British Journal of Nutrition (2004), **91**, 551–565
DOI: 10.1079/BJN20031079

Effects of dietary *n*-3 polyunsaturated fatty acids, breed and dietary vitamin E on the fatty acids of lamb muscle, liver and adipose tissue

G. Demirel[1], A. M. Wachira[2], L. A. Sinclair[2], R. G. Wilkinson[2], J. D. Wood[1] and M. Enser[1]*

[1]*Division of Farm Animal Science, School of Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU, UK*

[2]*ASRC, Harper Adams University College, School of Agriculture, Edgmond, Newport, Shropshire TF10 8NB, UK*

(Received 16 October 2002 – Revised 28 November 2003 – Accepted 1 December 2003)

The effect of feeding *n*-3 PUFA on the fatty acid composition of muscle, adipose tissue and liver of lambs was investigated. Groups of eight ram lambs per breed, Suffolk × Lleyn (24 kg live weight) and Scottish Blackface (18 kg live weight), were each fed one of six diets containing one of three fat sources (50 g fatty acids/kg DM; Megalac® (calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK) and formaldehyde-treated whole linseed (Trouw Nutrition UK, Northwich, Ches., UK) either alone or with fish oil (1:1, w/w) and either 100 or 500 mg α-tocopheryl acetate/kg DM. Feed was offered *ad libitum* until slaughter at approximately half breed mature live weight. The type of dietary fat had no effect on intake, growth rate or feed conversion ratio. The 3·0-fold higher concentration of 18 : 3*n*-3 in the linseed compared with the Megalac® diet approximately doubled ($P<0·001$) the concentration in the neutral and polar lipid fractions of musculus semimembranosus and liver, and in adipose tissue it increased 2·5-fold. Feeding protected linseed also increased ($P<0·001$) concentrations of 20 : 5*n*-3 and 22 : 5*n*-3 in muscle polar lipids and both lipid fractions of liver. The linseed–fish oil raised the 20 : 5*n*-3 concentrations above those for the linseed diet and also increased 22 : 6*n*-3. Scottish Blackface lambs had lower concentrations of 18 : 3*n*-3 in all lipids compared with Suffolk x Lleyn lambs, but more 20 : 5*n*-3 in the polar lipids of muscle and liver. High levels of dietary vitamin E were associated with small decreases in the concentration of monounsaturated fatty acids and increases in PUFA. Linseed raised the PUFA : saturated fatty acid ratios in liver and adipose tissue but not in muscle, and improved the *n*-6 : *n*-3 fatty acid ratio, as did the linseed–fish oil. Different combinations of dietary fatty acids and better protection against rumen biohydrogenation are required to improve muscle PUFA : saturated fatty acids ratios.

Polyunsaturated fatty acids: Breed: Sheep

Altering the lipid content and fatty acid composition of foods, without changing their organoleptic properties, is an effective way of helping consumers to meet nutritional guidelines by increasing PUFA : saturated fatty acid ratio and the content of long-chain *n*-3 PUFA. Raising the PUFA content of ruminant animal meat and milk by feeding animals PUFA is limited by the biohydrogenation of these fatty acids in the rumen. However, differences in dietary PUFA are reflected in the tissue lipids of ruminant animals (Marmer *et al.* 1984; Enser *et al.* 1998*b*) and it has been demonstrated that feeding linseed and fish oil increases the content of *n*-3 PUFA in beef muscle (Mandell *et al.* 1997; Scollan *et al.* 2001). We have obtained similar results with lambs fed linseed, fish oil or a combination of both (Wachira *et al.* 2002). Although the concentrations of the different dietary *n*-3 PUFA were increased 2- to 3-fold and would therefore meet the Committee on Medical Aspects of Food Policy recommendations (Department of Health, 1994), the efficiency of transfer from feed to muscle was poor.

Another potential route to increase PUFA deposition in ruminant animal tissues is to seek and exploit breeds with an increased capacity to deposit these fatty acids or to deposit *n*-3 PUFA in preference to those of the *n*-6 fatty acid series. Although many studies purport to demonstrate differences in tissue fatty acid composition between breeds, these effects stem mainly from differences in fatness. However, two studies have produced clear evidence for differences in the metabolism of PUFA between breeds: Malau-Aduli *et al.* (1998) found that Jersey cows had higher proportions of 20 : 5*n*-3 (EPA) and lower proportions of 20 : 4*n*-6 (arachidonic acid) in the phospholipids of the biceps brachii muscle compared with Limousin cows; Choi *et al.* (2000) found more *n*-3 PUFA and less *n*-6 PUFA in the longissimus muscle phospholipids of Welsh Black steers compared with Holstein–Friesians. In a study of lamb production systems, we observed higher concentrations of PUFA in the musculus semimembranosus of Soay lambs compared with Suffolk lambs at similar levels of total intramuscular fatty acids

* **Corresponding author:** Dr M. Enser, fax +44 117 928 9324, email m.enser@bristol.ac.uk

(Fisher *et al.* 2000). A controlled trial of these breeds on the same feed and slaughtered at the same stage of maturity confirmed these differences in fatty acid composition, although at a lower level (Wachira *et al.* 2002). The objectives of the present trial were to determine whether these breed differences (essentially between 'meat' and 'milk' producing breeds) occurred more widely, by comparing the Suffolk × Lleyn with the Scottish Blackface. The latter, a hardy hill breed, has not been selected for meat production and has a different fat distribution to the Suffolk, with less subcutaneous and more intermuscular fat (McClelland & Russel, 1972). The dietary PUFA sources selected were linseed and a mixture of linseed and fish oil. In an attempt to improve the rumen bypass of 18 : 3*n*-3 in the linseed, the whole seed was treated with formaldehyde to crosslink the protein surrounding the oil droplets and make it resistant to digestion in the rumen. Fish oil was included in the diet since our previous trials have indicted that the increments in 20 : 5*n*-3 and 22 : 6*n*-3 as a result of synthesis from 18 : 3*n*-3 were limited. We have also studied the effect of dietary vitamin E on fatty acid composition, since vitamin E protects PUFA from oxidation in body tissues. It was included in the feeds at two levels: 100 mg/kg DM to give an intake approximating a diet of fresh forage; a supra-nutritional level of 500 mg/kg DM, a concentration that is used to improve the colour and oxidative shelf-life of meat (Schaefer *et al.*1995). Finally we have investigated the effects of the dietary lipid and vitamin E on both the polar and neutral lipid fraction of muscle and liver to understand more clearly the overall changes in tissue fatty acid composition resulting from diet modification. Although liver is a minor part of the edible carcass, it was chosen in addition to the usual muscle and adipose tissue because of its high content of PUFA (Enser *et al.* 1998*a*) that might reveal differences in fatty acid metabolism more clearly than the other tissues. In an accompanying paper (Chikunya *et al.* 2004) the effects of rumen transit on the fatty acids of similar diets have been investigated in order to understand the post-rumen metabolism of fatty acids better.

Materials and methods

Experimental design and treatments

Ninety-six ram lambs (forty-eight Suffolk × Lleyn with an average live weight of 24 kg and forty-eight Scottish Blackface with an average live weight of 18 kg) were allocated by live weight to six treatment groups of eight animals per breed. Groups from both breeds were blocked according to live weight and fed one of six diets based on dried grass and molassed sugarbeet pulp, formulated to be isoenergetic and isonitrogenous with similar total fatty acid concentrations (Table 1). The diets contained three different fat mixtures with two levels each of vitamin E to give a 2 × 3 × 2 (breed × fat × vitamin E) factorial design. The fat of the control diet, Megalac® (Volac Ltd, Royston, Herts., UK) consisted of the calcium salts of palm oil fatty acids, mainly palmitic acid (16 : 0) and oleic acid (18 : 1*n*-9) with somewhat less linoleic acid (18 : 2*n*-6). The linseed diet contained formaldehyde-treated whole linseed (4 g/kg seed; Trouw Nutrition Ltd UK, Northwich, Ches., UK) rich in α-linolenic acid (18 : 3*n*-3) with smaller amounts of 18 : 1*n*-9 and 18 : 2*n*-6. The linseed–fish oil consisted of a mixture of formaldehyde-treated whole linseed and South American herring oil (Isaac Spencer and Co. Fleetwood Ltd, Lancs., UK) (1:1, w/w). The fish oil contributed the long-chain PUFA 20 : 5*n*-3 and 22 : 6*n*-3 to the diet. Diets were formulated with 100 and 500 mg α-tocopheryl acetate (Roche Vitamins (UK) Ltd, Heanor, Derby., UK)/kg DM.

Procedures and analyses

Lambs were housed individually in raised-floor pens and adapted to the experimental diet over a period of 2 weeks using a mixture of the three diets with the low level of vitamin E. The individual experimental diets were then offered *ad libitum* with refusals recorded three times each week. Fresh feed was offered daily and increased to 115 % of the previous recorded intake. Live weight was recorded weekly between 14.00 and 15.00 hours. Animals were slaughtered at approximately half the breed mature live weight (Suffolk × Lleyn 46 kg; Scottish Blackface 36 kg; mean time on trial 71·4 (SED 2·91) d) in the University of Bristol abattoir. After conventional slaughter and dressing, carcasses were chilled overnight, weighed and assessed for fatness and conformation using the fifteen-point European system (Fisher *et al.* 2000). The m. semimembranosus from the left hind leg and a section of full thickness loin subcutaneous adipose tissue were removed, vacuum packed, blast frozen and held at −20°C for lipid fatty acid analysis.

Samples of feeds were analysed for DM and organic matter (Association of Analytical Chemistry, 1995), neutral-detergent fibre (Van Soest *et al.* 1991) and N (Kjeltec 1035 analyser; Tecator AB, Hoganas, Sweden). The amount of N was multiplied by 6·25 to estimate crude protein. Feed fatty acids were obtained by hydrolysis with 5 mol KOH/l methanol–water (1:1, v/v) at 60°C for 3 h in the presence of 21 : 0 methyl ester as quantitative internal standard. The samples were acidified to pH 1·0 and heated for an additional 1 h before extraction of the fatty acids into light petroleum (40–60°C). Lipids were extracted from duplicate 10 g samples of muscle after blending in a food processor with chloroform-methanol (2:1, v/v) containing 2,6-di-*tert*-butyl-*p*-cresol (butylated hydroxytoluene) as an antioxidant (Folch *et al.* 1957). The lipid extract in chloroform (approximately 20 mg lipid) was applied to a 500 mg silicic acid column (Isolute Si; Jones Chromatography Ltd, Hengoed, UK) and the neutral lipids eluted with chloroform (10 ml) followed by methanol (20 ml) to elute the polar lipids. After the addition of 21 : 0 methyl ester as internal standard, the lipids were hydrolysed in 2 mmol KOH/l methanol–water (1:1, v/v), containing hydroquinone as antioxidant, at 60°C for 1 h. The solution was acidified and the fatty acids extracted into light petroleum (40–60°C) and methylated with a solution of diazomethane in diethyl ether. Methyl esters were analysed by GLC on a 50 m × 0·25 mm internal diameter Cp Sil 88 fatty acid methyl ester column (Chrompack UK, Ltd, London, UK).

Table 1. Raw materials and chemical composition of the experimental diets

Fat source...	Megalac®*		Linseed		Linseed–fish oil	
Vitamin E level...	Low	High	Low	High	Low	High
Ingredient (g/kg fresh weight)						
Dried grass	759	759	739	739	754	754
Sugarbeet pulp (molassed)	105	105	105	105	105	105
Megalac®*	35	35	-	-	-	-
Protected whole linseed	-	-	85	85	42	42
Fish oil	-	-	-	-	15	15
Soyabean meal	46	46	16	16	29	29
Molasses	25	25	25	25	25	25
Mineral and vitamin premix	20	20	20	20	20	20
Ammonium chloride	5	5	5	5	5	5
NaCl	5	5	5	5	5	5
α-Tocopheryl acetate (mg/kg)	100	500	100	500	100	500
Chemical composition (g/kg DM)						
DM	942	929	935	929	935	935
Organic matter	884	880	891	889	886	891
Crude protein (N × 6·25)	174	170	172	172	170	174
Neutral-detergent fibre	479	477	491	463	478	470
Total fatty acids	45	50	50	51	51	56
Vitamin E (mg/kg DM)	137	453	129	423	97	411
Se (mg/kg DM)	1·04	0·93	0·93	0·79	0·96	0·87
Fatty acids						
12 : 0 (lauric)	0·8	1·0	0·2	0·4	0·3	0·2
14 : 0 (myristic)	0·6	0·7	0·2	0·3	1·3	1·4
16 : 0 (palmitic)	15·1	16·5	5·5	6·6	6·8	7·8
16 : 1 (palmitoleic)	0·4	0·4	0·3	0·3	1·5	1·6
18 : 0 (stearic)	1·6	1·8	1·8	2·0	1·7	1·9
18 : 1*n*-9 (oleic)	10·4	11·7	6·8	7·9	6·1	6·7
18 : 1*n*-7 (*cis*-vaccenic)	0·3	0·4	0·3	0·4	0·6	0·7
18 : 2*n*-6 (linoleic)	6·3	6·9	7·2	7·9	6·4	7·4
18 : 3*n*-3 (linolenic)	7·3	8·2	25·7	23·5	18·3	20·3
20 : 5*n*-3 (eicosapentaenoic)	ND	ND	ND	ND	2·2	2·4
22 : 6*n*-3 (docosahexaenoic)	ND	ND	ND	ND	2·5	2·2

ND, not detected.
* Calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK.

The GC conditions were: carrier gas He; split mode injection 70:1; injector and flame ionisation detector temperature 250°C; initial oven temperature 180°C for 15 min, then increased at 1·5°C per min to 220°C, held at 220°C for 10 min. Saturated (fatty acid methyl ester 4) and monounsaturated (fatty acid methyl ester 5) fatty acid methyl ester standard mixtures (Thames Restek UK Ltd, Windsor, Berks., UK) were used to establish the response linearity of the system.

Adipose tissue samples were blended in a food processor and the lipid extracted into chloroform containing butylated hydroxytoluene as antioxidant. After drying with anhydrous sodium sulfate, samples were taken and fatty acid methyl esters prepared and analysed following the procedures described earlier for muscle lipids.

Fatty acid results are reported for major fatty acids and minor identifiable fatty acids relevant to the study. These comprise $\geqq 90\,\%$ total fatty acids. Branched-chain fatty acids were identified and estimated after hydrogenation of the methyl esters with H_2 gas in the presence of platinum black as a catalyst. As a result of incomplete resolution the *trans*-18 : 1 isomers are reported as a single value that does not include minor isomers (*trans*-13, *trans*-16-18 : 1) not resolved from *cis*-18 : 1*n*-9 and *cis*-18 : 1*n*-7. In addition to the minor cross-contamination of the latter two fatty acids, the fatty acid listed as *cis*-16 : 1 consists of *n*-9 and *n*-7 isomers and a contaminating branched-chain 17 : 0 fatty acid.

Vitamin E was determined as α-tocopherol on samples of m. longissimus lumborum by the method of Arnold *et al.* (1993).

Statistical analyses

Live-weight gain was estimated by regression of live weight *v.* time and is expressed in relation to metabolic live weight (live weight$^{0\cdot75}$). Data were subjected to ANOVA using a factorial design (Genstat 5; Lawes Agricultural Trust, Rothamsted, Herts., UK) with fat source, breed and vitamin E levels as the main effects.

Results

Animal performance and carcass classification

Type of dietary fat had no effect on feed intake, growth rate or feed conversion (Table 2). Suffolk lambs consumed significantly more feed than the Scottish Blackface (151·4 and 129·8 g/kg live weight$^{0\cdot75}$ respectively). However, feed utilization was more efficient in the Scottish Blackface, so that there was no difference in daily live-weight gain relative to live weight$^{0\cdot75}$. Although dietary vitamin E did not influence

Table 2. Effect of dietary fat and breed on performance, carcass weight and classification of lambs offered the experimental diets†‡
(Mean values)

	Fat source					Breed			
	Megalac®§	Linseed	Linseed–fish oil	SED	Statistical significance of effect	Suffolk × Lleyn	Scottish Blackface	SED	Statistical significance of effect
Animal performance									
Intake (g DM/kg $W^{0·75}$)	143·9	139·9	137·9	3·19	NS	151·4	129·8	2·6	***
DLWG (g/kg $W^{0·75}$)	20·3	18·9	19·8	0·85	NS	19·5	19·8	0·69	NS
FCR (kg feed/kg gain)	7·2	7·6	7·2	0·29	NS	7·9	6·7	0·23	**
Carcass characteristics									
Hot carcass wt (kg)	17·3	16·7	16·7	0·4	NS	19·5	14·2	0·33	***
Cold carcass wt (kg)	16·7	16·1	16·0	0·39	NS	18·9	13·7	0·32	***
Conformation score	7·3	6·7	6·6	0·33	*	8·1	5·6	0·27	***
Fat score	6·8	7·1	8·3	0·41	**	8·1	6·7	0·33	***

$W^{0·75}$, metabolic live weight; DLWG, daily live-weight gain; FCR, feed conversion ratio.
* $P<0·05$, ** $P<0·01$, *** $P<0·001$.
† For details of diets and procedures, see Table 1 and pp. 552–553.
‡ Megalac® *n* 32, linseed *n* 32, linseed–fish oil 31, Suffolk × Lleyn *n* 48, Scottish Blackface *n* 47.
§ Calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK.

lamb performance, intake of the high-vitamin E Megalac diet was significantly higher than the intake for the low-vitamin E Megalac diet (148·3 *v.* 136·9 g/kg live weight$^{0·75}$ per d; SED 4·0, $P<0·05$). Suffolk lambs had heavier carcasses, better conformation (higher scores) and were fatter than Scottish Blackface lambs (Table 2). Dietary fat source did not affect carcass weight, but mean conformation scores were higher for lambs fed Megalac compared with those fed linseed or linseed–fish oil. The presence of fish oil in the diet increased carcass fatness scores.

Muscle fatty acid composition

The effect of dietary fat and breed on the fatty acid composition of the neutral lipid fraction of the m. semimembranosus is shown in Table 3. The fatty acid composition was not affected by the amount of vitamin E in the diet and the type of dietary fat did not change the proportions of saturated fatty acids. Compared with Megalac, linseed doubled the proportion of 18 : 3*n*-3 whilst *cis*-16 : 1 and its metabolic product 18 : 1*n*-7 were reduced. The proportion of 18 : 3*n*-3 in the linseed–fish oil treatment was intermediate between the proportion in the Megalac and linseed treatments. However, linseed–fish oil also increased the proportions of 18 : 2*n*-6 and *trans*-18 : 1, mainly at the expense of 18 : 1*n*-9. The amounts of muscle neutral lipid fatty acids were higher in lambs fed either of the linseed-containing diets compared with Megalac ($P<0·001$). Breed mainly affected the proportion of saturated fatty acids with higher proportions of 12 : 0 and 14 : 0 in the Suffolk lambs and more 18 : 0 in the muscle of Scottish Blackface lambs. The latter also had significantly less

Table 3. Effect of dietary fat and breed on the fatty acid content and composition (g/100 g total fatty acids) of the neutral lipid fraction of lamb musculus semimembranosus†‡
(Mean values)

	Fat source					Breed			
	Megalac®§	Linseed	Linseed–fish oil	SED	Statistical significance of effect	Suffolk × Lleyn	Scottish Blackface	SED	Statistical significance of effect
12 : 0	0·26	0·26	0·24	0·021	NS	0·28	0·23	0·017	**
14 : 0	3·04	2·9	2·91	0·136	NS	3·07	2·82	0·111	*
16 : 0	25·6	24·9	25·7	0·43	NS	25·5	25·2	0·350	NS
cis-16 : 1	2·19	1·97	2·2	0·08	**	2·14	2·1	0·065	NS
18 : 0	13·6	13·8	13·7	0·324	NS	13·4	14·0	0·263	**
trans-18 : 1	2·03	2·16	3·93	0·273	***	2·74	2·65	0·223	NS
18 : 1*n*-9	43·8	42·2	37·3	1·051	***	41·1	41·2	0·856	NS
18 : 1*n*-7	1·18	0·81	1·04	0·096	***	1·05	0·97	0·078	NS
18 : 2*n*-6	1·52	1·45	2·15	0·206	***	1·68	1·72	0·168	NS
18 : 3*n*-3	1·18	2·54	1·72	0·139	***	1·97	1·65	0·113	**
Total fatty acids (g/kg muscle)	18·6	25·4	25·0	1·96	***	24·4	21·5	1·59	NS

* $P<0·05$, ** $P<0·01$, *** $P<0·001$.
† For details of diets and procedures, see Table 1 and pp. 552–553.
‡ Megalac® *n* 32, linseed *n* 32, linseed–fish oil *n* 31, Suffolk × Lleyn *n* 48, Scottish Blackface *n* 47.
§ Calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK.

18 : 3*n*-3 in their muscle neutral lipids. Breed did not significantly affect the total amount of fatty acids in this lipid fraction.

Muscle polar lipid fatty acid composition was affected by dietary fat, breed and the level of dietary vitamin E, but the concentration of polar lipid fatty acids was not significantly altered, having a mean value of 6·19 g/kg muscle (Table 4). Feeding *n*-3 PUFA as linseed or fish oil increased the proportions of these fatty acids in the polar lipid fraction except for 18 : 3*n*-3 in the linseed–fish oil diet which did not differ significantly from the proportion in animals fed the Megalac diet. Compared with the Megalac diet, the linseed diet almost doubled the concentration (g/100 g total fatty acids) from 4·6 to 8·2. The linseed diet also significantly increased the concentration of 20 : 5*n*-3 and 22 : 5*n*-3 compared with the Megalac diet, although 22 : 6*n*-3 was not altered. In addition to the deposition of 20 : 5*n*-3 and 22 : 6*n*-3 from the fish oil in the linseed–fish oil diet, lambs fed this diet had the highest concentrations of 20 : 4*n*-3 and 22 : 5*n*-3, neither of which was present in the feed in measurable quantities. Of the saturated fatty acids, lauric acid (12 : 0) and palmitic acid were unaffected by diet, and myristic and stearic acid were increased by the diets containing linseed, most markedly for myristic acid with levels (g/100 g total fatty acids) of 0·4, 0·5 and 0·6 (SED 0·042, $P>0·001$) for Megalac, linseed and linseed–fish oil respectively. The effects of dietary fat on monoenoic fatty acids varied: 16 : 1 and 18 : 1*n*-7 were lower in animals fed linseed with similar proportions in those fed Megalac or linseed–fish oil. Feeding Megalac resulted in the highest proportion of 18 : 1*n*-9 with lower and similar proportions produced by either diet containing linseed. The concentrations of *trans*-18 : 1 increased in the order Megalac < linseed < linseed–fish oil with mean values of (g/100 g total fatty acids) 0·53, 0·90, 2·11 (SED 0·015, $P<0·001$). Breed of sheep affected the proportion of seven fatty acids in the polar lipids of the m. semimembranosus. The saturated fatty acids 14 : 0 and 16 : 0 were higher in the Suffolks than in the Scottish Blackface as were the unsaturated fatty acids 18 : 2*n*-6 and 18 : 3*n*-3, whereas 18 : 1*n*-7 and 20 : 3*n*-6 were higher in the Scottish Blackface. The level of dietary vitamin E also affected the polar lipid fatty acids with 16 : 1 and 18 : 1*n*-9 being significantly higher in lambs given diets with the low level of vitamin E, whilst a high level of vitamin E increased the proportions of 18 : 2*n*-6, 20 : 5*n*-3 and 22 : 6*n*-3. There were significant interactions of type of dietary fat × vitamin E levels. For 18 : 2*n*-6, vitamin E at high levels only increased the proportions in Megalac-fed lambs and high vitamin E only raised the proportion of 20 : 5*n*-3 in lambs on linseed–fish oil.

Liver fatty acids

The fatty acid content (g/kg liver) and the fatty acid composition (g/100 g total fatty acids) of the liver neutral lipid fraction are shown in Table 5. The total fatty acid content of the liver neutral lipid fraction was unaffected by dietary fat, breed or vitamin E. The mean content was 22·1 (SED 0·6) g/kg liver. As in muscle the major fatty acids were 18 : 1*n*-9, 16 : 0, 18 : 0 and 18 : 2*n*-6, but unlike the muscle neutral lipids, measurable amounts of C_{20} and C_{22} PUFA were also present. The proportions of all the fatty acids reported, except for 12 : 0, varied according to the dietary fat. Feeding linseed more than doubled the concentration of 18 : 3*n*-3 in the liver neutral lipids compared to the Megalac diet, with linseed–fish oil producing an intermediate level; mean concentrations (g/100 g total fatty acids) were 3·8, 6·0 and 8·8 (SED 0·46, $P<0·001$). Feeding the linseed diet also increased 20 : 5*n*-3 and 22 : 5*n*-3, but the mean increase in 22 : 6*n*-3 was not significant. The linseed–fish oil diet more than doubled the concentrations of 20 : 5*n*-3 and 22 : 6*n*-3 compared with the Megalac diet, but for 22 : 5*n*-3 the increase was less. Both the linseed and linseed–fish oil diet decreased 20 : 4*n*-6 compared with the Megalac diet, but 18 : 2*n*-6 was only lower when fish oil was fed. Of the saturated fatty acids, 14 : 0 and 16 : 0 were highest in lambs that had been fed Megalac and 18 : 0 was highest in lambs fed the linseed diet. All of the *cis*-monoenoic fatty acids were highest in the neutral lipids from the Megalac-fed lambs. The concentration of 18 : 1*n*-9 in lambs fed linseed fell between that of the other two feeds. The mean values (g/100 g total fatty acids) were 30·4, 23·2 and 18·6 (SED 1·31, $P<0·001$) for lambs fed Megalac, linseed and linseed–fish oil respectively. *Trans*-18 : 1 changed in the opposite direction to 18 : 1 *n*-9, with the highest concentration of 4·7 g/100 g total fatty acids in the lambs fed linseed–fish oil followed by 3·2 g/100 g total fatty acids in those fed linseed and 2·5 g/100 g total fatty acids in Megalac-fed lambs (SED 0·43, $P<0·001$).

Breed affected the concentrations of all neutral lipid fatty acids reported except *trans*-18 : 1 and 18 : 1*n*-7. The saturated fatty acids 12 : 0, 14 : 0 and 16 : 0 were higher in the Scottish Blackface lambs, whilst 18 : 0 was highest in the Suffolk lambs. The concentrations of the monounsaturated fatty acids 16 : 1, 18 : 1*n*-9 and 20 : 1 were also higher in the Scottish Blackface, whereas 18 : 2*n*-6 and 18 : 3*n*-3 and all the C_{20} and C_{22} PUFA were present at higher levels in the Suffolk lambs. Vitamin E concentration in the feed also altered fatty acid composition. All monounsaturated fatty acids were present in higher concentrations in livers from lambs on the low-vitamin E diet, although the effect was only significant for 16 : 1, 18 : 1*n*-9 and 20 : 1. In contrast, the low vitamin E-fed lambs contained the lowest concentrations of all of the PUFA, although the difference was not significant for 20 : 5*n*-3 and 22 : 6*n*-3. The interactions between vitamin E and type of dietary fat differed according to the fatty acids involved. The differences between the high- and low-vitamin E diets were greatest for 18 : 0 and 20 : 4 in the Megalac-fed lambs, whereas for 18 : 2*n*-6 and 18 : 3*n*-3 the higher concentrations induced by the high-vitamin E diet were greatest in lambs fed the linseed diet. The only significant interaction between breed and vitamin E was for 20 : 4*n*-6. Dietary vitamin E level did not affect the 20 : 4*n*-6 level in Suffolk lambs, whereas Scottish Blackface lambs fed low-vitamin E diets had a lower concentration (g/100 g total fatty acids; high and low vitamin E-fed Suffolk lambs of 1·88 and 1·84 respectively and high and low vitamin E-fed Scottish Blackface lambs of 1·61 and 1·03 (SED 0·181, $P<0·05$)).

Table 4. Effect of dietary fat, breed and vitamin E on the fatty acid content and composition (g/100 g total fatty acids) of the polar lipid fraction of lamb musculus semimembranosus†‡ (Mean values)

Fat source…	Megalac®§				Linseed				Linseed–fish oil								
Vitamin E…	Low		High		Low		High		Low		High			Statistical significance of effect			
Fatty acid	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	SED	Fat	Breed	Vitamin E	Fat × Vitamin E‖
12 : 0	0·07	0·08	0·05	0·05	0·07	0·08	0·08	0·09	0·07	0·07	0·1	0·1	0·019	NS	NS	NS	NS
14 : 0	0·47	0·32	0·36	0·28	0·58	0·4	0·51	0·43	0·7	0·61	0·63	0·49	0·086	***	***	NS	NS
16 : 0	15·4	14·8	15·5	14·5	15·2	14·8	15·0	14·8	15·3	14·7	15·6	15·1	0·511	NS	**	NS	NS
16 : 1	1·6	1·6	1·5	1·3	1·1	1·2	1·0	1·1	1·5	1·5	1·3	1·3	0·169	***	NS	*	NS
18 : 0	10·3	10·7	10·4	10·4	12·1	12·6	12·2	13·3	12·3	13·5	13·1	12·3	0·734	***	NS	NS	NS
trans-18 : 1	0·6	0·51	0·54	0·49	1·00	0·74	1·00	0·82	2·30	1·60	2·00	2·50	0·316	***	NS	NS	NS
18 : 1*n*-9	22·4	23·7	22·4	20·2	16·7	19·3	15·8	17·8	17·3	17·3	16·0	15·1	1·344	***	NS	**	NS
18 : 1*n*-7	3·4	3·9	3·6	3·7	2·7	2·9	2·8	2·9	3·3	3·4	3·3	3·4	0·208	***	*	NS	NS
18 : 2*n*-6	12·3	11·4	12·6	13·5	11·1	9·1	12·4	9·9	9·2	7·5	9·6	8·4	0·812	***	***	**	*
18 : 3*n*-3	5·0	3·8	4·9	4·8	9·0	7·0	9·2	7·7	5·4	4·3	5·8	4·8	0·787	***	***	NS	NS
20 : 3*n*-6	0·57	0·68	0·58	0·69	0·52	0·52	0·51	0·52	0·58	0·61	0·54	0·56	0·038	***	**	NS	*
20 : 4*n*-6	5·6	5·7	6·0	6·2	4·7	4·6	5·3	3·9	3·4	3·2	3·6	3·3	0·482		NS	NS	NS
20 : 4*n*-3	0·14	0·14	0·15	0·15	0·29	0·26	0·24	0·28	1·09	1·32	1·25	1·69	0·195	***	NS	NS	NS
20 : 5*n*-3	4·0	4·0	3·9	4·4	5·6	5·5	5·4	6·0	6·5	7·8	7·3	9·1	0·432	***	***	**	**
22 : 5*n*-3	3·7	3·9	3·7	3·9	4·9	4·9	4·9	5·2	5·1	6·1	5·4	5·6	0·401	***	NS	NS	NS
22 : 6*n*-3	1·2	1·0	1·2	1·5	1·1	1·1	1·1	1·2	1·7	1·6	1·8	2·1	0·193	***	NS	**	NS
Total fatty acids (g/kg muscle)	6·33	6·03	5·97	5·25	6·05	5·89	6·05	6·98	6·83	6·68	6·27	5·99	0·46	NS	NS	NS	NS

*$P<0·05$, **$P<0·01$, ***$P<0·001$.
† For details of diets and procedures, see Table 1 and pp. 552–553.
‡ *n* 8 except for Blackface fed linseed–fish oil low vitamin E (*n* 7).
§ Calcium soap of palm oil distillate; Volac Ltd, Royston, Herts., UK.
‖ Other interactions: Fat × Vitamin E, 12 : 0, $P<0·05$; total fatty acids, $P<0·05$; Breed × Vitamin E, 22 : 6, $P<0·05$.

Table 5. Effect of dietary fat, breed and vitamin E on the fatty acid content and composition (g/100 g total fatty acids) of the neural lipid fraction from lamb liver†‡ (Mean values)

Fat source...	Megalac®§				Linseed				Linseed–fish oil								
Vitamin E...	Low		High		Low		High		Low		High			Statistical significance of effect			
Fatty acid	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	Suffolk	Scottish Blackface	SED	Fat	Breed	Vitamin E	Fat × Vitamin E‖
12 : 0	0·11	0·13	0·07	0·15	0·07	0·13	0·08	0·14	0·06	0·10	0·06	0·14	0·042	NS	***	NS	NS
14 : 0	1·15	1·3	0·89	1·1	0·75	0·98	0·66	1·0	0·76	0·89	0·61	1·0	0·157	***	***	NS	NS
16 : 0	18·0	18·3	16·6	18·9	13·1	14·8	13·1	14·0	13·5	13·4	11·6	14·7	1·406	***	*	NS	NS
16 : 1	2·7	3·4	2·3	2·9	1·8	2·6	1·6	2·2	1·7	2·2	1·8	2·2	0·300	***	***	*	NS
18 : 0	10·6	7·4	13·3	10·5	13·6	10·2	14·2	11·7	13·5	11·3	13·6	8·9	1·338	*	***	NS	*
trans- 18 : 1	2·5	2·4	3·0	1·8	3·6	3·2	3·6	2·3	6·0	4·3	4·0	4·6	0·862	***	NS	NS	NS
18 : 1*n*-9	29·9	35·0	25·6	31·1	21·5	25·6	21·3	24·3	17·6	21·3	15·4	20·2	2·626	***	***	*	NS
18 : 1*n*-7	2·5	2·5	2·2	2·4	1·7	1·9	1·6	1·7	1·7	1·7	1·6	1·7	0·229	***	NS	NS	NS
18 : 2*n*-6	5·8	3·9	6·4	5·3	5·4	4·0	7·1	6·5	4·7	4·2	4·8	3·9	0·606	***	***	***	**
18 : 3*n*-3	3·8	3·6	4·1	3·6	8·4	6·7	11·1	8·8	5·6	5·6	6·6	6·1	0·923	***	*	**	*
20 : 1	0·44	0·49	0·37	0·42	0·37	0·44	0·31	0·36	0·43	0·48	0·44	0·47	0·051	**	*	*	NS
20 : 4*n*-6	2·4	0·89	2·7	2·4	1·7	1·0	1·6	1·5	1·4	1·2	1·3	0·89	0·314	***	***	*	**
20 : 5*n*-3	2·9	1·8	3·8	2·3	5·6	3·6	5·4	4·4	7·1	6·5	8·3	5·9	0·932	***	***	NS	NS
22 : 5*n*-3	3·8	2·9	5·7	4·0	6·0	4·6	5·9	5·0	7·0	6·5	8·9	6·1	0·835	***	***	*	NS
22 : 6*n*-3	1·9	1·0	3·0	2·0	2·9	2·1	2·7	2·0	5·4	4·2	6·2	3·2	0·616	***	***	NS	NS
Total fatty acids (g/kg liver)	24·3	24·2	23·7	21·2	21·8	20·0	26·2	19·3	20·6	18·0	23·1	23·0	2·88	NS	NS	NS	NS

*$P<0·05$, **$P<0·01$, ***$P<0·001$.
† For details of diets and procedures, see Table 1 and pp. 552–553.
‡ *n* 8 except for Blackface fed linseed–fish oil low vitamin E (*n* 7).
§ Calcium soap of palm oil distillate; Volac Ltd, Royston, Herts., UK.
‖ Other interactions: Breed × Vitamin- E, 20 : 4n-6, $P<0·05$.

The fatty acids of the liver polar lipids resembled those of the neutral lipids much more than in muscle because of the long-chain PUFA present in the liver neutral lipids, in contrast to their virtual absence in the muscle neutral lipid fraction. Although dietary fat did not affect the total quantity of polar lipids, the amounts in the livers from Scottish Blackface lambs were greater than in Suffolk lambs: 35·5 *v.* 25·4 g/kg tissue (SED 2·18, $P<0·001$). However, there were marked differences in the content of the major fatty acids (Table 6). Dietary fat affected the concentration (g/100 g total fatty acids) of all fatty acids except 12 : 0 and 18 : 0. The effect of dietary fat on the concentration of individual fatty acids in the polar lipids was very similar to that in the neutral lipids of the liver. Compared with the Megalac diet, the linseed diet produced higher concentrations of 18 : 3*n*-3 and the longer-chain *n*-3 PUFA except for 22 : 6*n*-3. Lambs fed linseed–fish oil had even higher mean concentrations of the C_{20} and C_{22} *n*-3 PUFA, including 22: 6*n*-3, but the concentration of 18 : 3*n*-3 fell between that of the Megalac- and linseed-fed lambs. The increase in *n*-3 PUFA resulted in lower concentrations of the monoenoic fatty acids and 20 : 4*n*-6 in lambs fed the linseed diet. The effect of linseed–fish oil was similar, except that the concentration of 18 : 2*n*-6 was also significantly lower. In addition to the increase in *n*-3 PUFA, the *trans*-18 : 1 fatty acids were increased by the linseed diet and were highest in the lambs offered linseed–fish oil: mean concentrations (g/100 g total fatty acids) were 1·58, 2·13 and 3·67 respectively (SED 0·313, $P<0·001$).

Breed effects on the concentrations of fatty acids in the liver polar lipid fraction were limited to 16 : 1, 18 : 0, 18 : 3*n*-3 and 20 : 5*n*-3. The mean concentration of 16 : 1 was significantly higher in Scottish Blackface compared with Suffolk lambs, whereas 18 : 0 was higher in the Suffolk lambs. The latter breed also had more 18 : 3*n*-3 than the Blackface lambs: concentrations (g/100 g total fatty acids) of 5·40 and 4·69 (SED 0·415, $P<0·001$) respectively. The high concentration of 18 : 3*n*-3 in Suffolk lambs did not carry through into the longer-chain PUFA and 20 : 5*n*-3 was significantly higher in the Blackface lambs with respective concentrations of 6·76 and 7·53 (SED 0·304, $P<0·05$). The dietary concentration of vitamin E altered the fatty acid composition of the liver polar lipids in a similar way to the neutral lipids. However, the effects were less marked. Only 16 : 1 was significantly higher in the low-vitamin E group and 18 : 1*n*-7 was not affected. Of the PUFA, only 18 : 2*n*-6 was significantly higher in lambs on the high-vitamin E diets. However, there was a significant interaction between dietary fat and vitamin E such that the high-vitamin E diet only increased the proportion of 18 : 2*n*-6 in animals fed the Megalac and linseed diets.

Subcutaneous adipose tissue

Measurable PUFA in adipose tissue were limited to 18 : 2*n*-6 and 18 : 3*n*-3 (Table 7). Whereas the latter was highest in lambs fed the linseed diet and lowest in those fed Megalac, the former was highest in Megalac-fed lambs and lowest in those on the linseed–fish oil diet. The type of dietary fat had no significant effect on the concentrations of 10 : 0, 12 : 0 and 14 : 0, but the mean concentrations of 16 : 0 and 18 : 0 were significantly higher in the lambs fed linseed–fish oil than either of the other two diets. The odd-numbered fatty acids 15 : 0 and 17 : 0 and

Table 6. Effect of dietary fat and breed on the fatty acid content and composition (g/100 g total fatty acids) of the polar lipid fraction from lamb liver†‡

(Mean values)

	Fat source					Breed			
	Megalac®§	Linseed	Linseed–fish oil	SED	Statistical significance of effect	Suffolk × Lleyn‖	Scottish Blackface	SED	Statistical significance of effect
12 : 0	0·06	0·06	0·05	0·009	NS	0·05	0·06	0·007	NS
14 : 0	0·44	0·34	0·36	0·034	**	0·38	0·38	0·027	NS
16 : 0	12·9	10·8	11·4	0·280	***	11·8	11·6	0·229	NS
16 : 1	1·7	1·1	1·2	0·093	***	1·2	1·4	0·076	*
18 : 0	23·7	24·3	23·7	0·563	NS	24·8	22·9	0·460	***
trans-18 : 1	1·6	2·1	3·7	0·313	***	2·5	2·4	0·256	NS
18 : 1*n*-9	19·8	15·2	12·7	0·683	***	15·6	16·2	0·557	NS
18 : 1*n*-7	1·8	1·3	1·3	0·082	***	1·4	1·5	0·067	NS
18 : 2*n*-6	5·8	5·2	3·6	0·252	***	5·0	4·7	0·206	NS
18 : 3*n*-3	2·9	7·3	5·0	0·415	***	5·4	4·7	0·339	*
20 : 4*n*-6	4·8	2·4	1·7	0·173	***	3·0	3·0	0·141	NS
20 : 4*n*-3	0·24	0·35	0·99	0·078	***	0·48	0·57	0·064	NS
20 : 5*n*-3	4·6	7·6	9·3	0·373	***	6·8	7·5	0·304	*
22 : 5*n*-3	5·8	6·7	7·4	0·217	***	6·5	6·7	0·177	NS
22 : 6*n*-3	4·1	4·3	6·6	0·333	***	5·1	4·9	0·271	NS
Total fatty acids (g/kg liver)	30·8	31·7	29·0	2·67	NS	25·4	35·5	21·8	***

*$P<0·05$, **$P<0·01$, ***$P<0·001$.
† For details of diets and procedures, see Table 1 and pp. 552–553.
‡ Megalac® *n* 32, linseed *n* 32, linseed–fish oil *n* 31.
§ Calcium soap of palm oil distillate; Volac Ltd, Royston, Herts., UK.
‖ Interactions: Fat × Breed, 18 : 1*n*-7, $P<0·05$.

Table 7. Effect of dietary fat and breed on the fatty acid composition (proportion × 100) of lamb subcutaneous adipose tissue†‡ (Mean values)

	Fat source					Breed			
	Megalac®§	Linseed	Linseed–fish oil	SED	Statistical significance of effect	Suffolk × Lleyn	Scottish Blackface	SED	Statistical significance of effect
10 : 0	0·26	0·24	0·28	0·023	NS	0·3	0·22	0·019	***
12 : 0	0·16	0·13	0·14	0·017	NS	0·18	0·11	0·014	***
14 : 0	3·0	2·6	2·8	0·013	NS	3·1	2·5	0·164	***
15 : 0	1·8	1·4	1·0	0·119	***	1·4	1·4	0·098	NS
16 : 0	21·3	20·4	23·5	0·911	*	22·6	20·9	0·744	**
16 : 1	2·9	2·4	2·6	0·083	***	2·6	2·7	0·068	*
17 : 0	4·2	3·9	3·0	0·250	***	3·7	3·7	0·204	NS
18 : 0	9·2	10·4	12·8	0·661	***	11·2	10·3	0·54	NS
trans-18 : 1	1·5	1·7	4·0	0·337	***	2·5	2·3	0·275	NS
18 : 1*n*-9	30·1	27·8	27·8	0·654	**	28·0	29·3	0·534	*
18 : 1*n*-7	0·96	0·80	0·91	0·038	***	0·83	0·95	0·031	***
18 : 2*n*-6	1·9	1·7	1·1	0·089	***	1·6	1·5	0·072	NS
18 : 3*n*-3	1·7	4·3	2·0	0·228	***	2·9	2·4	0·186	*
Branched-chain fatty acids	15·6	13·6	8·1	1·443	***	11·8	13·1	1·178	NS

*$P<0·05$, **$P<0·01$, ***$P<0·001$.
† For details of diets and procedures, see Table 1 and pp. 552–553.
‡ Megalac® *n* 32, linseed *n* 32, linseed–fish oil *n* 31, Suffolk × Lleyn *n* 48, Scottish Blackface *n* 47.
§ Calcium soap of palm oil distillate; Volac Ltd, Royston, Herts., UK.
‖ Interactions: Fat × Breed, 18 : 1*n*-7, $P<0·05$.

the total branched-chain fatty acids were highest in adipose tissue from lambs on the Megalac diet and lowest in those fed linseed–fish oil. The concentrations of 16 : 0 and 18 : 0 were highest in the lambs fed linseed–fish oil as also were the *trans*-18 : 1 fatty acids and none were significantly different between the Megalac- and linseed-fed lambs. Breed had significant effects on the concentrations of fatty acids in the adipose tissue lipids. The fatty acids 10 : 0, 12 : 0, 14 : 0, 16 : 0 and 18 : 3*n*-3 were higher in Suffolk lambs and 16 : 1, 18 : 1*n*-9 and 18 : 1*n*-7 highest in Scottish Blackface lambs. However, for 18 : 1*n*-7 the difference was not significant for Scottish Blackface and Suffolk lambs fed the linseed diet, as shown by the significant interaction ($P<0·05$). For this fatty acid there was also a three-way interaction of breed × fat × vitamin E ($P<0·01$), with Scottish Blackface lambs having higher levels than Suffolk lambs on the low vitamin E Megalac diet and on the high vitamin E linseed–fish oil diet: 1·04 compared with 0·85 and 1·04 compared with 0·80 (SED 0·074) respectively. The significant interaction of fat × breed × vitamin E for *trans*-18 : 1 was the result of this fatty acid being significantly ($P<0·001$) higher in Scottish Blackface lambs fed linseed–fish oil high in vitamin E: 5·45 compared with 3·28 (proportion × 100, SED 0·673). For all other treatments, the values were higher in the Suffolk lambs, but not significantly so. The only individual effects of vitamin E stemmed from a higher proportion of 18 : 2*n*-6 (1·7 *v.* 1·5 (SED 0·072)) and 18 : 3*n*-3 (2·9 *v.* 2·5 (SED 0·186)) in lambs fed the high-vitamin E diet compared with the low-vitamin E diet.

Muscle vitamin E content

The vitamin E content of m. longissimus lumborum is shown in Table 8. Values ranged from 0·18–0·95 mg/kg muscle. Dietary fat and breed did not significantly affect vitamin E content, but dietary vitamin E concentration significantly affected tissue levels. Mean values were 0·27 and 0·52 mg/kg muscle (SED 0·076, $P<0·05$) for the low- and high-vitamin E diets respectively.

Table 8. Effect of dietary fat, breed and vitamin E on the vitamin E content (mg/kg) of musculus longissimus lumborum*‡
(Mean values for eight ram lambs per breed except Blackface, linseed–fish oil, low *n* 7)

		Breed	
Dietary fat	Vitamin E	Suffolk	Scottish Blackface
Megalac®	Low	0·20	0·18
	High	0·48	0·51
Linseed	Low	0·55	0·23
	High	0·51	0·29
Linseed–fish oil	Low	0·27	0·17
	High	0·37	0·95†

* For details of diets and procedures, see Table 1 and pp. 552–553.
† SED 0·186, dF 94, for low *v.* high vitamin E level, $P<0·05$.
‡ Megalac® *n* 32, linseed *n* 32, linseed–fish oil *n* 31, Suffolk × Lleyn *n* 48, Scottish Blackface *n* 47.

Human nutritional indices

The PUFA : saturated fatty acid ratios and 18 : 2*n*-6:18 : 3*n*-3 (*n*-6 : *n*-3 fatty acids) ratios are shown in Table 9. The PUFA : saturated fatty acid ratios were highest for the lambs fed the linseed diet. Compared with the Megalac diet, the greatest proportional change was in the subcutaneous adipose tissue followed by the liver with only a small effect in the muscle. The livers from Scottish Blackface lambs had a significantly higher PUFA : saturated fatty acid ratio than those from Suffolk lambs, but there

Table 9. Effect of dietary fat and breed on the PUFA:saturated fatty acid (S) and *n*-6:*n*-3 fatty acid ratios† (Mean values)

		Dietary fat					Breed			
Tissue	Ratio	Megalac®‡	Linseed	Linseed–fish oil	SED	Statistical significance of effect	Suffolk	Scottoish Blackface	SED	Statistical significance of effect
Muscle	PUFA:S§	0·17	0·19	0·15	0·011	*	0·17	0·17	0·009	NS
	n-6:*n*-3 fatty acids‖	2·11	0·93	1·54	0·097	***	1·42	1·63	0·079	*
Liver	PUFA:S§	0·26	0·42	0·30	0·018	***	0·31	0·35	0·014	***
	n-6:*n*-3 fatty acids‖	1·77	0·74	0·76	0·075	***	1·09	1·10	0·061	NS
Adipose tissue	PUFA:S§	0·11	0·18	0·08	0·010	***	0·13	0·12	0·008	NS
	n-6:*n*-3 fatty acids‖	1·22	0·41	0·57	0·035	***	0·69	0·77	0·028	**

*$P<0·05$, **$P<0·01$, ***$P<0·001$.
† For details of diets and procedures, see Table 1 and pp. 552–553.
‡ Calcium soap of palm oil distillate; Volac Ltd, Royston, Herts., UK.
§ PUFA:S is: $\frac{18:2n\text{-}6+18:3n\text{-}3}{12:0+14:0+16:0+18:0}$
‖ *n*-6:*n*-3 is $\frac{18:2n\text{-}6}{18:3n\text{-}3}$

were no significant breed differences in muscle or adipose tissue. Feeding linseed or linseed–fish oil decreased the *n*-6 : *n*-3 fatty acid ratios significantly in all three tissues. The ratios in liver and adipose tissue were similar for the two diets containing *n*-3 PUFA, but in muscle the linseed–fish oil diet was significantly less effective than linseed in lowering the ratio. The muscle and adipose tissue of Scottish Blackface lambs had significantly higher *n*-6 : *n*-3 fatty acid ratios, but breed had no effect on the ratio in the liver.

Discussion

The present trial has demonstrated that significant increases in the *n*-3 PUFA content of the lipids of lamb muscle, liver and adipose tissue can be obtained by feeding formaldehyde-treated linseed and a combination of this with fish oil. The linseed diet doubled the proportions of 18 : 3*n*-3 in tissues, but deposition of the longer-chain more unsaturated fatty acids synthesized from it decreased as the number of metabolic steps in their formation increased; tissue levels of 22 : 6*n*-3 were unaffected. Adding fish oil that contained 22 : 6*n*-3 and 20 : 5*n*-3 to the diet increased their deposition in tissue lipids. Formaldehyde treatment of the linseed resulted in a very limited increase in tissue levels of 18 : 3*n*-3 compared with our previous trial using bruised linseed (Wachira *et al.* 2002). The respective increments in the Suffolk lambs were 1·2-fold for muscle lipids and 1·6-fold for adipose tissue. This protection against rumen biohydrogenation by formaldehyde treatment of whole seed was much less than when protein-encapsulated lipid is treated with formaldehyde (Cook *et al.* 1970, 1972; Scott *et al.* 1971; Ashes *et al.* 1992). The effect of dietary lipids on the tissue lipids varied according to tissue and lipid fraction. C_{20} and C_{22} *n*-3 PUFA were not detected in adipose tissue or muscle neutral lipids, but the liver neutral lipids contained a wide range of such fatty acids as did the polar lipid fraction of the liver and m. semitendinosus. Suffolk × Lleyn lambs deposited significantly more 18 : 3*n*-3 in adipose tissue and muscle lipids than Scottish Blackface lambs. However, although the concentration of 18 : 3*n*-3 was also higher in the polar lipids of their livers, the tissue content was higher in the Scottish Blackface lambs, because of higher concentrations of liver polar lipids. A high dietary concentration of vitamin E resulted in some increases in tissue PUFA with lower proportions of monoenoic fatty acids.

Animal performance and carcass quality

The type of dietary fat had no significant effect on feed intake or daily live-weight gain (adjusted for metabolic body size) or the feed conversion ratio. However, the lower carcass conformation score for lambs fed the diets containing *n*-3 PUFA and the higher fat score for lambs fed linseed–fish oil clearly indicate effects on muscle and adipose tissue growth. Although breed did not affect daily live-weight gain (adjusted for metabolic body size), adjusted feed intake was high in the Suffolk lambs and their feed efficiency was poorer (more feed per unit of gain) than the Scottish Blackface, indicating a greater stage of maturity with associated increased deposition of body fat. Although the higher fatness score for the Suffolk lambs is consistent with such an interpretation, this assessment is based on subcutaneous fat cover and these breeds differ in fat partition with Blackface lambs preferentially depositing fat in intermuscular depots, whereas Suffolk lambs deposit it more extensively in the subcutaneous depot (McClelland & Russel, 1972). There were no main effects of vitamin E on animal performance and carcass quality (although both levels studied were higher than those used commercially), despite indications from the tissue fatty acid compositions that there was a loss of some PUFA on the low-vitamin E diets.

Neutral lipid fatty acids

The increments in the proportions of 18 : 3*n*-3 in tissue neutral lipids and adipose tissue of linseed-fed lambs

compared with those fed Megalac were similar, with a small preference for adipose tissue. The respective overall increases were 2·3-, 2·6- and 2·2-fold for liver, adipose tissue and m. semimembranosus respectively. Feeding linseed raised the proportion of 20 : 5*n*-3 and 22 : 5*n*-3 in liver neutral lipids compared with feeding Megalac: 1·8- and 1·3-fold respectively. The increases in these C_{20} and C_{22} *n*-3 PUFA and 18 : 3*n*-3 were accompanied by decreases in 20 : 4*n*-6 and in 16 : 1, 18 : 1*n*-9 and 18 : 1*n*-7 and would indicate a decrease in Δ^9-desaturase activity.

The quantity of 18 : 3*n*-3 in the linseed-fish-oil diet was at about the midpoint between the Megalac and linseed diets, and this was reflected in the flow of fatty acids entering the duodenum (Chikunya *et al.* 2004). However, the concentrations in the neutral lipids of the three tissues were closer to Megalac, indicating that as the dietary amount increased, more of the 18 : 3*n*-3 was used for metabolic processes. Although the C_{20} and C_{22} PUFA in the fish oil were not deposited in adipose tissue lipids or the neutral lipid fraction of the m. semimembranosus to any significant extent, their presence in the feed together with 18 : 3*n*-3 altered the concentrations of other fatty acids. In the m. semimembranosus 18 : 2*n*-6 was significantly higher, presumably an effect of the fish oil fatty acids, since there was no difference between the lambs fed Megalac or linseed. This appears to be a tissue-specific effect, since 18 : 2*n*-6 was present in lower proportions in the liver neutral lipids and adipose tissue of lambs fed linseed–fish oil compared with these fed linseed or Megalac. None of these effects on tissue levels of 18 : 2*n*-6 can be attributed to the levels in the diet or rumen metabolism since dietary concentrations and the proportions flowing into the duodenum were not significantly different between the diets (Chikunya *et al.* 2004). In subcutaneous adipose tissue fish oil also resulted in a significantly higher proportion of 16 : 0 and 18 : 0 in comparison with the other two diets. Dietary fatty acid levels cannot explain this effect. The proportion of 16 : 0 in the Megalac feed was twice that in linseed–fish oil and 18 : 0 levels were similar in the three feeds. Furthermore, the potential for 18 : 0 production in the rumen from unsaturated C_{18} fatty acids was much greater in the linseed than the linseed–fish oil, confirmed by the study of Chikunya *et al.* (2004). Again, this suggests a decrease in Δ^9-desaturase activity. Fish oil also raised the proportion of *trans*-18 : 1 fatty acids, an effect usually attributed to partial inhibition of biohydrogenation of 18 : 2*n*-6 and 18 : 3*n*-3 in the rumen (Czerkawski *et al.* 1975; Wachira *et al.* 2000). However, the proportions of *trans*-18 : 1 fatty acids were low compared with those we observed in earlier studies with lambs and beef cattle (Enser *et al.* 1999; Wachira *et al.* 2002). The failure of linseed to increase the proportions of *trans*-18 : 1 also contrasts with previous studies. This appears to result from post-digestive metabolism, since Chikunya *et al.* (2004) observed twice as much *trans*-18 : 1 flowing into the duodenum of the lambs fed the linseed diet compared with those fed Megalac. Another unusual feature of the adipose tissue lipids was the high concentration of branched-chain fatty acids, particularly in the Megalac-fed lambs. Small amounts of branched-chain fatty acids are synthesized by rumen bacteria from the C residues of branched-chain amino acids and by lamb adipose tissue from propionic acid via methylmalonyl-CoA (Tweedie *et al.* 1966; Scaife *et al.* 1978). However, when lambs are fed diets containing high levels of easily fermentable carbohydrate, such as ground barley, large quantities of branched-chain fatty acids are formed by adipose tissue from the propionic acid produced in the rumen (Garton *et al.* 1972). However, the diets were relatively low in rapidly fermentable carbohydrate and did not result in high concentrations of propionic acid, and the concentrations were not affected by the inclusion of fish oil (Chikunya *et al.* 2004).

Polar lipid fatty acids

There were distinct differences between the polar lipid fatty acid composition of the m. semimembranosus and liver; however, in both tissues the effects of including *n*-3 PUFA in the diets were similar, although the extent of changes varied for different fatty acids. The concentrations of 18 : 2*n*-6 and 18 : 3*n*-3 were twice as high in muscle lipids as in the liver lipids, but the liver contained higher proportions of all the C_{20} and C_{22} PUFA except for 20 : 4*n*-6 in the Megalac-fed lambs. The muscle fatty acid composition closely resembled that reported by Ashes *et al.* (1992) for sheep m. longissimus for 16 : 0, 18 : 0 and 18 : 1. However, the reported levels of 18 : 2*n*-6 and its metabolic product 20 : 4*n*-6 were twice those in our present Megalac-fed lambs. This probably stems from the high cereal content of the diet used by Ashes *et al.* (1992) that would have a high 18 : 2*n*-6:18 : 3*n*-3 ratio. The effects of cereals (high in 18 : 2*n*-6) and fresh forage (high in 18 : 3*n*-3) on the fatty acid composition of cattle muscle are well established (Marmer *et al.* 1984; Enser *et al.* 1998*b*). However, it is surprising that the amounts of 18 : 3*n*-3, 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3 in the muscle (mg/kg tissue) were virtually identical to those in the muscle of grass-finished lamb (Enser *et al.* 1998*b*), with a similar total content of intramuscular lipid. The 18 : 2*n*-6:18 : 3 ratio in grass is of the order 0·20–0·30, whereas in the Megalac diet it was 0·85. Although this difference did not affect the amounts of the major *n*-3 PUFA, lambs fed Megalac did have 0·25 more 18 : 2*n*-6 and 20 : 4*n*-3 in their muscles than the grass-fed lamb. These results indicate that the level of intake, 50 g lipid/kg DM in the present study compared to 10–20 g/kg DM in forage, as well as the 18 : 2*n*-6:18 : 3*n*-3 ratio are important in determining the relative proportions of longer-chain *n*-6 and *n*-3 fatty acids. The overall effect of the linseed diet was to increase the proportions of the C_{20} and C_{22} *n*-3 PUFA by just over one-quarter compared with the Megalac-fed lambs in both muscle and liver.

The failure of high levels of α-linolenic acid in the diet to increase tissue concentrations of 22 : 6*n*-3 is common to bovines, rats and human subjects, despite the fact that it is present in their tissues in the absence of a dietary supply (Sanders *et al.* 1989; Rustan *et al.* 1992; Scollan *et al.* 2001). The increases produced by feeding fish oils, as shown here and by others in sheep and beef cattle, demonstrate the capacity for their increased deposition when an increased amount is made available (Ashes *et al.*

1992; Scott *et al.* 1993; Mandell *et al.* 1997; Scollan *et al.* 2001). The extent of the increase in availability is not clear since evidence concerning the degree of biohydrogenation of the long-chain PUFA in fish oil is inconsistent. *In vitro* studies suggest biohydrogenation is minimal, but this appears to stem from a concentration-dependent inhibition of the process (Ashes *et al.* 1992; Palmquist & Kinsey, 1994; Gulati *et al.* 1999). Other studies based on pre- and post-rumen infusion of fish oils and incorporation of dietary fish oil fatty acids into tissues and milk indicate that extensive biohydrogenation occurs in cattle and sheep (Doreau & Chilliard, 1997; Chilliard *et al.* 2000; Wachira *et al.* 2000; Scollan *et al.* 2001). With similar diets to those used in the present study, we observed a disappearance of $>90\%$ 20 : 5*n*-3 and 22 : 6*n*-3 between feed and duodenum (Chikunya *et al.* 2004). If we accept that the extents of biohydrogenation of 20 : 5*n*-3 and 22 : 6*n*-3 are similar, there is a large preference for the incorporation of 20 : 5*n*-3 against 22 : 6*n*-3, since the linseed–fish oil feed contained 2·2 g 20 : 5*n*-3/kg and 2·4 g 22 : 6*n*-3/kg and the relative concentrations in muscle polar lipids were 7·7 and 1·8 g/100 g total fatty acids. Since 22 : 5*n*-3 was absent from the dietary fish oil, the increased proportions when fish oil was included in the diet most probably stem from chain-extension of 20 : 5*n*-3, although chain-shortening of 22 : 6*n*-3 cannot be ruled out.

The increased deposition of *n*-3 PUFA in the polar lipids of both muscle and liver when linseed or the linseed–fish oil were fed occurred at the expense of oleic acid and the *n*-6 PUFA 18 : 2*n*-6 and 20 : 4*n*-6. Since the concentration of 18 : 2*n*-6 was similar in all diets, effects on its tissue levels may be attributed to the changes in dietary *n*-3 PUFA. The 3-fold higher amount of 18 : 3*n*-3 in the linseed feed compared with Megalac decreased the proportions of 18 : 2*n*-6 much more in muscle than in liver polar lipids. As the proportionate increase in the deposition of 18 : 3*n*-3 was greater in liver than muscle this different response is unlikely to stem solely from direct competition between these two fatty acids for incorporation. A contributing factor may have been greater availability of 18 : 2*n*-6 in the liver since the proportion of 20 : 4*n*-6 was decreased more in liver than muscle. If this occurred through a decrease in synthesis then more 18 : 2*n*-6 would have been available for incorporation directly into lipids. That 18 : 2*n*-6 and 18 : 3*n*-3 compete as substrates for Δ^6-desaturase, the first step in the synthesis of 20 : 4*n*-6 from 18 : 2*n*-6, is well established (Brenner, 1989). EPA, a product of 18 : 3*n*-3 metabolism can also inhibit the desaturation of 18 : 2*n*-6 and hence its conversion to 20 : 4*n*-6. A decrease in the proportion of 20 : 4*n*-6 in response to feeding fish oils has been reported in other studies with ruminant animals (Mandell *et al.* 1997; Scollan *et al.* 2001), although Ashes *et al.* (1992) found no difference in sheep fed protected fish oil.

Changes in the saturated fatty acids of the polar lipids when feeding *n*-3 PUFA were tissue dependent. There were no significant diet effects on 12 : 0 in either muscle or liver, whereas 14 : 0 was higher in muscle and lower in the liver of lambs fed the linseed or linseed–fish oil compared with Megalac. The concentration of 16 : 0 was unchanged in muscle lipids, but was lower in liver lipid from lambs fed *n*-3 PUFA, but there was no difference between the linseed and linseed–fish oil. This differs from the findings of Ashes *et al.* (1992), who reported a decrease in 16 : 0 in animals fed 200 g protected fish oil/kg diet, but the level returned to the control value when 300 g protected fish oil/kg diet was fed. Mandell *et al.* (1997) reported an increase in 16 : 0 in beef muscle as a result of feeding fish meal (100 g fish oil/kg diet), as did Scollan *et al.* (2001) for both neutral and polar lipids of muscle when fish oil alone was fed but not with a mixture of linseed and fish oil. When they fed linseed, 16 : 0 was lower, but on a mixture of linseed and fish oil it was similar to that of the control Megalac diet. This result partially resembles the decreased proportion of 16 : 0 in the liver polar lipids of the linseed fed lambs here, but adding fish oil failed to reverse this effect. There is no consistency between the reported effects of fish oil on 18 : 0. Whereas we have found higher proportions in the polar lipids as a result of feeding linseed and linseed–fish oil, Ashes *et al.* (1992) reported a slight fall when fish oil was fed to sheep and in beef lower levels were observed from feeding linseed–fish oil or fish oil but not from feeding linseed alone (Scollan *et al.* 2001). These differences appear to stem from the effects of polar lipid fatty acid composition on the fatty acid combinations within the molecular species of different phospholipids. For example, an increase in 20 : 5*n*-3 in phosphatidylethanolamine would increase 18 : 0, whereas in phosphatidylcholine it would raise 16 : 0 since 18 : 0/20 : 5*n*-3 and 16 : 0/20 : 5*n*-3 are the major 20 : 5*n*-3-containing molecular species in sheep muscle (Scott *et al.* 1993).

Breed effects

The present trial demonstrates the ability of the Suffolk × Lleyn lambs to deposit higher proportions of 18 : 3*n*-3 in tissue lipids than Scottish Blackface lambs, results similar to our previous study in which the comparison was with Friesland lambs (Wachira *et al.* 2002). The effect was significant in all tissues and lipid fractions and was accentuated by feeding the linseed diet. The high amounts in the adipose tissue and liver and muscle neutral lipids indicate that supply exceeded metabolic requirements, but only in the liver neutral lipid fraction did this lead to an increase in 20 : 5*n*-3. On the linseed diet, the 20 : 5*n*-3:18 : 3*n*-3 ratio was higher in the Scottish Blackface lambs in liver and muscle polar lipids, indicating a more effective conversion or higher specificity for the incorporation of 20 : 5*n*-3 into the lipids. Differences between bovine breeds in the relationship between 18 : 3*n*-3 and 20 : 5*n*-3 have been reported. Compared with Limousin cows, Jersey cows deposited more 20 : 5*n*-3 in the polar lipids of m. longissimus, although the proportions of 18 : 3*n*-3 were the same (Malau-Aduli *et al.* 1998). This is closer to findings with the Scottish Blackface lambs than other studies in which higher proportions of 18 : 3*n*-3 are associated with higher levels of 20 : 5*n*-3 (Choi *et al.* 2000; Laborde *et al.* 2001). In fact the high 18 : 3*n*-3 in the Suffolk lambs failed to drive increased synthesis and deposition of 20 : 5*n*-3, unlike Welsh Black and Red Angus steers in the studies of Choi *et al.* (2000) and Laborde *et al.*

(2001) respectively. Although concentrations of 18 : 2*n*-6 and 20 : 4*n*-6 were lower in the muscle polar lipids of the Scottish Blackface lambs, there were no differences between the breeds in the proportions of these two fatty acids in the liver, suggesting that neither fatty acid affected the proportions of 20 : 5*n*-3 in competition for synthesis or incorporation into liver lipids. In the liver neutral lipids the proportion of all PUFA were lower in the Scottish Blackface lambs, possibly a reflection of the preferential use of these fatty acids for inclusion into polar lipids (35·5 g/kg liver in the Scottish Blackface compared with 25·4 g/kg liver in the Suffolk). Breed mean values for the liver concentration of 18 : 3*n*-3 in neutral lipids plus polar lipids were 2·92 g/kg for Suffolk and 2·90 g/kg for Scottish Blackface, confirming that the breed differences within lipid fractions stemmed mainly from differences in partition between unequal fractions. Breed effects on the saturated fatty acids of the subcutaneous adipose tissue and m. semimembranosus neutral lipids are contrary to expectations. The milk-derived fatty acids 10 : 0, 12 : 0 and 14 : 0 were lower in the Scottish Blackface despite this breed's lower fatness, which would have meant less dilution of these fatty acids by fatty acids synthesized *de novo*. This may be related to the difference in fat partition between the breeds, but in the absence of dissection results we are unable to assess total body fatness. The higher proportion of *cis*-monoenoic fatty acids in the adipose tissue of the Scottish Blackface lambs was also unexpected, since these are normally higher in fatter lambs with larger adipocytes containing more stearoyl-CoA desaturase (Barber *et al.* 2000). However, it is possible that the relationship differs between breeds as a result of differences in the cellularity of fat depots during growth. That the content of *cis*-monoenoic fatty acids in the livers of Blackface lambs followed the pattern for adipose tissue may occur because liver lacks the ability to synthesize these fatty acids and must import them from adipose tissue (St John *et al.* 1991). A similar result would have been expected for the saturated fatty acids 12 : 0 and 14 : 0, since the ruminant animal liver has a very low fatty acid synthetic ability (Hanson & Ballard, 1967; St John *et al.* 1991), but contrary to adipose tissue, these fatty acids were higher in the liver neutral lipids of Scottish Blackface lambs. Differences in the function of this lipid fraction between the tissues or differences in its component lipids may be the explanation.

Effects of vitamin E

The dietary levels of vitamin E in this trial were both in the supra-nutritional range; that is, they were much greater than the levels recommended as adequate for normal growth and reproduction (National Research Council, 1989; Agricultural Research Council, 1980). However, the higher intake did give additional protection against loss of PUFA by peroxidation as indicated by the higher proportion of PUFA and lower proportions of monoenoic fatty acids in muscle polar lipids, both lipid fractions in liver and in adipose tissue lipids. That such an effect occurred with these dietary levels of vitamin E was unexpected and probably resulted from the very low levels of muscle vitamin E observed (Table 8; 0·27 and 0·52 (SED 0·107) mg/kg respectively in lambs fed the low and high levels). The low deposition of vitamin E was unexpected since supplementation with 500 mg/kg feed has been reported to produce muscle concentrations of 5·5 mg/kg (Wulf *et al.* 1995). The poor deposition of vitamin E appears to be related to the dry pelleted feed, since it was less evident when grass silage was used as the basal diet (E Kasapidou, JD Wood, Sinclair LA, Wilkinson RG and Enser M, unpublished results).

Human nutrition

The PUFA:saturated fatty acid ratios of the muscle and adipose tissue of the Megalac-fed lambs were similar to those we have observed previously in grass-fed and commercial lamb samples (Enser *et al.* 1996, 1998*b*) and are low values compared with a minimum of 0·45 recommended for the whole diet (Department of Health, 1994). Whilst feeding formaldehyde-treated linseed improved the value in all tissues, it only approached 0·45 in the liver and the improvement in muscle was small. Although PUFA : saturated fatty acid ratios remained below the desirable level of 0·45, greater deposition of 18 : 3*n*-3 in adipose tissue would be undesirable because of its susceptibility to peroxidation, leading to the development of rancidity in the meat. The concentration of 18 : 3*n*-3 in the adipose tissue lipids at 5 g/100 g total fatty acids is in excess of the 3 g/100 g total fatty acids that is considered to be the maximum acceptable concentration to prevent rapid lipid peroxidation and colour loss in the meat (Sheard *et al.* 2000). The *n*-6 : *n*-3 fatty acid ratio of the tissues of Megalac-fed lambs were a little higher than those of lambs grazing grass (Enser *et al.* 1998*b*), but were well below the maximum recommended level of 4·0. Feeding the formaldehyde-protected linseed brought the ratio down to $<1{\cdot}0$, the dietary value thought to have been common during the evaluation of *Homo sapiens* (Weber *et al.* 1993).

In the Megalac-fed lambs, a 100 g serving of muscle would supply approximately 54 mg long-chain *n*-3 PUFA (20 : 5, 22 : 5 and 22 : 6) or 0·25 of the recommended daily human consumption, together with a similar amount of 18 : 3*n*-3. Feeding linseed or linseed–fish oil increased the long-chain *n*-3 PUFA to 0·33 and 0·48 of the daily recommendation respectively together with 116 and 76 mg 18 : 3*n*-3 respectively. The supply of *n*-3 PUFA from the supplemented lamb is potentially significant in human nutrition, since meat contributes approximately one-third of these fatty acids to the UK diet (Gregory *et al.* 1990).

Conclusion

The present trial has demonstrated that the *n*-3 PUFA content of lamb tissues can be significantly improved by feeding formaldehyde-treated whole linseed with or without fish oil. The concentrations achieved in liver can supply the recommended daily intake (Department of Health, 1994) in a single portion and the PUFA : saturated fatty acid ratio is acceptable. However, further increases are required in muscle and adipose tissue PUFA to meet this

objective. These will require improved systems to decrease rumen biohydrogenation and different combinations of dietary fatty acids. Suffolk × Lleyn lambs deposited more *n*-3 PUFA than Scottish Blackface lambs and this effect, although small, may offer scope for genetic improvement in meat fatty acid composition.

Acknowledgements

This investigation was funded by the Department for the Environment, Food and Rural Affairs, Roche Vitamins (UK) Ltd, the International Fish Oil and Meal Manufacturers Association and Trident Feeds. We are grateful to K. Hallett for technical assistance.

References

Agricultural Research Council (1980) *The Nutrient Requirements of Ruminant Livestock*. Farnham Royal, Surrey: Commonwealth Agricultural Bureaux.

Arnold RN, Arp SC, Scheller KK, Williams SW & Schaefer DM (1993) Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in cooked beef. *J Anim Sci* **71**, 105–118.

Ashes JR, Siebert BD, Gulati SK, Cuthbertson AZ & Scott TW (1992) Incorporation of *n*-3 fatty acids of fish oil into tissue and serum lipids of ruminants. *Lipids* **27**, 629–631.

Association of Official Analytical Chemists (1995) *Official Methods of Analysis*, 16th ed., Arlington, VA: AOAC.

Barber MC, Ward RJ, Richards SE, Salter AM, Buttery PJ, Vernon RG & Travers MT (2000) Ovine adipose tissue monounsaturated fat content is correlated to depot-specific expression of the stearoyl-CoA desaturase gene. *J Anim Sci* **78**, 62–68.

Brenner RR (1989) Factors influencing fatty acid chain elongation and desaturation. In *The Role of Fats in Human Nutrition*, pp. 46–49 [AJ Vergroesen and M Crawford, editors]. London: Academic Press.

Chikunya S, Demirel G, Enser M, Wood JD, Wilkinson RG & Sinclair LA (2004) Biohydrogenation of dietary *n*-3 PUFA and stability of ingested vitamin E in the rumen, and their effects on microbial activity in sheep. *Br J Nutr* **91**, 539–550.

Chilliard Y, Ferlay A, Mansbridge RM & Doreau M (2000) Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Ann Zootech* **49**, 181–205.

Choi NJ, Enser M, Wood JD & Scollan ND (2000) Effect of breed on the deposition in beef muscle and adipose tissue of dietary *n*-3 polyunsaturated fatty acids. *Anim Sci* **71**, 509–519.

Cook J, Scott TW, Faichney GJ & Lloyd Davies H (1972) Fatty acid interrelationships in plasma, liver, muscle and adipose tissues of cattle fed safflower oil protected from ruminal biohydrogenation. *Lipids* **7**, 83–89.

Cook LJ, Scott TW, Ferguson KA & McDonald IM (1970) Production of polyunsaturated ruminant body fats. *Nature* **228**, 178–179.

Czerkawski JW, Christie WW, Breckenridge G & Hunter MC (1975) Changes in the rumen metabolism of sheep given increasing amounts of linseed oil in their diet. *Br J Nutr* **34**, 25–44.

Department of Health (1994) *Nutritional Aspects of Cardiovascular Disease. Report on Health and Social Subjects* no. 46. London: H. M. Stationery Office.

Doreau M & Chilliard Y (1997) Digestion and metabolism of dietary fat in farm animals. *Br J Nutr* **78**, 57–64.

Enser M, Hallett KG, Hewitt B, Fursey GAJ & Wood JD (1996) Fatty acid content and composition of English beef, lamb and port at retail. *Meat Sci* **42**, 443–456.

Enser M, Hallett KG, Hewett B, Fursey GAJ, Wood JD & Harrington G (1998*a*) The polyunsaturated fatty acid composition of beef and lamb liver. *Meat Sci* **49**, 321–327.

Enser M, Hallett KG, Hewett B, Fursey GAJ, Wood JD & Harrington G (1998*b*) Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Sci* **49**, 329–341.

Enser M, Scollan ND, Choi NJ, Kurt E, Hallett K & Wood JD (1999) Effect of dietary lipid on the content of conjugated linoleic acid (CLA) in beef muscle. *Anim Sci* **69**, 143–146.

Fisher AV, Enser M, Richardson RI, Wood JD, Nute GR, Kurt E, Sinclair LA & Wilkinson RG (2000) Fatty acid composition and eating quality of lamb types derived from four diverse breed × production systems. *Meat Sci* **55**, 141–147.

Folch J, Lees M & Stanley GHS (1957) A simple method for the isolation and purification of lipides from animal tissues. *J Biol Chem* **226**, 497–509.

Garton GA, Hovell FD DeB & Duncan RW (1972) Influence of dietary volatile fatty acids on the fatty-acid composition of lamb triglycerides, with special reference to the effect of propionate on the presence of branched-chain components. *Br J Nutr* **28**, 409–416.

Gregory J, Foster K, Tyler H & Wiseman M (1990) *A Dietary and Nutritional Survey of British Adults. Office of Population, Censuses and Surveys, Social Survey Division*. London: H. M. Stationery Office.

Gulati SK, Ashes JR & Scott TW (1999) Hydrogenation of eicosapentaenoic and docosahexaenoic acids and their incorporation into milk fat. *Anim Feed Sci Technol* **79**, 57–64.

Hanson RW & Ballard F (1967) The relative significance of acetate and glucose as percursors for lipid synthesis in liver and adipose tissue of ruminants. *Biochem J* **105**, 529–536.

Laborde FL, Mandell IB, Tosh JJ, Wilton JW & Buchanan-Smith JG (2001) Breed effects on growth performance, carcass characteristics, fatty acid composition, and palatability attributes in finishing steers. *J Anim Sci* **79**, 355–365.

McClelland TJ & Russel AJF (1972) The distribution of body fat in Scottish Blackface and Finnish Landrace lambs. *Anim Prod* **15**, 301–306.

Malau-Aduli AEO, Siebert BD, Boltema CDK & Pitchford WS (1998) Breed comparison of the fatty acid composition of muscle phospholipids in Jersey and Limousin cattle. *J Anim Sci* **76**, 766–773.

Mandell IB, Buchanan-Smith JG, Holub BJ & Campbell PC (1997) Effect of fish meal in beef cattle diets on growth performance, carcass characteristics, and fatty acid composition of *longissimus* muscle. *J Anim Sci* **75**, 910–919.

Marmer WN, Maxwell RJ & Williams JE (1984) Effects of dietary regimen and tissue site on bovine fatty acid profiles. *J Anim Sci* **75**, 910–919.

National Research Council (1989) *Nutrient Requirements of Domestic Animals: The Nutrient Requirements of Dairy Cattle*, 6th ed., Washington, DC: National Academy of Sciences, National Research Council.

Palmquist DL & Kinsey DJ (1994) Lipolysis and biohydrogenation of fish oil by ruminal micro-organisms. *J Anim Sci* **72**, Suppl. 1, 350 Abstr.

Ruston AC, Christiansen EN & Drevon CA (1992) Serum lipids, hepatic glycerolipid metabolism and peroxisomal fatty acid oxidation in rats fed ω-3 and ω-6 fatty acids. *Biochem J* **283**, 333–339.

Sanders TAB, Hinds A & Pereira CC (1989) Influence of *n*-3 fatty acids on blood lipids in normal subjects. *J Int Med* **225**, 99–104.

Scaife JR, Wahle KWJ & Garton GA (1978) Utilisation of methylmalonate for the synthesis of branched-chain fatty acids by preparations of chicken liver and sheep adipose tissue. *Biochem J* **176**, 799–804.

Schaefer DM, Liu Q, Faustman C & Yin M-C (1995) Supranutritional administration of vitamins E and C improves oxidative stability of beef. *J Nutr* **125**, 1792S–1798S.

Scollan NG, Choi NJ, Kurt E, Fisher AV, Enser M & Wood JD (2001) Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *Br J Nutr* **85**, 115–124.

Scott TW, Ashes JR, Fleck E & Gulati SK (1993) Effect of fish oil supplementation on the composition of molecular species of choline and ethanolamine glycerophospholipids in ruminant tissues. *J Lipid Res* **34**, 827–835.

Scott TW, Cook LJ & Mills SC (1971) Protection of dietary polyunsaturated fatty acids against microbial hydrogenation in ruminants. *J Am Oil Chem Soc* **48**, 358–364.

Sheard PR, Enser M, Wood JD, Nute GR, Gill BP & Richardson RI (2000) Shelf life and quality of pork and pork products with raised *n*-3 PUFA. *Meat Sci* **55**, 213–221.

St John LC, Lunt DK & Smith SB (1991) Fatty acid elongation and saturation enzyme activities of bovine liver and subcutaneous adipose tissue microsomes. *J Anim Sci* **69**, 1064–1073.

Tweedie JW, Rumsby MG & Hawke JC (1966) Studies on rumen metabolism V. Formation of branched long chain fatty acids in cultures of rumen bacteria. *J Sci Food Agric* **17**, 241–244.

Van Soest PJ, Robertson JB & Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber and nonstarch polysacharides in relation to animal nutrition. *J Dairy Sci* **74**, 3583–3597.

Wachira AM, Sinclair LA, Wilkinson RG, Enser M, Wood JD & Fisher AV (2002) Effects of dietary fat source and breed on the carcass composition *n*-3 polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. *Br J Nutr* **88**, 697–709.

Wachira AM, Sinclair LA, Wilkinson RG, Hallett K, Enser M & Wood JD (2000) Rumen biohydrogenation of *n*-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. *J Agric Sci Camb* **135**, 419–428.

Weber PC, Sellmayer A & Hroboticky N (1993) Are we what we eat? Fatty acids and their diverse functions. In *Lifestyle Diseases and Human Diet: A Challenge to Future Food Production. Proceedings of the 44th Annual Meeting of the European Association for Animal Production*, pp. 19–27 Foulum: National Institute of Animal Science.

Wulf DM, Morgan JB, Sanders SK, Tatum JD, Smith GC & Williams S (1995) Effects of dietary supplementation of vitamin E on storage and case life properties of lambs retail cuts. *J Anim Sci* **73**, 399–405.