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Interactive effects of dietary protein / lipid level and oil source on growth, feed utilisation and nutrient and fatty acid digestibility of Atlantic salmon

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Abstract

Although the use of fish meal (FM) and fish oil (FO) has been extensive in Atlantic salmon culture, there is a growing need for less reliance on these commodities. Moreover, it is crucial for the aquafeed industry to optimise the use of dietary protein and to improve the protein utilisation in salmon diets. The interactive effects of the dietary protein/lipid level and rapeseed oil (RO) inclusion on growth, feed utilisation, nutrient and fatty acid (FA) digestibility and whole body chemical composition of large Atlantic salmon (*Salmo salar* L.), reared at summer water temperatures (11.6 ^{o}C), were investigated in a ten week feeding trial. The fish (initial

weight 2053g) were fed six isoenergetic diets in a factorial design containing 350 g kg^{-1} / 350 g kg^{-1}, 330 g kg^{-1} / 360 g kg^{-1}, 290 g kg^{-1} / 380 g kg^{-1} of protein / lipid for high protein (HP), medium protein (MP) and low protein (LP) diets, respectively. At all protein/lipid levels the oil source was either FO or RO (60% of the added oil). At the end of the trial the final weights ranged from 3340 – 3664g and the FCR from 0.99 – 1.10. The protein level did not affect significantly any of the growth parameters but the oil source had a significant effect on final weight, specific growth rate (SGR) and thermal growth goefficient (TGC), showing improved growth with RO inclusion. This could be explained by the significantly higher lipid digestibility of the fish fed the diets containing RO (86.1 vs. 92.2%) which was probably affected by the diet FA composition; the apparent digestibility coefficient (ADC) of saturated FA, and to a lesser extent of unsaturated FA and especially monoenes, was improved by RO inclusion. The protein ADC was significantly affected by the protein level indicating a higher ADC for the HP diets compared to the LP (80.1 vs. 77.7%, respectively). Regarding the whole body composition, moisture was significantly affected by both factors, the fat content was significantly affected only by the oil source, while significant interactions were shown for the protein content. In conclusion, the results of this study suggest that low protein / high lipid diets can be used with no negative effects on the growth, FCR and chemical composition of Atlantic salmon reared at high water temperatures. Moreover, the replacement of FO with RO can enhance the growth of the fish as well as the nutrient and FA digestibility of the diets.

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

1. Introduction

The use of marine-derived fish meals (FM) and fish oils (FO), as the key sources of protein and lipid, has been extensive in carnivorous fish nutrition over the past 30 years. However, as aquaculture, and particularly salmon culture, continues to grow rapidly, with a consequent growth in the demand for FM and FO, the need for less reliance on these commodities is also increasing. This is due to various reasons; namely, the lack of sustainable provision of FM and FO, the current and future increase in their price within the next few years, as the global production remains static or is reduced (Delgado, et al., 2003; Tacon and Metian, 2008; Trushenski, et al., 2006), and lastly the potential risk of contamination of FM and FO with organic pollutants, such as dioxins and PCBs, which can consequently affect the quality of the final product (Bell and Waagbø, 2008; SCAN, 2000; Sprague, et al., 2010).

Continuous development of salmon feeds has led to the use of energy dense diets containing low protein/ high lipid ratios (Einen and Roem, 1997; Hemre and Sandnes, 1999; Solberg, 2004). Low protein/high lipid diets are of potential benefit as they require less protein and consequently, less FM, however, such feed formulations shall potentially increase the requirements of aquaculture for FO; hence the need for sustainable oil alternatives is again emerging.

Low protein / high lipid diets in Atlantic salmon nutrition do not affect negatively the growth and feed utilization of the fish, while the sparing of protein by increased dietary oil has also been reported (Azevedo, et al., 2004a,b; Bendiksen, et al., 2003b; Einen and Roem, 1997; Hillestad, et al., 1998; Solberg, 2004). However, most of these studies used diets with relatively high dietary protein and low lipid contents. Hence, how much the dietary protein/lipid level can be further reduced is not currently known.

In terms of FO replacement, rapeseed oil (RO) has been widely used in diets for Atlantic salmon and other salmonids with generally no detrimental effects on growth and feed conversion ratio (FCR) (Bell and Waagbø, 2008; Glencross, 2009; Turchini, et al., 2009) while, notably, in some cases the use of RO was reported to enhance growth (Bendiksen, et al., 2003b; Karalazos, et al., 2007; Torstensen, et al., 2005).

It should be noted that although numerous studies have focused either on the investigation of sustainable alternatives to FM and FO or in the reduction of the dietary protein/lipid level very few have investigated the interactive effects such dietary changes may have on growth, feed utilization, nutrients and fatty acid (FA) digestibility and tissue chemical composition (Bendiksen, et al., 2003a,b; Karalazos, et al., 2007). Moreover, recent reviews have indicated that the concurrent reductions of FM and FO in fish diets could prove challenging (Bell and Waagbø, 2008; Glencross, 2009; Turchini, et al., 2009); however the knowledge on this area is limited.

In a previous study on the interactive effects of the dietary protein/fat ratio and the inclusion of RO, at the expense of FO, in the diets of salmon in cold (4.2 ± 0.8 °C) water temperatures a protein sparing effect was shown due to the RO inclusion, whereas growth was not negatively affected by low protein levels (Karalazos, et al., 2007). Given that, temperature plays a significant role in fish nutrition, nutrient metabolism and digestibility (Bendiksen, et al., 2003b; Bendiksen and Jobling, 2003; Ng, et al., 2004; Ruyter, et al., 2006) it became clear that further research was needed to assess the impact of even lower protein/lipid ratios and FO replacement, in Atlantic salmon reared at higher (summer) water temperatures.

Hence, the aim of this study was to investigate the interactive effects of three different, decreasing, protein/lipid ratios and the replacement of FO with RO, in a factorial two-way experimental design, on growth, feed utilization, whole body chemical composition and nutrients and FA digestibility of large Atlantic salmon at high water temperatures.

2. Materials and methods

2.1 Fish and facilities

Triplicate groups of Atlantic salmon (*Salmo salar*), in eighteen sea cages at the Fjord Research Station AS (Helgeland, Dønna, Norway, 66^{o}N), were used for the present trial. The fish were acclimatised to the trial cages for 63 days at 12^{o}C. During this period the fish were fed a commercial diet from BioMar AS (9 mm; declared composition 360 g kg^{-1} protein and 350 g kg^{-1} fat) according to the producer's recommendations. The sea cages were of 125 m^{3} (5x5x5m) with approximately 93 fish, of initial mean weight of 2053g, randomly distributed in each one. The average water temperature during the 10 week experimental period was 11.6 ± 1.1 $^{\circ}$C and the salinity 32.5 ± 0.4 g L^{-1}. During the experimental period the fish were subjected to natural photoperiod. Mortalities were recorded and dead fish were removed daily.

2.2 Experimental diets and feeding

The six experimental diets were produced at the BioMar TechCentre (Brande, DK) as practical-type extruded pellets (9 mm). The diets were formulated at three different dietary protein/fat levels. Specifically, the diets contained 350 g kg^{-1} / 350 g kg^{-1}, 330 g kg^{-1} / 360 g kg^{-1}, 290 g kg^{-1} / 380 g kg^{-1} of protein / lipid for high protein (HP), medium protein (MP) and low protein (LP) diets, respectively. Within each dietary protein/fat level the oil source was either FO or RO; in the RO diets crude RO

comprised 60% of the total added oil, the remainder of which was FO. The diets were isoenergetic with a gross energy content of 25 kJ g^{-1}. The formulations and the proximate compositions of the experimental diets are shown in Table 1 and the FA compositions are shown in Table 2. The diets were formulated to meet all known nutritional requirements of salmonid fish (NRC, 1993). All diets contained yttrium trioxide (Y_2O_3) as an inert marker to determine the apparent digestibility coefficients (ADC) of nutrients and FA. Each feed was fed daily to satiation by hand to triplicate groups (cages) of fish. Two daily meals were provided with a minimum of 4 hours between the meals. Uneaten feed was collected using a lift-up system and calculated on a daily basis in order to facilitate accurate calculations of feed intake and FCR.

2.3 Sampling procedure

At the start of the trial the fish were bulk weighed. An initial sample of six fish was taken to determine baseline values of whole body proximate composition. At the end of the trial (10th week) the fish were anaesthetized in MS-222 (metacain, 8mg/L) and individually weighed. Three fish per cage were selected at random from each cage to determine whole body proximate composition. The fish were killed with a sharp blow to the head. Fish were minced and homogenate sub-samples of each fish were obtained. The initial samples were pooled in pairs so three samples were finally obtained (n = 3) whereas the homogenates of the final sampling were pooled providing finally one sample per cage.

At the end of the trial faeces samples were obtained by stripping according to Austreng (1978). Fish were fed before sampling. The fish were anaesthetized and faeces were collected by gently squeezing the hindgut of the fish. One sample of faeces per cage was finally obtained by pooling faeces from an appropriate, varying number of fish from each cage until a minimum weight of 150 g (wet weight) of

faeces was collected. Faeces were separated by hand from other material i.e. excess water, urine and fish scales. Ethoxyquin (ETQ; 400 mg/L, 1 mL/60g wet faeces) was added to each sample which were then stored at -20 °C. The faeces samples were freeze dried prior to analysis. The freeze dried samples were also kept at -20 °C until further analysis. Lastly, samples were taken from the six experimental diets and stored at -20 °C until analyzed.

2.4 Proximate analysis

The nutrient compositions (moisture, crude protein, crude fat and ash content) of the six experimental diets and the whole body samples were determined by proximate analysis (thermal drying, Kjeldahl analyses, Soxhlet extraction and dry ashing, respectively) based on methods described in Karalazos et al. (2007). The gross energy content of diets and faeces was determined using an adiabatic bomb calorimeter using the Gallenkamp Autobomb system. Yttrium oxide (Y_2O_3) was determined by a spectrophotometric method after acid digestion. The diet or faeces samples were digested in concentrated nitric acid and then analysed by inductively coupled plasma-optical emission spectrometry (ICP-OES). All methods were based on those described in AOAC (1995).

2.5 Lipid extraction and fatty acid analysis

Extraction of total lipids of diets and faeces samples and preparation of fatty acid methyl esters (FAME) from total lipid were carried out as described in Karalazos et al. (2010). 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, UK) (Karalazos et al., 2010).

2.6 Calculations

Feed Conversion Ratio (FCR) was calculated as: feed intake (g) x wet weight gain^{-1} (g), Specific Growth Rate (SGR, %/day) as: 100 x [lnW_1 - lnW_0] x (days) $^{-1}$ and the Thermal Growth Coefficient (TGC, x 1000) as: 1000 x [$(W_1)^{1/3}$ - $(W_0)^{1/3}$] x (days x Temp. ^{o}C)$^{-1}$, where W is the weight of the sampled fish in grams, W_0 and W_1 are the initial and the final fish mean weights in grams. The Protein Productive Value, (PPV) was calculated as g protein gain x g protein ingested^{-1}, that is: (P_1W_1−P_0W_0) x (P_F x cumulative feed intake) $^{-1}$, where P_0 and P_1 are the initial and final protein concentrations of the fish, P_F is the protein concentration of the feed on a dry matter basis, and cumulative feed intake was determined in grams on a dry matter basis.

The %ADC (Apparent Digestibility Coefficient) of nutrients and FA was calculated as: 100 x [1 - (F x D^{-1}) x (Di x Fi^{-1})], where F is the concentration of the nutrient or FA (or kJ/g gross energy) in the faeces, D is the concentration of the nutrient or FA (or kJ/g gross energy) in the diet, Di is the concentration of the inert marker (Y_2O_3) in the diet and Fi is the concentration of the inert marker in the faeces.

2.7 Statistical analysis

The effects of the protein/fat ratio (protein level), dietary RO inclusion (oil source) and their interactions on growth, whole body proximate composition and ADC of nutrients and individual FA were analysed by factorial (two-way) ANOVA. When significant interactions of the two factors were observed, the main effects were not further discussed but instead, multiple comparison testing was performed to look at the simple main effects, that is the main effect of one factor at a given level of the other (Zar, 1999). The analysis of the simple main effects was done for both factors. Data which were identified as non-homogeneous (Levene's test) were subjected to square root, log or arcsin transformation before analysis. Differences were regarded as

significant when $P < 0.05$ (Zar, 1999). All the data are presented as means ± SD (n = 3) and all statistical analyses were performed using SPSS 14.0 (SPSS Inc, 2005). The graphs were created using Prism 4 (Graphpad Software Inc., San Diego, USA).

3. Results

3.1 Diet composition

The analysed proximate composition of the six experimental diets is shown in Table 1. All diets were isoenergetic with a gross energy value of 25.3 ± 0.1 (overall diet mean ± SD) kJ g^{-1}. The Digestible Protein / Digestible Energy (DP/DE) ratio was 14.5, 13.5 and 12.3 for the HP, MP and LP diets, respectively. DP and DE were calculated using the ADC values for protein and energy found in this trial (Table 4). The oil source of the diets was either 100% FO or a blend of 40% FO and 60% RO resulting in two different total lipid FA profiles (Table 2). Specifically, the inclusion of RO resulted in the reduction of 16:0, and consequently of the total saturated FA, by half compared to the FO diets. The total monoenes almost doubled in the RO diets, largely due to the high amount of 18:1n-9. The total n-6 PUFA in the RO diets increased 3-fold, mainly as 18:2n-6. EPA (20:5n-3) decreased by 70% and DHA (22:6n-3) by more than half and hence the total n-3 PUFA in the RO diets were reduced by half compared to the FO diets. The n-3/n-6 PUFA ratio was 7 and 1.2 for the FO and RO diets, respectively.

3.2 Growth

The initial mean weight of the fish was 2053.3 ± 25.4g (overall cage mean ± SD). After feeding the fish with the experimental diets for 10 weeks their weight ranged from 3340.2g to 3664.2g, for HP-FO and MP-RO, respectively (Table 3). Two way ANOVA showed a significant effect due to the oil source (P=0.032) with the fish

fed the RO diets having a significantly higher final weight compared to the FO diets. The same significant effect due to the oil source favouring the RO group was also observed in both SGR and TGC (0.87 vs. 0.94 and 3.46 vs. 3.81, respectively) which represented aprox. 8% increase in growth rate for the RO groups compared to the FO groups irrespective of the dietary protein level. The protein level had no significant effects on any of the growth parameters although a trend towards significance (P=0.085) was noted for lower final weight with decreasing protein level. In addition, no significant interactions between the two factors (protein level and oil source) were observed. Lastly, FCR ranged from 0.99 to 1.10 but was not significantly affected by the dietary treatments. The PPV ranged from 0.38 to 0.47 and the two-way ANOVA showed a significant effect both due to the protein level and the oil source. Specifically, the LP diets had significantly higher PPV than the other two groups (0.41, 0.43 and 0.47, for HP, MP and LP, respectively). The dietary inclusion of RO resulted in higher PPV compared to the FO groups (0.42 vs. 0.46, for FO and RO, respectively).

3.3. Apparent nutrient digestibility

The nutrient ADC are shown in Table 4. Significant interaction between dietary protein level and oil source was shown for the DM ADC. The LP diets had significantly lower DM ADC compared to HP and MP diets for both oil sources, while RO diets showed a significantly higher DM ADC for the HP diets only (Figure 1a). Significant interactions were also observed for energy ADC (Figure 1b). Specifically, MP groups had significantly higher energy ADC than HP and LP for the FO diets, whereas LP treatment showed a significantly lower energy ADC than HP and MP for the RO group. A significant difference between FO and RO energy ADC was shown only in the HP group. The protein ADC was only affected by the dietary

protein level; specifically, the increase of dietary protein level resulted in increased protein ADC. As there were no interaction effects, the means are represented as two parallel lines in Figure 1c. Lastly, there was a significant interaction of dietary protein level and oil source for fat ADC (Figure 1d). For the FO treatments HP had a significantly lower fat ADC compared to MP an LP, while there were no differences in the RO groups. RO resulted in significantly higher fat ADC at all protein levels.

3.4. Fatty acid apparent digestibility

The digestibility of each individual FA for each dietary treatment and the effects and interactions of the protein level and oil source are shown in Table 5. In general, the ADC of the individual FA decreased with increasing FA chain length. On the other hand, increasing the number of double bonds increased the FA ADC. For most of the saturated FA, ADC showed significant interactions between the protein level and oil source, hence, the simple main effects of the two factors were tested. For instance, for 16:0 (Figure 2a) HP diets had a significantly lower ADC compared to MP and LP for both oil sources while for the total saturates ADC (Figure 2b) the same effect was shown for FO only. RO dietary inclusion resulted in significantly higher ADC at all protein levels for 16:0 but only for HP for total saturates. Significant interactions were observed for the shorter chain monoenes (16 and 18 carbon atoms) and the total monoenes (Figure 2c, d and e). In general, the MP diet had higher ADC for the FO treatments while no significant effect due to the protein level was shown for the RO treatments. RO resulted in higher ADC at all protein levels, apart from the MP groups for 16:1n-7. The ADC of 20:1n-9. 22:1 and 24:1n-9 was significantly higher for RO compared to FO, and LP resulted in lower ADC. Protein level and oil source had significant effects on the ADC of 18:2n-6, 18:3n-3 and 18:4n-3 (MP > HP > LP and RO > FO). A significant interaction was shown for the ADC of the total n-6

PUFA (Figure 2f). EPA ADC was significantly affected only by the protein level (MP > HP > LP). On the contrary, DHA was significantly affected only by the oil source resulting in higher ADC for the FO groups compared to the RO. No significant effects were shown for the total n-3 PUFA ADC.

3.5. Whole body proximate composition

The initial proximate composition of the fish whole body was 36.1% DM, 17.2% crude protein, 16.2% crude fat and 1.8% ash (Table 6). At the end of the experimental period the DM varied from 38.6% to 40.7% and was significantly affected both by the protein level and the oil source (LP > HP, MP and FO < RO). Significant interactions between the two factors were shown for the protein content (15.8% - 16.6%) and the analysis of the simple main effects showed that for the FO groups decreased dietary protein resulted in lower whole body protein content, while no differences were shown for the RO groups. For the HP groups FO resulted in higher protein content, for the MP there were no differences between FO and RO, while for the LP groups RO had the highest protein content. The fat content was significantly affected by the oil source with RO groups having a higher fat content than the FO groups (21.3% vs. 19.4%) but was not influenced by the dietary protein level. No significant effects were shown on the ash content of the whole body, which varied from 1.6% – 1.8%.

4. Discussion

4.1. Growth

Previous studies have suggested that Atlantic salmon can grow efficiently when fed diets with high dietary fat and low protein content (Azevedo, et al., 2004a,b; Einen and Roem, 1997; Solberg, 2004). Such results were especially shown at low

water temperatures (Bendiksen, et al., 2003b; Hillestad, et al., 1998; Karalazos, et al. 2007), while at higher temperatures better growth has also been shown for fish fed low fat diets (Bendiksen, et al., 2003b) . However, most of the previous studies used high fishmeal feeds, designed for smaller fish, with higher dietary protein and lower lipid contents than the present study, ranging from greater than 500 g kg^{-1} to a minimum of approximately 340 g kg^{-1}, and varying lipid levels of as low as 200 g kg^{-1} up to 470 g kg^{-1}. In the present study the diets had a much lower protein/lipid ratio , ranging from 350/350 to 290/380 g kg^{-1}, or in terms of DP/DE from 15.4 to 12.3 and were fed to large Atlantic salmon (initial weight 2053g) at high water temperatures. Therefore, it is noteworthy that the reduced protein level had no significant negative effects on the final weight of the fish, SGR, TGC and FCR, while performance was very good for all treatments, even at protein levels below 300 g kg^{-1}. This suggests that fish can efficiently utilize high amounts of lipid for energy and tolerate relatively low dietary protein levels at high water temperatures, which could be of great significance for the Atlantic salmon industry.

In addition, a significant difference between the dietary treatments was shown on growth due to oil source. Specifically, the replacement of 60% of the dietary FO by RO resulted in higher final weight, SGR and TGC, but not FCR. Numerous previous studies on Atlantic salmon have concluded that the partial replacement of FO with RO or other vegetable oils has no negative effect on growth and FCR (Bell, et al., 2001; Bell, et al., 2003; Bell and Waagbø, 2008; Glencross, 2009; Rosenlund, et al., 2001; Torstensen, et al., 2004; Torstensen, et al., 2005; Turchini, et al., 2009). Nevertheless, the positive effect of RO on growth of juvenile and grower Atlantic salmon has also been shown previously (Bendiksen, et al., 2003b; Karalazos, et al., 2007) and a higher growth for larger Atlantic salmon fed with diets with a low level of n-3 FA has also

been reported (Menoyo, et al., 2003; Torstensen, et al., 2005). In these former studies the authors suggested that the increased growth was due to higher digestibility of the RO, and other vegetable oils, fatty acids, resulting in better utilization of the dietary oil for energy by the fish. The digestibility results of the present study, discussed below, confirm that theory. Moreover, since the effect on FA digestibility of increased chain length and unsaturation index at low rearing temperatures is well documented, the novel finding of improved FA digestibility at higher rearing temperatures in large Atlantic salmon is interesting and may point to an effect of RO not only on digestible function but maybe also on fish metabolism, possibly on FA oxidation level.

The protein sparing effect could also have been enhanced by both the higher dietary oil levels and the RO inclusion. This is supported by the PPV results, as the overall PPV 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 and Roem, 1997; Hillestad, et al., 1998, Karalazos, 2007), while interactions between dietary protein/fat, oil source and temperature may occour (Bendiksen, et al., 2003b). Interestingly, a clear positive effect on PPV was also shown due to the dietary inclusion of RO at the expense of FO. Information about the effects that dietary vegetable oils inclusion may have on protein sparing and the mechanisms involved is scarce. Data presented and discussed in detail in Karalazos et al. (2010) show that in the present trial the β-oxidation capacity was higher in the RO fish tissues compared to the FO ones, supporting the findings on increased PPV, as increased catabolism of FA for energy production may suggest a protein sparing effect.

4.2. Nutrients and fatty acid digestibility

The protein ADC was significantly lowered by the decreasing dietary protein/lipid level. The results of previous studies are contradictory; Einen and Roem (1997) and Azevedo et al. (2004b) reported no significant differences, whereas Bendiksen et al. (2003b) found that protein ADC was decreased when low protein / high fat diets were used. The latter also reported a positive effect of the VO on protein ADC, although such an effect was not shown in the present trial.

Nevertheless, lipid ADC was significantly higher for the RO treatments, compared to the FO, at all dietary protein levels. The lipid digestibility has been correlated to the dietary FA composition and the positive effect of the VO and especially RO has been reported previously (Bendiksen, et al., 2003b; Caballero, et al., 2002; Menoyo, et al., 2003; Ng, et al., 2004). In agreement with previous studies (Azevedo, et al., 2004b; Einen and Roem, 1997) the dietary protein level did not affect the lipid ADC, at least for the RO diets. However, in the FO treatments the lipid ADC of the HP diet was significantly lower compared to the MP and LP. This was probably due to the higher saturated FA content of this diet, the digestibility of which has been negatively correlated to their dietary level (Caballero, et al., 2002) and is also confirmed in the present study.

In line with previous studies (Azevedo, et al., 2004b), the energy ADC of the LP diets was lower for both oil sources, probably because of the respective lower protein ADC. Moreover, the lower lipid ADC of the HP-FO diet apparently reflected on its energy ADC. Lastly, RO inclusion resulted in higher energy ADC only for the HP diets, which could be due to the large difference in the two diets lipid ADC and more specifically in the ADC of individual FA, such as 16:0 and 18:1n-9, which are preferentially utilized for energy production by Atlantic salmon (Henderson and

Sargent, 1985). In contrast to these results, Ng et al. (2004) found no significant differences in the energy ADC in Atlantic salmon fed diets containing different amounts of palm oil; however, the diets used in that trial did not significantly differ in their levels of 16:0, 18:1n-9 or, generally, in total saturates and monoenes.

Previous studies have reported on the individual FA ADC in diets of salmonids (Caballero, et al., 2002; Menoyo, et al., 2003; Ng, et al., 2004; Torstensen, et al., 2000) suggesting that the digestibility of FA decreases with increasing chain length and saturation. This is in agreement with the results of the present study. Moreover, the digestibility of saturated FA, and to a lesser extent of unsaturated FA and especially monoenes, has been negatively correlated largely with their dietary level but also with dietary n-3 PUFA levels (Caballero, et al., 2002; Menoyo, et al., 2003; Ng, et al., 2004; Torstensen, et al., 2000). These authors suggested that at high levels of saturated FA, emulsion or micelle formation is impaired, although low levels are easily digested. In the current study, the ADC of 14:0 and 16:0 was significantly higher for the RO treatments compared to FO groups, which may be explained by the level of saturated FA in the RO diets being approximately half that in the FO diets. However, for the higher chain length saturated FA and the total saturates the difference between the oil sources was significant only for the HP treatments, resulting in very low total saturates ADC for the HP-FO group, probably due to the high content of total saturates and especially 16:0 in this diet. The RO diets had a significantly higher total monoene ADC than the FO diets, at all protein levels, suggesting a negative correlation between the dietary saturated FA content and the ADC of the monoenes. The protein level had no significant effect on the RO treatments but resulted in higher monoenes ADC for the MP - FO group. Taking into account that saturated FA and monoenes, largely 16:0 and 18:1n-9, are preferred

substrates for β-oxidation the significant increase in their ADC due to the RO apparently influenced positively the energy utilization and consequently enhanced the growth of the fish. It should be noted that RO diets had a much higher (approximately 4-fold) content of 18:1n-9 compared to the FO diets.

The n-6 and n-3 PUFA ADC were affected by the dietary treatments to a much smaller extent compared to the monoenes and saturated FA. The ADC of 18:2n-6 and 18:3n-3 was higher for the RO diets, while they were lower for the LP groups. Although, the rest of the n-6 PUFA were not affected by any of the factors, significant interactions were shown for total n-6 PUFA, similar to the monoenes. Again, this is probably because of the saturated FA levels in the diets influencing the digestibility of PUFA (Menoyo, et al., 2003). Lastly, ADC for EPA and DHA appeared more stable than most of the other FA with the former being affected by the protein level (EPA ADC order was MP ≥ HP ≥ LP), while DHA was significantly higher for the FO diets. It should be pointed out that DHA was the only FA for which the ADC was positively affected by the FO content of the diet. These results are in line with previous studies, as EPA and DHA ADC have been shown to be either higher for the FO diets or not affected by dietary vegetable oils inclusion (Caballero, et al., 2002; Ng, et al., 2004; Torstensen, et al., 2000). Menoyo et al. (2003) reported that EPA ADC was affected by the dietary saturated FA, while DHA ADC was affected by both the saturated FA and n-3 PUFA dietary levels.

4.3. Whole body proximate composition

Although the chemical composition of the fish body is influenced by the dietary composition this effect is in many cases only to a small extent. Previous studies, using either isoenergetic diets (Azevedo, et al., 2004a,b; Solberg, 2004) or diets with a respective increase in the energy content (Bendiksen, et al., 2003b; Einen

and Roem, 1997; Einen and Skrede, 1998; Hillestad and Johnsen, 1994), have shown that increasing dietary oil levels results in increased body lipid and usually DM content, in the fish body. However, the protein content is often decreased or in some cases unaffected and the ash content is also largely unaffected. In a previous study, with Atlantic salmon reared at low water temperatures it was reported that the dietary protein/lipid ratio did not affect the whole body chemical composition (Karalazos, et al., 2007). In the present study, carried out at higher water temperatures, the DM content was significantly affected by the protein level, resulting in higher DM content for the LP groups, whereas there was no significant effect on the lipid and ash of the carcass. However, a significant interaction was shown for the whole body protein, suggesting a decrease with decreasing dietary protein levels.

In agreement with previous studies, the present study demonstrated that the replacement of FO with RO in the diets of Atlantic salmon affected the whole body chemical composition (Bell, et al., 2001; Ruyter, et al., 2006; Torstensen, et al., 2004), although no effects on body composition due to use of vegetable oils have also been reported (Bendiksen, et al., 2003b; Karalazos, et al., 2007). Specifically, RO dietary inclusion resulted in increased DM and lipid content which may indicate a positive relationship between body fat contents and fish weight and/or growth rates, since RO fed fish grew better compared to FO fed fish. The protein content was higher for the FO groups at the HP level, not affected at the MP level and higher for the RO at the LP level. The latter suggests that RO could have a positive affect in the use of protein for growth when the fish are fed with low protein diets.

5. Conclusions

In conclusion, the results of this study suggest that firstly, low protein / high lipid diets can be used with no negative effects on the growth, FCR and chemical composition of Atlantic salmon reared at high water temperatures. Moreover, the partial replacement of FO with RO can enhance the growth of the fish as well as the nutrient and FA digestibility of the diets, showing a protein sparing effect. Understanding the mechanism for the putative positive effects of optimising oil unsaturation on protein utilisation has potentially large commercial implications and requires further investigation. Notably, significant interactions were shown between the dietary protein/lipid level and the RO inclusion especially for nutrients and FA digestibility underlying the importance of both factors on any changes in the diets for Atlantic salmon.

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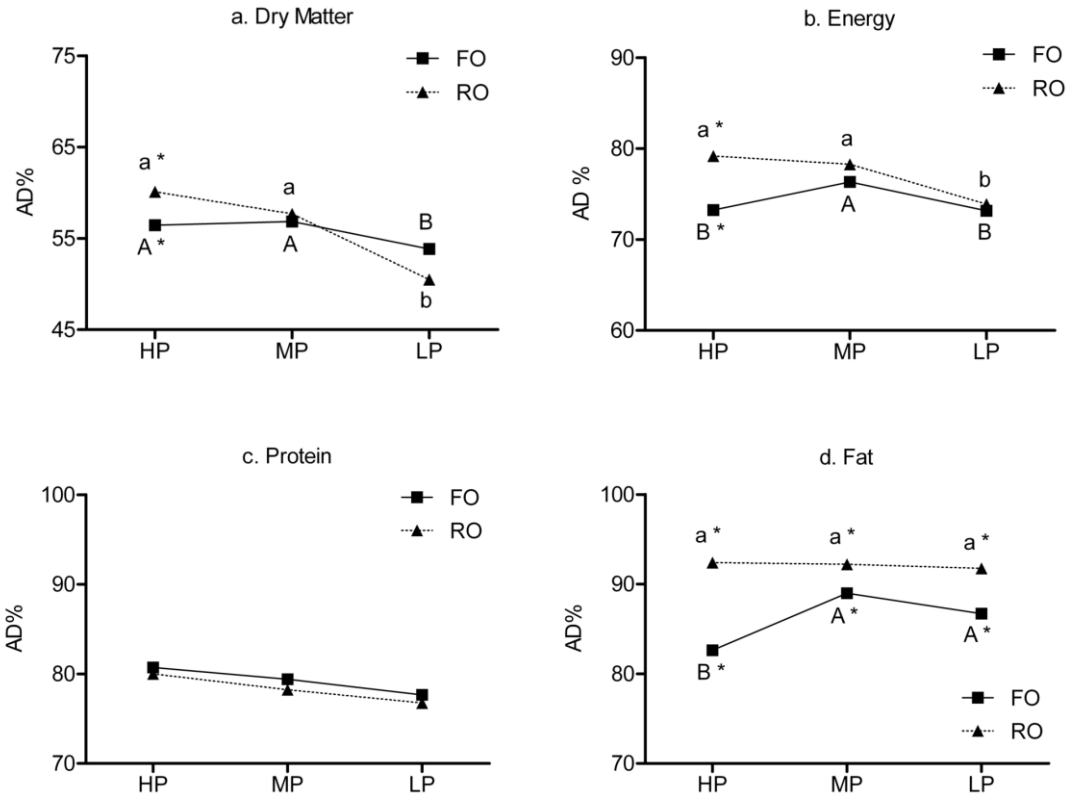

Figure 1

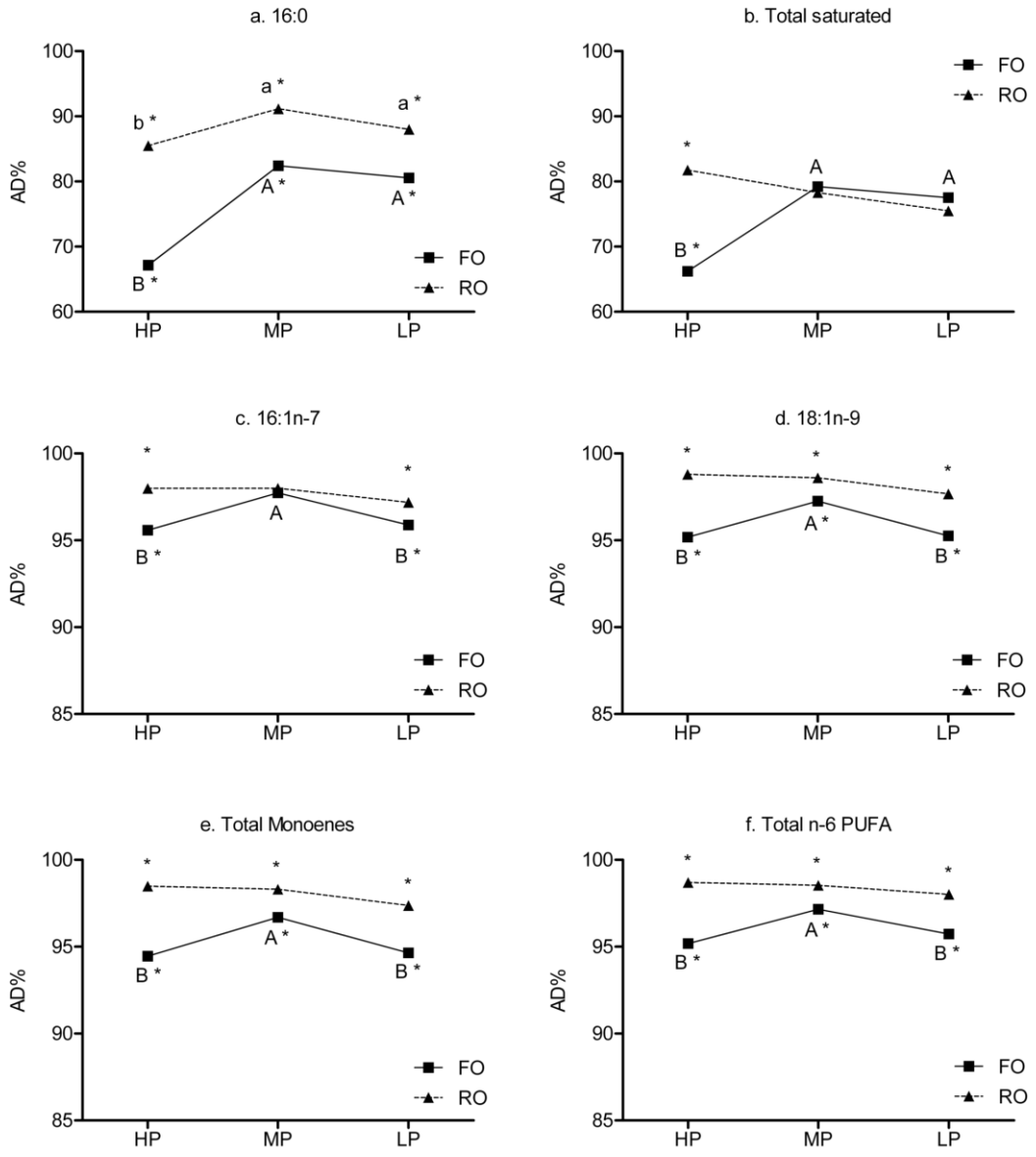

Figure 2

Table 1. Feed formulations and proximate compositions (g kg^{-1} on wet weight basis) of the experimental diets.

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO
Components						
Fishmeal[a]	402	340	268	402	340	268
Oil seed and legume seed meals	181	190	190	181	190	190
Binder	135	130	190	135	130	190
Fish oil	304	330	351	122	132	141
Rapeseed oil[b]	0	0	0	182	198	211
Premixes[c]	9	10	11	9	10	11
Analyzed composition						
Moisture	49	69	69	51	73	67
Dry Matter	951	931	931	949	927	933
Protein	353	338	291	345	328	296
Lipid	350	349	386	351	368	382
Ash	81	75	63	79	73	63
Gross Energy[d]	25.25	25.22	25.32	25.47	25.41	25.36
DP/DE[e]	15.4	14.0	12.3	13.7	13.0	12.3

[a] South-american, Anchoveta oil

[b] European, non-GM, double-low quality rapeseed oil

[c] Vitamin and mineral premixes prepared according to BioMar A/S commercial standards. Includes crystaline amino acids and Carophyl pink to provide 40mg/kg astaxanthin (DSM Roche, Basel, Switzerland)

[d] kJ/g

[e] Digestible Protein/Digestible Energy ratio was calculated using the ADC values for energy and protein found in the present study and presented in the Results section

HP, high protein; MP, medium protein; LP, low protein; FO, fish oil; RO, rapeseed oil.

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

Fatty Acid	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-R0
14:0	8.8	8.5	8.3	3.4	3.0	2.8
16:0	23.2	20.3	20.2	12.1	10.9	10.2
18:0	5.9	4.9	5.0	3.8	4.3	3.5
20:0	0.6	0.5	0.5	0.7	0.8	0.7
22:0	1.3	1.2	1.2	1.9	2.5	1.7
Total saturated[a]	40.4	35.9	35.7	22.0	21.7	19.1
16:1n-7	8.0	8.8	8.7	3.3	3.1	3.0
18:1n-9	9.5	10.9	11.3	37.7	37.7	40.0
18:1n-7	3.2	3.3	3.4	3.7	3.0	3.1
20:1n-9	1.5	1.4	1.3	1.6	1.5	1.5
22:1	1.7	1.7	1.5	1.3	1.1	0.9
24:1n-9	0.5	0.5	0.6	0.4	0.4	0.3
Total monoenes[b]	24.7	26.8	27.0	47.9	47.1	49.1
18:2n-6	2.1	2.4	2.7	12.5	13.0	13.8
20:2n-6	0.1	0.2	0.2	0.1	0.1	0.1
20:4n-6	1.1	1.1	1.1	0.4	0.5	0.4
22:5n-6	0.3	0.3	0.3	0.1	0.1	0.1
Total n-6 PUFA[c]	4.1	4.6	4.8	13.2	14.0	14.8
18:3n-3	0.6	0.7	0.8	6.2	6.3	6.7
18:4n-3	2.1	2.3	2.2	0.8	0.8	0.7
20:4n-3	0.6	0.7	0.6	0.2	0.2	0.2
20:5n-3	17.6	19.0	18.8	5.8	6.0	5.8
22:5n-3	1.9	2.0	2.0	0.7	0.7	0.6
22:6n-3	8.0	8.0	7.9	3.2	3.2	3.0
Total n-3 PUFA[d]	30.8	32.7	32.5	16.9	17.3	17.1
Total PUFA	34.9	37.3	37.3	30.1	31.3	31.9
(n-3) / (n-6)	7.5	7.1	6.8	1.3	1.2	1.2

[a] Includes 15:0

[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

HP, high protein; MP, medium protein; LP, low protein; FO, fish oil; RO, rapeseed oil.

Table 3. Growth and performance of Atlantic salmon fed the six experimental diets for 10 weeks.

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Start Weight, g	2031.7±8.3	2097.0±9.6	2031.7±6	2065.3±5	2055.7±2	2038.3±6			
End Weight, g	3340.2±0.0	3491.1±0.2	3352.9±0.2	3591.8±0.7	3664.2±0.0	3405.7±1	0.085	0.032	0.483
FCR[a]	1.07±6 0.0	1.10±9 0.0	1.06±5 0.0	0.99±5 0.0	1.02±5 0.0	1.09±1 0.0	0.587	0.262	0.376
SGR[b]	0.86±7 0.2	0.88±7 0.3	0.86±8 0.3	0.95±5 0.2	0.99±3 0.1	0.88±6 0.2	0.262	0.025	0.422
TGC[c]	3.41±9 0.0	3.54±2 0.0	3.44±4 0.0	3.85±5 0.0	4.04±7 0.0	3.53±6 0.0	0.202	0.021	0.414
PPV[d]	0.40±3	0.41±1	0.44±2	0.43±1	0.44±2	0.51±6	0.003	0.003	0.294

All values are mean ± S.D. (n=3)

[a] Feed Conversion Ratio

[b] Specific Growth Rate

[c] Thermal Growth Coefficient

[d] Protein Productive Value

HP, high protein; MP, medium protein; LP, low protein; FO, fish oil; RO, rapeseed oil.

Table 4. Apparent nutrients and energy digestibility (%) in Atlantic salmon fed the six experimental diets for ten weeks.

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Dry Matter	56.4±0.6	56.9±0.3	53.8±1.2	60.1±0.5	57.7±1.8	50.5±4.2	0.000	0.692	0.028
Protein	80.4±0.2	79.7±0.2	78.2±1.1	79.8±0.6	78.9±1.7	77.3±1.8	0.002	0.117	0.946
Lipid	82.3±2.0	89.1±0.7	87.0±1.7	92.3±0.7	92.4±0.9	92.0±1.0	0.005	0.000	0.003
Gross Energy	73.2±0.3	76.3±0.2	73.2±1.5	79.2±0.2	78.3±1.3	73.2±1.5	0.001	0.001	0.011

All values are mean ± S.D. (n=3).

HP, high protein; MP, medium protein; LP, low protein; FO, fish oil; RO, rapeseed oil.

Table 5. Apparent fatty acid digestibility (%) in Atlantic salmon fed the six experimental diets for ten weeks.

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
14:0	80.0±1.5	88.9±4	88.4±8	90.6±1.9	95.0±3	93.6±0.9	0.000	0.000	0.003
16:0	67.1±3.5	82.4±8	80.5±3	85.5±3.3	91.2±1	88.0±1.8	0.000	0.000	0.002
18:0	49.0±6.4	62.1±8	60.3±2	68.6±8	55.1±3	48.7±8.8	0.520	0.931	0.006
20:0	56.2±5.8	59.4±7	58.0±7	78.6±9.7	63.5±3	59.3±8.1	0.080	0.008	0.024
22:0	36.2±1	32.5±8	30.3±5	68.3±3	44.1±6	30.4±3	0.019	0.019	0.087
Total saturated[a]	66.2±3.7	79.2±0	77.5±8	81.8±6.1	78.3±4	75.4±4.3	0.070	0.023	0.001
16:1n-7	95.6±0.4	97.1±1	95.9±8	98.0±0.6	98.0±0	97.2±1.2	0.009	0.001	0.043
18:1n-9	95.2±0.1	97.4±4	95.3±9	98.8±0.4	98.6±1	97.7±1.1	0.006	0.000	0.029
18:1n-7	93.4±0.5	95.0±0	93.8±8	98.1±0.5	97.4±3	96.7±1.1	0.011	0.000	0.006
20:1n-9	93.6±0.2	95.2±6	92.7±1	97.2±0.8	97.0±2	95.9±1.7	0.016	0.000	0.319
22:1	92.7±0.5	94.4±4	91.6±0	95.9±1.1	96.2±4	93.9±1.6	0.002	0.000	0.453
24:1n-9	82.1±1.9	87.2±9	85.0±9	90.9±1.6	92.0±7	88.8±3.5	0.039	0.000	0.135
Total monoenes[b]	94.5±0.3	96.0±3	94.0±9	98.5±0.4	98.0±1	97.4±1.2	0.005	0.000	0.022
18:2n-6	95.2±0.3	96.0±7	94.1±0	98.8±0.4	98.0±1	98.1±0.7	0.012	0.000	0.062
20:2n-6	97.0±2.6	99.1±6	97.2±2	95.9±4.9	98.1±4	94.0±4.7	0.273	0.277	0.736
20:4n-6	97.8±0.6	98.0±4	97.0±8	97.9±0.2	98.0±3	97.9±0.8	0.082	0.975	0.180
22:5n-6	97.7±2.0	99.1±4	96.1±0	98.3±3.0	99.1±5	98.7±2.3	0.384	0.350	0.607
Total n-6 PUFA[c]	95.2±0.5	97.0±7	95.0±7	98.7±0.3	98.0±1	98.0±0.8	0.021	0.000	0.023
18:3n-3	97.1±0.3	98.0±4	96.0±8	99.3±0.3	99.0±0	98.7±0.6	0.015	0.000	0.081
18:4n-3	98.±0.3	99.±0.	97.±0.	99.±0.3	99.±0.	98.±0.4	0.013	0.003	0.082

	2	1 1	8 7	1	1 1	8			
	98.	98. 0.	97. 0.	98.	99. 0.	98.			
20:4n-3	6±1.3	9±2	5±7	8±1.1	5±9	7±1.2	0.166	0.159	0.702
	98.	99. 0.	97. 0.	98.	98. 0.	98.			
20:5n-3	3±0.5	1±2	7±8	9±0.3	7±2	6±0.5	0.045	0.091	0.087
	97.	98. 0.	97. 0.	97.	97. 0.	97.			
22:5n-3	7±0.7	4±3	0±9	9±0.1	7±1	3±0.9	0.058	0.826	0.310
	96.	97. 0.	95. 1.	95.	94. 0.	94.			
22:6n-3	0±1.2	1±6	5±0	7±1.0	4±7	4±1.8	0.391	0.025	0.207
Total n-3	97.	98. 0.	97. 0.	98.	98. 0.	97.			
PUFA[d]	6±0.7	5±3	1±8	4±0.4	0±2	9±0.8	0.098	0.230	0.160
	97.	98. 0.	96. 0.	98.	98. 0.	97.			
Total PUFA	3±0.7	3±3	9±8	5±0.3	3±1	9±0.8	0.064	0.024	0.168

All values are mean ± S.D. (n=3)

[a] Includes 15:0

[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

HP, high protein; MP, medium protein; LP, low protein; FO, fish oil; RO, rapeseed oil.

Table 6. Proximate composition of whole body from Atlantic salmon fed the experimental diets for ten weeks.

	START[a]	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
								protein	oil	prot x oil
Dry Matter	*36.0±1.9*	38.0±7.5	38.0±6.4	39.0±2.3	39.1±4.0	39.0±3.7	40.0±7.5	0.018	0.004	0.468
Protein	*17.0±2.3*	16.0±2.2	16.0±1.3	15.0±1.8	16.0±2.2	16.0±1.3	16.0±4.1	0.092	0.682	0.004
Lipid	*16.1±2.1*	19.1±1.0	19.0±7.1	19.0±4.1	21.0±5.9	20.0±9.7	21.0±6.7	0.808	0.000	0.274
Ash	*1.8±2.0*	1.7±1.0	1.7±1.0	1.8±1.0	1.8±1.0	1.6±1.0	1.7±1.0	0.093	0.542	0.428

All values are % of wet weight, mean ± S.D. (n=3).

[a] Values not included in the between means comparison

HP, high protein; MP, medium protein; LP, low protein; FO, fish oil; RO, rapeseed oil.