

Effect of diets enriched in $\Delta 6$ desaturated fatty acids (18:3n-6 and 18:4n-3), on growth, fatty acid composition and highly unsaturated fatty acid synthesis in two populations of Arctic charr (*Salvelinus alpinus* L.)

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Abbreviations: BHT - butylated hydroxytoluene; FAF-BSA - fatty acid-free bovine serum albumin; EO, echium oil; FO - fish oil; HBSS - Hanks balanced salt solution; HUFA - highly unsaturated fatty acids (carbon chain length $\geq C_{20}$ with ≥ 3 double bonds).

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Abstract

This study aimed to test the hypothesis that diets containing relatively high amounts of the $\Delta 6$ desaturated fatty acids stearidonic acid (STA, 18:4n-3) and γ -linolenic acid (GLA, 18:3n-6), may be beneficial in salmonid culture. The rationale being that STA and GLA would be better substrates for highly unsaturated fatty acid (HUFA) synthesis as their conversion does not require the activity of the reputed rate-limiting enzyme, fatty acid $\Delta 6$ desaturase. Duplicate groups of two Arctic charr (*Salvelinus alpinus* L.) populations with different feeding habits, that had been reported previously to show differences in HUFA biosynthetic capacity, were fed for 16 weeks on two fish meal based diets containing 47% protein and 21% lipid differing only in the added lipid component, which was either fish oil (FO) or echium oil (EO). Dietary EO had no detrimental effect on growth performance and feed efficiency, mortalities, or liver and flesh lipid contents in either population. The proportions of 18:2n-6, 18:3n-3, 18:3n-6, 18:4n-3, 20:3n-6 and 20:4n-3 in total lipid in both liver and flesh were increased by dietary EO in both populations. However, the percentages of 20:5n-3 and 22:6n-3 were reduced by EO in both liver and flesh in both strains, whereas 20:4n-6 was only significantly reduced in flesh. In fish fed FO, HUFA synthesis from both $[1-^{14}\text{C}]18:3\text{n-3}$ and $[1-^{14}\text{C}]20:5\text{n-3}$ was significantly higher in the planktonivorous Coulin charr compared to the demersal, piscivorous Rannoch charr morph. However, HUFA synthesis was increased by EO in Rannoch charr, but not in Coulin charr. In conclusion, dietary EO had differential effects in the two populations of charr, with HUFA synthesis only stimulated by EO in the piscivorous Rannoch morph, which showed lower activities in fish fed FO. However, the hypothesis was not proved as, irrespective of the activity of the HUFA synthesis pathway in either population, feeding EO resulted in decreased tissue levels of n-3HUFA and 20:4n-6. This has been observed previously in salmonids fed vegetable oils, and thus the increased levels of $\Delta 6$ desaturated fatty acids in EO did not effectively compensate for the lack of dietary HUFA.

1. Introduction

Omega-3 (or n-3) polyunsaturated fatty acids (PUFA) are important dietary nutrients for mammals including humans (Simopoulos, 2000), and fish are the major dietary source of n-3 highly unsaturated fatty acids (HUFA) (Ackman, 1980; Sargent and Tacon, 1999). An increasing proportion of fish is being provided by aquaculture (Tidwell and Allan, 2002) but, paradoxically, diets have traditionally been based on fish meal and fish oil (FO) as the major finfish species farmed in Europe are carnivorous (Sargent and Tacon, 1999; Tacon, 2004). For a variety of reasons, the sustainable development of aquaculture requires that dietary FO must be replaced, with vegetable oils (VO) as the primary candidate alternatives (Barlow, 2000; Sargent et al., 2002; Pike, 2005). Salmonids, including Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss* L.), can generally be grown on diets in which the FO has been replaced by VOs without compromising growth performance or significantly affecting fish health and welfare (Bell et al., 2001, 2002; Torstensen et al., 2000, 2006; Robin et al., 2003). However, this practice leads to increased C₁₈ PUFA and reduced n-3 HUFA in the flesh of the fish, potentially compromising the nutritional quality to consumers (Bell et al., 2001, 2002; Torstensen et al., 2000, 2006; Robin et al., 2003). Several studies have shown that feeding salmonid fish, including Arctic charr (*Salvelinus alpinus* L.), with VOs increased the activity of the hepatic HUFA biosynthesis pathway (Tocher et al., 2001, 2003; Zheng et al., 2005). However it is clear that the activity of this pathway is not sufficient to compensate for lack of dietary HUFA.

The mechanism underpinning an increased HUFA synthesis pathway in fish fed VO is not entirely clear. As HUFA such as arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are known to inhibit fatty acid desaturase activity (Brenner, 1981), possibly through suppressing gene expression (Tocher, 2003), it is assumed that the primary mechanism is increased gene expression of the fatty acid desaturases and/or elongases, as a consequence of reduced levels of dietary HUFA (Tocher, 2003). The role that increased fatty acid

substrate provision (18:2n-6 or 18:3n-3) plays is less clear (Tocher et al., 2003). Early work determined that the rate limiting step, at least for the production of ARA from 18:2n-6 in mammals, was $\Delta 6$ desaturase activity (Brenner, 1981). By definition, this would dictate that the activity of the entire pathway would be determined by the activity of this, the first enzyme in the chain. Certainly, high levels of dietary 18:3n-3, supplied by linseed oil, did not alleviate the problem of reduced n-3HUFA in salmon fed high VO (Tocher et al., 2002; Bell et al., 2003, 2004). However, logic implies that the production of HUFA could be increased if the rate-limiting step in the HUFA synthetic pathway was bypassed. The seed oils of some plants, particularly of the Boraginaceae species, can be relatively rich in the $\Delta 6$ desaturated fatty acid, γ -linolenic acid (GLA, 18:3n-6), but seed oils rich in the n-3 homologue, stearidonic acid (STA, 18:4n-3) are less common (Padley et al., 1986; Moine et al., 1992). However, some Boraginaceae species can contain high levels of STA with up to 21% being reported in *Echium asperrium* (Guil-Guerrero et al., 2001). Echium oil is thus similar to borage oil except that the levels of 18:4n-3 can exceed those of its n-6 series equivalent GLA.

In the present study, we aimed to test the hypothesis that feeding diets containing relatively high amounts of both $\Delta 6$ desaturated fatty acids, specifically STA and GLA, may be beneficial in salmonid culture. The rationale being that STA and GLA would be better PUFA substrates for HUFA synthesis in the fish as they does not require the activity of the reputed rate-limiting step enzyme, fatty acid $\Delta 6$ desaturase. In addition to determining basic growth performance parameters and tissue fatty acid compositions, the activity of the HUFA biosynthetic pathway in isolated hepatocytes was investigated to determine what effect a diet rich in $\Delta 6$ desaturated products had on this important pathway. Arctic charr were chosen for this trial as different populations or morphs with different feeding habits were available and these have been reported previously to show differences in HUFA biosynthetic capacity (Tocher et al., 2001).

2. Materials and methods

2.1. *Animals and diets*

The Arctic charr (*Salvelinus alpinus* L.) investigated were from two populations held in the FRS Fish Cultivation Unit, Aultbea, and were originally obtained from two locations. One was from Loch Rannoch, Perthshire, which is deep and contains three distinct charr morphs including a demersal, piscivorous population (Rannoch charr), and the other population was from Loch Coulin, Wester Ross, a shallow loch containing a planktonivorous morph (Coulins charr) (Tocher et al., 2001). Fish were distributed randomly into eight 1 m diameter circular fiberglass tanks of 500 L capacity at 30 fish per tank. The tanks were supplied with non-recirculated fresh water (average temperature, 5.0 ± 2.4 °C, range 1.4 – 10.8 °C) at 5 L/min. After a two-week acclimatization period, each population of charr were fed two diets in duplicate for a period of 16 weeks. Diets were fish meal based containing 47% crude protein and 21% crude lipid and differed only in the added lipid component which was either Northern hemisphere fish oil (FO) or echium oil (EO). The diets were prepared from a 1.5 mm base extrusion (Ecostart 17/47, BioMar Ltd., Grangemouth, UK) that were top coated with either FO or EO. The diet formulations and analysed proximate compositions are shown in Table 1, and fatty acid compositions in Table 2. The fish were fed a fixed ration of 3% of biomass per day with the ration being adjusted following assessment of biomass by bulk weighing every 28 days. After 16 weeks, fish were individually weighed and six fish per dietary treatment (three per tank) were killed by a blow to the head and samples of liver and muscle collected for lipid and fatty acid analyses. A further four fish per dietary treatment were used for the preparation of hepatocytes for the determination of highly unsaturated fatty acid synthesis.

2.2. *Proximate analyses of diets*

Dry matter in the diets was measured gravimetrically after oven drying of homogenised samples for 24 hours at 105°C. Crude protein was determined by combustion using the Kjeldhal method. Total lipid in

diets was measured gravimetrically after acid hydrolysis followed by Soxhlet extraction with petroleum ether.

2.2. Lipid extraction and fatty acid analyses

Total lipids of liver, flesh and diet samples were extracted by homogenization in chloroform/methanol (2:1, v/v) containing 0.01 % butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification using 2 ml of 1% H₂SO₄ in methanol plus 1 ml toluene as described by Christie (1982) and FAME extracted and purified as described previously (Tocher and Harvie, 1988). FAME were separated and quantified by gas-liquid chromatography (Fisons GC8600, Carlo Erba, Milan, Italy) using a 30 m x 0.32 mm capillary column (CP wax 52CB; Chrompak Ltd., London, U.K) utilizing on-column injection at 50°C and flame ionization detection at 250°C. Hydrogen was used as carrier gas and temperature programming was from 50°C to 180°C at 40°C/min and then to 225°C at 2°C/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman, 1980).

2.4. Hepatocyte HUFA synthesis

Isolated hepatocytes were prepared by collagenase treatment of chopped liver tissue and sieving through 100 µm nylon gauze essentially as described in detail previously for salmonid tissues including Arctic charr (Tocher et al., 2001). Viability, as assessed by trypan blue exclusion, was > 95% at isolation and decreased by less than 5% over the period of the incubation. One hundred µl of the hepatocyte suspensions were retained for protein determination according to the method of Lowry et al. (1951) following incubation with 0.4 ml of 0.25% (w/v) SDS/1M NaOH for 45 min at 60 °C. Five ml aliquots of each hepatocyte suspension were dispensed into 25 cm² tissue culture flasks and incubated at 20 °C for 2 h with 0.3 µCi (~ 1 µM) of either [1-¹⁴C]18:3n-3 or [1-¹⁴C]20:5n-3, added as complexes

with fatty acid-free bovine serum albumin in phosphate buffered saline prepared as described previously (Ghioni et al. 1997). After incubation, hepatocytes were harvested, washed, and lipid extracted as described previously (Zheng et al., 2005), and total lipid transmethylated and FAME prepared as described above. The FAME were separated by argentation (silver nitrate) thin-layer chromatography (Wilson and Sargent, 1992), located on the plate by autoradiography for 6 days, and quantified by liquid scintillation after being scraped from the plates all as described in detail previously (Stubhaug et al., 2005).

2.4. Materials

[1-¹⁴C]18:3n-3 and [1-¹⁴C]20:5n-3 (50-55 mCi/mmol) were obtained from NEN (Perkin Elmer LAS (UK) Ltd., Beaconsfield, U.K.). HBSS, Medium 199, HEPES buffer, collagenase (type IV), fatty acid-free bovine serum albumin, BHT and silver nitrate were obtained from Sigma-Aldrich Co. Ltd. (Poole, U.K.). Thin-layer chromatography (TLC) plates, precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK Ltd., Loughborough, England.

2.5 Statistical analysis

All the data are presented as means \pm SD with n = 6 (lipids), 4 (fatty acid metabolism) or 2 (growth performance). The effects of dietary oil and strain on all the data were determined by two-way ANOVA. Due to the highly significant interaction observed in the ANOVA of the HUFA synthesis data, the significance of dietary oil in each strain was also determined by the Student's t test. Percentage data or data that were identified as non-homogeneous (Bartlett's test) were subjected to either arcsine or log transformation before analysis. All statistical analyses were performed using Prism 3 (Graphpad Software, Inc., San Diego, USA). Differences were regarded as significant when $P < 0.05$ (Zar 1984).

3. Results

3.1 Dietary fatty acid compositions

The control diet FO, formulated with fish oil, contained approximately 25% total saturates, mainly 16:0, around 41% total monounsaturated fatty acids half of which were the long chain monoenes, 20:1 and 22:1, 5.5% n-6 fatty acids predominantly 18:2n-6, and almost 28% n-3 fatty acids, predominantly the n-3HUFA, EPA and DHA (Table 2). The $\Delta 6$ desaturated fatty acids, GLA (18:3n-6) and STA (18:4n-3) were present at 0.7 and 3.4%, respectively, totaling just over 4%. Replacement of the fish oil with echium oil resulted in an almost three-fold increase in the proportion of $\Delta 6$ desaturated fatty acids to 11.3%, with GLA and STA being increased to 4.5 and 6.8%, respectively (Table 2). Additionally, 18:3n-3 and 18:2n-6 were also increased with concomitant decreased proportions of n-3HUFA and long chain monoenes.

3.2 Effects of echium oil on growth performance and lipid content in Arctic charr

Echium oil had no effect on the growth performance of the charr as determined by final weight or SGR, and similarly no effect on feed efficiency (FCR) or mortalities (Table 3). The Coulin charr were larger than the Rannoch charr at the initiation of the trial and this resulted in that strain having increased weights at the end of the feeding period. Dietary echium oil had no effect on the lipid content of either muscle (flesh) or liver in either charr population (Table 3). However the Coulin charr were characterized by having significantly lower liver lipid than the Rannoch charr irrespective of diet.

3.3 Effects of echium oil on fatty acid composition of liver and flesh in the two strains of Arctic charr

The rank order of the major fatty acids from total lipid of liver from charr fed FO was $18:1n-9 > DHA > 16:0 > 16:1n-7 > EPA = 20:1n-9$, irrespective of population (Table 4). However, liver of Coulin charr was characterized by higher proportions of PUFA, including DHA and ARA and decreased proportions of monoenes, specifically $16:1n-7$ and $18:1n-9$, compared to liver from Rannoch charr. Some of the changes in fatty acid composition of the diets in response to replacement of FO with EO, described above, were reflected in the liver fatty acid compositions of both populations, including increased proportions of $18:2n-6$, GLA, $18:3n-3$ and STA and decreased proportions of long chain monoenes and DHA. In addition, the proportions of $20:3n-6$ and $20:4n-3$ but not ARA and EPA in liver were increased by EO in both populations.

The rank order of the major fatty acids from total lipid of flesh from charr fed FO was $DHA > 18:1n-9 = 16:0 > EPA > 22:1n-11 = 20:1n-9 = 16:1n-7$, irrespective of population (Table 5). In general, the effects of dietary EO on the fatty acid composition of the flesh were similar to those observed in liver. Thus, flesh of charr fed EO showed increased proportions of $18:2n-6$, GLA, $18:3n-3$ and STA and decreased proportions of long chain monoenes and DHA (Table 5). In addition, the proportions of $20:3n-6$ and, to a lesser extent, $20:4n-3$ were increased, whereas proportions of ARA and, especially, EPA were decreased by EO in flesh of both populations. In contrast to liver, there were few significant differences between the populations in the fatty acid composition of the flesh, irrespective of diet.

3.4 Effects of echium oil on HUFA biosynthesis from $[1-^{14}C]18:3n-3$ in hepatocytes from Arctic charr

The activity of the complete HUFA synthesis pathway in hepatocytes was determined by measuring the recovery of radioactivity in the summed desaturated products ($18:4$, $20:4$, $20:5$, $22:5$ and $22:6$) of $[1-^{14}C]18:3n-3$. The results of two-way ANOVA indicated that there was no significant effect of diet or strain but that there was significant interaction between the variables (Table 6). The interaction

masking the effects of diet and population was obvious in the graphical data, which showed that EO increased HUFA synthesis in Rannoch fish, but tended to have the opposite effect in Coulin fish (Fig.1). However, activity of the HUFA synthesis pathway in Coulin fish fed FO was significantly higher than in Rannoch fish as determined by t-test. These effects of diet and population were reflected in the production of specific individual products of $[1-^{14}\text{C}]18:3\text{n}-3$ metabolism. Thus, recovery of radioactivity in STA and EPA was significantly increased by EO in Rannoch fish and recovery of radioactivity in EPA and DHA significantly reduced by EO in Coulin fish (Fig.2). In fish fed FO, recovery of radioactivity in 18:4, 20:5 and 22:6 was higher in Coulin charr than Rannoch charr, whereas in fish fed EO, recovery of radioactivity in 20:5 was lower in Coulin charr than Rannoch charr.

3.5 Effects of echium oil on HUFA biosynthesis from $[1-^{14}\text{C}]20:5\text{n}-3$ in hepatocytes from Arctic charr

The production of DHA from EPA in hepatocytes was estimated by determining the conversion of $[1-^{14}\text{C}]20:5\text{n}-3$ to desaturated and elongated products by measuring the recovery of radioactivity in the products (22:5, 24:5n-3, 24:6n-3 and 22:6). Again, the results of two-way ANOVA indicated that there was a significant interaction masking the effects of diet and population (Table 6) that was obvious in the graphical data, which showed that EO increased conversion of EPA in Rannoch fish, but not in Coulin fish (Fig.3). However, overall conversion of EPA in Coulin fish was significantly higher than in Rannoch fish when fed FO, but significantly lower when fed EO, as determined by t-tests. Specifically focusing on desaturation of EPA (summed recovery in 24:6 and 22:6), EO increased desaturation of EPA in Rannoch fish, but not in Coulin fish (Fig.4) but, again, desaturation of EPA in Coulin fish was significantly higher than in Rannoch fish when fed FO. Thus, recovery of radioactivity in 24:6n-3 and DHA was significantly increased by EO in Rannoch fish, and significantly increased in Coulin fish fed FO compared to Rannoch fish fed FO (Fig.5).

4. Discussion

The conclusion of this study is that the hypothesis was not proved. Therefore, feeding charr EO diets containing relatively high amounts of the $\Delta 6$ desaturated fatty acids, STA and GLA, that do not require the activity of the reputed rate-limiting step enzyme, fatty acid $\Delta 6$ desaturase, for their conversion to HUFA, did not maintain tissue n-3HUFA or ARA at the same level as in fish fed FO. Thus, irrespective of the activity of the HUFA synthesis pathway in either Arctic charr population, feeding EO resulted in decreased tissue levels of n-3HUFA and ARA. This is a well-known effect that has been observed previously in several trials in which salmonids have been fed VOs (Torstensen et al., 2000, 2006; Bell et al., 2001, 2002, 2003; Robin et al., 2003), and thus the increased levels of $\Delta 6$ desaturated fatty acids in EO did not effectively compensate for the lack of dietary HUFA. This was, to some extent, an unexpected result as Arctic charr are known to be capable of producing EPA and DHA from 18:3n-3 (Tocher et al., 2001). By contrast, in human studies, STA in dietary EO was shown to be effectively converted to n-3HUFA, particularly EPA (Miles et al., 2004; Surette et al., 2004), and in one study directly comparing 18:3n-3 with STA, there was evidence that STA was more effective than 18:3n-3 in increasing erythrocyte and plasma phospholipids EPA concentrations (James et al., 2003).

With respect to the failed hypothesis, there was no difference between the two populations of Arctic charr. However, the data clearly showed that there were differences in lipid metabolism between the two populations of charr. The Rannoch charr had a higher lipid content in the liver which would undoubtedly be the result of higher neutral lipid stores, specifically triacylglycerol although lipid class composition was not determined in this trial. However, entirely consistent with higher liver triacylglycerol, the fatty acid composition in liver total lipid of Rannoch charr was characterized by higher monounsaturated fatty acids and lower PUFA, especially n-3HUFA compared to liver total lipid in Coulin charr. That the difference in fatty acid composition of the livers was due to underlying

differences in lipid class composition rather than effects of fatty acid metabolism such as HUFA synthesis was supported by the fact that muscle fatty acid compositions were identical between the two populations consistent with there being no difference in lipid content in that tissue. In a previous trial investigating HUFA synthesis in a range of salmonids, including these two populations of different charr morphs, the liver lipid contents observed in the charr were much lower than in the present study, at around 3% and there was no difference between the two populations (Tocher et al., 2001). The diets used in the present trial had a higher lipid content (21% v. 19%) and lower protein content (47% v. 54%), compared to the earlier trial although this is not likely to have such a large effect on liver lipid contents (Tocher et al., 2001). It is more likely that this is a seasonal effect as the earlier trial was carried out between June and September compared to the present trial that was run over the winter months, from October to February. The charr in the earlier trial were also older and larger fish (~200 g at end of trial compared to 25 g) and were showing signs of sexual maturation and so the lower liver lipid may be related to gonad formation. Early sexual maturation in Arctic charr culture is a known and significant problem (Jobling et al., 1998)

However, it is also possible that the difference in liver total lipid fatty acid composition was also, at least partly, influenced by endogenous HUFA synthesis capacity in the fish. Certainly, in hepatocytes in fish fed the FO diet, HUFA synthesis activity was higher in the Coulin charr than in the Rannoch charr. Therefore, the higher n-3HUFA, especially DHA, and ARA in liver of Coulin charr would be consistent with this. Additionally, the higher HUFA synthesis activity in Coulin charr compared to Rannoch charr when fed FO is perhaps expected, as the Coulin fish used are a planktonivorous morph and, as such, may be expected to be consuming a diet that was lower in n-3HUFA, especially DHA, than the piscivorous Rannoch morph (Bell et al., 1994; Ghioni et al., 1996). These data were also consistent with the results from our earlier trial, which also showed that HUFA synthesis activity was higher in hepatocytes from Coulin charr compared to Rannoch charr (Tocher et al., 2001). The higher HUFA synthesis in the Coulin charr when fed the FO diet was not reflected in higher HUFA contents

in flesh, although this is not entirely unexpected. Both populations were fed the same commercial trout pellet, containing FO, prior to initiation of the trial (Tocher et al., 2001) and, as observed in other trials with salmon, alterations in hepatic HUFA synthesis appear to have only minimal effects on final flesh fatty acid compositions (Bell et al., 2001, 2002, 2004; Tocher et al., 2002). Clearly, dietary fatty acid composition dominates over endogenous fatty acid conversions in determining flesh fatty acid compositions in salmonids (Sargent et al., 2002).

Although the HUFA synthesis activities in two populations fed FO was entirely consistent with our expectations, the effects of feeding EO were certainly not as they varied significantly between the two populations. As would have been predicted, hepatocyte HUFA synthetic activity was increased in fish fed EO compared to fish fed FO in Rannoch charr. This response has been demonstrated in many previous studies in which Atlantic salmon have been fed a variety of single VOs (Bell et al., 1997, 2001, 2002; Tocher et al., 2002; Ruyter et al., 2003) or VO blends (Tocher et al., 2000, 2003; Zheng et al., 2005). Indeed, in a trial comparing the effects of different VOs in Atlantic salmon, HUFA synthesis in hepatocytes was stimulated to greater extent by borage oil, containing high levels of GLA and so most similar to the EO used in the present study but without any n-3 PUFA, than sunflower or olive oils (Tocher et al., 1997). Increased HUFA synthesis in response to VO diets in salmonids has also been demonstrated in intestinal enterocytes, another site of significant HUFA synthetic activity (Tocher et al., 2002; Fonseca-Madrugal et al., 2005). In contrast, although there was no statistically significant difference between the HUFA synthesis activities in Coulin charr fed the two diets, the data tended to show at least a trend for lower activities in the fish fed EO. There is no precedent for this result, and in our earlier trial, hepatocyte HUFA synthesis activity was more than doubled in both Coulin and Rannoch charr fed a VO blend (linseed and rapeseed oils, 1:1) compared to fish fed FO (Tocher et al., 2001).

There is no obvious explanation for the lack of effect of dietary fatty acid composition on HUFA synthesis in Coulin charr. Dietary PUFA may influence desaturation enzymes quite directly at a

membrane level through alterations in fluidity or membrane microenvironments. However, dietary fatty acids are known to affect the expression of fatty acid desaturase genes in both mammals (Cho et al. 1999a,b) and rainbow trout (Seiliez et al. 2001). Recently it was demonstrated that the expression of fatty acid desaturase genes in Atlantic salmon was increased in fish fed VOs compared to fish fed FO (Zheng et al., 2004, 2005). PUFA can potentially affect gene transcription by a number of direct and indirect mechanisms and are known to bind and directly influence the activities of a variety of transcription factors (Jump et al., 1999), and desaturase genes have cis elements for a variety of transcription factors in their upstream sequences (Nakamura and Nara, 2004).

In conclusion, irrespective of the activity of the HUFA synthesis pathway in either population of Arctic charr, feeding EO resulted in decreased tissue levels of n-3HUFA and ARA, and thus the increased levels of the $\Delta 6$ desaturated fatty acids, GLA and STA, in EO did not effectively compensate for the lack of dietary HUFA.

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Legends to Figures:

Fig. 1. Effect of echium oil (EO) on highly unsaturated fatty acid (HUFA) synthesis from $[1-^{14}\text{C}]18:3\text{n}-3$ in hepatocytes isolated from Rannoch and Coulin populations of Arctic charr after feeding the experimental diets for 16 weeks. Results are means \pm SD ($n = 4$) and represent the rate of conversion ($\text{pmol.h}^{-1}.\text{mg protein}^{-1}$) of $[1-^{14}\text{C}]18:3\text{n}-3$ to all desaturated products (sum of radioactivity recovered as $18:4\text{n}-3$, $20:4\text{n}-3$, $20:5\text{n}-3$, $22:5\text{n}-3$ and $22:6\text{n}-3$). A single asterisk denotes a significant effect of EO for a specific population (Rannoch, $P = 0.0113$), and a double asterisk denotes a significant effect of strain for a particular diet (FO, $P = 0.0133$), as determined by Student's t tests. FO, fish oil.

Fig. 2. Individual fatty acid products of the metabolism of $[1-^{14}\text{C}]18:3\text{n}-3$ by desaturation and elongation in hepatocytes from Rannoch and Coulin populations of Arctic charr fed the experimental diets for 16 weeks. Results are means \pm SD ($n = 4$) and represent the rate production ($\text{pmol.h}^{-1}.\text{mg protein}^{-1}$) of individual fatty acid products as determined by the recovery of radioactivity in each fatty acid fraction. An asterisk denotes a significant effect of EO for a specific population (Rannoch $18:4$ and $20:5$ production, $P = 0.014$ and 0.009 , respectively; Coulin $20:5$ and $22:6$ production, $P = 0.018$ and 0.027 , respectively), and a double asterisk denotes a significant effect of population for a particular diet (FO $18:4$, $20:5$ and $22:6$ and $22:6$ production, $P = 0.0043$, 0.0113 and 0.0323 , respectively; EO $20:5$ production, $P = 0.0158$), as determined by Student's t tests.

Fig. 3. Effect of echium oil (EO) on metabolic conversion of $[1-^{14}\text{C}]20:5\text{n}-3$ in hepatocytes isolated from Rannoch and Coulin populations of Arctic charr after feeding the experimental diets for 16 weeks. Results are means \pm SD ($n = 4$) and represent the rate of conversion ($\text{pmol.h}^{-1}.\text{mg protein}^{-1}$) of $[1-^{14}\text{C}]20:5\text{n}-3$ to all desaturated and elongated products (sum of radioactivity recovered as $22:5\text{n}-3$,

24:5n-3, 24:6n-3 and 22:6n-3). An asterisk denotes a significant effect of EO for a specific population (Rannoch, $P = 0.001$), and double asterisks denote significant effects of population for a particular diet (FO, $P = 0.029$; EO, $P = 0.0097$), as determined by Student's *t* tests. FO, fish oil.

Fig. 4. Effect of echium oil (EO) on desaturation of $[1-^{14}\text{C}]20:5\text{n-3}$ in hepatocytes isolated from Rannoch and Coulin strains of Arctic charr after feeding the experimental diets for 16 weeks. Results are means \pm SD ($n = 4$) and represent the rate of conversion ($\text{pmol.h}^{-1}.\text{mg protein}^{-1}$) of $[1-^{14}\text{C}]20:5\text{n-3}$ to all desaturated and elongated products (sum of radioactivity recovered as 22:5n-3, 24:5n-3, 24:6n-3 and 22:6n-3). An asterisk denotes a significant effect of EO for a specific population (Rannoch, $P = 0.004$), and a double asterisk denotes a significant effect of population for a particular diet (FO, $P = 0.0007$), as determined by Student's *t* tests. FO, fish oil.

Fig. 5. Individual fatty acid products of the metabolism of $[1-^{14}\text{C}]20:5\text{n-3}$ by desaturation and elongation in hepatocytes from Rannoch and Coulin populations of Arctic charr fed the experimental diets for 16 weeks. Results are means \pm SD ($n = 4$) and represent the rate of production ($\text{pmol.h}^{-1}.\text{mg protein}^{-1}$) of individual fatty acid products as determined by the recovery of radioactivity in each fatty acid fraction. An asterisk denotes a significant effect of EO for a specific population (Rannoch 24:6 and 22:6 production, $P = 0.019$ and 0.004 , respectively), and a double asterisk denotes a significant effect of population for a particular diet (FO 24:6 and 22:6 production, $P = 0.0377$ and 0.0436 , respectively), as determined by Student's *t* tests. FO, fish oil.

Table 1

Formulations (g.kg⁻¹) and proximate compositions
(percentage of total diet) of experimental diets.

	Fish oil	Echium oil
Fishmeal ¹	500	500
Soya meal ²	100	100
Wheat ³	200	200
Micronutrients ⁴	14	14
Fish oil ⁵	186	40
Echium oil ⁶	-	146
<u>Proximate composition</u>		
Protein	47.5 ± 0.2	47.3 ± 0.1
Lipid	20.5 ± 0.1	20.8 ± 0.2
Ash	5.5 ± 0.1	5.6 ± 0.1
Moisture	8.7 ± 0.1	8.6 ± 0.1

Proximate compositions are means ± S.D. (n=3).

¹LT94, Norsemeal Ltd., London, UK.

²HiPro, Grosvenor Grain, Perth, UK. ³J.D. Martin, Tranent, UK.

⁴Vitamins and minerals (BioMar AS, Brande, Denmark)
provided at values in excess of the requirements for salmonid
fish (NRC, 1993).

⁵Northern hemisphere fish oil, BioMar Ltd., Grangemouth, UK.

⁶Technology Crops International, Braintree, UK.

Table 2

Fatty acid composition (g/100g of total fatty acids) of experimental diets.

	Fish oil	Echium oil
14:0	5.7 ± 0.3	3.5 ± 0.2
16:0	15.5 ± 0.3	12.8 ± 0.3
18:0	2.7 ± 0.1	3.1 ± 0.1
Total saturated ¹	24.9 ± 0.3	20.1 ± 0.3
16:1n-7 ²	5.5 ± 0.0	4.8 ± 0.4
18:1n-9	12.8 ± 0.0	14.1 ± 1.2
18:1n-7	2.7 ± 0.2	1.8 ± 0.1
20:1n-9 ³	8.2 ± 0.1	5.4 ± 0.1
22:1n-11 ⁴	11.1 ± 0.2	6.6 ± 0.4
Total monoenes	40.8 ± 0.2	31.9 ± 1.1
18:2n-6	3.7 ± 0.1	9.7 ± 0.2
18:3n-6	0.7 ± 0.0	4.5 ± 0.1
20:4n-6	0.6 ± 0.0	0.3 ± 0.0
Total n-6 PUFA ⁵	5.5 ± 0.1	14.7 ± 0.2
18:3n-3	2.7 ± 0.1	13.6 ± 0.1
18:4n-3	3.4 ± 0.0	6.8 ± 0.1
20:4n-3	0.7 ± 0.0	0.4 ± 0.0
20:5n-3	8.2 ± 0.2	5.1 ± 0.1
22:5n-3	0.9 ± 0.3	0.6 ± 0.0
22:6n-3	11.7 ± 0.3	7.3 ± 0.4
Total n-3 PUFA ⁶	27.6 ± 0.4	32.6 ± 0.4
Total PUFA ⁷	34.0 ± 0.3	47.9 ± 0.3

Results are means ± S.D. (n = 2).

¹, totals contain 15:0, 17:0, 20:0 and 22:0 present at up to 0.4%; ², contains n-9 isomer present at up to 0.1%; ³, contains n-11 and n-7 isomers present at up to 0.4%; ⁴, contains n-9 and n-7 isomers at up to 1.0%; ⁵, totals contain 20:2n-6, 20:3n-6 and 22:5n-6 present at up to 0.2%; ⁶, totals contain 20:3n-3 present at up to 0.1%; ⁷, contains C₁₆ PUFA present at up to 0.9%.

Table 3

Growth and biometric parameters for Arctic charr (*Salvelinus alpinus* L.) fed the experimental diets for four months

	Rannoch		Coulin		ANOVA		
	FO	EO	FO	EO	Diet	Pop.	Interact.
Initial weight (g)	5.4 ± 0.5	5.6 ± 0.4	6.7 ± 0.1	6.3 ± 0.3	0.712	0.017	0.301
Final weight (g)	23.9 ± 4.0	19.8 ± 1.5	28.0 ± 4.4	31.1 ± 2.3	0.840	0.029	0.195
SGR	1.3 ± 0.2	1.1 ± 0.0	1.2 ± 0.1	1.4 ± 0.0	1.000	0.275	0.065
FCR	2.0 ± 0.0	1.9 ± 0.1	1.9 ± 0.4	1.5 ± 0.1	0.171	0.171	0.374
Mortalities (n)	0	0	1	0			
<u>Lipid contents</u>							
Muscle	3.2 ± 0.5	3.6 ± 0.5	3.1 ± 0.4	3.2 ± 0.3	0.173	0.173	0.406
Liver	10.2 ± 2.7	9.6 ± 1.2	6.5 ± 0.8	8.7 ± 0.7	0.226	0.002	0.041

Data are presented as means ± SD (n = 2 for biometry and 6 for lipid contents). FCR, feed conversion ratio = feed consumed (kg) / weight gain (kg); SGR, specific growth rate (%/day) = $(\ln W_1 - \ln W_0)/T$, where W_1 = final weight, W_0 = initial weight and T = time in days; Significance (P values) of effects of diet, population, and their interaction were determined by two-way ANOVA as described in the Materials and Methods.

Table 4

Fatty acid composition (percentage of total fatty acids) of total lipid from liver of two strains of Arctic charr (*Salvelinus alpinus* L.) fed diets containing either fish oil (FO) or echium oil (EO)

Fatty acid	Rannoch		Coulin		ANOVA		
	FO	EO	FO	EO	Diet	Pop.	Interact.
14:0	2.6 ± 0.2	2.1 ± 0.1	2.4 ± 0.1	1.9 ± 0.2	<0.0001	0.026	1.000
16:0	11.0 ± 0.3	10.7 ± 0.8	11.1 ± 0.4	9.5 ± 0.9	0.013	0.117	0.069
18:0	2.1 ± 0.2	2.9 ± 0.3	2.2 ± 0.2	2.8 ± 0.2	<0.0001	1.000	0.400
Total saturated ¹	16.4 ± 0.3	16.2 ± 0.7	16.5 ± 0.3	14.6 ± 1.1	0.010	0.049	0.029
16:1n-7	9.6 ± 1.4	6.7 ± 0.5	6.9 ± 0.6	5.7 ± 0.6	0.001	0.001	0.070
18:1n-9	28.5 ± 1.8	28.3 ± 2.4	24.7 ± 1.6	27.4 ± 1.3	0.195	0.024	0.139
18:1n-7	3.1 ± 0.2	2.2 ± 0.1	2.9 ± 0.2	2.2 ± 0.1	<0.0001	0.230	0.230
20:1n-9	5.0 ± 0.2	3.6 ± 0.2	5.2 ± 0.5	3.8 ± 0.2	<0.0001	0.213	1.000
22:1n-11	3.7 ± 0.2	2.4 ± 0.2	3.8 ± 0.3	2.6 ± 0.1	<0.0001	0.183	0.646
24:1n-9	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.069	0.069	1.000
Total monoenes ²	53.1 ± 2.9	45.6 ± 2.4	46.8 ± 2.1	44.2 ± 1.0	0.001	0.005	0.047
18:2n-6	3.1 ± 0.3	5.8 ± 0.1	3.4 ± 0.1	6.6 ± 0.4	<0.0001	0.001	0.078
18:3n-6	0.6 ± 0.1	2.2 ± 0.2	0.6 ± 0.1	2.1 ± 0.1	<0.0001	0.464	0.464
20:3n-6	0.6 ± 0.1	1.7 ± 0.2	0.8 ± 0.1	2.0 ± 0.2	<0.0001	0.008	0.539
20:4n-6	0.8 ± 0.2	0.8 ± 0.2	1.2 ± 0.1	0.9 ± 0.2	0.208	0.009	0.208
Total n-6 PUFA ³	5.7 ± 0.5	11.1 ± 0.4	6.6 ± 0.2	12.2 ± 0.7	<0.0001	0.001	0.687
18:3n-3	1.7 ± 0.2	4.7 ± 0.3	1.9 ± 0.1	5.4 ± 0.4	<0.0001	0.007	0.093
18:4n-3	1.6 ± 0.1	3.6 ± 0.3	1.5 ± 0.2	3.4 ± 0.2	<0.0001	0.183	0.646
20:4n-3	1.1 ± 0.4	1.6 ± 0.4	1.0 ± 0.2	1.8 ± 0.3	0.002	0.771	0.389
20:5n-3	4.9 ± 0.6	4.6 ± 0.6	5.4 ± 0.4	4.3 ± 0.3	0.015	0.692	0.130
22:5n-3	1.7 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	1.3 ± 0.2	0.011	0.337	1.000
22:6n-3	13.9 ± 2.0	11.2 ± 1.4	18.8 ± 1.3	12.6 ± 0.6	<0.0001	0.001	0.029
Total n-3 PUFA ⁴	24.9 ± 2.7	27.3 ± 1.5	30.2 ± 1.8	29.1 ± 1.1	0.500	0.003	0.086
Total PUFA	30.6 ± 3.1	38.3 ± 1.8	36.8 ± 1.8	41.2 ± 1.6	0.0001	0.001	0.152

Results are means ± SD (n=4). PUFA, polyunsaturated fatty acids. ¹Totals include 15:0 and 20:0 present at up to 0.4%; ²Totals include 16:1n-9, 20:1n-11, 20:1n-7 and 22:1n-9 present at up to 0.5%; ³Totals include 20:2n-6 and 22:5n-6 present at up to 0.3%; ⁴Totals include 20:3n-3 present at up to 0.3%. Two-way ANOVA results are presented as P values.

Table 5

Fatty acid composition (percentage of total fatty acids) of total lipid from flesh of two strains of Arctic charr (*Salvelinus alpinus* L.) fed diets containing either fish oil (FO) or echium oil (EO)

Fatty acid	Rannoch		Coulin		ANOVA		
	FO	EO	FO	EO	Diet	Pop.	Interact.
14:0	3.3 ± 0.2	2.6 ± 0.2	3.6 ± 0.0	2.4 ± 0.0	<0.0001	0.493	0.004
16:0	15.5 ± 0.9	14.0 ± 0.0	15.2 ± 0.4	13.5 ± 0.1	<0.0001	0.132	0.693
18:0	2.5 ± 0.1	2.9 ± 0.1	2.5 ± 0.0	3.0 ± 0.1	<0.0001	0.271	0.271
Total saturated ¹	22.1 ± 0.7	20.1 ± 0.2	22.0 ± 0.5	19.4 ± 0.1	<0.0001	0.097	0.202
16:1n-7	4.9 ± 0.6	3.9 ± 0.1	5.2 ± 0.0	3.4 ± 0.1	<0.0001	0.529	0.023
18:1n-9	15.9 ± 1.9	16.9 ± 0.6	15.6 ± 0.4	15.9 ± 0.5	0.236	0.238	0.516
18:1n-7	2.4 ± 0.1	2.0 ± 0.0	2.6 ± 0.0	1.8 ± 0.0	<0.0001	1.000	<0.0001
20:1n-9	5.0 ± 0.2	3.8 ± 0.0	5.5 ± 0.2	3.6 ± 0.0	<0.0001	0.055	0.001
22:1n-11	5.6 ± 0.0	3.9 ± 0.3	5.9 ± 0.3	3.7 ± 0.1	<0.0001	0.655	0.041
24:1n-9	0.6 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.002	1.000	1.000
Total monoenes ²	36.7 ± 2.0	32.8 ± 1.1	38.0 ± 0.1	30.5 ± 0.4	<0.0001	0.405	0.001
18:2n-6	4.4 ± 0.9	8.4 ± 0.0	4.2 ± 0.2	8.5 ± 0.2	<0.0001	0.836	0.537
18:3n-6	0.2 ± 0.1	3.0 ± 0.2	0.2 ± 0.1	3.0 ± 0.0	<0.0001	1.000	1.000
20:3n-6	0.3 ± 0.2	0.9 ± 0.0	0.3 ± 0.1	1.0 ± 0.0	<0.0001	0.389	0.389
20:4n-6	0.7 ± 0.1	0.5 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	<0.0001	0.069	0.069
Total n-6 PUFA ³	6.3 ± 1.2	13.3 ± 0.2	5.9 ± 0.4	13.6 ± 0.2	<0.0001	0.88	0.301
18:3n-3	3.3 ± 1.4	8.9 ± 0.2	2.7 ± 0.6	9.3 ± 0.3	<0.0001	0.803	0.226
18:4n-3	2.7 ± 0.9	5.2 ± 0.2	2.5 ± 0.2	5.1 ± 0.0	<0.0001	0.537	0.836
20:4n-3	1.2 ± 0.2	1.3 ± 0.0	1.0 ± 0.1	1.2 ± 0.0	0.020	0.020	0.389
20:5n-3	6.7 ± 1.0	5.2 ± 0.1	6.8 ± 0.1	5.1 ± 0.0	<0.0001	1.000	0.699
22:5n-3	1.5 ± 0.1	1.2 ± 0.1	1.5 ± 0.0	1.1 ± 0.0	<0.0001	0.183	0.183
22:6n-3	18.5 ± 4.5	12.9 ± 0.1	18.7 ± 1.1	14.0 ± 0.1	0.001	0.585	0.705
Total n-3 PUFA ⁴	34.1 ± 3.1	35.0 ± 0.6	33.4 ± 0.4	36.1 ± 0.5	0.045	0.808	0.286
Total PUFA	40.8 ± 1.2	48.3 ± 0.8	39.6 ± 0.4	49.7 ± 0.7	<0.0001	0.813	0.008

Results are means ± SD (n=4). PUFA, polyunsaturated fatty acids. ¹Totals include 15:0 and 20:0 present at up to 0.4%; ²Totals include 16:1n-9, 20:1n-11, 20:1n-7 and 22:1n-9 present at up to 0.7%; ³Totals include 20:2n-6 and 22:5n-6 present at up to 0.3%; ⁴Totals include 20:3n-3 present at up to 0.3%. Two-way ANOVA results are presented as P values.

Table 6

Significance (P values) of combined effects of diet (echium oil, EO v. fish oil, FO) and charr population (Rannoch v. Coulin) as determined by two-way ANOVA analyses of the data in Figures 1-5 for highly unsaturated fatty acid (HUFA) synthesis from $[1-^{14}\text{C}]18:3\text{n-3}$ and $[1-^{14}\text{C}]20:5\text{n-3}$

	Diet	Population	Interaction
<u>$[1-^{14}\text{C}]18:3\text{n-3}$ substrate</u>			
Total desaturated	0.456	0.521	0.003
18:4 production	0.752	0.614	0.004
20:4 production	0.238	0.916	0.069
20:5 production	0.705	0.268	0.000
22:5 production	0.363	0.363	0.008
22:6 production	0.138	0.138	0.008
<u>$[1-^{14}\text{C}]20:5\text{n-3}$ substrate</u>			
Total converted	0.059	0.640	0.001
Total desaturated	0.057	0.592	0.000
22:5 production	0.340	0.260	0.081
24:5 production	0.732	0.807	0.732
24:6 production	0.850	0.461	0.013
22:6 production	0.003	0.624	0.005

Fig.1

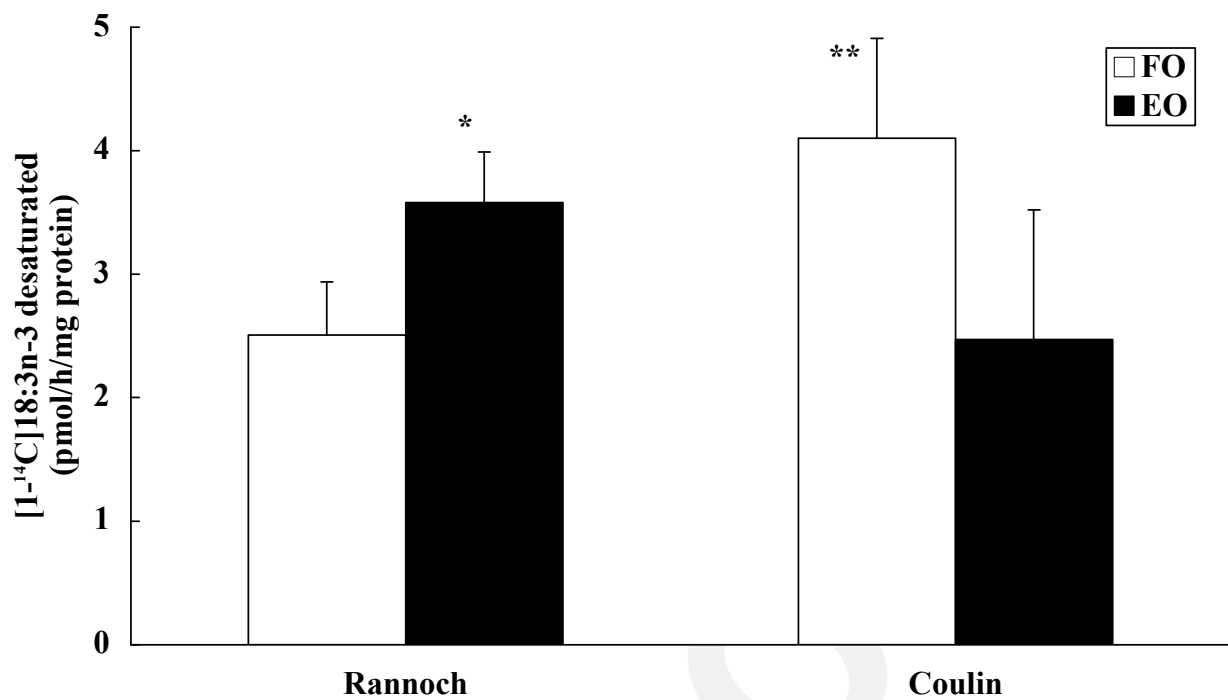


Fig.2

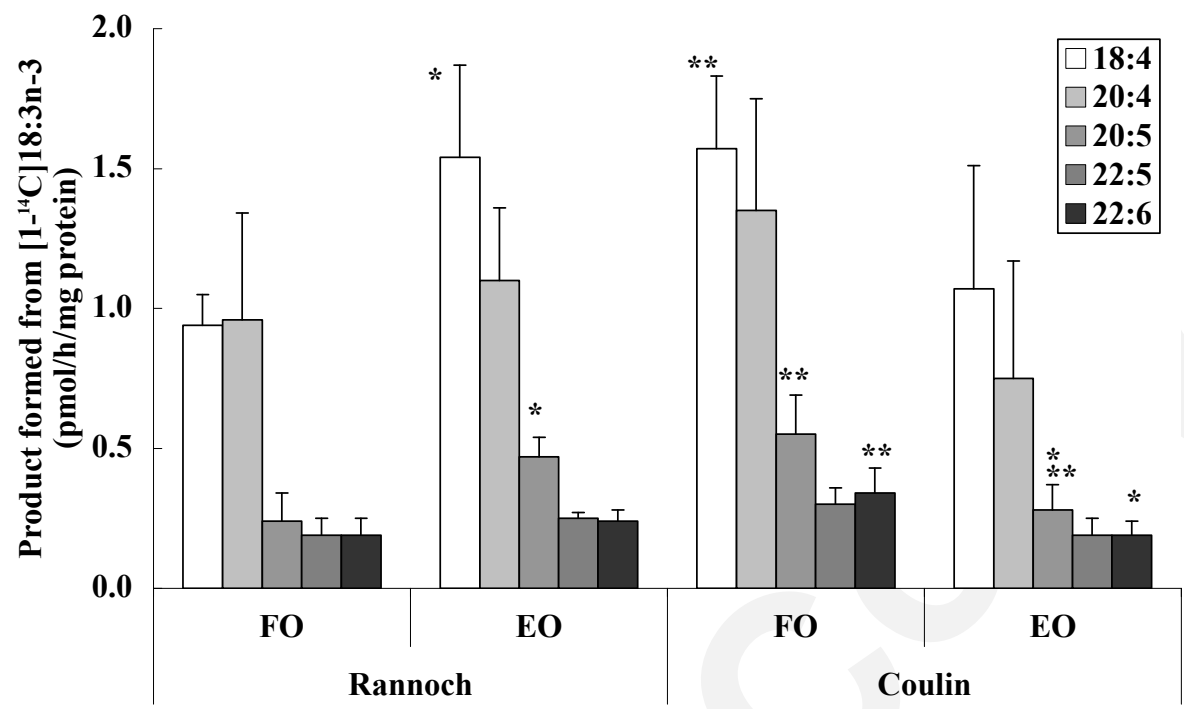


Fig.3

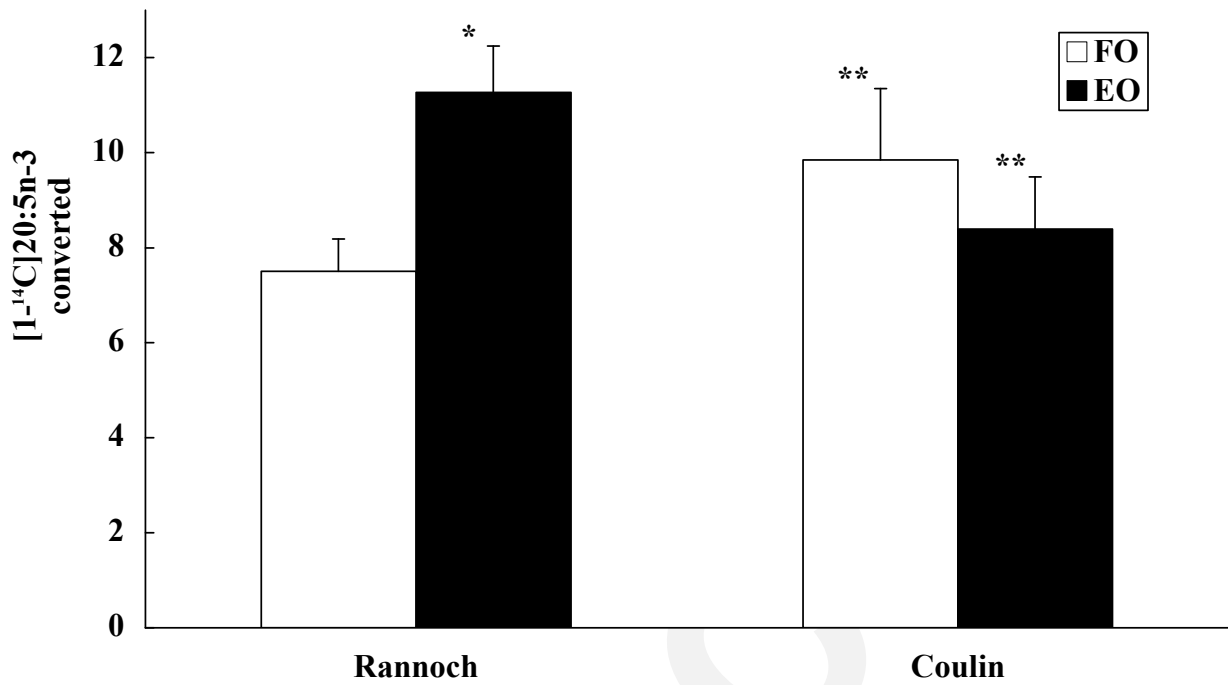


Fig.4

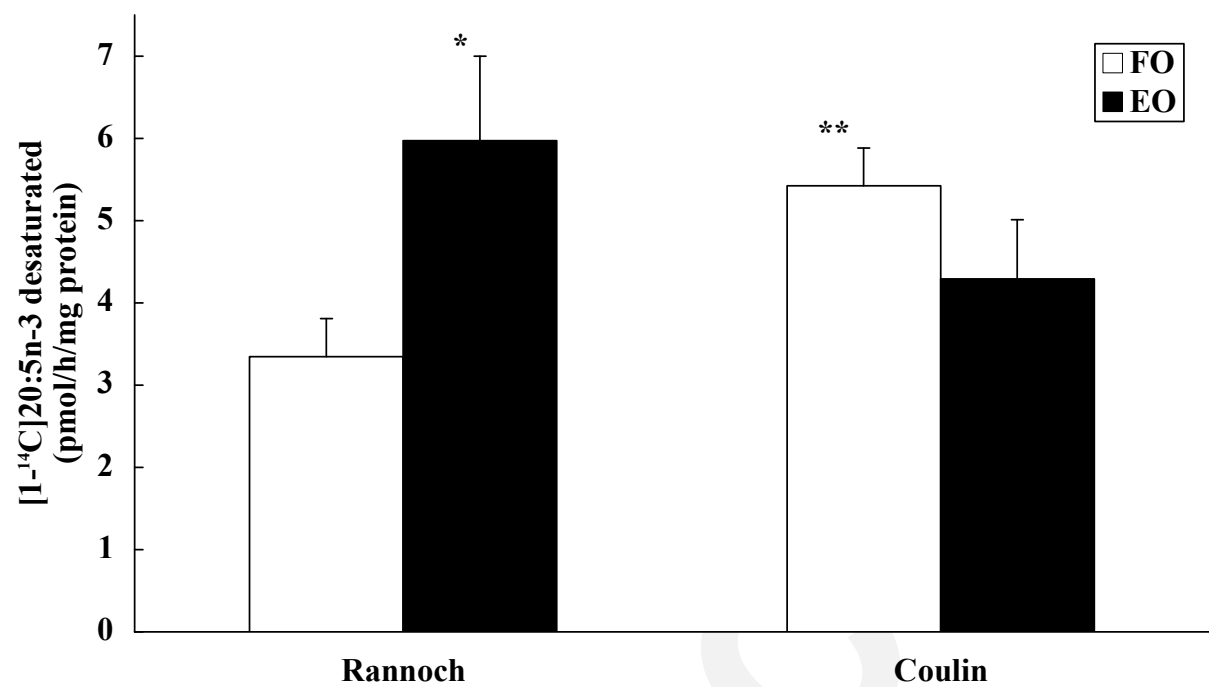


Fig.5

