, after 6 weeks and at the end of the trial (12 weeks). Mortalities were recorded and dead fish were removed daily.

Experimental diets

Six isoenergetic, practical-type extruded diets (9 mm) were formulated (BioMar TechCentre, Brande, DK) to provide either 390 g kg^{-1} protein and 320 g kg^{-1} fat (high protein (HP) diets) or 340 g kg^{-1} protein and 360 g kg^{-1} fat (low protein (LP) diets). Within each dietary fat and protein level crude RO comprised 0, 30 or 60% (R0, R30, R60) of the total added oil, the remainder of which was FO (Table 1). The diets were formulated to meet all the known nutritional requirements of salmonid fish (NRC, 1993). The proximate composition of the experimental diets is shown in Table 1 and the fatty acid compositions are shown in Table 2. Each feed was fed daily to satiation by hand to triplicate groups (cages) of fish. When sea temperature was below 5°C the fish were fed to satiation once a day. Above 5°C, two daily meals were provided with a minimum of 4 hours between the meals. In order to facilitate accurate calculations of feed intake and FCR, feed wastage was collected using a lift-up system and calculated on a daily basis.

Sampling procedure

Samples were taken from all diets and stored at -20 °C until analyzed. At the start of the experiment an initial sample of six fish was taken to determine baseline values of whole body proximate composition. At the end of the trial (12th week) three

fish per cage were sampled at random from the population in each cage for lipid and fatty acid composition and β-oxidation activities of liver and muscle. Another sample of three fish per cage was used for whole body proximate composition. Fish were killed with a sharp blow to the head and samples of liver were dissected and immediately placed in liquid nitrogen. Viscera, liver and heart weights from four fish per cage were recorded for measurement of viscero-somatic index (VSI), hepato-somatic index (HSI) and cardio-somatic index (CSI), respectively. For whole body analysis fish were minced and homogenate sub-samples of each fish were obtained. Initial whole body samples were pooled in pairs so three samples were finally obtained (n = 3) while 12 week whole body samples were pooled so there was one sample per cage. A muscle sample, representative of the edible portion, was obtained by cutting a steak between the dorsal and ventral fins (NQC). This section was then skinned, de-boned and homogenized. All samples were then stored at -20 °C until analyzed.

Proximate analysis

Proximate analysis was conducted to determine the nutrient composition of diets and whole body samples. Moisture was determined by thermal drying to constant weight in an oven at 110 °C for 24h. Crude protein contents were determined by Kjeldahl analyses (nitrogen x 6.25, Kjeltec Autoanalyser, Tecator). Crude fat was determined in diets by acid hydrolysis using a Soxtec System 1047 hydrolysing unit (Tecator Application note 92/87) followed by exhaustive Soxhlet extraction using petroleum ether (40-60°C, BP) on a Soxtec System HT6 (Tecator application note 67/83). Crude fat in whole body samples was determined by the above procedure but without the acid hydrolysis. Ash content was determined by dry ashing in porcelain

crucibles in a muffle furnace at 600 °C overnight. All methods are based on those described in AOAC (1995) and modified as described by Bell et al (2001).

Lipid extraction and fatty acid analyses

Total lipids of flesh, livers and diet samples were extracted by homogenization in 20 volumes of chloroform/methanol (2:1, v/v) containing butylated hydroxytoluene (0.01% w/w, BHT) as antioxidant, according to Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification using 2ml of 1% H_2SO_4 in methanol plus 1 ml toluene as described by Christie (1982) and FAME extracted and purified as described by Tocher & Harvie (1988). FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30 m x 0.32 mm capillary column (CP wax 52CB; Chrompak Ltd., London, U.K.). Hydrogen was used as carrier gas and temperature programming was from 50°C to 150°C at 40°C/min and then to 225°C at 2°C/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman, 1980).

Total β-oxidation capacity

Liver and red and white muscle were weighed and homogenized in 20% (w/v) ice-cold buffered sucrose solution containing 0.25M sucrose, 0.04M potassium phosphate buffer (pH 7.4), 0.15M KCl, 40mM KF and 1mM N-acetyl cysteine. The resulting total homogenates were then centrifuged at 1880 × *g* for 10 min at 2°C. The resulting post-nuclear fractions were collected, and portions were used immediately to determine total (mitochondrial and peroxisomal) β-oxidation capacity. The total β-oxidation capacity was determined as acid-soluble products using radiolabelled [1-^{14}C]-palmitoyl-CoA as a substrate as described by Frøyland *et al.* (1995).

196 *Calculations and statistical analysis*

197 The following formulae were applied to the data:

198 FCR (Feed Conversion Ratio) = (feed intake, g) x (wet weight gain, g) $^{-1}$

199 SGR (Specific Growth Rate), %/day = 100 x [lnW_1 - lnW_0] x (days) $^{-1}$

200 TGC (Thermal Growth Coefficient), x 1000 = 1000 x [$(W_1)^{1/3}$ - $(W_0)^{1/3}$] x (days x ^{o}C)$^{-}$
201 ^{1}

202 K (Condition Factor) = 100 x W x (fork length, cm) $^{-3}$

203 HSI % = 100 x (liver weight, g) x W $^{-1}$

204 VSI % = 100 x (viscera weight, g) x W $^{-1}$

205 CSI % = 100 x (heart weight, g) x W $^{-1}$

206 PRE (Protein retention efficiency, g protein gain x g protein ingested^{-1}), % = 100 x
207 [(P_1W_1−P_0W_0) x (P_F x cumulative feed intake) $^{-1}$]

208 In the above formulae W is the weight of the sampled fish in grams, W_0 and
209 W_1 are the initial and the final fish mean weights in grams, P_0 and P_1 are the initial
210 and final protein concentrations of the fish, P_F is the protein concentration of the feed
211 on a dry matter basis, and cumulative feed intake was determined in grams on a dry
212 matter basis.

213 All the data are presented as means ± SD (n = 3) and all statistical analyses
214 were performed using SPSS 13 (SPSS Inc, 2004). The effects of dietary RO, the
215 fat/protein ratio and their interactions on growth, tissue fatty acid compositions, and
216 β-oxidation were analysed by two-way ANOVA. Percentage data and data which
217 were identified as non-homogeneous (Levene's test) were subjected to square root or

log transformation before analysis. Differences were regarded as significant when $P < 0.05$ (Zar, 1996).

Results

There were no significant differences in initial cage mean weights of the fish (Table 3). Following 12 weeks of feeding, the cage mean weight ranged between 1711g and 1784g and the final length of the fish varied from 49.9 to 51.8 cm. No significant effects or interactions of dietary protein level and oil source were identified in final weight and length by two-way ANOVA. However, there was a significant effect of oil source on growth performance (SGR and TGC). Specifically, the inclusion of RO resulted in higher SGR (0.49 vs. 0.56 % day^{-1}) and TGC (4.45 vs. 5.13). Feed conversion ratios (FCR) were good for all treatments and ranged from 0.81 to 0.87. No significant overall effects and interactions of dietary protein level and oil source on FCR were identified, although there was a trend ($P<0.10$) of lower FCR for the fish fed the HP diets compared to LP diets. K ranged from 1.30 to 1.34 and no significant effects and interactions of dietary protein level and oil source were seen. The organ-to-whole-body indices are shown in Table 3. VSI varied from 11.7% to 12.6%, HSI from 1.4% to 1.5%, and similar CSIs were found for all groups (1.3%).Two-way ANOVA showed no significant effects and interactions of dietary protein level and oil source.

The PRE were high for all groups (42-47%) and the there was a significant overall effect of dietary protein and fat level ($P<0.05$), with higher overall PRE for the LP groups compared to the HP groups.

The proximate composition of whole body is shown in Table 4. Whole body moisture, protein and ash contents were very similar in all groups (approximately 662,

242 162 and 15 g kg^{-1}, respectively), whereas final lipid content ranged from 145 to 164 g
243 kg^{-1}. Two-way ANOVA did not reveal any significant overall effects or interactions

244 The replacement of increasing proportions of FO with RO in the diets resulted
245 in significant changes in dietary fatty acid compositions (Table 2). FO diets had
246 approximately 30% total saturates of which two thirds was 16:0, and about 35% total
247 monoenes with around 13% 18:1n-9. Long chain monoenes, 20:1 and 22:1,
248 comprising more than 10% of the diet, and 5% n-6PUFA predominantly 18:2n-6, and
249 approximately 30% n-3PUFA, with over 20% as the n-3HUFA (mainly EPA and
250 DHA). Graded inclusion of RO resulted in decreased 16:0, 20:1n-9, 20:4n-6, 20:5n-3
251 and 22:6n-3 (approximately 10%, 2.5%, 0.2%, 3.5% and 4.5% respectively in diets
252 containing 60% RO) and increased 18:1n-9, 18:2n-6 and 18:3n-3 (approximately
253 43%, 14.5% and 6% respectively in diets containing 60% RO) within both dietary
254 protein levels. The n-3/n-6 ratio decreased from 6.0 in diets containing 100% FO to
255 1.0 in diets containing 60% RO.

256 The total lipid content and the fatty acid compositions of muscle and liver are
257 shown in Tables 5 & 6. Total lipid content ranged from 92.6 to 117.8 mg lipid g^{-1}
258 tissue and from 49.4 to 81.0 mg lipid g^{-1} tissue for muscle and liver, respectively. RO
259 inclusion increased the total lipid content in liver but not in muscle. RO inclusion
260 affected significantly tissue FA compositions. However, there were no significant
261 interactions between dietary protein level and RO inclusion either in muscle or in liver
262 and in most cases the overall protein level effect was not significant. Specifically,
263 both in muscle and liver a reduction was seen in 16:0, total saturates, 20:1n-9, 22:1,
264 20:4n-6, 20:5n-3, 22:6n-3, total n-3 PUFAs and n-3/n-6 ratio as RO inclusion
265 increased within both dietary fat and protein levels. Conversely, 18:1n-9, total
266 monoenes, 18:2n-6, total n-6 PUFAs and 18:3n-3 increased in muscle and liver with

graded RO inclusion. However, 20:2n-6 did not reflect the dietary content, as it increased both in liver and muscle with increased dietary RO inclusion.

The total palmitoyl-CoA oxidation capacity in muscle and liver is shown in Table 7. The values shown for muscle represent the total β-oxidation capacity of a combined red and white muscle fraction. β-oxidation capacity ranged from 0.45 to 0.61 pmol/min/mg protein in muscle and from 3.28 to 5.13 pmol/min/mg protein in liver. However, no significant overall effects of dietary protein/fat level or oil source were shown by two-way ANOVA.

Discussion

This study aimed to investigate the effects and interactions of the replacement of FO with RO at two different protein/lipid ratios at low water temperatures. Several previous studies have shown that replacement of FO with RO, blends of RO and other vegetable oils or other vegetable oils alone, such as linseed oil, (LO) or palm oil, (PO) in diets of salmon, has no negative effects on fish growth (Bell *et al.*, 2001; Rosenlund *et al.*, 2001; Bell *et al.*, 2002; Bell *et al.*, 2003a; Bell *et al.*, 2003b; Bendiksen *et al.*, 2003; Ng *et al.*, 2004; Torstensen *et al.*, 2004a; Torstensen *et al.*, 2004b). Moreover, when low protein feeds were compared to high protein feeds growth was not significantly affected (Azevedo *et al.*, 2004; Solberg, 2004), especially at low temperatures (Hillestad *et al.*, 1998; Bendiksen *et al.*, 2003). It is likely that the effect of low temperature masked any potential effects of feed treatment, and that diet-related growth differences observed at the higher temperature were diminished at the lower temperature (Bendiksen *et al.*, 2003). In line with the previous studies, the current experiment showed no significant effects due to dietary protein level in final weights SGR, TGC and FCRs. Nevertheless, oil source had a significant positive effect on SGR and TGC. Graded inclusion of RO, at the expense

of FO, resulted in increased SGR and TGC. This is in accordance with other studies and could be a result of enhanced protein utilisation, arising from improved use of oil for energy, due to superior fatty acid availability from vegetable oils at low water temperatures, although the exact mechanism of this effect is currently unclear (Bendiksen *et al.*, 2003). Although no significant interactions were found, the effect of oil source seemed to differ between LP and HP series. This indicates that the lower SAFAs RO oil increased the digestible energy content of the feed, resulting in improved growth performance of the fish when the dietary protein and amino acids where in excess (HP feeds).

The protein sparing effect could have also been enhanced by higher dietary oil levels. This is supported by the PRE results, as the overall PRE was significantly improved when the fish were fed the LP diets. Previous studies have also suggested a positive effect of increased dietary lipid content on protein retention and, hence, on protein sparing (Einen & Roem, 1997; Hillestad *et al.*, 1998; Bendiksen *et al.*, 2003).

In this study, the different dietary treatments had no influence on the chemical composition of the whole carcass, either due to dietary protein level or due to the oil source. The moisture, protein and ash content of the carcass were almost constant between the groups and there were only minor differences in the lipid content. This is in agreement with the findings of Hillestad et al. (1998) who reported that the fillet and dressed carcass fat content was not influenced by dietary lipid level, although significant differences in tissue fat content were shown, due to the dietary energy content. Other studies have shown that when the dietary oil increases, tissue lipid, and usually moisture, increases, while protein decreases (Hillestad & Johnsen, 1994; Einen & Roem, 1997; Einen & Skrede, 1998; Hemre & Sandnes, 1999; Bendiksen *et al.*, 2003; Azevedo *et al.*, 2004; Solberg, 2004). However, in most of these studies the

lipid content of the fillet, carcass or whole body was possibly influenced by the differences in the dietary energy content, as only Azevedo et al. (2004) and Solberg (2004) used isoenergetic diets. In the present study no significant differences were observed due to dietary oil source. This is in agreement with the results reported by Bendiksen et al. (2003) when FO was replaced with a blend of VO at low water temperatures (2^{o}C). By contrast, other studies have shown that when RO replaced FO in diets of Atlantic salmon, the chemical composition of tissues is significantly influenced, to a small extent, although these studies were of longer duration than the present study (Bell *et al.*, 2001; Torstensen *et al.*, 2004b). It has been shown that in Atlantic salmon fat deposition increases as the fish grow larger (Jobling & Johansen, 2003). This was clearly demonstrated in the present study where an increase in lipid content, along with a decrease in crude protein and ash, was observed between the initial and final sampling.

In the present trial K, VSI, HSI and CSI were not affected by the different dietary treatments. This is in agreement with other studies, as changes in dietary protein / fat ratio in Atlantic salmon have not been associated with changes in K, VSI and HSI (Einen & Roem, 1997; Solberg, 2004) or with the replacement of FO with RO and LO (Rosenlund *et al.*, 2001; Bendiksen *et al.*, 2003). Few reports on diet effects on the CSI are available although a reduced CSI was observed in one study where salmon were fed diets where sunflower oil was used as a FO replacement (Bell *et al.*, 1991).

The fatty acid compositions of tissue lipids of Atlantic salmon are known to be highly influenced by dietary fatty acids (Torstensen *et al.*, 2000; Rosenlund *et al.*, 2001) and linear correlations exist between individual fatty acids in tissue total lipid and their concentrations in dietary lipid (Bell *et al.*, 2001; Bell *et al.*, 2003a; Tocher *et*

342 *al.*, 2003a). In the present study, the differences in the dietary fatty acid compositions
343 resulting from the graded inclusion of RO, at the expense of FO, were not affected by
344 dietary protein level. Hence, diets including similar proportions of RO had very
345 similar fatty acid compositions irrespective of their protein/lipid ratio. As a result the
346 oil source affected significantly liver and muscle FA compositions while no
347 significant effects on the tissue FA compositions were shown due to the dietary
348 protein level. The results are in line with previous studies showing that dietary fatty
349 acid compositions are reflected in tissue FA compositions.
350 However, previous studies have shown that although dietary fatty acids
351 correlated to fatty acids deposited in flesh, specific fatty acids were selectively
352 utilized or retained (Bell *et al.*, 2001; 2003a; Torstensen *et al.*, 2004a). This was also
353 demonstrated in the present study. Dietary 18:1n-9 increased more than 3-fold and
354 18:2n-6 and 18:3n-3 more than 4-fold in diets containing 60% RO compared to FO
355 diets, whereas in muscle and liver these fatty acids increased only around 2-fold.
356 These data confirm that when certain fatty acids are provided to the fish in high
357 concentrations, they are readily metabolised, largely catabolism by β-oxidation,
358 although they may also be subject to limited desaturation and elongation (Bell *et al.*,
359 2003a). On the contrary, n-3 HUFAs were selectively deposited and retained in flesh.
360 Tissue DHA was reduced only by 30% - 40% and EPA by less than 45% when fish
361 were fed diets containing 60% RO compared to FO groups, whereas in 60% RO diets
362 these HUFAs were only 30% of the concentrations in 0% RO diets. Apart from the
363 selective deposition and retention of these FAs, the moderate reductions in EPA and
364 DHA could have also been affected, even to small extent, by the hepatic desaturation
365 and elongation of dietary α-linolenic acid, which can be increased by inclusion of

366 vegetable oils in the diets (Tocher *et al.*, 2000; Tocher *et al.*, 2001; Tocher *et al.*,
367 2003a; Tocher *et al.*, 2003b).

368 It is well documented that n-3 HUFAs, particularly EPA and DHA and a high
369 n-3 / n-6 ratio, in human diets are beneficial for various aspects of human health
370 including preventive or protective effects in coronary heart disease, rheumatoid
371 arthritis, cancer, neurodevelopmental and mood disorders etc (De Deckere *et al.*,
372 1998; Horrocks & Yeo, 1999; Simopoulos, 1999; Hunter & Roberts, 2000; ISSFAL,
373 2000; Simopoulos, 2003). At present, intensive culture of Atlantic salmon uses marine
374 FO resulting in a highly nutritious and healthy product, as it is rich in n-3 HUFAs and
375 has a high n-3 / n-6 ratio(Bell *et al.*, 1998). However, in recent times there has been a
376 desire to investigate more sustainable alternatives to fish meal and fish oil for use in
377 aquaculture feeds. Clearly, any changes towards use of vegetable alternatives to
378 marine FO should not be at the expense of the quality and nutritional value of the final
379 product. In this regard the present study showing moderate reduction of EPA and
380 DHA in fish fed diets containing RO, at levels as high as 60% even at low protein
381 levels, could be significant, although it should be remembered that this was a
382 relatively short trial compared to the whole production cycle for Atlantic salmon.

383 The total β-oxidation capacity, including mitochondrial and peroxisomal β-
384 oxidation activity, was measured in liver and a combined red and white muscle
385 fraction. No significant effects were observed either in liver or muscle. These results
386 are in line with other studies which showed that when FO was replaced by RO or
387 other VO, in diets for Atlantic salmon, β-oxidation capacity was not affected (Tocher
388 *et al.*, 2003b; Stubhaug *et al.*, 2005).

389 The results of this study showed no negative effects on growth and feed
390 conversion, no major detrimental effects on lipid and fatty acid metabolism in Atlantic

391 salmon and an enhanced protein sparing effect, when fish were fed with lower protein
392 feeds where RO replaced FO up to 60% of the total oil. In conclusion, the results of
393 this study suggest that more sustainable, lower protein diets, in which a high
394 proportion of the dietary protein and lipid is of non-marine origin, with high rapeseed
395 oil inclusion, can be used in Atlantic salmon culture at low water temperatures.

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Table 1 Feed components and proximate compositions (g kg^{-1}) of the experimental diets

	HP-R0	HP-R30	HP-R60	LP-RO	LP-R30	LP-R60
Components						
Fishmeal	390	390	390	310	310	310
Oil seed and legume seed meals	225	225	225	235	235	235
South American fish oil	280	196	112	318	222	126
Rapeseed oil [a]	0	84	168	0	96	192
Binder	120	120	120	130	130	130
Premixes [b]	18	18	18	23	23	23
Analysed composition [c]						
Moisture	43	47	44	67	58	56
Protein	387	388	391	344	347	342
Lipid	327	323	321	353	347	361
Ash	79	78	78	70	70	69
Gross Energy, kJ g^{-1} [d]	25.2	25.1	25.2	25.3	25.6	25.5
Protein/Energy ratio [e]	15.3	15.5	15.3	13.6	13.4	13.2

[a] Double-low quality rapeseed oil

[b] Vitamin and mineral premixes prepared according to BioMar A/S commercial standards. Includes crystaline amino acids and Carophyl pink to provide 40mg/kg astaxanthin.

[c] Wet weight

[d] Estimated from caloric values of 39.5, 23.6 and 17.2 kJ g^{-1} for fat, protein and carbohydrate, respectively

[e] Calculated g protein kJ^{-1}

Table 2 Fatty acid compositions (% by weight of total fatty acids) of the experimental diets

Fatty Acid	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60
14:0	5.9	3.5	1.8	5.4	3.1	1.9
16:0	20.3	15.2	10.4	19.6	14.3	10.4
18:0	3.6	3.0	2.5	3.5	2.9	2.5
Total saturated[a]	31.1	23.1	15.9	29.9	21.6	16.4
16:1n-7	6.6	4.2	2.3	6.1	4.2	2.2
18:1n-9	13.4	29.0	42.5	13.2	28.0	42.9
18:1n-7	2.5	2.8	3.0	2.5	3.1	2.6
20:1n-9	4.4	3.5	2.6	4.6	3.4	2.5
22:1	6.5	4.8	2.5	6.7	4.3	2.5
24:1n-9	0.8	0.6	0.4	0.9	0.9	0.5
Total monoenes[b]	34.6	45.1	53.4	34.7	44.5	53.4
18:2n-6	3.7	9.4	14.5	3.5	9.4	14.3
20:2n-6	0.2	0.2	0.1	0.3	0.2	0.1
20:4n-6	0.5	0.3	0.2	0.6	0.4	0.2
22:5n-6	0.3	0.2	0.1	0.3	0.2	0.1
Total n-6 PUFA[c]	5.0	10.4	15.1	5.0	10.4	14.9
18:3n-3	1.3	3.7	5.8	1.4	3.9	5.9
18:4n-3	2.9	1.8	1.0	3.0	2.0	1.0
20:4n-3	0.7	0.4	0.2	0.7	0.5	0.3
20:5n-3	10.4	6.7	3.6	11.0	7.4	3.5
22:5n-3	2.0	0.7	0.4	1.2	0.8	0.3
22:6n-3	11.9	8.0	4.6	12.8	8.7	4.3
Total n-3 PUFA[d]	29.3	21.4	15.5	30.3	23.4	15.3
Total PUFA	34.3	31.8	30.7	35.4	33.8	30.2
(n-3) / (n-6)	5.9	2.1	1.0	6.0	2.2	1.0

[a]Includes 15:0, 20:0 & 22:0.

[b]Includes 16:1n-9 & 20:1n-7.

[c]Includes 18:3n-6, 20:3n-6 & 22:4n-6.

[d]Includes 20:3n-3 & 22:4n-3.

Table 3 Growth and performance of Atlantic salmon fed the experimental diets for 12 weeks

Parameter	HP-R0	HP-R30	HP-R60	LP-RO	LP-R30	LP-R60	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Initial Weight (g)	1168.4 ± 32.3	1184.6 ± 16.1	1152.4 ± 21.0	1162.8 ± 24.4	1171.9 ± 14.6	1168.4 ± 24.4			
Final Weight (g)	1711.3 ± 79.8	1772.0 ± 36.5	1784.3 ± 48.5	1721.7 ± 47.6	1760.3 ± 27.0	1767.7 ± 57.6	0.812	0.149	0.894
Final Length (cm)	50.5 ± 0.4	50.9 ± 1.1	50.7 ± 1.0	50.7 ± 0.3	49.9 ± 0.6	51.8 ± 0.6	0.829	0.130	0.077
Mortalities[1]	5	1	2	3	0	5			
FCR	0.86 ± 0.01	0.84 ± 0.02	0.81 ± 0.01	0.86 ± 0.02	0.87 ± 0.04	0.85 ± 0.03	0.077	0.160	0.349
SGR	0.49 ± 0.03	0.52 ± 0.01	0.56 ± 0.01	0.50 ± 0.02	0.52 ± 0.01	0.53 ± 0.03	0.723	0.002	0.128
TGC	4.45 ± 0.27	4.75 ± 0.16	5.13 ± 0.11	4.59 ± 0.20	4.78 ± 0.07	4.87 ± 0.28	0.742	0.005	0.227
PRE	42.32 ± 3.45	42.55 ± 1.16	44.03 ± 1.44	45.56 ± 1.85	46.33 ± 1.63	46.08 ± 5.26	0.045	0.799	0.868
K (%)	1.31 ± 0.01	1.30 ± 0.02	1.32 ± 0.04	1.31 ± 0.06	1.30 ± 0.01	1.34 ± 0.01	0.609	0.274	0.941
VSI (%)	12.03 ± 1.00	12.01 ± 0.69	12.41 ± 0.98	12.57 ± 0.27	11.67 ± 0.28	11.90 ± 0.49	0.749	0.515	0.395
HSI (%)	1.34 ± 0.12	1.32 ± 0.02	1.45 ± 0.20	1.36 ± 0.10	1.36 ± 0.07	1.31 ± 0.07	0.646	0.822	0.371
CSI (%)	0.15 ± 0.03	0.13 ± 0.01	0.13 ± 0.00	0.13 ± 0.01	0.14 ± 0.03	0.12 ± 0.01	0.358	0.556	0.522

All values are mean ± S.D. (n=3).

[1] Total number

Table 4 Proximate composition (g kg^{-1} of wet weight) of whole body from Atlantic salmon fed the experimental diets for 12 weeks

	Start [1]	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60	TWO WAY ANOVA *P*		
								protein	oil	prot x oil
Moisture	666.1 ± 16.5	664.7 ± 12.6	661.7 ± 12.9	665.3 ± 11.5	666.5 ± 6.2	665.8 ± 13.9	650.8 ± 10.9	0.602	0.522	0.350
Protein	176.4 ± 2.2	162.2 ± 4.9	162.1 ± 4.8	162.9 ± 2.5	162.2 ± 2.5	162.2 ± 4.2	162.3 ± 2.0	0.925	0.976	0.983
Lipid	128.9 ± 16.1	150.3 ± 10.8	155.1 ± 6.9	151.9 ± 11.9	145.2 ± 6.7	149.5 ± 13.2	164.5 ± 10.5	0.901	0.250	0.259
Ash	17.5 ± 0.7	14.9 ± 0.3	15.4 ± 1.0	15.0 ± 0.4	16.0 ± 0.7	15.4 ± 1.2	15.1 ± 1.0	0.376	0.672	0.421

All values are mean ± S.D. (n=3).

[1]Values not included in the two-way ANOVA

Table 5 Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of muscle from Atlantic salmon fed the experimental diets for 12 weeks

	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Total Lipid	117.8 ± 8.5	101.9 ± 6.4	102.8 ± 4.1	92.6 ± 13.9	101.7 ± 17.1	110.0 ± 9.6	0.259	0.748	0.057
Fatty acid									
14:0	4.7 ± 0.4	4.1 ± 0.1	3.4 ± 0.3	5.1 ± 0.4	4.3 ± 0.1	3.6 ± 0.4	0.064	0.000	0.739
16:0	16.0 ± 0.8	15.2 ± 0.9	13.5 ± 1.3	16.2 ± 1.4	14.6 ± 1.1	13.3 ± 1.1	0.649	0.004	0.857
18:0	3.1 ± 0.2	3.1 ± 0.3	3.1 ± 0.4	3.1 ± 0.3	2.9 ± 0.3	2.8 ± 0.3	0.299	0.635	0.825
Total saturated[1]	24.5 ± 1.4	23.2 ± 1.5	21.0 ± 2.5	25.3 ± 2.4	22.7 ± 1.7	20.8 ± 2.1	0.971	0.015	0.874
16:1n-7	6.6 ± 0.1	5.5 ± 0.2	4.4 ± 0.1	6.8 ± 0.0	5.6 ± 0.1	4.6 ± 0.1	0.019	0.000	0.474
18:1n-9	15.7 ± 0.5	23.3 ± 1.1	29.7 ± 0.9	15.9 ± 0.3	22.9 ± 0.4	27.9 ± 1.9	0.197	0.000	0.340
18:1n-7	3.2 ± 0.1	3.4 ± 0.1	3.1 ± 0.1	3.2 ± 0.2	3.3 ± 0.1	3.1 ± 0.3	0.766	0.070	0.613
20:1n-9	7.8 ± 0.5	7.2 ± 0.3	6.7 ± 0.2	8.0 ± 0.2	7.2 ± 0.2	6.8 ± 0.2	0.577	0.000	0.968
22:1	8.3 ± 0.4	7.4 ± 0.5	6.0 ± 0.6	8.5 ± 0.1	7.3 ± 0.2	6.4 ± 0.4	0.573	0.000	0.566
24:1n-9	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.0	0.407	0.002	0.531
Total monoenes[2]	42.9 ± 1.2	48.0 ± 1.4	51.0 ± 0.5	43.8 ± 0.3	47.4 ± 0.2	49.9 ± 1.0	0.426	0.000	0.270
18:2n-6	3.7 ± 0.1	6.3 ± 0.1	8.2 ± 0.9	3.6 ± 0.1	6.3 ± 0.5	8.2 ± 0.6	0.922	0.000	0.942
20:2n-6	0.4 ± 0.0	0.5 ± 0.0	0.8 ± 0.2	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.179	0.000	0.076
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.870	0.274	0.481
20:4n-6	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.843	0.000	0.756
Total n-6 PUFA[3]	5.1 ± 0.1	7.5 ± 0.2	9.6 ± 0.8	5.0 ± 0.2	7.6 ± 0.6	9.5 ± 0.7	0.817	0.000	0.912
18:3n-3	1.2 ± 0.0	2.2 ± 0.1	3.0 ± 0.4	1.2 ± 0.1	2.2 ± 0.2	3.0 ± 0.3	0.743	0.000	0.968
18:4n-3	1.8 ± 0.1	1.3 ± 0.2	1.0 ± 0.1	1.8 ± 0.1	1.4 ± 0.1	1.1 ± 0.0	0.302	0.000	0.418
20:4n-3	1.5 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	0.936	0.000	0.166
20:5n-3	7.4 ± 0.4	5.4 ± 0.9	4.2 ± 0.4	7.2 ± 0.7	5.7 ± 0.3	4.6 ± 0.2	0.485	0.000	0.679
22:5n-3	2.5 ± 0.1	1.9 ± 0.2	1.6 ± 0.2	2.5 ± 0.3	1.9 ± 0.2	1.7 ± 0.0	0.505	0.000	0.734
22:6n-3	12.9 ± 0.9	9.3 ± 1.2	7.5 ± 0.7	11.9 ± 1.1	9.7 ± 0.5	8.2 ± 0.1	0.898	0.000	0.252
Total n-3 PUFA[4]	27.5 ± 1.1	21.4 ± 2.6	18.4 ± 1.9	26.0 ± 2.3	22.3 ± 1.3	19.8 ± 0.5	0.668	0.000	0.422
Total PUFA	32.6 ± 1.2	28.8 ± 2.8	28.0 ± 2.7	31.0 ± 2.6	29.8 ± 1.9	29.3 ± 1.2	0.766	0.081	0.535
(n-3) / (n-6)	5.4 ± 0.1	2.9 ± 0.3	1.9 ± 0.1	5.2 ± 0.2	2.9 ± 0.1	2.1 ± 0.1	0.557	0.000	0.263

Values are mean ± S.D. (n=3).

[1]Includes 15:0, 20:0 & 22:0.

[2]Includes 16:1n-9 & 20:1n-7.

[3]Includes 18:3n-6, 20:3n-6 & 22:4n-6.

[4]Includes 20:3n-3 & 22:4n-3.

Table 6 Total lipid (mg lipid g-1 tissue) and fatty acid compositions (% by weight of total fatty acids) of liver from Atlantic salmon fed the experimental diets for 12 weeks

	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Total lipid	62.7 ± 16.6	49.4 ± 6.6	81.0 ± 37.0	52.5 ± 16.7	54.9 ± 7.9	69.3 ± 16.2	0.422	0.037	0.519
Fatty acid									
14:0	2.4 ± 0.4	1.8 ± 0.0	1.2 ± 0.1	2.7 ± 0.3	1.8 ± 0.3	1.2 ± 0.1	0.543	0.000	0.714
16:0	15.2 ± 1.7	12.6 ± 1.1	10.0 ± 0.9	14.2 ± 1.0	13.2 ± 1.1	9.1 ± 1.0	0.418	0.000	0.450
18:0	6.1 ± 0.6	5.1 ± 0.3	4.8 ± 0.5	5.5 ± 0.1	4.9 ± 0.5	4.0 ± 0.2	0.022	0.000	0.537
Total saturated[1]	24.4 ± 2.5	20.1 ± 1.4	16.5 ± 1.4	23.2 ± 1.2	20.4 ± 1.2	15.1 ± 1.5	0.359	0.000	0.611
16:1n-7	3.7 ± 0.5	2.6 ± 0.2	1.9 ± 0.2	4.1 ± 0.2	2.6 ± 0.3	1.9 ± 0.2	0.327	0.000	0.268
18:1n-9	15.9 ± 4.0	23.0 ± 3.9	35.7 ± 3.9	16.7 ± 2.0	21.3 ± 2.1	37.1 ± 4.8	0.928	0.000	0.729
18:1n-7	3.1 ± 0.3	2.9 ± 0.2	3.1 ± 0.1	3.4 ± 0.3	3.0 ± 0.3	3.2 ± 0.5	0.234	0.300	0.946
20:1n-9	4.4 ± 0.6	4.2 ± 0.8	5.0 ± 0.2	4.7 ± 0.3	4.0 ± 0.5	5.3 ± 0.7	0.692	0.025	0.615
22:1	1.4 ± 0.2	1.1 ± 0.1	0.9 ± 0.0	2.1 ± 0.4	1.4 ± 0.3	1.0 ± 0.0	0.007	0.000	0.238
24:1n-9	1.0 ± 0.3	0.9 ± 0.2	0.6 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	0.737	0.011	0.299
Total monoenes[2]	29.8 ± 5.2	35.0 ± 5.1	47.3 ± 4.0	32.1 ± 2.8	33.6 ± 3.4	49.3 ± 6.2	0.661	0.000	0.746
18:2n-6	2.1 ± 0.4	4.9 ± 0.5	8.2 ± 0.4	2.5 ± 0.3	4.9 ± 0.5	9.0 ± 0.5	0.124	0.000	0.396
20:2n-6	0.6 ± 0.1	1.3 ± 0.2	2.1 ± 0.1	0.6 ± 0.1	1.2 ± 0.1	2.1 ± 0.2	0.695	0.000	0.600
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.853	0.001	0.215
20:4n-6	2.2 ± 0.5	1.9 ± 0.4	1.0 ± 0.3	1.9 ± 0.2	2.0 ± 0.3	1.0 ± 0.2	0.524	0.000	0.413
Total n-6 PUFA[3]	5.7 ± 0.1	8.6 ± 0.4	11.9 ± 0.3	5.7 ± 0.3	8.7 ± 0.1	12.5 ± 0.4	0.100	0.000	0.094
18:3n-3	0.7 ± 0.1	1.6 ± 0.2	2.7 ± 0.1	0.9 ± 0.1	1.6 ± 0.2	2.9 ± 0.1	0.047	0.000	0.242
18:4n-3	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.032	0.000	0.879
20:4n-3	1.4 ± 0.2	1.1 ± 0.1	0.8 ± 0.0	1.7 ± 0.1	1.1 ± 0.1	0.8 ± 0.0	0.208	0.000	0.096
20:5n-3	10.4 ± 1.8	8.8 ± 1.3	5.2 ± 0.9	10.3 ± 0.8	9.4 ± 0.8	4.9 ± 1.4	0.921	0.000	0.808
22:5n-3	3.3 ± 0.7	2.9 ± 0.3	1.5 ± 0.2	3.7 ± 0.3	2.5 ± 0.2	1.4 ± 0.3	0.671	0.000	0.370
22:6n-3	23.7 ± 4.6	21.2 ± 3.4	13.4 ± 3.1	21.9 ± 2.1	22.0 ± 2.0	12.1 ± 3.7	0.621	0.000	0.763
Total n-3 PUFA[4]	40.2 ± 5.9	36.3 ± 4.1	24.3 ± 4.2	39.0 ± 3.1	37.3 ± 2.3	23.0 ± 5.2	0.814	0.000	0.877
Total PUFA	45.9 ± 6.0	45.0 ± 3.7	36.2 ± 4.2	44.7 ± 3.1	46.0 ± 2.1	35.5 ± 4.8	0.899	0.002	0.893
(n-3) / (n-6)	7.0 ± 1.0	4.2 ± 0.6	2.1 ± 0.4	6.9 ± 0.7	4.3 ± 0.3	1.8 ± 0.5	0.757	0.000	0.911

Values are mean ± S.D. (n=3).

[1]Includes 15:0, 20:0 & 22:0.

[2]Includes 16:1n-9 & 20:1n-7.

[3]Includes 18:3n-6, 20:3n-6 & 22:4n-6.

[4]Includes 20:3n-3 & 22:4n-3.

Table 7 Total β-oxidation capacity (pmol/min/mg protein) of muscle and liver from Atlantic salmon fed the experimental diets for 12 weeks

Tissue	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Muscle[1]	0.49 ± 0.02	0.61 ± 0.10	0.57 ± 0.12	0.48 ± 0.09	0.45 ± 0.07	0.49 ± 0.07	0.062	0.575	0.340
Liver	3.28 ± 1.19	4.72 ± 1.33	5.13 ± 1.77	3.86 ± 0.16	4.09 ± 0.61	4.42 ± 0.79	0.631	0.196	0.544

Values are mean ± S.D. (n=3).
[1]Includes red and white muscle