Replacement of Dietary Fish Oil with Increasing Levels of Linseed Oil: Modification of Flesh Fatty Acid Compositions in Atlantic Salmon (*Salmo salar)* Using a Fish Oil Finishing Diet.

J.Gordon. Bell*, R.James. Henderson, Douglas.R. Tocher and John.R. Sargent.

Lipid Nutrition Group, Institute of Aquaculture, University of Stirling, FK9 4LA, Scotland, U.K.

Running title; FLESH FATTY ACID COMPOSITIONS IN ATLANTIC SALMON

Corresponding author; Dr J.G. Bell, Lipid Nutrition Group, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, U.K. Tel. 44 1786 467997; Fax. 44 1786 472133; email g.j.bell@stir.ac.uk.

1 *To whom all correspondence should be addressed at Lipid Nutrition Group, Institute of
2 Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, U.K.
3 Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil;
4 HUFA, highly unsaturated fatty acid; LO, linseed oil.
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ABSTRACT: Five groups of Atlantic salmon smolts, of initial mean weight 127 ± 3g, were fed increasing levels of dietary linseed oil (LO) in a regression design. The control diet contained capelin oil (FO) only and the same oil was blended with LO to provide the experimental diets. After an initial growth period of 40 weeks all treatment groups were switched to a finishing diet containing only FO for a further 24 weeks. Growth, flesh total lipid content and astaxanthin content were not affected by dietary oil composition. The fatty acid compositions of flesh total lipids were linearly correlated with dietary fatty acid compositions (r^2 = 0.88-1.00, $P < 0.0001$). Inclusion of the LO at 50% of added dietary lipid reduced flesh docosahexaenoic (22:6n-3; DHA) and eicosapentaenoic acids (20:5n-3; EPA) to 65 and 58%, respectively, of the concentrations in fish fed FO. When inclusion of dietary LO reached 100% the flesh DHA and EPA concentrations were reduced to 38 and 30%, respectively, of values in fish fed FO. Differences between diet fatty acid concentration and flesh fatty acid concentration showed that 16:0, 18:1n-9 and especially DHA were preferentially retained by salmon whereas 18:2n-6, 18:3n-3 and 22:1n-11 were selected against and presumably utilised for energy production. Feeding a finishing diet containing FO alone for 16 weeks restored flesh DHA and EPA concentrations in fish previously fed 50 and 100% LO to around 80% of their values in fish fed FO throughout. Flesh DHA and EPA concentrations in fish fed up to 50% LO were in excess of recommended intake values for these fatty acids. By utilising FO finishing diets for at least 16 weeks similar flesh DHA and EPA concentrations could be achieved in fish previously fed up to 100% LO for 40 weeks. This study suggests that LO can be used as a substitute for FO in salmon feeds during seawater growth and that any reductions in DHA and EPA can be overcome by feeding FO for a period before harvest.

The importance of the n-3 PUFA, and specifically the n-3 highly unsaturated fatty acids (HUFA) docosahexaenoic (22:6n-3; DHA) and eicosapentaenoic acids (20:5n-3, EPA) in human nutrition, is now widely recognised (1,2). There is also increasing evidence that a range of conditions currently prevalent in the developed world, including cardiovascular disease, diabetes, rheumatoid arthritis, neurological disorders and the metabolic syndrome, are influenced by changes in dietary fatty acid intake (3).

Fish, particularly those with high levels of body oil, like salmon, herring and sardines, represent a rich source of EPA and DHA for the human consumer. Due to increased population pressure worldwide demand for seafood is increasing yet traditional food-grade fisheries are unable to match demand with supply (4). Increased demand has resulted in rapid expansion of aquaculture production to alleviate this potential seafood deficit (5,6). However, the feed-grade fisheries that have supplied the raw materials for aquaculture feeds have reached sustainable limits and it is estimated that by 2010 > 85% of world fish oil supplies will be used for aquaculture production (7). Consequently, continued expansion of the aquaculture industry can only occur if sustainable alternatives to fish oil (FO), derived from terrestrial plants, are researched and implemented.

The lipids in feeds for Atlantic salmon must meet both energy and EFA requirements to allow the rapid growth and development required in modern aquaculture production (8,9). Earlier studies on β-oxidation activity suggest that salmonid fish have a preference for 16:0, 16:1, 18:1n-9 and 22:1n-11 as well as 18:2n-6 (10,11) although more recent studies with salmon suggest that 18:3n-3 and 20:5n-3 may also be utilised for energy production when present at higher concentrations (12-14). The high latitude FO, that are currently favoured in salmon production, are very rich in 20:1n-9 and 22:1n-11 while different

vegetable oils are rich in 16:0, 18:1n-9, 18:2n-6 and 18:3n-3. Several recent studies have shown that salmon can be cultured for up to 30 weeks using diets formulated with up to 100% replacement of added FO by vegetable oils (15-18). The ability to convert C_{18} PUFA to their long chain HUFA products has been confirmed in Atlantic salmon as has the up-regulation of these pathways in hepatocytes and enterocytes of fish fed vegetable oil-containing diets (19,20). However, the increased flux through the fatty acid desaturation and elongation pathway is unlikely to compensate fully for reduced dietary EPA, DHA and possibly arachidonic acid requirements and, thus, sufficient dietary supply of these HUFA will need to be provided (12,13).

Atlantic salmon fed diets containing raw materials of predominantly marine origin are of high nutritional quality being rich in EPA and DHA and with an n-3/n-6 ratio of around 4:1 (21,22). Recent studies have shown that salmon cultured using diets where > 50% of added FO has been replaced with vegetable oil show significant reductions in flesh EPA and DHA concentrations (14-17). Obviously, from a human health perspective, any changes to n-3 HUFA content should be kept to a minimum and any FO replacement should provide sufficient energy alternatives to long-chain monoenes, maximise conversion of dietary 18:3n-3 and preserve endogenous n-3 HUFA levels. In this regard linseed oil is an important candidate for FO replacement, not only because it is rich in 18:3n-3, the precursor for n-3 HUFA but, 18:3n-3 is also a favoured substrate for β-oxidation both in salmonid fish and in mammals (12-14,23). Furthermore, increasing salmon flesh concentrations of 18:3n-3 should not be perceived as detrimental, from a human nutrition perspective, as 18:3n-3 has been shown to be beneficial in cardiovascular

disease and some forms of cancer (24), possibly due to inhibition of 18:2n-6 conversion to 20:4n-6 and inhibition of cyclooxygenase (25,26).

In the present study, Atlantic salmon post-smolts were stocked into 5 seawater pens one of four diets containing 25, 50, 75 or 100% LO, or a control diet containing only FO. The fish were grown for 40 weeks before sampling and thereafter all treatments were switched to a diet containing only FO for a further 24 weeks to follow accumulation of DHA and EPA and dilution of 18:3n-3 and 18:2n-6 from LO.

MATERIALS AND METHODS

Fish and diets

Atlantic salmon post-smolts (n = 3000) of initial mean weight 127 ± 3g (individual weights of 50 fish/pen) were distributed into five net pens (5 x 5m; 600 fish/pen) in Loch Duich, Lochalsh, Scotland, in June 2001. The temperature over the experimental period (June 2001-December 2002) ranged from 5.0-16.8ºC with a mean temperature of 10.8 ± 2.2ºC. The fish were fed one of four experimental diets in which the FO component was replaced by LO or a control diet containing only FO (capelin oil). Specifically the five diets were 100% FO (FO), FO/LO, 3:1 w/w (25% LO); FO/LO, 1:1 w/w (50% LO); FO/LO, 1:3 w/w (75% LO) and 100% LO. Feeds were fed to satiation by hand for a period of 40 weeks and all feed fed was recorded. After this time samples of fish were collected for analysis and the remaining fish were all switched to the FO diet for a further 24 weeks. The 5 practical type diets were formulated (Nutreco Aquaculture Research Centre, Stavanger, Norway) to fully satisfy the nutritional requirements of salmonid fish and differed only in their oil composition (27). The main dietary components were

fishmeal, 338g kg^{-1}, capelin oil 0-258g kg^{-1} and/or linseed oil 0-258g kg^{-1}, maize gluten 200g kg^{-1}, soya meal 100g kg^{-1} and micronutrients 25g kg^{-1}. The diets were produced in 3, 6 and 9 mm sizes and had average proximate compositions of 44.1 ± 0.3% crude protein, 29.4 ± 0.6% crude lipid, 7.1 ± 0.45% ash and 5.9 ± 0.3% moisture. The fatty acid compositions of the experimental diets are shown in Table 1. The FO diet containing capelin oil contained about 20% saturates, mainly 16:0 and almost 60% monoenes of which 50% were the long chain 20:1n-9 and 22:n-11. The FO diet contained 5% n-6 fatty acids, mostly 18:2n-6 and 16% n-3 fatty acids, dominated by EPA and DHA in almost equal amounts and with 18:3n-3 less than 1%. Addition of increasing levels of dietary LO resulted in increasing levels of 18:3n-3, 18:2n-6 and 18:1n-9 and decreasing levels of EPA, DHA, 16:0, total saturates, 20:1n-9, 22:1n-11 and total monoenes. Thus, the 100% LO diet contained 10% total saturates, 21% total monoenes, 15% 18:2n-6, 50% 18:3n-3 with EPA and DHA accounting for only 2.5% of total dietary fatty acids.

Sampling procedures

After 40 weeks, 18 fish were selected from each treatment and killed by a blow to the head after anaesthetising using MS222 (Sigma-Aldrich, Poole, England). A sample of flesh, representative of the edible portion was obtained by cutting a steak between the leading and trailing edges of the dorsal fin. The samples were combined as six pools of 3 steaks in each pool. The steaks were frozen on dry ice and stored at –40ºC until processed. The steaks were thawed, skinned, deboned and the flesh homogenised in a food processor, after removal of the dorsal fat body. The homogenate was frozen immediately and stored at –40ºC prior to analysis. During the period where fish from all treatments were returned to a FO diet samples were collected after 4, 8, 16 and 24 weeks

following reintroduction of FO diets. At each time point nine fish were sampled from each treatment group and were combined as three pools of 3 steaks in each pool. The steaks were processed and stored as described above.

Lipid extraction and fatty acid analysis. Total lipid was extracted from 2g of homogenised muscle by homogenising in 20 volumes of chloroform/methanol (2:1, v/v) in an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). Total lipid was prepared according to the method of Folch et al. (28) and non-lipid impurities removed by washing with 0.88% (w/v) KCl. The weight of lipid was determined gravimetrically after evaporation of solvent and overnight desiccation *in vacuo*. Fatty acid methyl esters were prepared by acid-catalysed transesterification of total lipid according to the method of Christie (29). Extraction and purification of fatty acid methyl esters was performed as described by Ghioni et al. (30). Fatty acid methyl esters were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) 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 50ºC to 150ºC at 40ºC min^{-1} and then to 230ºC at 2.0ºC min^{-1}. Individual methyl esters were identified by comparison with known standards and by reference to published data (30). Data were collected and processed using the Chromcard for Windows (version 1.19) computer package (Thermoquest Italia S.p.A., Milan, Italy).

Statistical analysis. Significance of difference ($P < 0.05$) between dietary treatments was determined by one-way analysis of variance (ANOVA). Differences between means were determined by Tukey's test. Data identified as non-homogeneous, using Bartlett's test, were subjected to log or arcsin transformation before applying the ANOVA. ANOVA

and regression analysis was performed using a Graphpad Prism ™ (version 3.0) statistical package (Graphpad Software, San Diego, CA).

RESULTS

After feeding the five diets for 40 weeks the final weights of the fish were 1.79 ± 0.40 kg (FO), 1.89 ± 0.34 kg (25% LO), 1.9 ± 0.33 kg (50% LO), 1.87 ± 0.35 kg (75% LO) and 1.87 ± 0.33 kg (100% LO) and there were no significant differences between dietary treatments ($P = 0.156$, $n = 87$). Specific growth rates were very similar (% weight gain day^{-1}) varying from 0.94 (FO) to 0.98 (25% LO) and feed conversion ratios (dry feed consumed/wet weight gain) varied from 1.29 (25 & 75% LO) to 1.41 (50% LO). Flesh lipid content varied from 7.4 ± 1.4% (FO) to 8.8 ± 1.7% (25% LO) and flesh astaxanthin content from 3.31 ± 0.58 mg kg^{-1} (FO) to 3.60 ± 0.49 mg kg^{-1} (50% LO). These values showed no significant differences between dietary treatments.

Flesh fatty acid compositions; 40 weeks feeding LO-containing diets The fatty acid compositions of flesh total lipid reflected the changes in dietary fatty acids due to increasing inclusion of LO (Table 2). Total saturated fatty acids decreased significantly with increasing LO inclusion, largely due to significant reductions in both 14:0 and 16:0, although 18:0 was increased with addition of LO. Total monoene fatty acids were reduced by more than half in fish fed 100% LO compared to those fed FO due to significant reductions in 16:1n-7, 20:1n-9 and 22:1n-11 with each addition of dietary LO. Total n-6 fatty acids were increased by more than 2-fold, largely due to 18:2n-6 increasing from 3.9 g/100g in flesh from FO fish to 13.1 g/100g in fish fed 100% FO. Flesh 20:4n-6 concentrations were significantly lower in fish fed 75 and 100% LO

compared to those fed FO. Total n-3 fatty acids were increased significantly with each addition of LO, largely due to increased flesh deposition of 18:3n-3, although the elongation and desaturation products, 20:3n-3 and 20:4n-3 were also increased significantly. However, both EPA and DHA were significantly reduced in flesh from fish fed LO with values for EPA and DHA in fish fed 100% LO being reduced to 30 and 38% of values in fish fed FO. The n-3/n-6 ratios were similar for all treatments being in the range of 3.2-3.6.

Plotting fatty acid concentration (g/100g) in flesh lipid (Table 2) against fatty acid concentration in dietary lipid (Table1) resulted in straight line graphs and some examples are shown in Figs. 1 and 2. These graphs demonstrate that concentrations of dietary fatty acids were linearly correlated with flesh fatty acid concentrations, with r^2 values in the range 0.966-0.999, and that different slope values indicate that the relationship between dietary and flesh fatty acid concentrations is different for each fatty acid (Table 3). This is shown more clearly in Table 3 by comparing the differences (Δ values) between the concentration of individual fatty acids in dietary lipid and flesh lipid for fish fed the FO, 50% LO and 100% LO. This shows that, for all the PUFA and HUFA listed in Table 3, only DHA was present in flesh at a greater concentration than that in the diet for all three treatments. This situation is in contrast with both 18:3n-3, which was always present in flesh at a lower concentration than in diets, and 20:5n-3 which only had a higher value in flesh compared to diet when diet concentrations were low. Interestingly, 16:0, total saturates and 18:1n-9 all showed positive Δ values for each of the three diets suggesting both saturated fatty acids and 18:1n-9 were being preferentially retained by the fish. In contrast, 18:2n-6, 18:3n-3, 20:1n-9 and 22:1n-11 were generally discriminated against in

terms of flesh deposition particularly when present at high concentrations in the diet. This suggests that these four fatty acids, in comparison to the saturates or 18:1n-9, were selectively utilised for metabolism, probably for energy production.

Flesh fatty acid compositions; 24 weeks refeeding FO-containing diets to all dietary treatments By returning all experimental treatments to a FO-containing diet for 24 weeks, following the initial 40 week feeding phase, the differences between flesh fatty acid compositions were reduced (Table 4). The concentrations of 16:0 and total saturates were only significantly higher in fish fed FO throughout compared to those previously fed 75 and 100% LO. A similar result was seen for 16:1n-7 while 20:1n-9 and 22:1n-11 were still significantly different for all treatment groups except that fish previously fed 75% LO were not different from those fed 50 or 100% LO. Concentrations of total monoenes showed similar differences but the concentration in fish previously fed 100% LO was restored to 80% of the value in fish fed FO throughout. The 18:2n-6 and total n-6 fatty acid concentrations were not different between fish fed either FO or 25% LO and these two treatments were significantly lower than fish previously fed 75 and 100% LO. Concentrations of flesh 20:4n-6 were restored to similar values for all dietary treatments. The flesh concentrations of 18:3n-3 were significantly higher in fish previously fed 75 and 100% LO, compared to fish previously fed 50% or 25% LO or FO. The concentration of EPA was significantly higher in fish fed FO throughout compared to those previously fed 50, 75 and 100% LO while concentrations of 22:5n-3 were significantly higher in fish fed FO throughout compared to all other treatments, which has similar concentrations. The concentration of flesh DHA was significantly higher in fish fed FO throughout compared to fish previously fed 50 and 100% LO. Concentrations of

total n-3 fatty acids were significantly higher in fish previously fed 75 and 100% LO compared to fish fed the other three diets.

After feeding the experimental diets for 40 weeks the flesh18:2n-6 concentration was significantly different between fish fed FO, 50% LO and 100% LO (Fig. 3A) with the highest values in fish fed 100% LO being 236% higher than in fish fed FO. After feeding the FO-containing finishing diet to all treatments the 18:2n-6 concentrations were still significantly different between the treatments after 56 and 64 weeks. However, after 64 weeks the 18:2n-6 concentrations in fish previously fed the 50% and 100% LO diets had reduced by 31% and 44%, respectively, following the 24 week FO refeeding period (Fig. 3A). A similar result was observed for 18:3n-3 such that the concentrations of in fish previously fed the 50 and 100% LO diets were still significantly different, both from each other and, from fish fed FO after 16 and 24 weeks on a FO diet (Fig. 3B). However, the concentrations of 18:3n-3, in fish previously fed 50% and 100% LO, had reduced by 64% and 66%, respectively, following 24 week FO refeeding period.

After the initial 40 week feeding period the flesh concentration of EPA was significantly lower in fish fed 50 and 100% LO compared to those fed FO (Fig. 4A). After refeeding FO for 16 and 24 weeks, the flesh EPA concentration increased such that fish previously fed the 50 and 100% LO diets had similar EPA concentrations but they were still significantly lower than fish fed FO throughout. However, the flesh concentrations of EPA, in fish previously fed 50% and 100% LO, were restored to 88% and 83%, respectively, of the concentration in fish fed FO throughout, following the 24 week FO refeeding period. A very similar result was seen with flesh DHA concentrations in fish previously fed 50% and 100% LO whereby DHA concentrations were restored to

80% and 83%, respectively, of the concentration in fish fed FO throughout, following the 24 week refeeding period. Interestingly, the flesh EPA and DHA concentrations were restored to 81 and 74% and 85 and 79%, respectively, for fish previously fed 50% and 100% LO diets, respectively, following refeeding the FO diet for only 16 weeks.

DISCUSSION

Salmon produced using diets containing raw materials with high levels of marine fish oils currently provide a rich source of the n-3 HUFA, EPA and DHA, that are relatively cheap and highly nutritious (21,22). However, the growth in aquaculture required to augment dwindling seafood stocks can only be sustained if alternatives to marine fish oils (FO) can be found (5,7). In the present study graded levels of linseed oil were used to replace FO in the culture of Atlantic salmon to market size with no obvious detriment in terms of growth and health of the fish. Although a number of previous studies have looked at FO substitution in salmon most have used smaller fish or that were grown on the experimental feeds for relatively short periods (16,17,32,33,34). However, the growth results obtained with the present study using LO compare favourably with studies utilising soya and rapeseed oils as FO replacements and suggests that the energy requirements of Atlantic salmon can be satisfied by vegetable oils with variable fatty acid compositions (18,35). A few studies have investigated LO alone, or blended with other oils, in salmon feeds and these have also confirmed no obvious effects on growth parameters (14,33,34).

Atlantic salmon store high amounts of lipid in their flesh and in market size salmon (2-5kg) lipid levels would normally be in the range of 10-20% of wet weight (21,22).

Changing the fatty acid composition of salmon diets can affect the composition and quantity of flesh lipid stores and previous studies using rapeseed and palm oils, to replace FO, observed reduced flesh lipid at replacement levels above 50% (16,17). In the present study, fish of ~2kg had lipid levels of 7.4-8.8% and there were no significant differences between treatments. In a previous study with salmon harvested at a similar size, and fed diets with varying levels of FO, LO and rapeseed oil, no differences in flesh lipid content were observed (14). However, lipid concentrations in the liver of fish from the present dietary trial showed significantly increased lipid deposition in fish fed diets with > 50% LO, suggesting that changes in diet fatty acid composition can affect adiposity in specific tissues (19).

It is well documented that tissue fatty acid compositions in salmonid fish are closely related to dietary fatty acid compositions and that feeding high levels of vegetable oils will strongly influence flesh fatty acid compositions (14-18). The results from the present study confirm this relationship in which linear correlations between dietary fatty acid concentrations and flesh fatty acid concentrations are clearly demonstrated. In previous studies similar linear relationships have been observed where rapeseed, palm and blends of rapeseed and linseed oil were used, along with FO, in salmon feed formulations (14,16,17). The present study has confirmed that individual fatty acids, within a blend of dietary fatty acids, are selectively retained or metabolised depending on their concentration in the diet and the biological function of the specific fatty acids. In practical terms, these linear correlations (Figs. 1 & 2) can be used to predict the flesh concentration of a particular fatty acid when present in a mixture of fatty acids, derived from blends of LO and FO, fed to salmon in sea water.

Additional information on selective retention or metabolism of different dietary fatty acids, present in different oil blends, can be obtained from Figs 1 & 2 and in Tables 2 & 5. One of the most striking effects is the preferential deposition and retention of DHA in flesh lipids, regardless of concentration present in the diet. The Δ values in Table 5 show positive values in the range 1.6-3.1 for 100% LO, 50% LO and FO treatments indicating that, regardless of dietary concentration, DHA was selectively deposited and retained in salmon flesh. This selectivity presumably reflects the specificity of the fatty acyl transferase enzymes that perform the incorporation of fatty acids into flesh TAG and phospholipids and has been observed in previous studies with salmon fed different combinations of vegetable oils (14,16,17).

By comparison, the other PUFA and HUFA tended to be directed towards metabolism, presumably largely for energy production, rather than deposition, especially when present at high concentrations. When present at low concentrations only EPA showed a positive Δ value while both 18:2n-6 and especially 18:3n-3 were selected against in terms of flesh deposition. The tendency towards preferential metabolism of C_{18} PUFA by β-oxidation has been observed, not only in salmonid fish (12,13), but also in humans where 18:3n-3 was preferred over 18:2n-6 as an oxidative substrate (23). However, it should also be noted that both 18:2n-6 and 18:3n-3 are substrates for Δ6-desaturase and it has been reported that Atlantic salmon hepatocytes tend to favour desaturation and elongation of 18:3n-3 over 18:2n-6 (20,34). In addition to PUFA, the long-chain monoene fatty acids, that are characteristic of high latitude fish oils (9), are thought to be important catabolic substrates. This appeared to be confirmed in the present study where the preference

appeared to be for 22:1n-11 over 20:1n-9 even though the former was less abundant in the diets than the latter.

The literature suggests that 22:1n-11 and 18:2n-6 are preferred substrates for β-oxidation, along with 16:0, 16:1 and 18:1n-9 (11,36,37). However, in the present study 16:0, and especially 18:1n-9, had positive Δ values (Table 5) indicating that these two fatty acids were being selectively retained in flesh lipids rather than being metabolised for energy production. This is in contrast to two previous studies which showed that 18:1n-9 was used for energy production by the β-oxidation pathway in salmon fed diets containing blends of LO and rapeseed oil although both 18:2n-6 and 18:3n-3 appeared to be preferred over 18:1n-9 (14,35). Perhaps these differences can be explained in part by genetic differences in salmon stocks utilised in the different trials. The selection of 18:1n-9 and 16:0 for deposition, rather than mobilisation, in flesh may reflect the structural function of these fatty acids in membrane phospholipids where they are often located in the *sn*-1 position of phospholipids, especially of PC and PE, with HUFA being favoured in the *sn*-2 position (9,38).

The diet of early humans pursuing a hunter/gatherer lifestyle was probably considerably lower in fat and had an n-6/n-3 ratio of around 1:1 in comparison to the current diet in the developed world with an n-6/n-3 ratio of 10-20:1 (39). The health benefits of diets rich in EPA and DHA, that can reduce this high n-6/n-3 ratio, are well documented and the outcome of a number of conditions prevalent in the developed world, including cardiovascular disease, immune dysfunction, diabetes and other inflammatory disorders, can be improved as a result (40-42). While outcomes of clinical trials with 18:3n-3 have been less clear than with n-3 HUFA there is evidence that diets promoting

increased tissue levels of 18:3n-3 can also be beneficial to health (24). Benefits of increased 18:3n-3 for cardiovascular disorders, including rhythm disorders, myocardial infarctions, sudden cardiac death and coronary thrombosis have been reported (43-45). In addition, some studies have reported low tissue levels of ARA as risk factors for both breast and prostate cancers (46,47).

Atlantic salmon presently cultured using only marine fish oils are rich in n-3 HUFA and as such represent a valuable source of EPA and DHA for the human consumer (21,22). The present study suggests that salmon cultured with LO replacing up to 50% of FO results in moderate reduction of n-3 HUFA, but increased deposition of 18:3n-3, and only moderate deposition of 18:2n-6, means that flesh n-3/n-6 ratios of > 3 are maintained. Furthermore, even salmon fed 100% LO provide n-3 HUFA levels of 0.12g EPA and 0.28g DHA, as well as 3.48g 18:3n-3 and 1.12g 18:2n-6, per 100g of salmon flesh. These values are close to the recommended 0.22g/day each for EPA and DHA, 2.22g/day for 18:3n-3, and are well below the maximum recommended 18:2n-6 intake of 6.67g/day (39). However, it is also recommended to increase the dietary n-3/n-6 ratio and especially to increase n-3 HUFA of marine origin (39,40).

This study has demonstrated mechanisms by which the ratio of DHA/EPA/18:3n-3/18:2n-6 can be manipulated in salmon flesh so that precise "tailored" EFA compositions can be delivered to human consumers. This can be achieved via the initial dietary input, for the majority of the ongrowing phase, and by the use of a FO finishing diet to restore the values of the aforementioned EFA to the desired concentrations. The latter procedure restored both EPA and DHA concentrations in flesh to > 80% of the value in fish fed FO throughout after 24 weeks while 18:2n-6 and 18:3n-3 concentrations

were 74% and 13-fold higher than in fish fed FO. These results are similar to previous studies with Atlantic salmon and gilthead seabream which showed that restoration of flesh EPA and DHA concentrations can be achieved relatively easily with finishing diets but that C_{18} PUFA concentrations still remain elevated (14,48).

In conclusion, this study suggests that Atlantic salmon can be cultured, during the marine phase of their life cycle, using diets in which the FO is replaced by LO without apparent reduction in growth rates or fish health. In addition, this study has shown that salmon cultured with diets containing up to 50% LO, or up to 100% LO followed by a period of 16-24 weeks on a FO finishing diet, can provide a carcass EFA composition that is highly beneficial for human health. While the goal of minimising reliance on marine raw materials cannot be achieved overnight this study suggests that salmon can be cultured on diets with minimal FO input yet still retain a high functional nutritional value that cannot be replicated in terrestrial farmed foodstuffs.

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Figure legends.

FIG. 1. Relationship between dietary fatty acid concentration and muscle fatty acid concentration for 22:6n-3 (A), 20:5n-3 (B) and 18:3n-3 (C) in total lipids of Atlantic

salmon fed diets containing blends of fish oil (FO) and linseed oil (LO). The additional line represents the line of equality.

FIG. 2. Relationship between dietary fatty acid concentration and muscle fatty acid concentration for 18:2n-6 (A), 20:1n-9 (B) and 22:1n-11 (C) in total lipids of Atlantic salmon fed diets containing blends of fish oil (FO) and linseed oil (LO). The additional line represents the line of equality.

FIG. 3. Linoleic (18:2n-6) [A] and linolenic acid (18:3n-3) [B] concentration in total lipid from salmon flesh after feeding diets containing 100% fish oil (FO), 50% linseed oil (50% LO) or 100% LO for 40 weeks and after feeding a 100% FO diet for a further 16 and 24 weeks to all treatment groups. Columns assigned a different letter, within each time point, are significantly different ($P < 0.05$).

FIG. 4. Eicosapentaenoic (20:5n-3) [A] and docosahexaenoic acid (22:6n-3) [B] concentration in total lipid from salmon flesh after feeding diets containing 100% fish oil (FO), 50% linseed oil (50% LO) or 100% LO for 40 weeks and after feeding a 100% FO diet for a further 16 and 24 weeks to all treatment groups. Columns assigned a different letter, within each time point, are significantly different ($P < 0.05$).

TABLE 1.

Fatty acid compositions (g/100g total fatty acids) and astaxanthin concentrations (mg/kg) in experimental diets.

Fatty acid	FO	25% LO	50% LO	75% LO	100% LO
14:0	6.3	4.7	3.4	2.0	0.4
16:0	12.1	10.6	9.3	8.1	6.1
18:0	1.1	1.7	2.1	2.7	3.1
Total saturates[1]	19.9	17.2	15.1	13.0	10.5
16:1n-7	8.1	6.1	4.2	2.3	0.5
18:1n-9	11.9	13.6	15.1	16.0	17.0
18:1n-7	3.3	2.6	2.2	1.6	1.0
20:1n-9	17.9	13.1	9.0	5.0	1.1
22:1n-11	13.3	10.1	7.1	4.3	1.1
22:1n-9	2.1	1.5	1.0	0.5	0.1
Total monoenes[2]	58.4	48.4	39.6	30.5	21.1
18:2n-6	4.2	7.4	9.8	12.3	15.1
20:4n-6	0.2	0.2	0.1	0.1	0.1
Total n-6[3]	5.0	8.0	10.2	12.6	15.2
18:3n-3	0.9	14.0	25.6	37.8	50.4
18:4n-3	2.9	2.1	1.6	0.9	0.2
20:5n-3	5.9	4.6	3.5	2.2	1.0
22:6n-3	5.0	4.0	3.4	2.4	1.5
Total n-3[4]	15.7	25.6	34.6	43.7	53.3
Total PUFA	21.7	34.4	45.3	56.5	68.5
n-3/n-6	3.1	3.2	3.4	3.5	3.5
Astaxanthin (mg/kg)	56.9	61.8	66.6	72.2	68.7

[1]Includes 15:0, 17:0, 20:0 and 22:0. [2]Includes 16:1n-9, 20:1n-11, 20:1n-7, 22:1n-13 and 24:1. [3]Includes 20:2n-6, 20:3n-6 and 22:5n-6. [4]Includes 20:3n-3, 20:4n-3 and 22:5n-3.

TABLE 2.
Total lipid fatty acid compositions (g/100g total fatty acids) of flesh from Atlantic salmon fed the linseed oil experimental diets for 40 weeks.

Fatty acid	FO	25% LO	50% LO	75% LO	100% LO
14:0	4.7 ± 0.2^{a}	3.9 ± 0.1^{b}	2.9 ± 0.1^{c}	0.8 ± 0.1^{d}	0.7 ± 0.1^{d}
16:0	12.9 ± 0.2^{a}	11.7 ± 0.2^{b}	10.7 ± 0.3^{c}	9.6 ± 0.2^{d}	8.3 ± 0.2^{e}
18:0	1.9 ± 0.1^{e}	2.3 ± 0.0^{d}	2.8 ± 0.1^{c}	3.1 ± 0.0^{b}	3.6 ± 0.1^{a}
Total saturates[1]	19.9 ± 0.3^{a}	18.1 ± 0.2^{b}	16.5 ± 0.4^{c}	14.7 ± 0.2^{d}	12.7 ± 0.3^{e}
16:1n-7	7.5 ± 0.2^{a}	5.5 ± 0.1^{b}	4.0 ± 0.1^{c}	2.3 ± 0.1^{d}	0.8 ± 0.0^{e}
18:1n-9	16.0 ± 0.4^{c}	17.4 ± 0.3^{b}	17.6 ± 0.3^{b}	17.6 ± 0.3^{b}	18.6 ± 0.3^{a}
18:1n-7	3.8 ± 0.0^{a}	3.4 ± 0.3^{b}	2.5 ± 0.1^{c}	2.2 ± 0.1^{d}	1.3 ± 0.1^{e}
20:1n-9	16.6 ± 0.3^{a}	11.9 ± 0.2^{b}	8.7 ± 0.1^{c}	4.8 ± 0.0^{d}	1.6 ± 0.1^{e}
22:1n-11	9.9 ± 0.1^{a}	7.3 ± 0.3^{b}	5.4 ± 0.1^{c}	3.0 ± 0.0^{d}	1.1 ± 0.1^{e}
Total momoenes[2]	57.1 ± 0.6^{a}	48.4 ± 0.6^{b}	40.3 ± 0.4^{c}	31.5 ± 0.3^{d}	24.2 ± 0.4^{e}
18:2n-6	3.9 ± 0.1^{e}	6.8 ± 0.1^{d}	8.6 ± 0.1^{c}	11.0 ± 0.1^{b}	13.1 ± 0.2^{a}
20:2n-6	0.4 ± 0.0^{d}	0.5 ± 0.0^{c}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	0.7 ± 0.0^{a}
20:4n-6	0.3 ± 0.1^{a}	0.2 ± 0.0^{ab}	0.2 ± 0.0^{ab}	0.1 ± 0.1^{b}	0.1 ± 0.0^{b}
Total n-6[3]	4.9 ± 0.2^{e}	7.8 ± 0.1^{d}	9.8 ± 0.2^{c}	11.8 ± 0.1^{b}	14.0 ± 0.1^{a}
18:3n-3	0.8 ± 0.2^{e}	11.5 ± 0.3^{d}	20.1 ± 0.3^{c}	30.1 ± 0.4^{b}	38.7 ± 0.8^{a}
18:4n-3	1.5 ± 0.1^{a}	1.3 ± 0.1^{b}	1.3 ± 0.1^{b}	1.2 ± 0.1^{b}	1.2 ± 0.1^{b}
20:3n-3	0.1 ± 0.0^{e}	0.7 ± 0.0^{d}	1.4 ± 0.1^{c}	2.1 ± 0.1^{b}	2.7 ± 0.1^{a}
20:4n-3	1.2 ± 0.0^{d}	1.4 ± 0.0^{c}	1.5 ± 0.0^{bc}	1.6 ± 0.0^{b}	1.8 ± 0.1^{a}
20:5n-3	4.3 ± 0.2^{a}	3.0 ± 0.1^{b}	2.5 ± 0.1^{c}	1.8 ± 0.1^{d}	1.3 ± 0.1^{e}
22:5n-3	1.5 ± 0.0^{a}	1.1 ± 0.1^{b}	1.0 ± 0.0^{b}	0.6 ± 0.1^{c}	0.4 ± 0.1^{d}
22:6n-3	8.1 ± 0.7^{a}	6.1 ± 0.7^{b}	5.3 ± 0.4^{bc}	4.3 ± 0.5^{c}	3.1 ± 0.2^{d}
Total n-3	17.5 ± 0.7^{e}	25.2 ± 0.7^{d}	33.1 ± 0.6^{c}	41.7 ± 0.3^{b}	49.1 ± 0.6^{a}
Total PUFA	22.9 ± 0.8^{e}	33.4 ± 0.7^{d}	43.1 ± 0.7^{c}	53.7 ± 0.3^{b}	66.1 ± 0.7^{a}
n-3/n-6	3.6 ± 0.4	3.2 ± 0.3	3.4 ± 0.3	3.5 ± 0.2	3.5 ± 0.3

Values are mean ± SD, n = 6. [1]Includes 15:0, 17:0, 20:0 and 22:0. [2]Includes 16:1n-9, 20:1n-11, 20:1n-7, 22:1n-13 and 24:1. [3]Includes 18:3n-6, 20:3n-6 and 22:5n-6. SD values

<0.05 are shown as 0.0. Values in the same row with a different superscript letter are significantly different ($P < 0.05$).

TABLE 3.
Correlation coefficients and slopes from plots of dietary fatty acid concentrations vs. flesh fatty acid concentrations including the difference (Δ) between diet and flesh fatty acid values for salmon fed 100% FO, 50% LO and 100% LO diets.

Fatty acid	Correlation coefficient (r^2)	Slope	Δ100% FO	Δ50% LO	Δ100% LO
16:0	0.997	0.78 ± 0.02	0.8	1.4	2.2
Total saturates	0.998	0.77 ± 0.02	0.0	1.4	2.2
18:1n-9	0.876	0.43 ± 0.09	4.1	2.5	1.6
20:1n-9	0.999	0.89 ± 0.02	-1.3	-0.3	0.5
22:1n-11	0.997	0.73 ± 0.02	-3.4	-1.7	0.0
18:2n-6	0.999	0.85 ± 0.02	-0.3	-1.2	-2.0
18:3n-3	0.999	0.77 ± 0.01	-0.1	-5.5	-11.7
20:5n-3	0.966	0.59 ± 0.06	-1.6	-1.0	0.3
22:6n-3	0.979	1.37 ± 0.12	3.1	1.9	1.6

Fatty acid concentrations are g fatty acid/100g total fatty acids in muscle and diets.

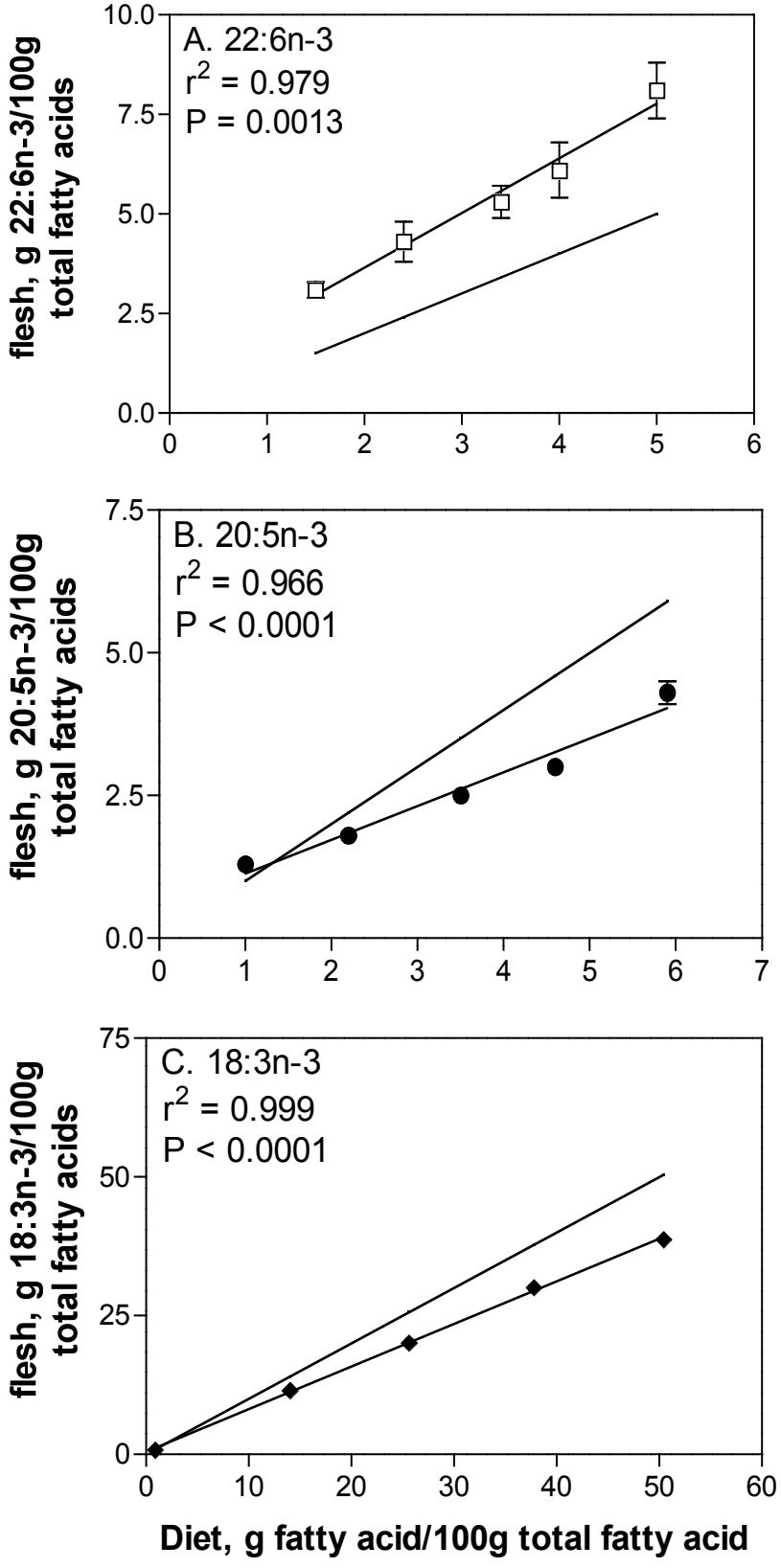
Negative Δ values indicate lower values in muscle compared with diet, whereas positive values indicate accumulation in muscle relative to diet.

TABLE 4.
Total lipid fatty acid compositions (g/100g total fatty acids) of flesh from Atlantic salmon fed the linseed oil experimental diets for 40 weeks, followed by re-feeding a 100% FO diet for 24 weeks.

Fatty acid	FO	25% LO	50% LO	75% LO	100% LO
14:0	4.8 ± 0.1^{a}	4.5 ± 0.1^{b}	3.9 ± 0.1^{c}	3.8 ± 0.1^{c}	3.6 ± 0.3^{c}
16:0	12.6 ± 0.2^{a}	12.1 ± 0.0^{ac}	12.3 ± 0.3^{a}	11.3 ± 0.3^{bc}	11.2 ± 0.5^{b}
18:0	2.1 ± 0.1^{b}	2.2 ± 0.1^{b}	2.3 ± 0.0^{bc}	2.5 ± 0.1^{ac}	2.6 ± 0.1^{a}
Total saturates[1]	19.9 ± 0.2^{a}	19.0 ± 0.1^{ac}	18.7 ± 0.5^{ab}	17.8 ± 0.5^{bc}	17.5 ± 0.8^{b}
16:1n-7	7.5 ± 0.1^{a}	7.0 ± 0.1^{ab}	6.5 ± 0.3^{b}	5.8 ± 0.1^{c}	5.3 ± 0.4^{c}
18:1n-9	16.2 ± 0.2^{b}	16.6 ± 0.1^{ab}	16.9 ± 0.1^{a}	16.7 ± 0.2^{a}	16.9 ± 0.3^{a}
18:1n-7	3.9 ± 0.1^{b}	4.1 ± 0.1^{ab}	4.3 ± 0.2^{a}	3.4 ± 0.1^{c}	3.1 ± 0.2^{c}
20:1n-9	16.7 ± 0.0^{a}	14.9 ± 0.1^{b}	13.2 ± 0.4^{c}	12.1 ± 0.2^{cd}	11.1 ± 0.8^{d}
22:1n-11	10.3 ± 0.1^{a}	9.2 ± 0.3^{b}	8.2 ± 0.3^{c}	7.7 ± 0.3^{cd}	7.2 ± 0.6^{d}
Total monoenes[2]	57.9 ± 0.3^{a}	55.7 ± 0.7^{a}	53.0 ± 0.7^{b}	48.8 ± 0.6^{c}	46.4 ± 1.9^{c}
18:2n-6	4.2 ± 0.2^{d}	5.1 ± 0.1^{cd}	5.9 ± 0.3^{bc}	6.7 ± 0.2^{ab}	7.3 ± 0.7^{a}
20:2n-6	0.4 ± 0.0^{b}	0.5 ± 0.1^{ab}	0.5 ± 0.0^{ab}	0.5 ± 0.0^{ab}	0.6 ± 0.1^{a}
20:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
Total n-6[3]	5.2 ± 0.1^{d}	6.2 ± 0.2^{cd}	6.9 ± 0.4^{bc}	7.7 ± 0.3^{ab}	8.4 ± 0.7^{a}
18:3n-3	0.9 ± 0.4^{c}	4.0 ± 0.4^{bc}	7.3 ± 0.5^{b}	10.9 ± 0.7^{a}	13.0 ± 2.6^{a}
18:4n-3	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.0
20:3n-3	0.1 ± 0.0^{d}	0.3 ± 0.1^{cd}	0.5 ± 0.1^{bc}	0.8 ± 0.1^{ab}	1.0 ± 0.2^{a}
20:4n-3	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
20:5n-3	4.1 ± 0.1^{a}	3.8 ± 0.1^{ab}	3.6 ± 0.1^{bc}	3.4 ± 0.1^{c}	3.4 ± 0.2^{c}
22:5n-3	1.9 ± 0.1^{a}	1.6 ± 0.0^{b}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}
22:6n-3	6.6 ± 0.1^{a}	6.1 ± 0.1^{ab}	5.3 ± 0.6^{b}	5.7 ± 0.1^{ab}	5.5 ± 0.6^{b}
Total n-3	16.3 ± 0.3^{c}	18.6 ± 0.6^{bc}	20.9 ± 0.8^{b}	25.1 ± 0.9^{a}	27.2 ± 2.0^{a}
Total PUFA	22.0 ± 0.4^{c}	25.1 ± 0.7^{bc}	28.1 ± 1.1^{b}	33.1 ± 1.1^{a}	35.9 ± 2.7^{a}
n-3/n-6	3.1 ± 0.3	3.0 ± 0.2	3.0 ± 0.3	3.3 ± 0.2	3.2 ± 0.4

Values are mean ± SD, n = 3. [1]Includes 15:0, 17:0, 20:0 and 22:0. [2]Includes 16:1n-9, 20:1n-11, 20:1n-7, 22:1n-13 and 24:1. [3]Includes 18:3n-6, 20:3n-6 and 22:5n-6. SD values <0.05 are shown as 0.0. Values in the same row with a different superscript letter are significantly different ($P < 0.05$).

Fig. 1.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B. 20:5n-3
r2 = 0.966
P < 0.0001
flesh, g 20:5n-3/100g total fatty acids
C. 18:3n-3
r2 = 0.999
P < 0.0001
flesh, g 18:3n-3/100g total fatty acids
Diet, g fatty acid/100g total fatty acid

Fig. 2.

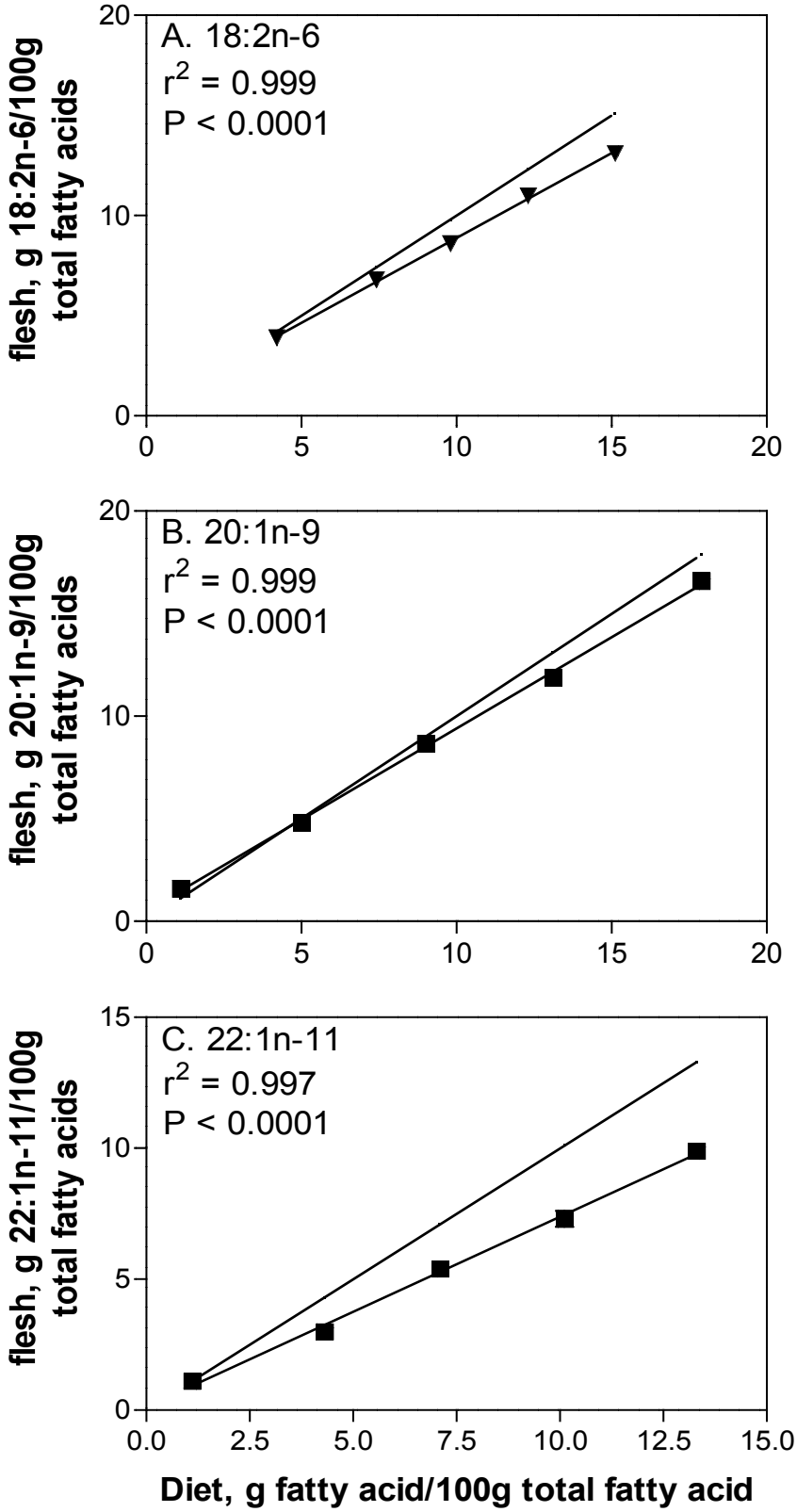

A. 18:2n-6
r2 = 0.999
P < 0.0001
flesh, g 18:2n-6/100g total fatty acids
B. 20:1n-9
r2 = 0.999
P < 0.0001
flesh, g 20:1n-9/100g total fatty acids
C. 22:1n-11
r2 = 0.997
P < 0.0001
flesh, g 22:1n-11/100g total fatty acids
Diet, g fatty acid/100g total fatty acid

Fig. 3.

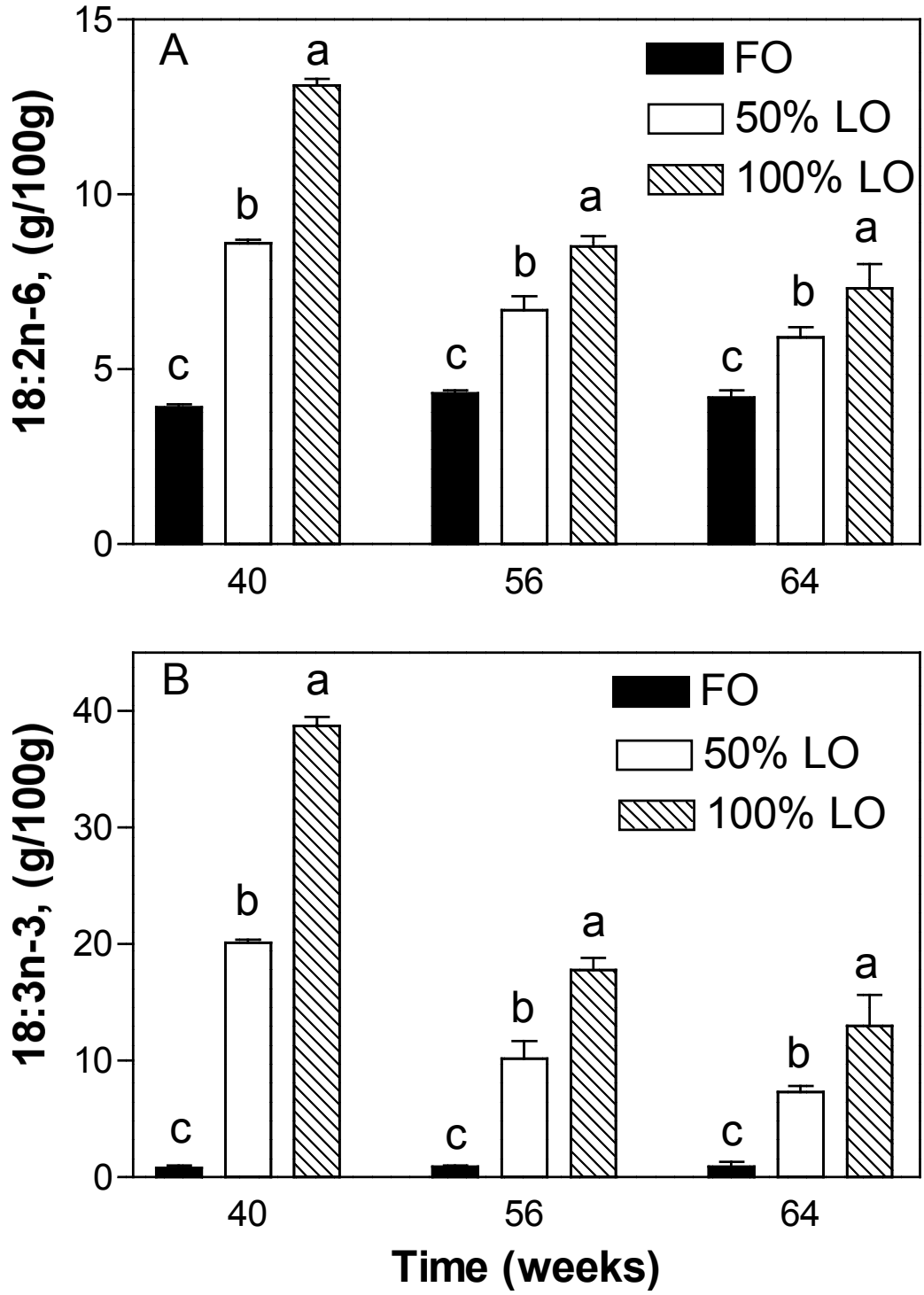
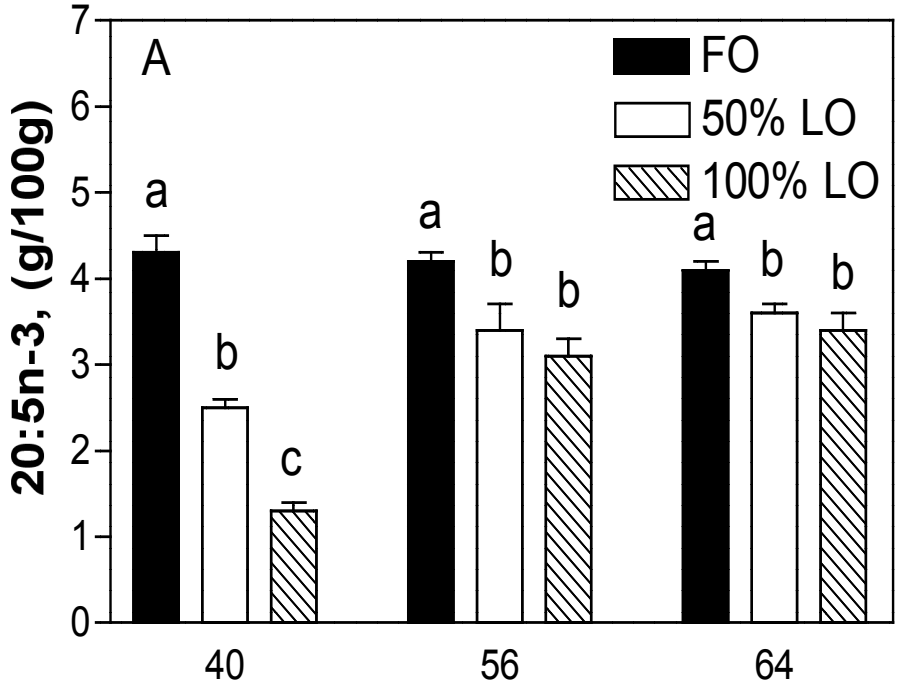
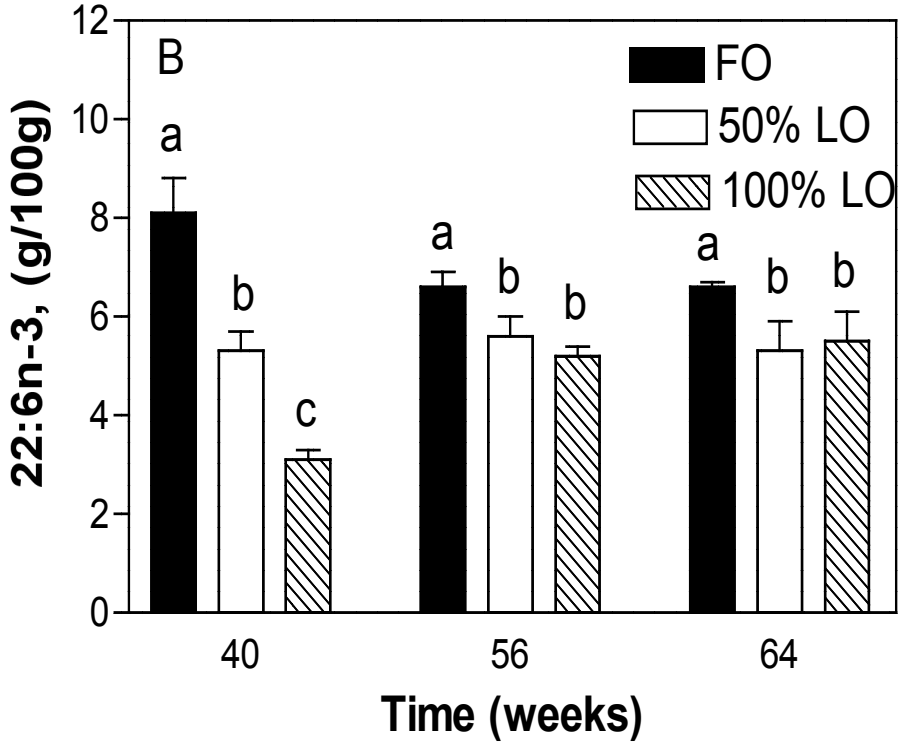

A
B
FO
50% LO
100% LO
18:2n-6, (g/100g)
18:3n-3, (g/100g)
Time (weeks)
40
56
64
a
b
c

Fig. 4


A
FO
50% LO
100% LO
20:5n-3, (g/100g)
0
1
2
3
4
5
6
7
a
b
c
a
b
b
a
b
b
40
56
64


B
FO
50% LO
100% LO
22:6n-3, (g/100g)
0
2
4
6
8
10
12
a
b
c
a
b
b
a
b
b
40
56
64
Time (weeks)