Effects of different blends of alternative protein sources as alternatives to dietary fishmeal on growth performance and body lipid composition of Atlantic salmon (*Salmo salar* L.)

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Abstract

Recently, we reported that growth of Atlantic salmon was reduced as dietary fishmeal (FM) was lowered from 25 % to 5 % in dual-substituted feeds compared to a control diet, formulated to represent the current upper levels of substitution of FM and fish oil. In the present study, the effects of different alternative protein blends and binders on growth of salmon fed dual-substituted feeds containing only 11 % FM, and with 60 % of dietary fish oil replaced by rapeseed oil were investigated. Salmon of initial weight 1.3 kg were grown to market size (> 3 kg) over a period of 19 weeks. Salmon fed the diets with reduced FM showed lower final weight, SGR and TGC, associated with reduced feed intake. There was a tendency for increased FCR in fish fed the diets containing reduced FM although this was not significant, and there was no effect on PER. There were no significant effects on digestibility of protein or fat but the two parameters varied reciprocally and there were clear trends of increased protein and lower fat digestibilities in fish fed diets with reduced FM. Although lipid and fatty acid compositions did not vary greatly between diets there were significant effects on fish tissue compositions. Thus, liver lipid was generally reduced in fish fed diets with lower FM, significantly so in two of the four treatments. The proportions of monoenes were significantly lower and those of polyunsaturated fatty acids (PUFA) significantly higher in flesh and liver of fish fed diets with reduced levels of FM. The increased proportions of PUFA were due to increased percentages of 20:4n-6, 20:5n-3, 22:5n-3 and, although not consistently significant, 22:6n-3. The mechanisms for these unexpected effects of diet on tissue lipids and fatty acids are discussed.

1. Introduction

The supply of marine raw materials such as fishmeal (FM) and fish oil (FO), the predominant sources of protein and lipid for carnivorous fish feeds, has become a limiting factor for expansion of aquaculture due to the pressure on feed-grade fisheries (Naylor et al., 2009). Production of these marine raw materials cannot increase above current levels and, coupled with the increasing demand driving prices upwards, it is no longer feasible to use FM and FO at the current inclusion levels (FAO, 2009). This has led to the investigation of new, cost-efficient protein and oil sources as alternatives to FM and FO in aquafeeds (Hardy, 2010; Turchini et al., 2009). Previous studies indicated that replacement of FM and FO with protein and lipid sources from terrestrial plant and animal sources would be possible provided amino acid and fatty acid requirements are met (Glencross et al., 2007; Webster et al., 2007)

Several land animal products including poultry by-products, meat, bone and blood meals have been investigated as substitutes for FM in fish diets (Smith et al., 1995; Robaina et al., 1997; Webster et al., 2000). However, plant products potentially offer more sustainable protein sources for aquafeeds although they often contain anti-nutritional factors, which can affect growth performance and fish health (Francis et al., 2001; Gatlin et al., 2007). Thus rapeseed, soybean and sunflower meals as well as various legumes (beans and peas) are less expensive and readily available in high quantities, although they have variable desirable and undesirable characteristics that both support and limit their use (Francis et al. 2001; Gill et al., 2006; Krogdahl et al., 2010). Corn gluten meal has been shown to be a good alternative for FM replacement in salmon feeds, being low in anti-nutritional factors (Mente et al., 2003), and wheat gluten has high digestibility and palatability although prices tend to fluctuate due to production restrictions and demand (Hardy, 1996). Soy protein concentrates (SPC) have been reported as good sources for partial substitution of FM for many species of fish including salmon without reducing growth (Refstie et al., 2001), although problems

with gut damage have been reported at inclusion > 20 % of some soybean products in salmon (Baeverfjord and Krogdahl, 1996; Knudsen et al., 2007). Legumes including field peas and pea protein have been shown to be good protein sources for Atlantic salmon (Aslaksen et al., 2007).

Partial replacement of FM with plant meals at a variety of different levels of substitution has been studied in several fish species including salmonids (Kaushik et al., 1995; Espe et al., 2006; Torstensen et al., 2008). Generally, the replacement of up to 30 - 40 % FM with single plant meals does not compromise growth of fish (Nengas et al., 1996; Refstie et al., 2001; Lozano et al., 2007). However, replacement of greater than 70 % of dietary FM by blends of plant meal resulted in negative effects on growth of various fish including salmonids (Gomes et al., 1995; Espe et al., 2006; Torstensen et al., 2008). Although complete replacement of FM by plant meals has generally not been very successful, substitution of close to 100 % of dietary FM by blends of plant proteins was possible in salmonids with no major negative effects on growth if the amino acid profile in the feed was well balanced, and if feed intake was comparable to a high FM diet (Barrows et al., 2007; Espe et al., 2007). This demonstrated the potential of replacing dietary FM with mixtures of alternative protein sources in Atlantic salmon.

In a recent study, we reported the effects of progressive reduction in dietary FM in dual-substituted feeds for Atlantic salmon. Compared to a control diet, formulated to represent the current upper levels of substitution of FM and FO, growth of Atlantic salmon was progressively reduced as the FM content of the diet was reduced from 25 % to 5 % (Pratoomyot et al., 2010). In the present study, we tested the hypothesis that the negative effects on growth were due to factors associated with the specific protein replacers used and that alternative combinations of replacers could avoid these effects. Therefore, different alternative protein blends (mixtures of sunflower meal, corn gluten meal, soybean meal, wheat gluten, pea protein and blood meal) and binders (faba and kidney beans) were investigated in salmon fed dual-substituted feeds with almost 90 % of FM replaced and 60 % of dietary FO replaced by rapeseed oil. Atlantic salmon of

initial weight of 1.3 kg were grown to market size (> 3 kg) on the different feeds over a period of 19 weeks and the effects on growth performance, feed utilization efficiency, protein and fat digestibility, and lipid and fatty acid compositions of flesh and liver investigated.

2. Materials and methods

2.1. Diets and animals

Five diets were formulated to satisfy the nutritional requirements of salmonid fish (NRC, 1993) and manufactured at BioMar Tech-Centre, Brande, Denmark. All diets contained 35 % crude protein and 28 % crude lipid. The control diet was formulated to contain 25 % FM, the minimum level of FM inclusion currently used in commercial salmon culture, and the other experimental diets contained 11 % FM. The protein ingredients of the control diet (25F) contained 25 % FM and 45 % alternative proteins (a blend of plant proteins including sunflower meal, corn gluten, soybean protein concentrate and faba bean). The basal ingredients of the other four experimental diets were 11% FM and 55 % alternative proteins that were, diet 11FW (qualitatively similar blend to 25F plus wheat gluten), 11FP (qualitatively similar blend to 11FW plus pea protein), 11FB (qualitatively similar blend to 11FW plus blood meal) and 11FK (qualitatively similar blend to 11FW plus kidney bean). All diets were coated with a 60:40 blend of rapeseed oil and northern hemisphere FO and all the experimental diets were supplemented with crystalline indispensible amino acids, lecithin and carophyll pink to meet requirement levels for amino acids, phospholipid and pigments (Table 1).

Two thousand two hundred and fifty Atlantic salmon of initial mean weight 1.3 ± 0.1 kg were randomly distributed among 15 cages of 125 m^3 with 150 fish/cage at the Marine Harvest Fish Trials Unit, Ardnish, Scotland. The fish were fed one of five diets

in triplicate cages. The experiment was conducted over 19 weeks from October 2007 to February 2008 under natural photoperiod with average water temperature ranging from 7 to 11 ^{o}C. Fish were fed to apparent satiation by automatic feeders (Sterner Arvo-tec UK, Inverness, Scotland). Mortalities, feed consumption and waste feed were recorded daily.

2.2 Sampling protocols

Fish were bulk weighed at the initiation, at the end of week 8 and at the termination of the trial, week 19. At the end of the trial, 2 fish per pen (6 fish per dietary treatment) were anaesthetized with metacaine sulphonate (MS222; 50 mg/L) and killed by a blow to the head. Flesh samples were taken using the Norwegian Quality Cut and were homogenized in a food processor after removal of skin and bones and stored at -20 ^{o}C prior to lipid analysis. Livers were also collected from the six fish and a 1-2g sample placed into glass vials containing chloroform/methanol (2:1, by vol.) for analysis of lipid class and fatty acid composition, and the remaining portion immediately frozen on dry ice (for lipid content). Both liver samples were then stored at -20 ^{o}C prior to analysis.

At the end of the trial faeces samples were obtained by stripping according to Austreng (1978). The fish were anaesthetized and faeces collected by gently squeezing the hindgut of the fish. One sample per cage was obtained by pooling faeces from an appropriate number of fish until a minimum wet weight of 150 g was collected. Water, urine and fish scales were removed, ethoxyquin (400 mg/L, 1 mL/60g wet faeces) added, and the samples freeze-dried before analysis. Briefly, faeces samples were placed into pre-weighed dishes and the weight recorded prior to being frozen at -70^{o}C for 6 h. The faeces were freeze-dried at -50^{o}C under vacuum for at least 16 h until

constant weight and the moisture content determined. The freeze-dried faeces samples were stored at -20 °C until further analysis.

2.3. Proximate composition analyses

Diets were ground prior to determination of proximate composition according to standard procedures (AOAC, 2000). Moisture contents were obtained after drying in an oven at 110 ^{o}C for 24 h and ash content determined after incineration at 600 ^{o}C for 16 h. Crude protein content was measured by determining nitrogen content (N × 6.25) using automated Kjeldahl analysis (Tecator Kjeltec Auto 1030 analyzer, Foss, Warrington, U.K), and crude lipid content determined after acid hydrolysis followed by Soxhlet lipid extraction (Tecator Soxtec system 2050 Auto Extraction apparatus, Foss, Warrington, U.K).

2.4. Apparent digestibility analyses

Yttrium oxide (Y_2O_3) was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). The diet (0.2-0.5g) or faeces (0.1g) were weighed into pre-cleaned beakers and 4 ml of concentrated nitric acid added. The beakers were covered with clean watch glasses and placed in a fume cupboard for 24h. The partially digested samples were placed on a hotplate and boiled for 1h before being transferred quantitatively to pre-cleaned 25 ml volumetric flasks and made to volume with 2% v/v nitric acid. The digested samples were then analysed by ICO-OES using a Varian 725-ES instrument. Standards of between 0.5 and 120 mg/L Y were prepared as calibrants and the Y signal was monitored at two different wavelengths. Apparent digestibility coefficients (ADC) were estimated according to the formula:

$$ADC = 100-100*((Y_{feed}/Y_{faeces})*(N_{faeces}/N_{feed}))$$

where Y_{feed} = Yttrium oxide in feed, Y_{faeces} = Yttrium in faeces, N_{faeces} = nutrient in faeces, N_{feed} = nutrient in feed. All data were based on calculated dry weight of the samples.

2.5. Lipid and fatty acid analysis

Total lipid of flesh and liver was extracted according to the method of Folch et al. (1957). Approximately 1 g of flesh homogenate or liver was placed in 20 ml of ice-cold chloroform/methanol (2:1, by vol) and homogenized with an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). The aqueous and lipid layers were separated by addition of 5 ml of 0.88 % (w/v) KCl and allowed to separate on ice for 1 h. The upper aqueous layer was aspirated and the lower lipid layer dried under oxygen-free nitrogen. The lipid content was determined gravimetrically after drying overnight in a vacuum desiccator.

Lipid class composition of diet and tissues was determined by high-performance thin-layer chromatography (HPTLC). Approximately 10 μg of total lipid was applied to a 10 x 10 cm HPTLC plate (VWR, Lutterworth, England) as 2 mm streaks, 1 cm from the bottom, and the plates developed in methyl acetate/isopropanol/ chloroform/methanol/0.25 % aqueous KCl (25:25:25:10:9, by vol.) to two-thirds up the plate to separate polar lipid classes. After desiccation for 20 min, the plate was fully developed with isohexane/diethyl ether/acetic acid (85:15:1, by vol.) to separate neutral lipid classes and placed in a vacuum desiccator for 20 min. The lipid classes were visualized by charring at 160 ^{o}C for 15 min after spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid and quantified by densitometry using a CAMAG-3 TLC scanner (version Firmware 1.14.16) (Henderson and Tocher,

1992). Scanned images were recorded automatically and analyzed by computer using winCATS Planar Chromatography Manager, version 1.2.0).

Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification at 50 ^{o}C for 16 h according to the method of Christie (1993). Extraction and purification of FAME was carried out as described by Tocher and Harvie (1988). The FAME were separated and quantified by gas-liquid chromatography (ThermoFisher, UK) using a 30m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-column injection. Hydrogen was used as carrier gas and temperature programming was from 50 ^{o}C to 150 ^{o}C at 40 ^{o}C min^{-1} and then to 230 ^{o}C at 2.0 ^{o}C min^{-1}. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980; Tocher and Harvie, 1988). Data were collected and processed using Chromcard for Windows (version 1.19)

2.6. Formulae, calculations and statistical analysis

Feed consumption (g/day) = feed intake (g) x [number of fish x days]$^{-1}$

Feed Conversion Ratio (FCR) = feed intake (g) x [final biomass – initial biomass + mortalities]$^{-1}$

Hepatosomatic Index (HSI, %) = 100 × [weight of liver (g)] x [weight of fish (g)]$^{-1}$

Protein efficiency ratio (PER) = [final mean weight (g) - initial mean weight (g)] x [crude protein fed (g)]$^{-1}$

Specific growth rate (SGR, % day) = 100 × [ln (final mean weight) – ln (initial mean weight)] x days^{-1}

Thermal growth coefficient (TGC) = 1000 x [(final wt)$^{1/3}$ – (initial wt)$^{1/3}$ x (degree days)$^{-1}$

Visceromatic Index (VSI, %) = 100 × [weight of viscera (g)] x [weight of fish (g)]$^{-1}$

All data are presented as means ± SD (n value as stated). The effects of dietary treatment on growth performance were analyzed by one-way analysis of variance (ANOVA) followed, where appropriate, by Tukey's post hoc test. Percentage data and data identified as non-homogeneous (Lavene's test) or non-normality (Shapiro-Wilks's test) were subjected to arcsine transformation before analysis. Statistical analysis was performed using a SPSS Statistical Software System version 14 (SPSS inc, 2005). Differences were regarded as significant when $P < 0.05$ (Zar, 1999).

3. Results

3.1. Diet compositions

The main differences in proximate compositions of the diets were that lipid and the nitrogen-free extract (NFE) were lower and higher, respectively, in the diets with highest FM replacement, with levels in the 11F diets generally being significantly different to those in diets 25F (Table 1). The lipid component of the diets was 90 – 92 % neutral lipid, predominantly triacylglycerol (TAG), and there were no significant differences between the diets in total polar and neutral lipid levels (Table 2). There were some small but significant differences in the relative proportions of the different phospholipid classes including phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylinositol (PI) / phosphatidylserine (PS), and in the percentage of sterol in the diets (Table 2). All diets contained approximately 51 - 55 % total monoenes, predominantly 18:1n-9, with around 15 - 17 % saturated fatty acids, mainly 16:0, and 30 - 32 % polyunsaturated fatty acids (PUFA), with half of that being 18:2n-6 and the remainder being n-3 PUFA, 18:3n-3, and the long-chain PUFA (LC-PUFA),

eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) (Table 3). All fatty acids showed significant differences between diets with some clear trends. There were trends for increased saturated fatty acids and decreased monenes, particularly C20 and C22, in the diets with lower fishmeal inclusion. Although DHA tended to be lower in the diets with reduced fishmeal, EPA was , if anything, increased as was 18:3n-3 (Table 3)

3.2. Growth performance and biometry

There was no difference in initial weights of the fish and virtually no mortality in the entire trial (data not shown). Growth of salmon fed the diets with reduced FM was lower as evidenced by lower SGRs (Fig.1), TGC and final weights (Fig. 2). The reduced growth was associated with reduced feed intake in fish fed the low FM diets (Fig. 1). There was a trend for increased FCR in fish fed the diets containing reduced FM although this was not significant, and there was no effect on PER (Figs. 1 and 2). The HSI was 1.1 to 1.2 with no effect of dietary treatment, and VSI averaged 12 % and was also unaffected by diet other than being slightly higher in fish fed diet 11FW (13.2 %) compared to fish fed diets 11FP (11.4) and 11 FK (11.5).

3.3. Protein and lipid digestibilities

The apparent digestibility coefficients (ADC) of protein and fat showed no significant differences between the dietary treatments. However, there were consistent trends with the numerical values for protein ADC generally being higher, and those for fat ADC generally lower in fish fed the diets with the reduced levels of FM (Fig. 3). Thus, the ADC of protein and fat in diet 25F were 82.6 and 94.6, respectively, whereas they

varied between 83.6 – 84.5, and 90.3 – 93.7, in fish fed the diets formulated with 11 % FM.

3.5. Lipid and fatty acid compositions of salmon flesh and liver

Lipid content of flesh varied from 10.5 to 13.2 % with fish fed diet 11FP showing a higher level compared to fish fed diet 11 FK (Fig. 4). Liver lipid showed a clear trend with lower contents in fish fed the diets with lower inclusion of FM, significantly so in the case of fish fed the 11FB and 11FK diets (Fig. 4). Lipid in flesh was around 90 % neutral lipid, predominantly TAG, and there were no significant differences in the proportions of any of the major neutral or polar lipids (Table 4). Liver showed higher levels of phospholipids and there were some differences in the relative proportions of the major classes including PC, PE, PS and PI with phospholipids highest in fish fed diet 11FB (Table 4). There was a trend for neutral lipids and TAG to be generally lower in liver of salmon fed the diets with reduced FM.

The proportions of monoenes were significantly lower and those of n-3 PUFA significantly higher in flesh of fish fed the diets with reduced levels of FM (Table 5). The increased n-3 was primarily due to increased proportions of EPA and 22:5n-3 although there was also a consistent trend for higher DHA in fish fed the reduced FM diets. The effects of dietary treatments on liver fatty acid compositions were similar to those observed in flesh but much more pronounced (Table 6). Thus, the proportions of total monoenes, particularly 18:1n-9, 20:1n-9 and 22:1n-11, were significantly lower, and those of total PUFA significantly higher, in liver of fish fed the reduced FM diets. The increased proportions of total PUFA were due to increased percentages of arachidonic acid (ARA, 20:4n-6) and total n-3 PUFA, particularly EPA, 22:5n-3 and, although not consistently statistically significant, DHA (Table 6).

4. Discussion

The present study was performed to investigate dual-substituted feeds for Atlantic salmon, that is, feeds with high levels of substitution of both of the main marine ingredients, FM and FO. The control feed contained 25 % FM whereas the experimental feeds contained 11 % FM, with all feeds having 60 % of FO replaced by rapeseed oil. The primary aim of the study was to test the hypothesis that the negative effects on growth of very high replacement of FM in feeds, previously observed in Atlantic salmon (Espe et al., 2006; Torstensen et al., 2008; Pratoomyot et al., 2010), could be mitigated by different including combinations of FM replacers. However, none of the tested blends of FM replacers, which included sunflower meal, corn gluten meal, soybean meal, wheat gluten, pea protein and blood meal, prevented the reduction in growth when the FM level of the feeds was reduced from 25 % to 11 % and so the hypothesis was not proved.

Obviously, the amino acid composition of the feeds will vary with the different formulations, however the feed formulations in the present trial supplied all indispensible amino acids at or above levels reported to satisfy the nutritional requirements of Atlantic salmon (NRC, 1993). This was ensured by the addition of appropriate amounts of crystalline amino acids as indicated in Table 1. Therefore it is not likely that the effect of growth was due to any limitation in amino acid content of the feeds.

Reduced feed intake as the level of dietary FM decreased had been identified as a factor affecting growth and resulting in lowest SGR in our previous study (Pratoomyot et al., 2010). In the present trial, essentially similar results were obtained with all the

combinations resulting in lower feed intake, weight gain, SGR and TGC. The final weights were 3.8 Kg for fish fed the control diet and 3.3 - 3.5 Kg for fish fed the low FM diets. Calculating the difference in feed intake over the whole trial between the fish fed the control feed and those fed the low FM feeds was actually 439 ± 59 g/fish. Therefore, the difference in feed intake essentially balanced the difference in final weights (~ 400 g). Therefore, reducing FM from 25 to 11 % lowered growth associated with reduced feed intake and there were no significant differences between any of the combinations of alternative ingredients tested. There were no significant effects on ADC, FCR or PER to suggest any other major effects contributed to the reduced growth performance. The reduced feed intake may have been the result of an effect on palatability that resulted in the fish simply consuming less of the very low FM feeds. We can only speculate as to whether the reduced intake was due higher levels of negative "bitter" tastes or reduced levels of attractants although this is something that can be investigated in future by supplementing feeds with attractants. It was noteworthy that the feed with LAP (i.e. bloodmeal) did not yield improved performance. At the outset, the 11FB feed was possibly expected to be the most promising combination for preventing effects on growth but this was not the case and, in actual fact this feed (11FB) gave the lowest feed intake and one of the higher FCR values. Different binders (faba and kidney beans) were also investigated but kidney beans showed no difference compared to identical feed with Faba bean (11FW and 11FK) although FCR possibly even higher.

In a recent study on salmon, reduced growth was observed when diets containing 80% plant protein and either 35 or 70 % VO (9 and 17 % growth reduction respectively) were used although growth was not reduced in the control 62 % FM / 25 % FO group or in the 40 % plant protein / 70 %VO groups (Torstensen et al., 2008).

The lack of growth reduction in the 40 % plant protein / 70 % VO group was similar to that seen in earlier FO replacement studies (Rosenlund et al., 2001; Bell et al., 2002; Torstensen et al., 2005) and suggests that the problem relates to high levels of plant protein inclusion. The reduced growth observed in the study by Torstensen et al. (2008) was related to reduced feed intake during both the initial and final growth periods and may be linked to feed palatability as in the current study and seen in other studies with high FM replacement (Gomes et al., 1995; Espe et al., 2006). The inclusion of krill meal, often used to improve feed acceptance, in the diets used by Torstensen et al. (2008) did not improve feed intake.

In contrast to the growth performance data that showed few effects of different combinations of alternative ingredients, there were clear effects on lipid and fatty acid metabolism that were unexpected. Of prime interest among these effects were the increased levels of n-3 LC-PUFA in fish consuming the feeds with reduced FM, despite the primary lipid components of the feeds being essentially unchanged other than slightly increased added oils to account for the lower inclusion of FM. As a result, the lipid contents and fatty acid compositions of the feeds were unchanged other than a slight trend for increased EPA but lower DHA in feeds with very low FM. Therefore, considering the very similar fatty acid compositions of the feeds, the very significant increases in proportions of EPA, DPA and DHA (and ARA) suggest that there is a strong metabolic response in fish given feeds with very low FM. Possibly reflecting this, the greatest effects were observed in liver, the main lipid metabolic organ (Tocher, 2003). However, the effects were also observed in the flesh, which is important because it means that the feeds have an impact on the nutritional quality of the fish (Torstensen et al., 2005; Fountoulaki et al., 2009; Rosenlund et al., 2010). Although not quantitatively as great as in liver, the increased proportions in flesh of n-3 LC-PUFA,

amounting to 2 - 3 % of total fatty acids depending upon ingredient combination, were biologically significant and should be investigated further.

Although effects of dietary protein source on lipid metabolism have been reported previously in European seabass (*Dicentrachus labrax*) (Dias et al., 2005), the results of the present study in salmon are nonetheless surprising and the unexpected effects on tissue fatty acid compositions require some explanation. Metabolic pathways that could contribute to the observed effects include decreased oxidation and increased retention of dietary LC-PUFA, and possibly increased endogenous synthesis from C_{18} PUFA (Tocher, 2003; Bell and Tocher, 2009). Based on existing knowledge of fatty acid metabolism, we would not expect that the fatty acid oxidation and LC-PUFA synthesis pathways would be greatly influenced by the different feeds considering the very marginal differences fatty acid content and composition between the feeds (Leaver et al., 2008a; Turchini et al., 2009; Torstensen and Tocher, 2010). However, LC-PUFA synthesis (fatty acid desaturation and elongation) is affected by dietary cholesterol in mammals (Garg et al., 1986) with reduced ARA observed in liver of cholesterol supplemented rats and guinea pigs related to down-regulated Δ6 and Δ5 fatty acid desaturase activities (Muriana et al., 1992; Huang et al., 1993). The 11% FM diets used in the present study would contain less cholesterol than the control diet and probably higher levels of phytosterols derived from the plant protein component (Gilman et al., 2003). In a study conducted by Bjerkeng et al., (1999), salmon fed a cholesterol supplement had significantly lower levels of both ARA and DHA in their liver lipids as well as reduced liver lipid levels compared to fish fed no cholesterol supplement. Therefore the increased proportions of ARA and DHA in liver of fish fed the low FM diets may be related to the lower cholesterol content of these feeds. The potential

effects of altered sterol content and composition of alternative feeds required further investigation.

A further mechanism for the increased levels of n-3 LC-PUFA, at least in liver, may be lower tissue lipid deposition perhaps associated with lower growth (Karalazos et al., 2006). Certainly lower levels of lipid and TAG were observed in liver of fish fed the low FM feeds and so higher LC-PUFA in liver may be partly a consequence of the higher proportions of polar lipids (phospholipids). Consistent with this, the highest levels of DHA, n-3PUFA and total PUFA were observed in liver of fish fed 11FB, which also showed one of the lowest liver lipid contents and the highest polar lipid and lowest TAG. This mechanism was not so clear in flesh as lipid content was not reduced and there was no change in polar : neutral lipid ratios or TAG contents, and so the increased EPA in flesh appeared to be independent of lipid/TAG content. In addition to the biochemical mechanisms discussed above, it is also probable that the differences in protein composition of the feeds could affect metabolic pathways by altering gene expression that have consequences for lipid metabolism (Taggart et al., 2008; Leaver et al., 2008b, 2010). These molecular mechanisms warrant further investigation.

5. Conclusion

Our hypothesis that the negative effects on growth of very high FM replacement could be reduced with different combinations of protein replacers was not proved as none of the alternative feeds with only 11 % FM could equal the growth of salmon fed the feed with 25 % FM. The reduced growth (SGR) was associated with reduced feed intake but there were no major effects on FCR or PER. Surprisingly, the proportions of n-3 PUFA, EPA, 22:5n-3 and DHA, and ARA were generally increased in both liver and flesh. This was probably the result of a combination of metabolic effects including reduced

lipid deposition, in liver at least, possibly as a result of lower growth as well as possibly increased retention and/or synthesis from C_{18} fatty acid precursors supplied by rapeseed oil. This was a highly unexpected result considering that dietary lipid and fatty acid compositions did not varying greatly between treatments. Furthermore, the magnitude of the effect was quantitatively important and sufficient to warrant further investigation in future trials.

6. Acknowledgements

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Figure legends

Fig.1. Feed intake, specific growth rate (SGR) and feed conversion efficiency (FCR) in Atlantic salmon fed the experimental diets. Values are mean ± SD (n = 3). Values for each parameter with different superscript letters are significantly different as determined by ANOVA ($p < 0.05$). Diet 25F was formulated with 25 % fishmeal, and diets 11FB, 11FK, 11 FP and 11FW were formulated with 11 % fishmeal and various combinations of alternative protein sources as described in detail in the Methods section.

Fig.2. Final weight (kg), thermal growth coefficient (TGC) and protein efficiency (PER) in Atlantic salmon fed the experimental diets. Values are mean ± SD (n = 3). Values for each parameter with different superscript letters are significantly different as determined by ANOVA ($p < 0.05$). Diet 25F was formulated with 25 % fishmeal, and diets 11FB, 11FK, 11 FP and 11FW were formulated with 11 % fishmeal and various combinations of alternative protein sources as described in detail in the Methods section.

Fig. 3. Apparent digestibility coefficients (ADC%) for total protein and fat in salmon fed the diets containing 25% (25F), 18% (18F), 11% (11F) and 5% (5F) fishmeal. Values are mean ± SD (n = 3). Values (columns) for each nutrient with different superscript letters are significantly different as determined by ANOVA ($p < 0.05$). Diet 25F was formulated with 25 % fishmeal, and diets 11FB, 11FK, 11 FP and 11FW were formulated with 11 % fishmeal and various combinations of alternative protein sources as described in detail in the Methods section.

Fig. 4. Lipid contents (percentage of wet weight) of flesh and liver of Atlantic salmon. Values are mean ± SD (n = 6). Values for each tissue with different superscript letters are significantly different as determined by ANOVA ($p < 0.05$). Diet 25F was formulated with 25 % fishmeal, and diets 11FB, 11FK, 11 FP and 11FW were formulated with 11 % fishmeal and various combinations of alternative protein sources as described in detail in the Methods section.

Table 1. Feed formulation (mg kg^{-1}) and proximate composition (%) of the diets

Feed ingredients	25F	11FW	11FP	11FB	11FK
Fishmeals[1]	250	110	110	110	110
Sunflower expeller	115	40	4	108	40
Corn gluten	85	175	130	100	175
Soy concentrate[2]	85	175	130	100	175
Wheat gluten	-	18	17	11	18
Pea protein	-	-	130	-	-
Haemoglobin meal	-	-	-	84	-
Field beans	160	160	160	160	-
Kidney beans	-	-	-	-	160
Rapeseed oil[3]	173	178	176	180	178
Fish oil[4]	116	118	117	120	118
Micronutrients[5]	11.95	23.59	25.48	21.49	23.59
Crystalline amino acids[6]	1.19	5.43	4.88	2.32	5.43
Lecithin	5.0	5.0	5.0	5.0	5.0
Astaxanthin[7]	0.40	0.40	0.40	0.40	0.40
Antioxidant[8]	4.25	4.25	4.25	4.25	4.25
Proximate composition					
Protein	34.3 ± 0.4^{c}	35.0 ± 0.1^{bc}	34.5 ± 0.3^{b}	36.1 ± 0.2^{a}	35.3 ± 0.1^{b}
Lipid	29.8 ± 0.1^{a}	27.9 ± 0.1^{c}	27.5 ± 0.3^{c}	28.8 ± 0.3^{bd}	28.3 ± 0.3^{cd}
Moisture	6.7 ± 0.1^{a}	6.2 ± 0.0^{b}	6.2 ± 0.1^{b}	6.5 ± 0.1^{a}	5.5 ± 0.1^{c}
Ash	6.0 ± 0.1^{a}	5.2 ± 0.1^{bc}	5.4 ± 0.1^{b}	5.0 ± 0.0^{c}	5.3 ± 0.1^{b}
NFE [9]	23.2 ± 0.3^{b}	25.7 ± 0.2^{a}	26.4 ± 0.3^{a}	23.6 ± 0.2^{b}	25.6 ± 0.3^{a}

[1] Peruvian fishmeals produced from Anchoveta

[2] Soy protein concentrate (SPC60; 60% crude protein) produced from defatted soy flakes

[3] Non-GM double-low rapeseed oil

[4] North-Atlantic standard fish oil

[5] Vitamin and mineral premixes with limestone carrier added according to the commercial standards of BioMar AS

[6] Highly purified (99%) crystalline amino acids

[7] Carophyll Pink CWS 10 %

[8] Blend of antioxidants and starch carrier added according to the commercial standards of BioMar

[9] NFE (nitrogen free extract) calculated by subtraction, 100 - (crude protein + crude fat + moisture + ash)

Table 2. Lipid class composition (percentage of total lipid) of the diets

	25F	11FW	11FP	11FB	11FK
PC	2.8 ± 0.2 [a]	2.1 ± 0.3 [ab]	2.7 ± 0.6 [ab]	2.4 ± 0.4 [ab]	1.9 ± 0.2 [b]
PE	3.5 ± 0.6 [c]	3.9 ± 0.6 [bc]	5.0 ± 0.8 [ab]	5.4 ± 0.2 [a]	4.5 ± 0.8 [abc]
PI/PS	1.5 ± 0.3 [b]	3.2 ± 0.6 [a]	1.9 ± 0.4 [b]	1.7 ± 0.8 [b]	2.3 ± 0.3 [ab]
Sphingomyelin	0.2 ± 0.0 [a]	0.1 ± 0.1 [b]	0.1 ± 0.0 [b]	0.2 ± 0.1 [a]	0.1 ± 0.1 [b]
Lyso-PC	0.1 ± 0.0 [b]	tr	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]	0.2 ± 0.0 [a]
Total PL	8.2 ± 0.8	9.3 ± 1.4	9.9 ± 1.1	10.1 ± 0.9	9.1 ± 1.0
Total NL	91.8 ± 0.8	90.4 ± 1.4	90.1 ± 1.1	89.9 ± 0.9	90.9 ± 1.0
Triacylglycerol	74.2 ± 1.8	73.9 ± 1.4	74.6 ± 0.9	73.2 ± 1.4	73.8 ± 1.3
Sterol	8.5 ± 0.6 [a]	6.9 ± 0.4 [b]	7.2 ± 0.7 [b]	7.1 ± 0.7 [b]	8.6 ± 0.6 [a]
Free fatty acid	9.1 ± 1.5	9.9 ± 0.8	8.5 ± 0.2	9.6 ± 0.4	8.6 ± 0.4
Steryl ester	tr	tr	tr	tr	tr

Results are means ± SD (n = 4). Values within a row with different superscript letters are significantly different as determined by ANOVA. NL, neutral lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, polar lipid; PS, phosphatidylserine; tr, trace.

Table 3. Fatty acid compositions (percentage of total fatty acids) of the diets.

Parameters	25F	11FW	11FP	11FB	11FK
14:0	2.6 ± 0.0 ^{ab}	2.5 ± 0.0 ^{b}	2.5 ± 0.0 ^{b}	2.6 ± 0.0 ^{ab}	2.7 ± 0.0 ^{a}
16:0	8.7 ± 0.1 ^{c}	9.1 ± 0.2 ^{b}	9.1 ± 0.1 ^{b}	9.3 ± 0.0 ^{ab}	9.4 ± 0.1 ^{a}
18:0	2.7 ± 0.1 ^{c}	2.6 ± 0.1 ^{c}	3.0 ± 0.1 ^{b}	3.2 ± 0.0 ^{a}	3.0 ± 0.1 ^{b}
22:0	0.9 ± 0.0 ^{b}	0.8 ± 0.1 ^{b}	1.1 ± 0.1 ^{a}	1.1 ± 0.0 ^{a}	1.1 ± 0.2 ^{a}
Total saturated[1]	15.5 ± 0.3 ^{c}	15.7 ± 0.1 ^{c}	16.3 ± 0.1 ^{b}	16.9 ± 0.1 ^{a}	16.9 ± 0.3 ^{a}
16:1n-7	3.0 ± 0.0 ^{a}	2.8 ± 0.1 ^{b}	2.8 ± 0.0 ^{b}	2.8 ± 0.0 ^{b}	2.9 ± 0.0 ^{b}
18:1n-9	38.4 ± 0.3 ^{b}	41.1 ± 0.7 ^{a}	40.5 ± 0.5 ^{a}	39.1 ± 0.1 ^{b}	39.2 ± 0.2 ^{b}
18:1n-7	2.7 ± 0.0 ^{b}	2.7 ± 0.2 ^{b}	2.7 ± 0.1^{b}	2.9 ± 0.0 ^{a}	2.9 ± 0.0 ^{a}
20:1n-9	4.5 ± 0.0 ^{a}	3.6 ± 0.0 ^{b}	3.3 ± 0.0 ^{c}	2.8 ± 0.0 ^{e}	2.9 ± 0.0 ^{d}
22:1n-11	4.6 ± 0.0 ^{a}	3.4 ± 0.0 ^{b}	3.0 ± 0.0 ^{c}	2.3 ± 0.0 ^{d}	2.3 ± 0.1 ^{d}
Total monoenes[2]	54.6 ± 0.1 ^{a}	54.8 ± 1.0 ^{a}	53.5 ± 0.5 ^{a}	51.1 ± 0.1 ^{b}	51.5 ± 0.3 ^{b}
18:2n-6	15.0 ± 0.1 ^{c}	15.1 ± 0.3 ^{bc}	14.5 ± 0.2 ^{d}	15.9 ± 0.2 ^{a}	15.5 ± 0.1 ^{ab}
20:3n-6	0.2 ± 0.0 ^{b}	0.3 ± 0.0 ^{a}	0.2 ± 0.0 ^{b}	0.3 ± 0.0 ^{a}	0.2 ± 0.0^{b}
20:4n-6	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.3 ± 0.0 ^{a}	0.3 ± 0.0 ^{a}
Total n-6PUFA[3]	15.7 ± 0.1 ^{b}	15.8 ± 0.3 ^{b}	15.2 ± 0.2 ^{c}	16.6 ± 0.1 ^{a}	16.3±0.2 ^{a}
18:3n-3	5.6 ± 0.1 ^{c}	5.8 ± 0.2 ^{bc}	6.1 ± 0.1 ^{ab}	6.1 ± 0.0 ^{ab}	6.3 ± 0.2 ^{a}
18:4n-3	1.0 ± 0.1 ^{a}	0.8 ± 0.1 ^{b}	0.9 ± 0.0 ^{ab}	0.9 ± 0.0 ^{ab}	0.8 ± 0.0 ^{b}
20:5n-3	4.1 ± 0.1 ^{c}	3.9 ± 0.3 ^{c}	4.5 ± 0.1 ^{b}	4.9 ± 0.0 ^{ab}	5.0 ± 0.2 ^{a}
22:5n-3	0.4 ± 0.0 ^{b}	0.4 ± 0.0 ^{b}	0.5 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}
22:6n-3	3.0 ± 0.1 ^{a}	2.5 ± 0.2 ^{b}	2.8 ± 0.1 ^{ab}	2.8 ± 0.0 ^{ab}	2.6 ± 0.1 ^{b}
Total n-3PUFA[4]	14.2 ± 0.3 ^{ab}	13.7 ± 0.8 ^{b}	15.0 ± 0.4 ^{a}	15.3 ± 0.1 ^{a}	15.4 ± 0.5 ^{a}
Total PUFA	29.9 ± 0.4 ^{c}	29.4 ± 1.1 ^{c}	30.2 ± 0.5 ^{bc}	31.9 ± 0.2 ^{a}	31.7 ± 0.6 ^{ab}

Results are means ± SD (n = 6). Values within a row with different superscript letters are significantly different as determined by ANOVA. [1]Totals include 15:0 and 20:0 present at up to 0.6 %; [2]Totals include 16:1n-9, 20:1n-7, 22:1n-9 and 24:1n-9 present at up to 0.7 %; [3]Totals include 18:3n-6, 20:2n-6 and 22:5n-6 present at up to 0.1 %; [4]Totals include 20:4n-3 present at up to 0.2 %.

Table 4. Lipid class compositions (percentage of total lipid) of flesh and liver of salmon fed the experimental diets for 19 weeks

	25F	11FW	11FP	11FB	11FK
Flesh					
PC	4.4 ± 0.3	4.7 ± 0.7	4.4 ± 0.7	4.3 ± 0.7	5.0 ± 0.5
PE	4.4 ± 0.9	4.6 ± 0.9	3.9 ± 0.7	3.7 ± 0.7	4.1 ± 0.6
PI/PS	0.9 ± 0.2^{ab}	1.0 ± 0.1^{ab}	0.4 ± 0.4^{c}	0.4 ± 0.4^{c}	1.2 ± 0.4^{a}
SM	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ±0.1	0.3 ± 0.2
Lyso-PC	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Total PL	10.0 ± 1.1^{ab}	10.8 ± 1.4^{a}	8.9 ± 0.9^{ab}	8.7 ± 1.5^{b}	10.9 ± 1.1^{a}
TotalNL	90.0 ± 1.1^{ab}	89.2 ± 1.4^{b}	91.0 ± 0.9^{ab}	91.3 ± 1.5^{a}	89.1 ± 1.1^{b}
TAG	78.9 ± 1.1	78.7 ± 2.6	80.0 ± 2.3	80.6 ± 1.5	78.4 ± 1.9
Sterol	5.8 ± 0.6	5.8 ± 0.7	6.3 ± 0.8	5.8 ± 0.4	5.7 ± 0.7
FFA	5.4 ± 0.6	4.7 ± 1.0	4.7 ± 0.9	4.8 ± 0.6	4.9 ± 0.9
Steryl ester	tr	tr	tr	tr	tr
Liver					
PC	16.0 ± 3.9	16.5 ± 3.1	19.4 ± 2.9	19.4 ± 2.7	17.5 ± 1.7
PE	10.2 ± 2.5^{b}	10.7 ± 2.1^{ab}	12.9 ± 2.1^{ab}	14.0 ± 2.0 ^{a}	11.1 ± 1.3^{ab}
PI	3.6 ± 1.0 ^{b}	3.4 ± 1.1 ^{b}	4.1 ± 1.0^{ab}	5.6 ± 1.6 ^{a}	4.5 ± 0.6^{ab}
PS	2.3 ± 0.9 ^{b}	2.4 ± 0.9 ^{b}	2.9 ± 0.9^{ab}	4.6 ± 1.7 ^{a}	3.4 ± 0.6^{ab}
SM	1.8 ± 0.6	1.8 ± 0.5	2.2 ± 0.3	3.3 ± 0.7	2.0 ± 0.3
Lyso-PC	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
UPL	2.3 ± 1.4	2.4 ± 0.9	2.7 ± 0.6	3.1 ± 1.3	3.0 ± 0.6
Total PL	36.4 ± 9.5 ^{b}	37.4 ± 8.3 ^{b}	44.5 ± 7.6 ^{ab}	49.6 ± 8.7 ^{a}	41.9 ± 4.8^{ab}
Total NL	63.6 ± 9.5 ^{a}	62.6 ± 8.3 ^{a}	55.5 ± 7.6 ^{ab}	50.4 ± 8.7 ^{b}	58.1 ± 4.8^{ab}
TAG	49.0 ± 12.7	46.0 ± 11.7	36.9 ± 8.9	33.2 ± 11.1	41.1 ± 5.2
Sterol	10.4 ± 2.1	12.7 ± 2.2	13.1 ± 2.2	13.4 ± 1.6	12.1 ± 1.3
FFA	0.9 ± 0.9^{d}	1.8 ± 0.2^{cd}	2.5 ± 0.9^{abc}	3.4 ± 1.0^{a}	2.2 ± 0.4^{bcd}
Steryl ester	tr	tr	tr	tr	tr
UNL	3.3 ± 0.8^{a}	3.4 ± 1.3^{a}	2.9 ± 1.7 ^{a}	0.6 ± 0.3^{b}	2.7 ± 1.2 ^{a}

Results are means ± SD (n = 6). Values within a row with different superscript letters are significantly different as determined by ANOVA. FFA, free fatty acid; NL, neutral lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, polar lipids; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol; tr, trace; UNL, unidentified neutral lipid; UPL, unidentified polar lipid.

Table 5. Fatty acid compositions (percentage of total fatty acids) of flesh of salmon fed the experimental diets for 19 weeks

	25F	11FW	11FP	11FB	11FK
14:0	2.9 ± 0.1	3.0 ± 0.3	3.1 ± 0.3	3.0 ± 0.2	2.9 ± 0.2
16:0	10.5 ± 0.4 ^{b}	11.2 ± 0.5^{ab}	11.4 ± 0.6 ^{a}	11.3 ± 0.4 ^{a}	11.1 ± 0.3 ^{ab}
18:0	2.8 ± 0.1	2.8 ± 0.2	2.9 ± 0.2	2.9 ± 0.1	2.8 ± 0.1
Total saturated[1]	16.8 ± 0.5	17.6 ± 0.8	18.0 ± 1.1	17.8 ± 0.6	17.4 ± 0.6
16:1n-7	3.4 ± 0.1	3.6 ± 0.3	3.7 ± 0.3	3.6 ± 0.2	3.5 ± 0.3
18:1n-9	35.4 ± 1.0	34.4 ± 1.8	35.1 ± 1.3	34.8 ± 1.0	34.2 ± 1.2
18:1n-7	3.1 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	3.3 ± 0.2
20:1n-9	5.3 ± 0.2 ^{a}	4.5 ± 0.3 ^{b}	4.1 ± 0.2 ^{bc}	3.5 ± 0.1 ^{d}	3.8 ± 0.2 ^{cd}
22:1n-11	3.7 ± 0.3 ^{a}	2.6 ± 0.1 ^{b}	2.4 ± 0.2 ^{b}	1.9 ± 0.1 ^{c}	2.0 ± 0.1 ^{c}
Total monoenes[2]	52.6 ± 1.5 ^{a}	49.9 ± 1.9 ^{b}	50.1 ± 1.4 ^{b}	48.4 ± 0.7 ^{b}	48.3 ± 1.2 ^{b}
18:2n-6	12.1 ± 0.5 ^{ab}	11.4 ± 0.6 ^{b}	11.3 ± 0.8 ^{b}	12.6 ± 0.5 ^{a}	12.5 ± 0.5 ^{a}
20:2n-6	1.0 ± 0.1 ^{b}	1.2 ± 0.1 ^{a}	1.0 ± 0.1 ^{b}	1.0 ± 0.1 ^{b}	1.1 ± 0.1 ^{ab}
20:3n-6	0.4 ± 0.0 ^{b}	0.5 ± 0.1 ^{a}	0.5 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}	0.5 ± 0.1 ^{a}
20:4n-6	0.3 ± 0.1 ^{b}	0.4 ± 0.1 ^{a}	0.4 ± 0.0 ^{a}	0.5 ± 0.0 ^{a}	0.5 ± 0.1 ^{a}
Total n-6 PUFA[3]	14.1 ± 0.4 ^{ab}	13.9 ± 0.6 ^{ab}	13.5 ± 0.9 ^{b}	14.9 ± 0.5 ^{a}	14.9 ± 0.5 ^{a}
18:3n-3	4.0 ± 0.2 ^{ab}	3.9 ± 0.3 ^{b}	4.0 ± 0.3 ^{ab}	4.1 ± 0.2 ^{ab}	4.3 ± 0.3 ^{a}
18:4n-3	0.7 ± 0.1 ^{b}	0.8 ± 0.1 ^{ab}	0.8 ± 0.1 ^{ab}	0.9 ± 0.0 ^{a}	0.7 ± 0.0 ^{b}
20:4n-3	0.7 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.1
20:5n-3	3.8 ± 0.3 ^{b}	4.8 ± 0.7 ^{a}	4.6 ± 0.6 ^{a}	4.8 ± 0.3 ^{a}	4.9 ± 0.5 ^{a}
22:5n-3	1.6 ± 0.2 ^{b}	2.2 ± 0.3 ^{a}	2.1 ± 0.2 ^{a}	2.1 ± 0.1 ^{a}	2.4 ± 0.4 ^{a}
22:6n-3	5.2 ± 0.9	5.7 ± 0.8	5.7 ± 0.5	5.9 ± 0.5	5.8 ± 0.5
Total n-3 PUFA[4]	16.4 ± 1.3 ^{b}	18.6 ± 1.7 ^{a}	18.4 ± 1.4^{ab}	19.0 ± 0.7 ^{a}	19.4 ± 1.1 ^{a}
Total PUFA	30.5 ± 1.4 ^{c}	32.5 ± 1.5^{abc}	31.8 ± 1.9^{bc}	33.9 ± 0.7 ^{ab}	34.3 ± 0.8 ^{a}

Results are means ± SD (n = 6). Values within a row with different superscript letters are significantly different as determined by ANOVA. [1]Totals include 15:0, 20:0 and 22:0 present at up to 0.3 %; [2]Totals include 16:1n-9, 20:1n-7, 22:1n-9 and 24:1n-9 present at up to 0.7 %; [3]Totals include 18:3n-6, 22:4n-6 and 22:5n-6 present at up to 0.2 %; [4]Totals include 18:4n-3 present at 0.4 %.

Table 6. Fatty acid compositions (percentage of total fatty acids) of liver of salmon fed the experimental diets for 19 weeks

	25F	11FW	11FP	11FB	11FK
14:0	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.3	1.4 ± 0.1	1.4 ± 0.1
16:0	8.7 ± 1.7	9.5 ± 1.9	12.0 ± 3.0	11.7 ± 2.7	11.3 ± 2.7
18:0	4.0 ± 0.8	4.1 ± 0.3	4.7 ± 0.8	4.9 ± 0.3	4.9 ± 0.4
Total saturated[1]	14.6 ± 2.4	15.5 ± 2.3	18.6 ± 3.8	18.4 ± 2.9	18.0 ± 2.8
16:1n-7	2.5 ± 0.6	2.3 ± 0.5	2.0 ± 0.4	1.9 ± 0.2	1.8 ± 0.2
18:1n-9	35.8 ± 5.5 [a]	33.1 ± 4.9 [ab]	27.3 ± 4.6 [ab]	26.1 ± 4.[9 b]	27.1 ± 4.7 [b]
18:1n-7	3.4 ± 0.3 [a]	3.3 ± 0.2 [a]	2.9 ± 0.2 [b]	3.1 ± 0.2 [ab]	3.1 ± 0.2 [ab]
20:1n-9	7.5 ± 1.2 [a]	5.7 ± 1.0 [ab]	4.0 ± 1.1 [bc]	3.5 ± 1.1 [c]	3.7 ± 1.2 [c]
22:1n-11	1.6 ± 0.2 [a]	1.1 ± 0.1 [b]	0.8 ± 0.2 [c]	0.5 ± 0.1 [d]	0.6 ± 0.1 [cd]
Total monoenes[2]	52.4 ± 7.5 [a]	47.1 ± 6.6 [a]	38.5 ± 6.2 [b]	36.5 ± 6.5 [b]	37.9 ± 6.2 [b]
18:2n-6	8.7 ± 0.6	8.4 ± 0.5	7.9 ± 0.9	8.4 ± 0.9	8.5 ± 0.9
20:2n-6	2.1 ± 0.3	2.1 ± 0.3	1.6 ± 0.3	1.9 ± 0.5	1.8 ± 0.5
20:3n-6	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
20:4n-6	0.9 ± 0.4 [b]	1.3 ± 0.4 [ab]	1.8 ± 0.4 [a]	1.9 ± 0.4 [a]	2.0 ± 0.4 [a]
Total n-6PUFA[3]	12.5 ± 0.6	12.8 ± 0.5	12.2 ± 0.7	13.3 ± 1.0	13.3 ± 1.0
18:3n-3	2.5 ± 0.3	2.6 ± 0.3	2.5 ± 0.4	2.5 ± 0.2	2.8 ± 0.3
20:3n-3	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
20:4n-3	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
20:5n-3	5.5 ± 1.6 [b]	7.3 ± 1.6 [ab]	9.0 ± 2.0 [a]	8.5 ± 0.6 [a]	8.7 ± 1.0 [a]
22:5n-3	1.4 ± 0.2 [b]	2.2 ± 0.5 [ab]	2.8 ± 0.6 [a]	2.9 ± 0.9 [a]	2.9 ± 1.0 [a]
22:6n-3	9.7 ± 4.2 [b]	10.9 ± 2.9 [ab]	14.8 ± 2.5 [ab]	16.5 ± 3.5 [a]	14.8 ± 3.1 [ab]
Total n-3PUFA[4]	20.5 ± 5.5 [b]	24.7 ± 4.8 [ab]	30.7 ± 4.4 [a]	31.9 ± 4.6 [a]	30.8 ± 4.5 [a]
Total PUFA	33.0 ± 5.4 [c]	37.4 ± 4.8 [bc]	42.9 ± 4.0 [ab]	45.1 ± 3.7 [a]	44.1 ± 3.7 [ab]

Results are means ± SD (n = 6). Values within a row with different superscript letters are significantly different as determined by ANOVA. [1]Totals include 15:0 and 20:0 present at up to 0.2 %; [2]Totals include 16:1n-9, 20:1n-7, 22:1n-9 and 24:1n-9 present at up to 0.7 %; [3]Totals include 18:3n-6 and 22:5n-6 present at up to 0.3 %; [4]Totals include 18:4n-3 present at 0.2 %.

Fig.1

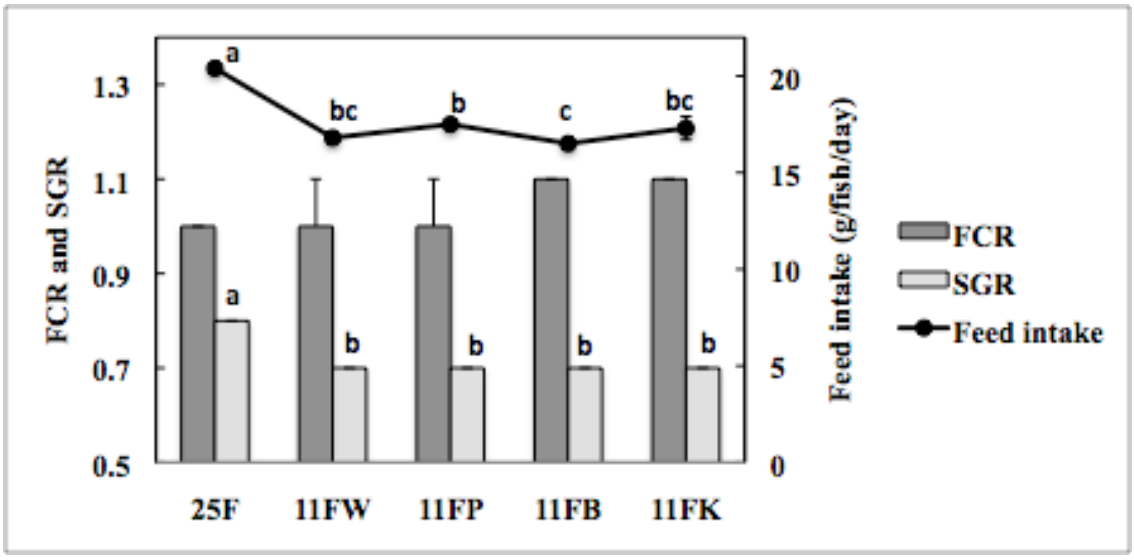

FCR and SGR
Feed intake (g/fish/day)
1.3
1.1
0.9
0.7
0.5
20
15
10
5
0
a
bc
b
c
bc
a
b
b
b
b
25F
11FW
11FP
11FB
11FK
FCR
SGR
Feed intake

Fig. 2.

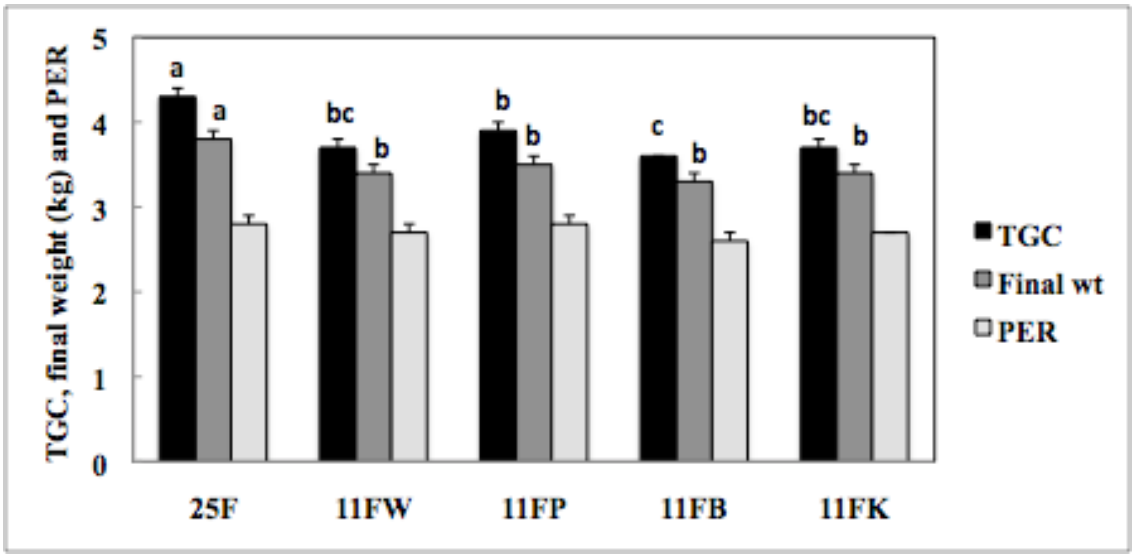

TGC, final weight (kg) and PER
5
4
3
2
1
0
a
a
bc
b
b
b
c
b
bc
b
25F
11FW
11FP
11FB
11FK
TGC
Final wt
PER

Fig.3.

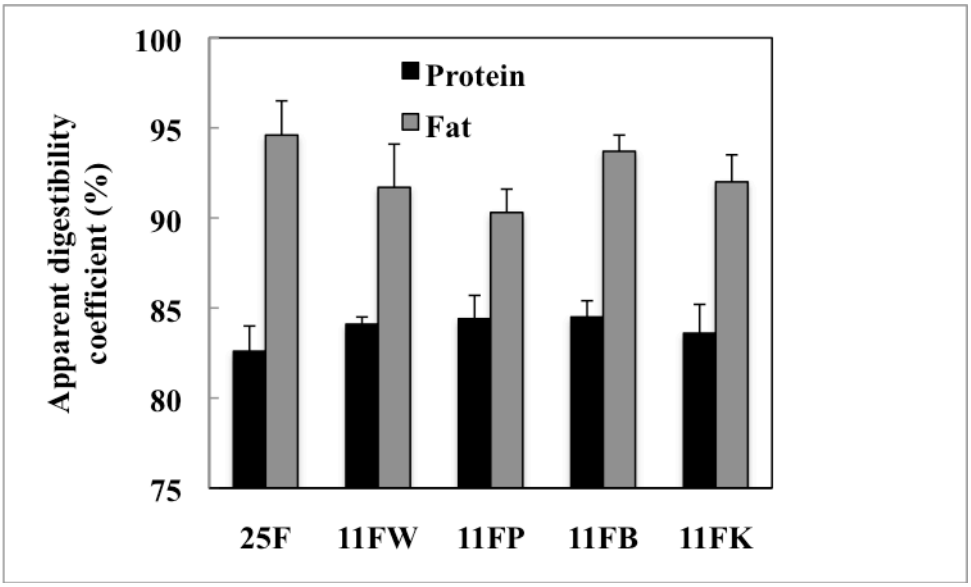
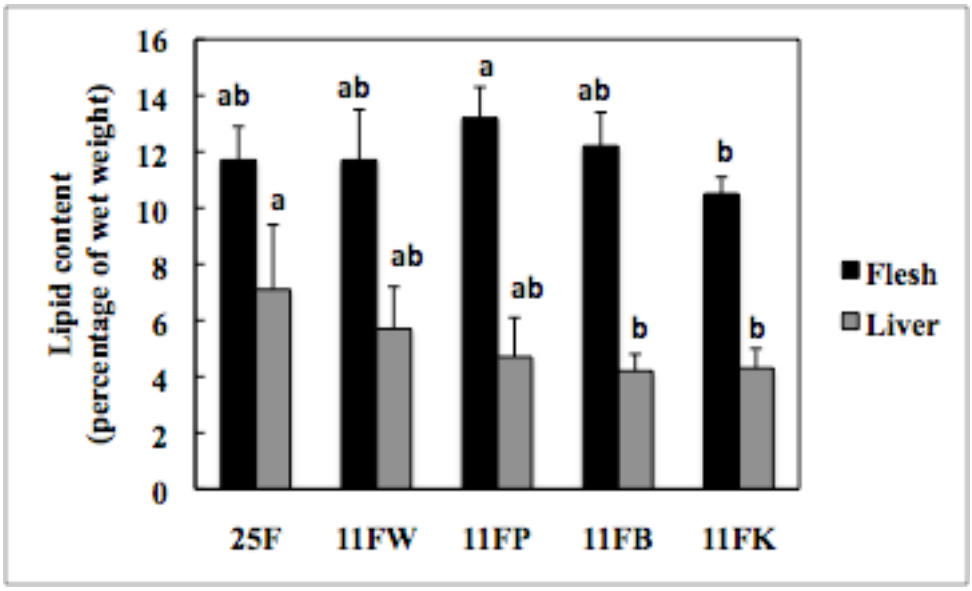

Apparent digestibility coefficient (%)
100
95
90
85
80
75
Protein
Fat
25F
11FW
11FP
11FB
11FK

Fig.4.


Lipid content
(percentage of wet weight)
16
14
12
10
8
6
4
2
0
ab
a
ab
ab
a
ab
ab
b
ab
b
b
25F
11FW
11FP
11FB
11FK
Flesh
Liver