

Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle

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Enhancing the *n*-3 polyunsaturated fatty acid (PUFA) content of beef is important in view of the generally saturated nature of fatty acids in ruminant meats and the negative effect this can have on human health. This study examined the effects of different sources of dietary *n*-3 PUFA on the performance of steers and the fatty acid composition of *m. longissimus thoracis* muscle and associated subcutaneous adipose tissue. Animals were fed *ad libitum* on grass silage plus one of four concentrates (60:40 forage:concentrate on a DM basis) containing differing sources of lipid: Megalac (16:0), lightly bruised whole linseed (18:3*n*-3), fish oil (20:5*n*-3 and 22:6*n*-3) and a mixture of linseed and fish oil (1:1, on an oil basis). Diets were formulated so that total dietary oil intake was 6 %, approximately half of which was from the experimental test oil. Linseed feeding not only increased the levels of 18:3*n*-3 in muscle phospholipid from 9.5 to 19 mg/100 g muscle but also enhanced the synthesis of 20:5*n*-3, the level of which increased from 10 to 15 mg/100 g muscle. Linseed also increased the proportion of 18:3*n*-3 in muscle neutral lipid and in adipose tissue lipids by a factor of 1.64 and 1.75 respectively. Fish oil feeding doubled the proportion of 20:5*n*-3 and 22:6*n*-3 in muscle phospholipids. The proportion of 18:1 *trans* in muscle neutral lipid was higher on the *n*-3 PUFA diets than the control diet, 0.04 and 0.02 respectively. Despite the implied modification to rumen metabolism, lipid source did not affect feed intake, growth rate, cold carcass weight or carcass fatness, but carcass conformation score was higher on fish oil treatments ($P < 0.05$). However, total muscle fatty acid content was not different between treatments and ranged from 3.5–4.3 % of tissue weight. The increase in *n*-3 PUFA in the meat produced by feeding linseed or fish oil lowered the *n*-6:*n*-3 ratio but had little effect on the P:S ratio.

Beef: Fatty acids: Health

High levels of fat consumption and particularly of saturated fatty acids are considered to predispose man to several of the so-called 'Diseases of Western Civilisation', notably coronary heart disease (Department of Health, 1994). Current recommendations are that the contributions of fat and saturated fatty acids to dietary energy intake should be reduced to below 0.35 and 0.10 of total intake, respectively, the ratio of the unsaturated to saturated fatty acids should be around 0.45 and intakes of *n*-3 polyunsaturated fatty acids (PUFA) should be increased relative to *n*-6 PUFA (Department of Health, 1994). The precursor for the *n*-3 series is α -linolenic (18:3*n*-3), man having the capacity to synthesise the C_{20} PUFA, eicosapentaenoic acid (20:5*n*-3) and, to a lesser extent, docosahexaenoic acid (22:6*n*-3), from 18:3*n*-3. The Department of Health (1994) recommends

an increase in the consumption of these *n*-3 fatty acids to overcome the imbalance in the ratio of *n*-6:*n*-3 PUFA in the current diets (10:1) compared to primitive man (1:1, Eaton *et al.* 1996), which results from the consumption of plant oils rich in linoleic acid (18:2*n*-6). Meat, fish, fish oils and eggs are the only significant sources of these *n*-3 C_{20} PUFA for man. Although meat has lower concentrations of these fatty acids compared to oily fish, it is a very significant source for many people, since fish consumption is low. Hence, enhancing the levels of these fatty acids in meat would help to combat the negative image of beef attributed to its highly saturated nature.

The low PUFA concentrations and the high concentrations of saturated fatty acids in ruminant tissues result from the biohydrogenation of dietary PUFA in the rumen

Abbreviation: PUFA, polyunsaturated fatty acid.

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(Harfoot & Hazlewood, 1988). Efforts to increase the PUFA content of ruminant tissues have used different procedures to attempt to bypass ruminal biohydrogenation. These have involved the feeding of chemically or physically modified oils or oil seeds mainly supplying 18:2*n*-6 (Scott & Ashes, 1993). The potential to enrich ruminant tissues with C₂₀ *n*-3 PUFA was highlighted when it was demonstrated that ruminal microorganisms *in vitro* did not hydrogenate 20:5*n*-3 and 22:6*n*-3 to any significant extent (Ashes *et al.* 1992). It may also be feasible to enhance these fatty acids by encouraging *de novo* synthesis from 18:3*n*-3 in the tissues, by incorporating linseed, which is high in 18:3*n*-3, into the diet. The objective of this experiment was to investigate the impact of feeding different sources of long chain PUFA on animal performance and on the intramuscular fatty acid composition of beef. A summary of this study was presented previously (Scollan *et al.* 1997).

Materials and methods

Experimental design and treatments

Thirty-two Charolais steers with an initial live weight of 436 kg (SE 3.36) were randomly allocated to one of four treatments, resulting in eight animals per treatment. The formulation and chemical composition of the four experimental concentrates is given in Table 1. The concentrates, which were based on barley and sugarbeet pulp, contained differing sources of lipid: Diet 1, Megalac (experimental control, high in the saturated palmitic acid, 16:0, from palm oil); Diet 2, whole linseed (high in α -linolenic acid, 18:3*n*-3); Diet 3, fish oil (high in eicosapentaenoic acid, 20:5*n*-3 and docosahexaenoic acid, 22:6*n*-3); and Diet 4, linseed plus fish oil in equal amounts on an oil basis. Diets were

formulated so that total dietary oil intake was 6 % of which approximately 3 % was from the experimental test oil. Vitamin E content of the concentrates was 345 IU/kg.

Procedures and measurements

Animals were housed in a well-ventilated barn and those within a treatment group were penned together and bedded on wood shavings. Animals were individually fed via Calan Broadbent gates and had free access to fresh water. At the beginning of the experiment, during a 14 d covariate period, all animals were fed on grass silage *ad libitum* and rolled barley was allocated individually to provide 0.3 of DM intake. The animals were then introduced to their allocated dietary treatment over a 7 d period. Feed levels were adjusted to grass silage *ad libitum* and concentrate at 60:40, forage: concentrate on a DM basis. During the 14 d covariate period feed intake was recorded and live weight measurements were made on five occasions. During the main experimental period, feed intake was recorded and weekly live weights were recorded. All weighings were conducted at the same time of day (13:30 h) to reduce variations due to diurnal patterns of feed intake.

Grass silage was fed at 14:00 h daily, and the concentrates in two equal portions at 09:00 h and 16:30 h. The grass silage and concentrates were sampled three times per week for DM analysis in a forced-air oven at 105°C. Silage was offered at 110 % of the previous consumption. Concentrates feed levels were adjusted weekly, based on the previous week's silage consumption. Samples of silage and concentrate were accumulated over 4-week periods and chemical composition assessed as described by Dewhurst *et al.* (1999). Feed refusals were measured three times per week.

Table 1. Formulation and chemical composition of the components of the experimental diets
(Values are g/kg fresh weight for ingredients and g/kg DM for chemical composition)

Ingredient	Silage	Control	Concentrates		
			Linseed	Fish oil	Linseed–fish oil
Barley		638	489	648	569
Sugarbeet pulp		213	213	213	213
Molasses		64	64	64	64
Megalac		64	–	–	–
Fish oil		–	–	54	27
Linseed		–	213	–	106
Mineral and vitamin premix		21	21	21	21
Chemical composition					
	258	904	902	906	901
pH	3.7	NA	NA	NA	NA
Total nitrogen	22.6	19.4	25.0	18.6	22.1
Ammonia-nitrogen	1.44	NA	NA	NA	NA
Acid detergent insoluble nitrogen	0.74	0.81	0.79	0.58	0.69
Organic matter	921.0	927.8	933.9	941.3	933.5
Neutral detergent fibre	475	225	218	211	206
Acid detergent fibre	283	86	108	92	100
Water-soluble carbohydrates	14.5	ND	ND	ND	ND
Acid hydrolysis ether extract	42.7*	77.3	106.5	82.4	96.9
Acetic acid	29.6	NA	NA	NA	NA

NA, not applicable; ND, not determined.

* Silage value is based on ether extract only and not acid hydrolysis ether extract.

Animals remained on treatment on average 120 d. Eight animals were slaughtered each week over a 4-week period. Animals were selected for slaughter on a live weight basis; the two heaviest animals in each group were selected for slaughter each week. Animals were transported to Bristol on the day before slaughter. Cold carcass weights (excluding kidney knob and channel fat), external fatness and conformation scores were assessed as described by Kempster *et al.* (1986), by the same individual.

Complete cross-sections of the *m. longissimus thoracis* 10–12 rib level, 15 mm thick were freed of epimysium and adhering adipose tissue. The muscle was blended in a food processor and the lipids extracted from duplicate 10 g samples using chloroform:methanol (2:1, v/v; Folch *et al.* 1957). Neutral lipid and phospholipids were isolated from the lipid extract using silicic acid chromatography. Extracted lipid, 20–30 mg in approximately 1.0 ml chloroform was applied to a 500 mg silicic acid column (Isolute Si, Jones Chromatography, Hengoed, Glamorgan, UK) previously washed extensively with chloroform. The neutral lipid fraction was eluted with 10 ml chloroform and the phospholipid fraction (polar lipids) with 20 ml methanol. After the addition of the fatty acid standard, heneicosanoic acid methyl ester (Sigma, Gillingham, UK), the solvents were removed under nitrogen and the lipids hydrolysed with 2 M potassium hydroxide in methanol:water (1:1, v/v), containing 1 g/l hydroquinone as antioxidant, at 60°C for 1 h. After dilution with water and removal of non-saponifiables by three extractions with petroleum spirit (BP 40–60°C), the hydrolysate was acidified and the fatty acids extracted into petroleum spirit. After neutralisation and drying, with solid sodium hydrogencarbonate and anhydrous sodium sulphate, the fatty acids were methylated with a solution of diazomethane in diethyl ether and their composition determined by gas-liquid chromatography. Samples were injected in the split mode, 70:1, onto a CP Sil 88, 50 m × 0.25 mm fatty acid methyl esters (FAME) column (Chrompack UK Ltd, London) with helium as the carrier gas. The output from the flame ionization detector was quantified using a computing integrator (Spectra Physics 4270) and linearity of the system was tested using saturated (FAME4) and monounsaturated (FAME5) methyl ester quantitative standards (Thames Restek UK Ltd, Windsor, UK).

Adipose tissue fatty acid composition was determined in the subcutaneous adipose tissue over the *m. longissimus thoracis* at the 10th–12th rib. A sample of the complete thickness of the tissue was blended in a food processor. Lipid was extracted by homogenizing duplicate 1 g samples in chloroform containing 2,6-di-tert-butyl-*p*-cresol (100 mg/l) as antioxidant and then adding anhydrous sodium sulphate to remove water. After filtration, samples were taken and hydrolysed and the fatty acids processed and analysed as above.

Lipids were extracted from the silage by the method of Bauchart *et al.* (1984) and then converted to methyl esters as described for tissue lipids. Concentrate fatty acids were attained by direct hydrolysis in 5 M potassium hydroxide in aqueous methanol (1:1, v/v) for 3 h at 60°C after addition of 21:0 methyl ester as an internal standard. After

acidification the samples were heated for an additional 1 h before extraction of the fatty acids.

Fatty acid results are given as proportion times 100, or as mg of fatty acid per 100 g wet tissue quantified by reference to the internal standard. Only the major fatty acids and minor components readily identified and relevant to the study are reported, representing over 90 % of the total fatty acids present. The fatty acid reported as 16:1 *cis* consists of both the *n*-9 and *n*-7 isomers and contaminating branched seventeen-carbon fatty acids. The *trans* 18:1 isomers are incompletely resolved by this column and are reported as one value. In view of the complexity of the chromatogram in this area as a result of the wide range of 18:1 isomers in ruminant tissues (Hay & Morrison, 1972), some minor cross-contamination of the listed 18:1 isomers may be present.

Statistical analysis

Live weight growth rates (g/d) for each animal were estimated from regression of live weight on time. Average daily feed intakes of silage and concentrate were calculated for the experimental period. The visual scores for carcass fatness and conformation of the EU Carcass Classification Scheme were converted to numerical values by multiplying the values for estimated fat and conformation by 10 (Kempster *et al.* 1986). All the data were subjected to analysis of variance (Genstat 5; Lawes Agricultural Trust, 1994).

Results

Animal performance and carcass classification

The animal performance and carcass classification data are presented in Table 2. Lipid source did not influence feed intake or growth rate. Cold carcass weights were similar across treatments, average 333.7 kg. Carcass conformation was highest on the fish oil treatments in comparison to either the control or linseed ($P < 0.05$), but no significant differences were noted for carcass fatness.

Fatty acid composition

The fatty acid composition of neutral lipid and phospholipid fatty acids expressed as proportion times 100 are shown in Tables 3 and 5 and the tissue content of the two lipid fractions expressed as mg per 100 g of muscle in Tables 4 and 6, respectively. Intramuscular fatty acid content was not significantly different between treatments with neutral lipid fatty acids varying from 2.9 to 3.9 g per 100 g tissue and phospholipid fatty acids from 0.45 to 0.50 g per 100 g tissue.

Neutral lipids

The three major fatty acids in the neutral lipids were 18:1 n -9, 16:0 and 18:0 whose combined proportions were approximately 0.75. There were marked treatment effects on the proportions of these fatty acids although these were not apparent when the results were expressed as mg fatty

Table 2. Animal performance and carcass classification in Charolais steers fed on diets containing different sources of lipid (Values are means and standard error for eight animals per diet; residual degrees of freedom 31)

	Control	Linseed	Fish oil	Linseed–fish oil	SEM	<i>P</i> value†
Silage DM intake (kg/d)	5.43	5.43	5.12	5.37	0.124	NS
Concentrate DM intake (kg/d)	3.61	3.62	3.46	3.60	0.082	NS
Live weight gain (kg/d)	1.29	1.25	1.38	1.36	0.056	NS
<i>Carcass weight and classification</i>						
Cold carcass weight (kg)	333.1	330.1	334.4	337.3	4.56	NS
Conformation†	91.3	83.8	105.6	105.6	5.60	*
External fatness†	84.4	79.7	85.3	81.6	4.32	NS

NS, not significant $P > 0.05$.*, ** and *** refer to significance levels $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.† Fatness and conformation scores were converted to numerical values using the procedure of Kempster *et al.* (1986).

acid per 100 g muscle because of the large variability in the quantity of marbling fat between animals. Compared with fat from animals fed the control diet, the fat from animals fed the linseed diet had lower proportions of 16:0 and 18:0 and the fat from animals on the fish oil diet had a higher proportion of 16:0 and lower proportions of 18:0 and 18:1*n*-9. The concentration and proportion of 18:1 *trans* was much higher in the steers given the unsaturated lipid supplements ($P < 0.001$) with an average proportion of 0.044 compared with 0.019. The proportion of 18:2*n*-6 was decreased by feeding the *n*-3 PUFA in linseed and fish oils ($P < 0.001$) whereas the concentration and proportion of 18:3*n*-3 was almost doubled by feeding linseed.

Phospholipids

The concentration of phospholipid fatty acids was not affected significantly by diet; the mean value across treatments being 462 mg per 100 g muscle. As with the neutral lipids, the major fatty acids were 18:1*n*-9 followed by 16:0 and then 18:0 but their proportions were one-third or more less than in the neutral lipid fraction. The effect of diet on these three fatty acids in the phospholipids was also similar to that in the neutral lipid. There was a lower proportion of 16:0 acid as a result of feeding the linseed diet compared with the control diet but the proportion of 18:0 was not different. However, compared to the control

diet, fish oil did not alter the proportion of 16:0 but produced lower proportions of 18:0 and 18:1*n*-9. The quantity of 18:1 *trans* contributed to muscle lipids by the phospholipid fraction was on average 6.6 mg compared with 123 mg in the neutral lipids. The concentration and proportion of 18:1 *trans* was higher in the phospholipids of animals fed the unsaturated fatty acids compared with the control animals fed megalac, with the fish oil diet producing the highest concentration, threefold higher than in the controls. The concentrations of 18:2*n*-6 were not significantly different between treatments but the proportions were significantly lower ($P < 0.05$) on the diets containing fish oil. Feeding linseed doubled the amount and proportion of 18:3*n*-3 with the mixed linseed–fish oil diet producing intermediate levels. The major long-chain PUFA with twenty or more carbon atoms were 20:4*n*-6 followed by 22:5*n*-3 and 20:5*n*-3 in muscles from megalac-fed steers. The diets containing the *n*-3 PUFA resulted in higher proportions of the long-chain *n*-3 PUFA and lower proportions of long-chain *n*-6 PUFA except for 22:5*n*-3, the proportions of which were very slightly affected by the fish oil diet only. The linseed diet resulted in significant increases in the proportion of 20:4*n*-3 (twofold) and 20:5*n*-3 (1.5-fold) but not 22:6*n*-3 or 22:4*n*-3 whereas the fish oil diet also increase 22:6*n*-3 (twofold) and 22:4*n*-3 (fourfold). Interestingly, the proportions of 22:6*n*-3 were similar with the fish oil and linseed–fish oil mixed diet.

Table 3. Fatty acid composition (proportion $\times 100$) of the neutral lipids of *m. longissimus thoracis* from Charolais steers fed on diets containing different sources of lipid (Values are means and standard errors for eight animals per diet; residual degrees of freedom 31)

	Control	Linseed	Fish oil	Linseed–fish oil	SEM	<i>P</i> value
12:0 lauric	0.12	0.12	0.12	0.12	0.017	NS
14:0 myristic	3.72	4.10	4.57	4.36	0.421	NS
16:0 palmitic	30.0	27.1	32.0	29.0	0.931	***
16:1 <i>cis</i>	3.73	4.02	4.18	4.21	0.235	NS
18:0 stearic	15.8	14.2	13.2	12.9	0.684	***
18:1 <i>trans</i>	1.93	3.76	4.43	4.95	0.459	***
18:1 <i>n</i> -9 oleic	36.0	35.7	30.1	33.2	1.57	**
18:1 <i>cis</i> vaccenic	0.92	1.04	0.97	0.91	0.103	NS
18:2 <i>n</i> -6 linoleic	0.93	0.79	0.57	0.72	0.075	***
18:3 <i>n</i> -3 α -linolenic	0.36	0.59	0.38	0.41	0.043	***

NS, not significant $P > 0.05$.** $P < 0.01$; *** $P < 0.001$.

Table 4. Fatty acid content, (mg per 100 g muscle) of the neutral lipids of *m. longissimus thoracic* from Charolais steers fed on diets containing different sources of lipid

(Values are means and standard errors for eight animals per diet; residual degrees of freedom 31)

	Control	Linseed	Fish oil	Linseed–fish oil	SEM	<i>P</i> value
12:0 lauric	3.5	3.8	4.4	3.7	0.92	NS
14:0 myristic	112	130	178	131	30.4	NS
16:0 palmitic	872	851	1247	853	167	NS
16:1 <i>cis</i>	110	126	162	125	23.7	NS
18:0 stearic	449	451	521	374	82.4	NS
18:1 <i>trans</i>	57	120	175	139	26.4	***
18:1 <i>n</i> -9 oleic	1039	1150	1171	975	206	NS
18:1 <i>cis</i> vaccenic	27	32	38	27	6.2	NS
18:2 <i>n</i> -6 linoleic	27	25	23	20	4.9	NS
18:3 <i>n</i> -3 α -linolenic	10	19	15	12	2.9	*
Total fatty acids	2903	3180	3900	2922	567	NS

NS, not significant, $P > 0.05$.* $P < 0.05$; *** $P < 0.001$.*Subcutaneous adipose tissue*

The effect of dietary lipid on the fatty acid composition of the subcutaneous adipose tissue (proportion times 100) and on the total fatty acid content of adipose tissue (mg per 100 g tissue) are shown in Table 7. Adipose tissue from the steers given the feeds containing fish oil had a slightly lower ($P < 0.05$) content of lipid than the animals on the control feed. As in muscle the major fatty acids were 18:1*n*-9, 16:0 and 18:0, which together contributed 0.78 of the total fatty acids in animals on the control diet. Compared with the latter, linseed feeding gave lower 16:0 but did not affect 18:1*n*-9 or 18:0 but doubled the proportion of 18:1 *trans* and increased the proportion (times 100) of 18:3*n*-3 from 0.37 to 0.65. The adipose tissue from animals fed the fish oil diet differed most from the control with significantly high proportions of 12:0, 14:0, 16:0 and 18:1 *trans*. However, the proportion of 18:1*n*-9 and 18:2*n*-6 were less. The diet containing a mixture of linseed and fish oil gave a fatty acid composition between that of the two

dietary lipids fed separately. No C₂₀ or C₂₂ PUFA were detected in the adipose tissue lipid.

Nutritional indices

Fatty acid ratios related to healthy human nutrition are shown in Table 8. The results for the muscle were calculated from the combined neutral lipid and phospholipid fractions. In muscle P:S ratios tended to be higher in the control and linseed treatments than in fish or mixture treatments. However, in adipose tissue the P:S ratios of the linseed treatments were significantly higher than those of the control, with the lowest values for the fish oil diet. The 18:2*n*-6 to 18:3*n*-3 ratio was lower in all three unsaturated treatments compared to the control ($P < 0.001$) in both muscle and adipose tissue. When the ratio was calculated as total *n*-6:total *n*-3 for muscle the ratios were further improved falling to half the control value for the fish oil diet.

Table 5. Fatty acid composition (proportion \times 100) of the phospholipids of *m. longissimus thoracis* from Charolais steers fed on diets containing different sources of lipid

(Values are means and standard errors for eight animals per diet; residual degrees of freedom 31)

	Control	Linseed	Fish oil	Linseed–fish oil	SEM	<i>P</i> value
12:0 lauric	0.05	0.04	0.05	0.04	0.012	NS
14:0 myristic	0.74	0.58	0.94	0.71	0.205	NS
16:0 palmitic	17.2	14.7	17.5	15.5	0.894	*
16:1 <i>cis</i>	2.20	2.01	2.28	2.30	0.190	NS
18:0 stearic	10.5	10.4	9.7	9.6	0.335	*
18:1 <i>trans</i>	0.59	1.31	1.85	1.78	0.227	***
18:1 <i>n</i> -9 oleic	23.4	21.6	17.4	20.7	1.63	**
18:1 <i>cis</i> vaccenic	1.67	1.75	2.28	1.94	0.182	*
18:2 <i>n</i> -6 linoleic	11.4	10.1	8.4	9.2	1.03	*
18:3 <i>n</i> -3 α -linolenic	2.13	4.34	2.37	3.49	0.33	***
20:3 <i>n</i> -6	1.50	1.23	1.10	0.98	0.131	**
20:4 <i>n</i> -6 arachidonic	5.09	4.47	2.96	3.87	0.377	***
20:4 <i>n</i> -3	0.48	0.97	3.46	1.42	0.341	***
20:5 <i>n</i> -3 eicosapentaenoic	2.31	3.55	4.87	3.60	0.440	***
22:4 <i>n</i> -6	0.46	0.30	0.21	0.26	0.051	***
22:4 <i>n</i> -3	0.10	0.13	0.41	1.02	0.106	***
22:5 <i>n</i> -3 docosapentaenoic	4.26	4.64	4.81	4.51	0.286	NS
22:6 <i>n</i> -3 docosahexaenoic	0.55	0.63	1.08	1.22	0.117	***

NS, not significant, $P > 0.05$.* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 6. Fatty acid content (mg per 100 g muscle) of the phospholipids of *m. longissimus thoracis* from Charolais steers fed on diets containing different sources of lipids

(Values are means and standard errors for eight animals per diet; residual degrees of freedom 31)

	Control	Linseed	Fish oil	Linseed–fish oil	SEM	<i>P</i> value
12:0 lauric	0.24	0.15	0.26	0.17	0.070	NS
14:0 myristic	4.0	2.7	5.0	3.3	1.490	NS
16:0 palmitic	81	65	89	70	11.94	NS
16:1 <i>cis</i>	10	8.8	12	10	1.564	NS
18:0 stearic	44	46	49	44	5.81	NS
18:1 <i>trans</i>	3.0	5.9	9.7	7.9	1.484	***
18:1 <i>n</i> -9 oleic	107	96	87	94	13.68	NS
18:1 <i>cis</i> vaccenic	7.5	7.7	11.4	8.6	1.104	**
18:2 <i>n</i> -6 linoleic	51	44	40	41	4.12	NS
18:3 <i>n</i> -3 α -linolenic	9.5	19	12	16	1.568	***
20:3 <i>n</i> -6	6.5	5.3	5.4	4.4	0.514	**
20:4 <i>n</i> -6 arachidonic	22	19	14	17	1.518	***
20:4 <i>n</i> -3	2.1	4.3	17.9	6.4	2.049	***
20:5 <i>n</i> -3 eicosapentaenoic	10	15	24	16	2.257	***
22:4 <i>n</i> -6	2.0	1.3	1.0	1.2	0.199	***
22:4 <i>n</i> -3	0.46	0.59	5.3	1.8	0.664	***
22:5 <i>n</i> -3 docosapentaenoic	19	20	24	20	1.382	**
22:6 <i>n</i> -3 docosahexaenoic	2.5	2.7	5.3	5.5	0.490	***
Total fatty acids	456	438	500	451	44.2	NS

NS, not significant $P > 0.05$.** $P < 0.01$; *** $P < 0.001$.**Table 7.** Fatty acid composition (proportion $\times 100$) and content of the subcutaneous adipose tissue of Charolais steers fed on diets containing different sources of lipids

(Values are means and standard errors for eight animals per diet; residual degrees of freedom 31)

	Control	Linseed	Fish oil	Linseed–fish oil	SEM	<i>P</i> value
12:0 lauric	0.07	0.08	0.10	0.09	0.012	*
14:0 myristic	4.0	3.9	5.4	4.5	0.400	**
16:0 palmitic	28.5	24.4	30.7	27.2	0.663	***
16:1 <i>cis</i>	5.3	5.4	4.9	5.1	0.388	NS
18:0 stearic	12.4	10.5	12.4	11.3	0.847	NS
18:1 <i>trans</i>	2.3	4.7	5.8	5.9	0.464	***
18:1 <i>n</i> -9 oleic	36.7	35.4	26.1	30.7	1.520	***
18:1 <i>cis</i> vaccenic	1.3	1.3	1.1	1.2	0.094	NS
18:2 <i>n</i> -6 linoleic	0.94	0.86	0.67	0.73	0.046	***
18:3 <i>n</i> -3 α -linolenic	0.37	0.65	0.44	0.45	0.036	***
Total fatty acids (g/100 g adipose tissue)	90.4	88.4	85.9	87.4	1.44	*

NS, not significant, $P > 0.05$.* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.**Table 8.** Fatty acid ratios related to healthy nutrition in Charolais steers fed on diets containing different sources of lipid

(Values are means and standard errors for eight animals per diet; residual degrees of freedom 31)

	Control	Linseed	Fish oil	Linseed–fish oil	SEM	<i>P</i> value
<i>Muscle</i>						
P:S*	0.067	0.067	0.045	0.051	0.008	NS
(18:2 <i>n</i> -6)/(18:3 <i>n</i> -3)†	3.84	1.86	2.58	2.17	0.177	**
(<i>n</i> -6)/(<i>n</i> -3)‡	2.00	1.19	0.91	1.11	0.106	**
<i>Adipose tissue</i>						
P:S*	0.028	0.035	0.021	0.024	0.002	***
(18:2 <i>n</i> -6)/(18:3 <i>n</i> -3)†	2.54	1.34	1.52	1.65	0.120	***

* P:S calculated as (18:2*n*-6 + 18:3*n*-3)/(12:0 + 14:0 + 16:0 + 18:0).† *n*-6:*n*-3 calculated as (18:2*n*-6)/(18:3*n*-3).‡ *n*-6:*n*-3 calculated as (18:2*n*-6 + 20:3*n*-6 + 20:4*n*-6)/(18:3*n*-3 + 20:4*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3).

Discussion

Animal performance and carcass characteristics

Incorporation of lipid into the diet did not influence DM intake, although there was a tendency for intake of the fish oil diet to be lower. Chilliard & Doreau (1997) reported a reduction of 1.6 kg DM per d when dairy cows fed on maize and concentrates (at 0.65:0.35 on a DM basis) were supplemented with 300 ml of fish oil (menhaden type) and similar effects have been observed elsewhere (Wonsil *et al.* 1994; Mandell *et al.* 1997). It is thought that these effects are mediated by specific fatty acids produced as a result of rumen biohydrogenation rather than a negative effect of the fatty acids of fish oil on rumen function, since the fish oil does not disturb ruminal fibre digestion (Wonsil *et al.* 1994; Chilliard & Doreau, 1997). Since intakes were similar across treatments of similar nutrient balance, no differences in live weight gain or carcass fatness were expected (Table 2). Surprisingly, carcass conformation was significantly higher on the fish oil and mix concentrates ($P < 0.05$). This result is difficult to explain although it could indicate a positive effect of fish oil on muscle development.

Tissue fatty acid composition

Feeding *n*-3 PUFA supplements as bruised linseed or fish oil resulted in significant increases in their deposition in muscle lipids. However, the increases were much less than those obtained when formaldehyde-treated, protein-encapsulated lipids were fed (Cook *et al.* 1972; Kimoto *et al.* 1974; McDonald & Scott, 1977; Ashes *et al.* 1992). Milk from cows fed formaldehyde-protected linseed oil contained 20% 18:3*n*-3 in the lipid compared with 1% in milk from cows fed unprotected linseed oil. This illustrates well the extent to which ruminant PUFA are amenable to manipulation if they escape rumen biohydrogenation. Our results suggest that the seed coat of linseed had little effect in protecting 18:3*n*-3 as confirmed in the associated digestion experiment (Scollan *et al.* submitted). The increase in adipose tissue 18:3*n*-3 observed (Table 7) was similar to feeding steam-flaked linseed, which resulted in a doubling of 18:3*n*-3 in perirenal adipose tissue of bulls (Cliquart *et al.* 1991). Processing oilseeds (whole, crushing, rolling) is generally far less effective than feeding protected lipid supplements (Kennelly, 1996). The increase in the concentration of 20:5*n*-3 on the linseed diet (Tables 5 and 6) suggests that increasing the availability of 18:3*n*-3 has resulted in enhanced synthesis of 20:5*n*-3 from its precursor 18:3*n*-3. In light of the increasing pressures to reduce the use of fish products in animal feeds this finding is particularly noteworthy, as an alternative means of enhancing the 20:5*n*-3 levels in ruminant product.

In ruminants the long-chain C₂₀ and C₂₂ PUFA are found mainly in the tissue phospholipids and not in the triacylglycerol fraction of muscle and adipose tissue (Ashes *et al.* 1992). The increases in the concentration of 20:5*n*-3 and 22:6*n*-3 on feeding either fish meal or fish oil is similar to other studies with poultry, pigs and cattle (Ratnayake *et al.* 1989; Morgan *et al.* 1992; Cant *et al.* 1997; Mandell *et al.* 1997; Mansbridge & Blake, 1998).

In this study, the concentration and proportion of 20:5*n*-3 and 22:6*n*-3 are similar to that quoted by Marmer *et al.* (1984) and by Mandell *et al.* (1997), who also reported that both the amount and duration of fish meal feeding increased intramuscular deposition of these fatty acids. However, the increases in 20:5*n*-3 and 22:6*n*-3 were much less than the responses observed when sheep were fed a rumen-protected fish oil supplement (Ashes *et al.* 1992), suggesting high levels of ruminal biohydrogenation of these fatty acids (Scollan *et al.* submitted). Surprisingly, 22:6*n*-3 was incorporated as effectively from the mix treatment (linseed–fish oil) as from the fish oil alone, so that the concentration was similar, on average 4.75 mg/100 g muscle.

Since phospholipids constitute a relatively constant component of tissues, increased deposition of *n*-3 PUFA can only occur through displacement of another similar fatty acid usually of the *n*-6 series (Ratnayake *et al.* 1989). The decreases in the concentration of 20:3*n*-6 and 20:4*n*-6 on the unsaturated treatments compared to the control is similar to that noted in other studies using fish meal or fish oil, with both ruminants and non-ruminants (Morgan *et al.* 1992; Van Elswyck *et al.* 1992; Mandell *et al.* 1997). In rats, fish oils were more effective than α -linolenic acid in depressing levels of 20:4*n*-6 (Hwang *et al.* 1988) similar to the greater reduction in 20:4*n*-6 by the fish oil treatments compared to the linseed (Tables 4 and 5). The *n*-3 fatty acids also interfere with the synthesis of 20:4*n*-6 from 18:2*n*-6 (Ratnayake *et al.* 1989). However, fish oil supplementation in the work by Ashes *et al.* (1992) did not affect arachidonic acid, 20:4*n*-6, in serum or muscle phospholipid, which the authors attributed to adequate Δ -6 desaturase activity ensuring conversion of 18:2*n*-6 to 20:4*n*-6.

The proportions of 12:0, 14:0 and 16:1 were not influenced by treatment but the proportion of palmitic acid was decreased by the linseed diet in both neutral lipids and phospholipids of muscle. However, fish oil significantly increased the proportion of 16:0 in the neutral lipids and Mandell *et al.* (1997) also found that fish meal feeding increased 16:0, contrary to the lack of effect observed by Mills *et al.* (1992) feeding fish meal and Ashes *et al.* (1992) who fed ruminally-protected fish oil. Although the concentrations of 18:0 in the muscle were not affected by treatment the proportions in animals fed fish oil or the mixture were lower than in the control animals. In the phospholipids this could result from replacement of 18:0 by 18:1 *trans* which behaves like a saturated fatty acid *in vivo*. Lower 18:0 has been reported after fish meal supplementation (Mills *et al.* 1992; Mandell *et al.* 1997) but not after feeding fish oil (Ashes *et al.* 1992) in sheep. In the muscle neutral lipids the proportions of 18:0 (Table 2) were decreased by all the diets with added *n*-3 PUFA but the sum of the 18:0 plus 18:1 *trans* was similar across all feeds. These compositions are markedly different from the ratios of 18:0 to 18:1 *trans* in the flow to the duodenum, which were 6.6, 1.8, 0.8 and 0.8 respectively for the four feeds (Scollan *et al.* submitted). This indicates extensive catabolism of 18:1 *trans* and that fatty acids derived from intestinal absorption may have minimal effects on the composition of depot triacylglycerols, at least in the case of

saturated and monounsaturated fatty acids. It is noteworthy that the highest amounts of 18:1 *trans* occurred in steers fed the fish oil containing concentrates despite a lower total PUFA level in the feed compared with the linseed concentrate indicating that long-chain fish oil *n*-3 PUFA or a degradation product are more inhibitory to the biohydrogenation than 18:3*n*-3. In the phospholipids the proportion of 18:1*n*-9 was decreased by the fish-oil-containing concentrates apparently through substitution by 20:5*n*-3 and 22:6*n*-3. Although the duodenal flow of 18:1*n*-9 was low, 3–4 % of total fatty acids (Scollan *et al.* submitted), it was the major fatty acid in both lipid fractions, so that attribution of any effects on tissue levels of differences between the feeds must be tenuous, at least for the neutral lipid fraction.

The proportions of 18:2*n*-6 in both lipid fractions were significantly lowered by fish oil feeding with a smaller non-significant effect of linseed ($P < 0.05$; Tables 3 and 5). The similarity between the effects in the neutral and phospholipid fractions suggests that they may result from intestinal availability rather than changes in 20:5*n*-3 and 22:6*n*-3 since the latter fatty acids are not present in the neutral lipids. Tissue levels of 18:2*n*-6 respond very readily to increases in post-rumen availability when protected lipid is fed (Cook *et al.* 1972) and the tissue levels resulting from the different diets in our study reflect the amounts entering the duodenum in the associated study (Scollan *et al.* submitted). Much higher deposition of 20:5*n*-3 and 22:6*n*-3 than in the present study was needed to displace 18:2*n*-6 from phospholipids (Dawson *et al.* 1991; Ashes *et al.* 1992).

The fatty acid composition of the subcutaneous adipose tissue resembled that of the intramuscular neutral lipids very closely (Tables 4 and 7) but was marginally less saturated with less 16:0 and 18:0. In both cases there was little increase in the proportion of 18:3*n*-3 present in animals fed the linseed–fish oil mixture compared with those fed fish oil alone, whereas the expected intermediate value occurred in the phospholipids. No obvious explanation is available for this finding. Overall, the fatty acid composition for the adipose tissue lipid is similar to that reported by Marmer *et al.* (1984) with the values for 18:2*n*-6 and 18:3*n*-3 in the control group falling between their values for grass and grain finished steers in line with the composition of the basal feed.

Impact on human nutrition

The COMA report on the nutritional aspects of cardiovascular disease (Department of Health, 1994) recommended the P:S ratio should remain at 0.45, which is considered appropriate for the human diet as a whole. Hence, the P:S ratios in this study for the total muscle fatty acids (Table 8) were lower than the recommended ratio, and lower than reported for typical retail beef in the UK (0.11; Enser *et al.* 1996) and fish-meal-supplemented Charolais steers (0.11–0.13; Mandell *et al.* 1997), even when calculated on the same basis. However, imbalances in the P:S can be addressed elsewhere in the diet so of more significance is the *n*-6:*n*-3 ratio, which was beneficially at or below the recommended value of 2.0 for all treatments. The ratio for

the three unsaturated fatty acid treatments was lower than that for the control, on average 1.07 and 2.0 respectively (Table 8) and similar to the diet of primitive man (1.0; Weber *et al.* 1993). This is in accord with the recommendations of the COMA report (Department of Health, 1994), which advised no change in the intake of *n*-6 PUFA and an increase in the intake of *n*-3 PUFA.

The recommended daily consumption of *n*-3 fatty acids is 100–200 mg/d, mainly as 20:5*n*-3 and 22:6*n*-3 (Department of Health, 1994). However, in the USA, the consumption of 1 g/d of *n*-3 fatty acids is recommended with 300–400 mg/d as preformed 20:5*n*-3 and 22:6*n*-3. Indeed, consumption of up to 1 g/d of 20:5*n*-3 and 22:6*n*-3 has been advised and in patients with cardiovascular disease positive benefits may be gained from consuming at least 3 g/d (Barlow *et al.* 1990). The main reason for incorporating 20:5*n*-3 and 22:6*n*-3 as preformed fatty acids, rather than relying on converting the body tissues from their precursor, α -linolenic acid, 18:3*n*-3, is that there are concerns over efficiency and speed of conversion in man (Nettleton, 1991).

Based on the results in this experiment (Tables 4 and 6) and assuming that 100 g/d is an appropriate figure for beef consumption (Enser *et al.* 1996), then it is feasible to calculate the contribution of *n*-3 fatty acids to the human diet. Consumption of 100 g/d of beef muscle from cattle fed on the control, linseed, fish oil and mix treatments would provide 55.2, 82.4, 77.6 and 70.9 mg/d of *n*-3 fatty acids, including 13.2, 18.4, 27.6 and 19.9 mg/d as 20:5*n*-3 and 22:6*n*-3, respectively. Hence, beef from the linseed and fish oil treatments could supply between 41–82 and 39–78 % of the daily recommended consumption of *n*-3 fatty acids, (100–200 mg/d, respectively). Further enrichment of the *n*-3 fatty acids in beef seems possible, since the deposition of 20:5*n*-3 in Japanese (Koizumi *et al.* 1991) and Australian (Mitchell *et al.* 1991) cattle exceeds the combined 20:5*n*-3 and 22:6*n*-3 deposition in this experiment. Although the levels of 18:1 *trans* were more than doubled as a result of feeding the concentrates containing *n*-3 PUFA they would not cause the COMA recommendation of a maximum of 2 % of calories to be exceeded. Furthermore, there is doubt that ruminant 18:1 *trans*, mainly *trans*-vaccenic acid, are a significant risk factor for cardiovascular disease compared with *trans* fatty acids arising from chemical hardening of oils (Willett *et al.* 1993). Since the dietary PUFA supplements used in this study also increased tissue concentrations of conjugated linoleic acid (Enser *et al.* 1999) its reported anti-atherogenic action may counteract the effects of increased *trans* 18:1 (Banni & Martin, 1998). Further work is required to examine methods of protecting the *n*-3 PUFA, particularly from linseed, from rumen biohydrogenation and which would incidentally decrease the production of *trans* fatty acids. Greater knowledge of the genetic and enzymatic mechanisms influencing the synthesis and deposition of long-chain *n*-3 PUFA is also required.

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