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Effects of including a ruminally protected lipid supplement in the diet on the fatty acid composition of beef muscle

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Enhancing the polyunsaturated fatty acid (PUFA) and decreasing the saturated fatty acid content of beef is an important target in terms of improving the nutritional value of this food for the consumer. The present study examined the effects of feeding a ruminally protected lipid supplement (PLS) rich in PUFA on the fatty acid composition of *longissimus thoracis* muscle and associated subcutaneous adipose tissue. Animals were fed *ad libitum* on grass silage plus one of three concentrate treatments in which the lipid source was either Megalac (rich in palmitic acid; 16 : 0) or PLS (soyabean, linseed and sunflower-seed oils resulting in an 18 : 2*n*-6:18 : 3*n*-3 value of 2·4:1). Treatment 1 contained 100 g Megalac/kg (Mega, control); treatment 2 (PLS1) contained 54 g Megalac/kg with 500 g PLS/d fed separately; treatment 3 (PLS2) contained no Megalac and 1000 g PLS/d fed separately. The PLS was considered as part of the overall concentrate allocation per d in maintaining an overall forage:concentrate value of 60:40 on a DM basis. Total dietary fat was formulated to be 0·07 of DM of which 0·04 was the test oil. Total intramuscular fatty acids (mg/100 g muscle) were decreased by 0·31 when feeding PLS2 compared with Mega ($P<0·05$). In neutral lipid, the PLS increased the proportion of 18 : 2*n*-6 and 18 : 3*n*-3 by 2·7 and 4·1 on diets PLS1 and PLS2 *v*. Mega, respectively. Similar responses were noted for these fatty acids in phospholipid. The amounts or proportions of 20 : 4*n*-6, 20 : 5*n*-3 or 22 : 6*n*-3 were not influenced by diet whereas the amounts and proportions of 22 : 4*n*-6 and 22 : 5*n*-3 in phospholipid were decreased with inclusion of the PLS. The amounts of the saturated fatty acids, 14 : 0, 16 : 0 and 18 : 0, in neutral lipid were on average 0·37 lower on treatment PLS2 compared with Mega. Feeding the PLS also decreased the proportion of 16 : 0 in neutral lipid. The amount of 18 : 1*n*-9 ($P=0·1$) and the amount and proportion of 18 : 1 *trans* ($P<0·01$) were lower on treatments PLS1 and PLS2 in neutral lipid and phospholipid. Conjugated linoleic acid (*cis*-9, *trans*-11) was not influenced by diet in the major storage fraction for this fatty acid, neutral lipid. The PUFA:saturated fatty acids value was increased markedly (×2·5) with inclusion of the PLS ($P<0·001$) while the Σn-6 : *n*-3 value increased slightly (×1·2; $P=0·015$). The results suggest that the protected lipid used, which was rich in PUFA, had a high degree of protection from the hydrogenating action of rumen micro-organisms. The PLS resulted in meat with a lower content of total fat, decreased saturated fatty acids and much higher 18 : 2*n*-6 and 18 : 3*n*-3. The net result was a large shift in polyunsaturated: saturated fatty acids, 0·28 *v*. 0·08, on feeding PLS2 compared with Mega, respectively.

Beef: Rumen: Fatty acids: Healthy eating

The last 30 years have been characterized by an increase in consumer interest in the nutritional aspects of health, which has resulted in the development of specific health messages by governments for some food components (Simopoulos, 2001), including fats. The relationships between dietary fat and incidence of lifestyle diseases, particularly CHD, are well established and this has contributed towards advice that the contribution of fat and saturated fatty acids (SFA) to dietary energy intake should not exceed 0·35 and 0·10 of total intake, respectively, polyunsaturated fatty acids (PUFA):SFA (P:S) should be around 0·45 and the *n*-6 : *n*-3 PUFA value should be less than 4 (Department of Health, 1994). The last seeks to address the large increase in *n*-6 at the expense of *n*-3 PUFA, which has occurred from the Palaeolithic period to the present. Currently, the *n*-6:*n*-3 PUFA value is between 15:1 and 20:1 in Western Europe and the USA, whereas during our evolution it was 1:1 or less (Simopoulos, 2001). Meat and meat products are an important part of today's diet and make an important contribution to nutritional intakes (British Nutrition Foundation, 1999). Lean beef has an intramuscular fat content of around 5 % or less with approximately 47, 42 and 4 % of total fatty acids as SFA, monounsaturated fatty acids and PUFA, respectively (Moloney *et al*. 2001). Of the total SFA, 30 % are represented by stearic acid (18 : 0), which is considered to be neutral in its effect on plasma

Abbreviations: CLA, conjugated linoleic acid; Mega, Megalac control treatment; PLS, protected lipid supplement; P:S, polyunsaturated fatty acids:saturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

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cholesterol (Willett *et al*. 1993). P:S for beef is typically low at around 0·1, except for double-muscled animals, which are very lean (< 1 % intramuscular fat) where P:S is typically 0·5–0·7 (Raes *et al.* 2001). The *n*-6:*n*-3 value for beef is beneficially low, typically less than 3 (Choi *et al*. 2000; Scollan *et al*. 2001*a*).

Increasing the dietary supply of *n*-3 PUFA is one of the most important strategies to increase the PUFA composition of beef. Hence, feeding grass or linseed as a rich source of α-linolenic acid (18 : 3*n*-3) or fish oil or meal as a source of the long-chain C_{20} PUFA eicosapentaenoic acid (20 : 5*n*-3) and docosahexaenoic acid (22 : 6*n*-3) increases the levels of these PUFA in the meat (Choi *et al*. 2000; Moloney *et al*. 2001; Scollan *et al.* 2001*a*). Feeding linseed resulted in not only an increase in 18 : 3*n*-3 but also 20 : 5*n*-3 by promoting *de novo* synthesis from 18 : 3*n*-3 in the tissues. These studies have been successful in manipulating *n*-6:*n*-3 in beef but have had little impact on P:S. The latter reflects the high level of biohydrogenation of dietary PUFA by rumen micro-organisms, resulting in low amounts of dietary PUFA relative to SFA bypassing the rumen (Demeyer & Doreau, 1999). Effective ruminal protection of dietary fatty acids, such as that provided by encapsulation of PUFA in formaldehyde-treated protein, may ameliorate this situation (Scott & Ashes, 1993). This product resists proteolysis in the rumen and thereby protects the polyunsaturated oil droplets against microbial hydrogenation. In the acidic secretions of the abomasum, however, the formaldehyde–protein complex is hydrolysed, thus making the PUFA available for digestion and absorption in the small intestine.

The objective of the present study was to investigate the effects of feeding a ruminally protected lipid supplement (PLS) in the diet of Charolais steers on animal performance, carcass characteristics and the fatty acid composition of *longissimus thoracis* muscle. The target was to increase P:S and maintain *n*-6:*n*-3 (typically 0·06–0·1 and 2–3, respectively). To help achieve this aim, the supplement was formulated to attain an 18 : 2*n*-6:18 : 3*n*-3 value of 2:1, based on the achievements of Scollan *et al*. (2001*a*) in beneficially decreasing *n*-6:*n*-3 when feeding linseed compared with a saturated fat source, Megalac.

Materials and methods

Experimental design and treatments

Twenty-four Charolais steers with an initial mean live weight of 528 (SE 6·3) kg were randomly allocated to one of three dietary treatments, each consisting of eight animals. The three diets consisted of *ad libitum* grass silage plus one of three concentrate treatments in which the lipid source was either Megalac (high in palmitic acid, 16 : 0 from palm oil; Volac Ltd, Royston, Herts., UK) or a ruminally PLS. Treatment 1 contained Megalac (Mega, experimental control); treatment 2 (PLS1) contained Megalac and 500 g PLS/d (fresh weight) fed separately and treatment 3 (PLS2) contained a concentrate containing no Megalac and 1000 g PLS/d (fresh weight) fed separately. The PLS was not included in the concentrate to avoid any potential negative effect that the manufacturing process may have had on protection mechanism. The PLS was considered as part of the overall concentrate allocation per d in maintaining an overall forage:concentrate value of 60:40 on a DM basis. To ensure that total dietary fat and protein intakes were balanced across the three treatment groups, the three concentrates differed in formulation and hence chemical composition (Table 1). The diets were formulated so that the total dietary fat content was approximately 70 g/kg DM (40 g/kg DM of which was the test oil) and protein content was approximately 150 g/kg DM. Vitamin E was added to the concentrates at a level of (350 mg/kg). The PLS comprised soyabean (0·70), linseed (0·22) and sunflower-seed oils (0·08), mixed to achieve an 18 : 2*n*-6:18 : 3*n*-3 value of 2:1. The PLS was protected from ruminal biohydrogenation by encapsulating the lipids in a matrix of rumen-inert protein using the procedures outlined by Scott & Ashes (1993). The degree of ruminal protection of the PLS was 0·75, as measured *in vitro* using the methods described by Gulati *et al*. (1997).

Procedures and measurements

Animals were housed in a well-ventilated barn and penned in groups of five and bedded on wood shavings. The animals were individually fed via Hoko feeders (Insentec, Marknesse, The Netherlands) and had free access to fresh water. At the beginning of the experiment, during a 21 d covariate period, all animals were fed on grass silage *ad libitum* and a standard commercial beef concentrate to

Table 1. Formulation (g/kg fresh weight) and the chemical composition (g/kg dry matter) of the silage and concentrates used in the experimental diets

		Concentrates		
	Silage	Mega	PLS1	PLS2
Barley		504	575	651
Molassed sugarbeet pulp		191	221	251
Molasses		56	65	76
Soyabean meal		127	63	0
Megalac		100	54	0
Mineral and vitamin premix*		22	22	22
Oven-dried matter (g/kg fresh matter)	NA	889	888	868
Freeze-dried matter (g/kg fresh matter)	360	NA	NA	NA
Organic matter (g/kg)	914	909	917	924
pH	4·2	NA	NA	NA
Crude protein (6·25 × N)	172	147	129	109
NH_3-N	2·4	NA	NA	NA
Neutral-detergent fibre	530	186	189	205
Acid-detergent fibre	319	67	70	78
Water-soluble carbohydrates	8	10	10	11
Acetic acid	15	NA	NA	NA
Propionic acid	0·5	NA	NA	NA
Butyric acid	0·9	NA	NA	NA
Valeric acid	0·04	NA	NA	NA
Lactic acid	67	NA	NA	NA
Diethyl-ether extract	37	NA	NA	NA
Acid hydrolysis diethyl ether extract	NA	108	65	19
Gross energy (MJ/kg DM)	21	NA	NA	NA

Mega, Megalac; PLS, protected lipid supplement; NA, not applicable.
*Roche Vitamins (UK) Ltd, Heanor, Derbyshire, UK.

provide 0·4 of DM intake. At the end of the covariate period the animals were weighed on three consecutive days and were then introduced to their allocated dietary treatment over a 7 d period and maintained on treatment for on average a further 83 d. During the main experimental period, daily feed intake and live weights at 30 d intervals (on two consecutive days) were recorded. All weighings were conducted at the same time of day (13.30 hours) to minimize variations due to diurnal patterns of feed intake.

Grass silage was fed at 09.30 hours daily and the concentrates in one portion at 15.00 hours. The PLS was mixed in with the daily concentrate allocation. The grass silage and the concentrate were sampled three times per week for DM analysis in a forced-air oven at 105°C. Silage was offered at 0·1 in excess of the previous week's daily consumption. Concentrate feed levels were adjusted each week based on the previous week's silage consumption. Samples of the silage, concentrate and the PLS were accumulated over a 3-week period and chemical composition assessed as described by Choi *et al*. (2000).

Eight animals were slaughtered each week over a 3-week period. Each week the two heaviest animals in each group were selected along with two further animals selected on weight from two of the treatment groups (Mega, PLS1 or PLS2). Animals were moved from the Institute of Grassland and Environmental Research to Bristol University on the day before slaughter. Assessment of cold carcass weights (excluding kidney knob and channel fat), carcass fatness and conformation scores was carried out as described by Kempster *et al*. (1986). Briefly, fatness was scored on a scale of 1–15 from 1, being extremely lean (practically devoid of fat cover), to 15 meaning extremely fat (substantial fat cover over entire surface). Conformation was also scored on a fifteen-point scale where 1 was extremely thinly fleshed (concave carcass profiles) to 15, being extremely thickly fleshed (convex carcass profiles). The same trained operative assessed the carcasses on each occasion.

The carcasses were held in chill for 48 h before butchering and sampling. Complete cross-sections of the *longissimus thoracis* muscle at the 10–12th rib level and subcutaneous adipose tissue over the same site were taken as described by Scollan *et al*. (2001*a*). The fatty acid composition of muscle neutral lipids and phospholipids and adipose tissue was assessed as described by Scollan *et al*. (2001*a*).

Fatty acid composition results are given as proportion times 100 and muscle fatty acid content as mg fatty acid/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 present study are reported, representing around 0·9 of the total fatty acids present. As mentioned by Scollan *et al*. (2001*a*), 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 were incompletely resolved by the column used and are reported as one value.

Statistical analysis

Live-weight gain (g/d) for each animal was estimated from regression of live weight on time for the period from the introduction of experimental diets until slaughter. Average daily feed intakes of silage and concentrates were calculated for the experimental period. Visual scores for carcass fatness and conformation using the European Union system were converted to numerical values as described by Kempster *et al*. (1986). All data were subjected to general one-way ANOVA with diet as the main factor (Genstat 5; Lawes Agricultural Trust, 2000).

Results

Diet composition

The fatty acid composition of the experimental diets and the PLS are given in Tables 2 and 3, respectively. Total lipid fatty acids in the silage consisted of 0·54 α-linolenic acid. The dominant fatty acids in the experimental concentrates were 16 : 0, 18 : 1*n*-9 and 18 : 2*n*-6, derived from the cereals and Megalac. The PLS was rich in protein and oil and the measured 18 : 2*n*-6:18 : 3*n*-3 value was 2·4:1.

Animal performance and carcass composition

The animal performance and carcass composition data are presented in Table 4. Diet did not influence DM intake,

Table 2. Fatty acid composition of the components of the experimental diets (g/kg dry matter)

		Concentrates		
	Silage	Mega	PLS1	PLS2
12 : 0 (Lauric acid)	0·2	0·3	0·1	0·4
14 : 0 (Myristic acid)	0·1	0·9	0·5	0·2
16 : 0 (Palmitic acid)	2·4	37·1	20·6	6·4
18 : 0 (Stearic acid)	0·2	3·7	1·9	0·5
18 : 1 *trans*	0·03	0·6	0·4	0·2
18 : 1*n*-9 (Oleic acid)	0·4	22·2	11·7	3·1
18 : 2*n*-6 (Linoleic acid)	2·1	6·1	4·7	4·0
18 : 3*n*-3 (α-Linolenic acid)	6·9	0·4	0·4	0·3
20 : 0	0·1	0·3	0·2	0·1
Total fatty acids	12·7	71·5	40·4	15·1

Mega, Megalac; PLS, protected lipid supplement.

Table 3. Chemical composition (g/kg dry matter) of the protected lipid supplement

	Protected lipid supplement
Oven-dried matter (g/kg fresh weight)	920
Organic matter	950
Crude protein (6·25 × N)	299
Neutral-detergent fibre	210
Acid-detergent fibre	137
Acid hydrolysis diethyl ether extract	366
12 : 0 (Lauric acid)	ND
14 : 0 (Myristic acid)	0·2
16 : 0 (Palmitic acid)	22·6
18 : 0 (Stearic acid)	12·0
18 : 1 *trans*	0·2
18 : 1*n*-9 (Oleic acid)	65·8
18 : 2*n*-6 (Linoleic acid)	115·7
18 : 3*n*-3 (α-Linolenic acid)	48·5
20 : 0	0·9
Total fatty acids	266·0

ND, not determined.

Table 4. Effect of diet on animal performance and carcass composition*

(Residual degrees of freedom 21)

	Treatment				
	Mega	PLS1	PLS2	SED	*P*
Intake (kg/d)					
Total DM	10·06	9·96	9·62	0·338	NS
Forage DM	6·07	6·05	5·79	0·244	NS
Concentrate DM	3·98	3·45	2·91	0·120	0·001
Lipid supplement DM	0	0·46	0·92	NA	NA
Live-weight gain (kg/d)	1·43	1·37	1·39	0·100	NS
Carcass weight (kg)	359	358	360	8·51	NS
Conformation (1–15 scale)	8·9	9·6	9·9	9·12	NS
Fat class (1–15 scale)	8·8	8·8	7·4	7·30	NS

Mega, Megalac; PLS, protected lipid supplement; NA, not applicable.
* For details of diets and procedures, see Tables 1 to 3 and p. 710.

growth rate, carcass weight or conformation. Carcass fat score tended to be lower on the PLS2 treatment compared with Mega ($P=0·08$).

Fatty acid composition

The fatty acid compositions of the neutral lipid and phospholipid (expressed as both mg/100 g muscle and as proportion × 100) are given in Tables 5 and 6, respectively. Intramuscular fatty acid content was significantly lower on the PLS2 diet compared with Mega and across all three diets was 3607, 3389 and 2604 mg/100 g tissue for Mega, PLS1 and PLS2, respectively. The decrease was related to lower amounts of neutral lipid.

Neutral lipids

The major fatty acids in the neutral lipids were 18 : 1*n*-9, 16 : 0 and 18 : 0, which together accounted for 0·79 of total neutral fatty acids. The amounts of the SFA, 14 : 0, 16 : 0 and 18 : 0, were on average 0·37 lower on the PLS2 treatment compared with feeding Mega. Feeding the PLS decreased the proportion of 16 : 0. Similarly, the amount of oleic acid, 18 : 1*n*-9, was much decreased ($P=0·1$) in animals fed the PLS diets and the amount ($P=0·003$) and proportion ($P=0·005$) of 18 : 1 *trans* were lower on both levels of PLS. On average, the PLS increased the amount of 18 : 2*n*-6 and 18 : 3*n*-3 (× 2·6); the increases were not different between PLS1 and PLS2. The proportions of 18 : 2*n*-6 and 18 : 3*n*-3 were increased on PLS1 and PLS2 treatments compared with Mega (× 2·7 and 4·1, respectively). Neither the amount nor proportion of conjugated linoleic acid (CLA; *cis*-9, *trans*-11) was influenced by diet.

Phospholipids

The concentration of phospholipid fatty acids was not affected by diet and averaged across treatments was 489 mg/100 g muscle. The major fatty acids noted in neutral lipid, 18 : 1*n*-9, 16 : 0 and 18 : 0, were also important in phospholipid but at much lower proportions, whereas the PUFA, particularly 18 : 2*n*-6, appeared at much higher proportions. The effect of diet on most of the fatty acids was similar to that noted in neutral lipid. The amount ($P=0·021$) and proportion ($P=0·006$) of 16 : 0 decreased with increasing PLS in the diet and in contrast the proportion of 18 : 0 increased ($P<0·001$). The amount

Table 5. Effect of diet on the fatty acid composition in neutral lipid of the *longissimus thoracis* muscle*

(Residual degrees of freedom 21)

	Treatment				
	Mega	PLS1	PLS2	SED	*P*
Muscle fatty acid content (mg/100 g muscle)					
14 : 0 (Myristic acid)	106·4	86·2	68·0	16·58	0·092
16 : 0 (Palmitic acid)	926	802	573	121·4	0·026
16 : 1 *cis*	130·5	119·1	84·6	21·17	NS
18 : 0 (Stearic acid)	467	383	299	62·4	0·046
18 : 1 *trans*	69·8	51·7	37·1	8·33	0·003
CLA (*cis*-9, *trans*-11)	15·9	14·5	10·1	2·79	NS
18 : 1*n*-9 (Oleic acid)	1121	1135	767	185·1	0·100
18 : 1*n*-7	29·7	31·7	21·5	5·24	NS
18 : 2*n*-6 (Linoleic acid)	33·9	81·4	87·3	8·71	0·001
18 : 3*n*-3 (α-Linolenic acid)	13·0	32·9	34·8	3·79	0·001
Total fatty acids	3101	2916	2116	451	0·091
Proportion of total neutral lipid fatty acids (×100)					
14 : 0 (Myristic acid)	3·45	2·93	3·15	0·314	NS
16 : 0 (Palmitic acid)	30·18	27·56	26·9	0·868	0·003
16 : 1 *cis*	4·21	4·01	3·90	0·279	NS
18 : 0 (Stearic acid)	14·95	13·29	14·41	0·663	0·059
18 : 1 *trans*	2·28	1·80	1·80	0·147	0·005
CLA (*cis*-9, *trans*-11)	0·51	0·51	0·49	0·064	NS
18 : 1*n*-9 (Oleic acid)	35·92	38·63	35·96	1·088	0·032
18 : 1*n*-7	0·95	1·07	1·01	0·047	0·072
18 : 2*n*-6 (Linoleic acid)	1·12	2·93	4·38	0·348	0·001
18 : 3*n*-3 (α-Linolenic acid)	0·41	1·18	1·74	0·128	0·001

Mega, megalac; PLS, protected lipid supplement; CLA, conjugated linoleic acid.
* For details of diets and procedures, see Tables 1 to 3 and p. 710.

Table 6. Effect of diet on the fatty acid composition in phospholipid of the *longissimus thoracis* muscle*
(Residual degrees of freedom 21)

	Treatment				
	Mega	PLS1	PLS2	SED	*P*
Muscle fatty acid content (mg/100 g muscle)					
14 : 0 (Myristic acid)	1·91	2·04	1·61	0·335	NS
16 : 0 (Palmitic acid)	81·2	73·3	69·0	4·09	0·021
16 : 1 *cis*	9·60	5·00	4·26	0·688	0·001
18 : 0 (Stearic acid)	51·00	50·50	53·00	1·99	NS
18 : 1 *trans*	2·85	1·42	1·89	0·306	0·001
CLA (*cis*-9, *trans*-11)	0·88	0·58	0·57	0·106	0·012
18 : 1*n*-9 (Oleic acid)	103·6	50·1	36·3	7·08	0·001
18 : 1*n*-7	9·83	8·16	7·56	0·552	0·001
18 : 2*n*-6 (Linoleic acid)	76·3	132·9	156·1	8·01	0·001
18 : 3*n*-3 (α-Linolenic acid)	12·67	16·05	15·56	1·725	NS
20 : 3*n*-6	9·64	9·32	8·21	0·614	0·072
20 : 4*n*-6 (Arachidonic acid)	31·25	29·24	31·58	2·257	NS
20 : 5*n*-3 (Eicosapentaenoic acid)	11·16	9·71	9·78	1·146	NS
22 : 4*n*-6	2·62	1·84	1·70	0·292	0·010
22 : 5*n*-3 (Docosapentaenoic acid)	18·59	15·30	14·39	0·816	0·001
22 : 6*n*-3 (Docosahexaenoic acid)	2·52	1·91	2·04	0·338	NS
Total fatty acids	506	473	488	18·8	NS
Proportion of total phospholipid lipid fatty acids (×100)					
14 : 0 (Myristic acid)	0·38	0·43	0·33	0·066	NS
16 : 0 (Palmitic acid)	16·07	15·49	14·12	0·552	0·006
16 : 1 *cis*	1·89	1·06	0·87	0·120	0·001
18 : 0 (Stearic acid)	10·08	10·69	10·88	0·175	0·001
18 : 1 *trans*	0·56	0·30	0·39	0·061	0·001
CLA (*cis*-9, *trans*-11)	0·17	0·12	0·12	0·019	0·010
18 : 1*n*-9 (Oleic acid)	20·42	10·62	7·40	1·271	0·001
18 : 1*n*-7	1·95	1·73	1·55	0·097	0·002
18 : 2*n*-6 (Linoleic acid)	15·12	28·04	32·10	1·294	0·001
18 : 3*n*-3 (α-Linolenic acid)	2·49	3·39	3·20	0·282	0·010
20 : 3*n*-6	1·91	1·98	1·69	0·128	0·082
20 : 4*n*-6 (Arachidonic acid)	6·22	6·22	6·47	0·492	NS
20 : 5*n*-3 (Eicosapentaenoic acid)	2·20	2·05	2·01	0·205	NS
22 : 4*n*-6	0·52	0·39	0·35	0·063	0·036
22 : 5*n*-3 (Docosapentaenoic acid)	3·68	3·25	2·95	0·16	0·001
22 : 6*n*-3 (Docosahexaenoic acid)	0·50	0·40	0·42	0·073	NS

Mega, Megalac; PLS, protected lipid supplement; CLA, conjugated linoleic acid.
* For details of diets and procedures, see Tables 1 to 3 and p. 710.

and proportion of 18 : 1*n*-9 ($P<0·001$), 18 : 1 *trans* ($P<0·001$) and *cis*-9, *trans*-11 CLA ($P<0·01$) were decreased with increasing level of PLS. On average, the PLS increased ($P<0·001$) the amount and proportion of 18 : 2*n*-6 by 0·37 and 1·04 and 0·85 and 1·12 for PLS1 and PLS2 compared with Mega, respectively. Similarly, the PLS increased the amount and proportion of 18 : 3*n*-3 on average by 0·25 and 0·32, respectively; the increases were not different between PLS1 and PLS2. The amounts or proportions of 20 : 4*n*-6, 20 : 5*n*-3 (eicosapentaenoic acid) or 22 : 6*n*-3 (docosahexaenoic acid) were not influenced by diet whereas the amounts and proportions of 22 : 4*n*-6 and 22 : 5*n*-3 (docosapentaenoic acid) were decreased with inclusion of the PLS.

Subcutaneous adipose tissue

The effects of dietary lipid on the fatty acid composition of the subcutaneous adipose tissue (proportions × 100) and on total fatty acid content of adipose tissue (mg/100 g tissue) are shown in Table 7. The content of lipid in adipose tissue was similar across treatments. As in muscle, the major fatty acids were 18 : 1*n*-9, 16 : 0 and 18 : 0, which together contributed 0·76 of the total fatty acids in animals fed on Mega. Diets containing the PLS resulted in decreases in 16 : 0 ($P<0·001$), 18 : 0 ($P<0·05$), 18 : 1 *trans* ($P=0·002$) and 18 : 1*n*-9 ($P<0·05$). The proportions of 18 : 2*n*-6 ($P<0·001$) and 18 : 3*n*-3 ($P<0·001$) were increased by 1·9 and 3·4 and 2·3 and 4·0 on PLS1 and PLS2 compared with Mega, respectively.

Nutritional indices

Fatty acid values related to human health and 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 values were significantly increased ($P<0·001$) by approximately 1·4 and 2·6 on treatments PLS1 and PLS2 compared with Mega, respectively. The *n*-6:*n*-3 value when calculated as 18 : 2*n*-6:18 : 3*n*-3 was not influenced by diet but when calculated as Σn-6:*n*-3 was higher on diets PLS1 and PLS2 compared with Mega ($P=0·015$). The trends for these values in subcutaneous adipose tissue were similar to muscle.

Table 7. Effect of diet on fatty acid composition in subcutaneous adipose tissue (proportion × 100)*

(Residual degrees of freedom 21)

	Treatment				
	Mega	PLS1	PLS2	SED	*P*
14 : 0 (Myristic acid)	4·12	3·70	4·24	0·344	NS
15 : 0	1·98	2·39	2·39	0·273	NS
16 : 0 (Palmitic acid)	28·94	26·45	25·98	0·733	0·001
18 : 0 (Stearic acid)	11·86	9·73	11·15	0·822	0·050
18 : 1 *trans*	2·77	2·09	2·07	0·190	0·002
CLA (*cis*-9, *trans*-11)	0·72	0·82	0·71	0·084	NS
18 : 1*n*-9 (Oleic acid)	35·04	36·47	33·89	0·980	0·049
18 : 1*n*-7	1·11	1·25	1·07	0·096	NS
18 : 2*n*-6 (Linoleic acid)	1·11	3·20	4·85	0·484	0·001
18 : 3*n*-3 (α-Linolenic acid)	0·39	1·29	1·95	0·187	0·001
Total (g/100 g tissue)	89·5	88·6	88·7	1·78	NS

Mega, Megalac; PLS, protected lipid supplement; CLA, conjugated linoleic acid.

* For details of diets and procedures, see Tables 1 to 3 and p. 710.

Discussion

Incorporating the PLS in the diet did not influence DM intake and since the diets were of similar nutrient content no differences in live-weight gain were expected. Earlier studies with protected lipids reported reductions in voluntary intake and live-weight gain (for example, see Scott & Ashes, 1993). However, these authors concluded that when cattle were fed less than 15 % of the supplement (less than 6 % fat in the diet), no effects on animal performance were noted. Interestingly, carcasses of the PLS2 treatment had lower fat scores than either Mega or PLS1 (8·8 *v.* 7·4; $P = 0{\cdot}08$), which is further discussed later (p. 714).

A most notable effect of feeding the PLS was a large decrease in intramuscular concentration of neutral lipid. Phospholipid concentrations were unaffected. Since the supplement did not influence voluntary intake (Table 4), it is probable that this is related to the action of dietary PUFA in repartitioning of fatty acids away from triacylglycerol synthesis and toward oxidation (Clarke, 2000). This was first noted by Allmann & Gibson (1969), who observed that the induction of hepatic lipogenesis associated with carbohydrate feeding could be inhibited by inclusion of 18 : 2*n*-6 in the diet while the addition of 16 : 0 had no effect. Since then, numerous studies have demonstrated that the ingestion of fats rich in *n*-6, and particularly *n*-3 PUFA, influence fuel metabolism (suppress hepatic lipogenesis, decrease hepatic triacylglycerol output, enhance ketogenesis and induce fatty acid oxidation) accompanied by a decrease in body fat deposition (Clark, 2000). In ruminants, supplementing lambs with protected lipid depressed lipogenesis in adipose tissue both *in vivo* and *in vitro* and the effect was particularly large for supplements rich in 18 : 2*n*-6 compared with 18 : 0 (Hood *et al.* 1980).

Previous studies have demonstrated that linseed as a rich source of 18 : 3*n*-3, or fish oil or meal as a source of long-chain C_{20} PUFA (eicosapentaenoic acid and docosahexaenoic acid) increase the levels of these PUFA in the meat (Mandell *et al.* 1997; Choi *et al.* 2000, Moloney *et al.* 2001; Scollan *et al.* 2001*a*). However, the efficiency of transfer of dietary PUFA from diet through to product is not high (typically less than 5 %) which relates to the high degree of biohydrogenation of the PUFA in the rumen. However, infusing *n*-3 PUFA directly into the small intestine (and hence bypassing the rumen) as linseed oil significantly increased the percentage of 18 : 3*n*-3 in milk compared with feeding an equivalent amount of 18 : 3*n*-3 in the diet (13·9 *v.* 1·0 % in milk, respectively; Petit *et al.* 2002). Feeding the PLS (treatment PLS2 compared with Mega) resulted in significant increases in both 18 : 2*n*-6 and 18 : 3*n*-3 deposition in muscle lipids (× 3·9 and 2·9 for 18 : 2*n*-6 and 18 : 3*n*-3 in muscle neutral lipid, respectively. The proportions of these fatty acids in muscle were much higher than levels achieved in previous studies using unprotected lipids (Clinquart *et al.* 1991; Choi *et al.* 2000; Scollan *et al.* 2001*a*; NJ Choi, ND Scollan, JD Wood and M Enser, unpublished results). The total dietary intake of 18 : 2*n*-6 and 18 : 3*n*-3 on diet PLS2 was approximately 130 (of which 0·82 was from the lipid supplement) and 84·4 g/d (0·52 from the lipid supplement) and respective proportions (× 100) in muscle neutral lipid were 4·4 and 1·72. This compares with similar animals fed a concentrate-rich diet containing either full-fat soya or lightly crushed linseed consuming approximately 385 g 18 : 2*n*-6 and 403 g 18 : 3*n*-3/d resulting in proportions (× 100) in muscle neutral lipid of 1·72 and 0·91, respectively (NJ Choi, ND Scollan, JD Wood and M Enser, unpublished results). Similarly, inclusion of a protected linseed-oil product in the diet of dairy cows (providing an intake of 446 g 18 : 3*n*-3/d) resulted in 8·3 % 18 : 3*n*-3 in milk fat compared with 0·64 % in the control (Gulati *et al.* 2002). Similar results for milk were attained by Goodridge *et al.* (2001).

In neutral lipid, the proportions of 18 : 2*n*-6 and 18 : 3*n*-3 increased in a linear manner with increasing inclusion of the PLS. However, in phospholipid the incremental response between PLS1 and PLS2 was much less than

Table 8. Effect of diet on the values of fatty acids related to human nutrition*

(Residual degrees of freedom 21)

	Treatment				
	Mega	PLS1	PLS2	SED	*P*
Muscle					
P:S†	0·077	0·187	0·270	0·0276	0·001
P:S‡	0·110	0·270	0·405	0·0460	0·001
n-6:*n*-3§	4·64	4·39	4·74	0·483	NS
Σ *n*-6:*n*-3‖	2·75	3·25	3·59	0·263	0·015
Adipose tissue					
P:S†	0·032	0·107	0·157	0·0163	0·001
P:S‡	0·042	0·140	0·214	0·0243	0·001
n-6:*n*-3‡§	2·92	2·49	2·49	0·1718	0·029

Mega, Megalac; PLS, protected lipid supplement; P:S, polyunsaturated fatty acids:saturated fatty acids.

* For details of diets and procedures, see Tables 1 to 3 and p. 710.

† Calculated as (18 : 2*n*-6+18 : 3*n*-3):(12 : 0+14 : 0+16 : 0+18 : 0).

‡ Calculated as (18 : 2*n*-6+18 : 3*n*-3):(14 : 0+16 : 0+18 : 1 *trans*).

§ Calculated as (18 : 2*n*-6):(18 : 3*n*-3).

‖ Calculated as (18 : 2*n*-6+20 : 3*n*-6+20 : 4*n*-6+22 : 6*n*-6):(18 : 3*n*-3+20 : 4*n*-3+20 : 5*n*-3+22 : 5*n*-3+22 : 6*n*-3).

between PLS1 and Mega for 18 : 2*n*-6. In phospholipid there was no difference in the proportion of 18 : 3*n*-3 between PLS1 and PLS2 suggesting deposition had reached a maximum in this lipid fraction but more probably is related to the well-recognized preferential deposition of 18 : 2*n*-6 compared with 18 : 3*n*-3 in phospholipid (Ratnayake *et al*. 1989). In the neutral lipid and phospholipid fractions, the increases in PUFA (18 : 2*n*-6 and 18 : 3*n*-3) were primarily associated with decreases in 16 : 0 and 18 : 1*n*-9, respectively. Since phospholipids constitute a relatively constant component of tissues, the decrease in other fatty acids is to be expected since increased deposition of PUFA can only occur through displacement of another fatty acid. Hence feeding more *n*-3 PUFA by feeding linseed was associated with a decrease in 18 : 1*n*-9, 20 : 3*n*-6 and 20 : 4*n*-6 (Scollan *et al*. 2001*a*). However, in the same study, increasing long-chain C_{20} PUFA by feeding fish oil was more effective in decreasing levels of these fatty acids than 18 : 3*n*-3 provided from linseed.

Inclusion of the PLS had little effect on the amounts or proportions of long-chain C_{20} PUFA except for decreases in 22 : 4*n*-6 and 22 : 5*n*-3, particularly on diet PLS2. In our previous studies, feeding 18 : 3*n*-3 was associated with increases in not only 18 : 3*n*-3 in muscle but also 20 : 5*n*-3 in muscle phospholipid, reflecting an increased availability of the precursor of the *n*-3 series, 18 : 3*n*-3 (Choi *et al*. 2000; Scollan *et al*. 2001*a*). Similarly, feeding soya rich in 18 : 2*n*-6 increases the synthesis of the longer-chain C_{20} *n*-6 series (NJ Choi, ND Scollan, JD Wood and M Enser, unpublished results). In the present study, the lack of response in either longer-chain C_{20} *n*-6 or *n*-3 series to increased levels of their C_{18} precursors may be associated with the deposition of the precursor in phospholipid membranes rather than in chain elongation.

The proportion of 14 : 0 was not affected by treatment but the proportion of 16 : 0 in neutral lipid and phospholipid (and 16 : 1 in phospholipid) was decreased on inclusion of the PLS. Reductions in 16 : 0 were also noted by Scollan *et al*. (2001*a*) when feeding unprotected linseed and in milk fat when feeding dairy cows a PLS (Goodridge *et al*. 2001; Gulati *et al*. 2002). The proportion of 18 : 0 tended to be lower in neutral lipid ($P=0{\cdot}059$) and higher in phospholipid but the changes were relatively small compared with the effects of feeding unprotected lipids, in particular fish oil (Scollan *et al*. 2001*a*) or fishmeal (Mills *et al*. 1992; Mandell *et al*. 1997). When feeding unprotected lipids, the decreases in 18 : 0 are usually associated with increases in 18 : 1 *trans*. The increase in 18 : 1 *trans* is a reflection of incomplete biohydrogenation in the rumen as a result of an inhibition by PUFA of the conversion from 18 : 1 *trans* to 18 : 0 (Demeyer & Doreau, 1999; Scollan *et al*. 2001*b*). In the present study, 18 : 1 *trans* was decreased on diets PLS1 and PLS2 relative to the control, suggesting a decrease in ruminal biohydrogenation as the PLS was highly resistant to biohydrogenation as measured *in vitro*. The proportion of 18 : 1 *trans* averaged across PLS1 and PLS2 in neutral lipid was 1·8 % compared with 3·8 and 4·4 % in the muscle neutral lipid of similar animals fed a concentrate containing unprotected linseed and fish oil, respectively (Scollan *et al*. 2001*a*). CLA (*cis*-9, *trans*-11) was mostly associated with neutral lipid (approximately 0·95 of total muscle CLA) and this fraction was not affected by dietary treatment. In comparison, Enser *et al*. (1999) noted that CLA was increased in the muscle of animals fed unprotected linseed and fish oil and that there was a strong positive relationship between total intramuscular 18 : 1 *trans* and CLA. Originally it was thought that the *cis*-9, *trans*-11 CLA in ruminant products, meat or milk was derived from biohydrogenation in the rumen but it is now recognized that conversion of 18 : 1 *trans* primarily to CLA by Δ^9-desaturase is more important (Bauman *et al*. 1999).

The fatty acid composition of the subcutaneous adipose tissue was similar to that of the intramuscular neutral lipids and the overall fatty acid composition is similar to that reported by Scollan *et al.* (2001*a*). The notable responses were reduction in 16 : 0, 18 : 0 and 18 : 1 *trans* and increase in 18 : 2*n*-6 and 18 : 3*n*-3 with inclusion of the PLS.

The Committee on the Medical Aspects of Food's report on the nutritional aspects of cardiovascular disease (Department of Health, 1994) recommended that P:S should remain at 0·45 for the human diet as a whole. P:S observed in the present study for total muscle fatty acids (Table 8) showed a major increase from 0·08 to 0·28 on the Mega and the PLS2 treatments, respectively. Enser *et al*. (1996) reported that typical retail beef in the UK has a value of 0·11 while in studies feeding animals with unprotected lipid sources (linseed, fish oil, fishmeal) values ranged from 0·045 to 0·13 (Mandell *et al*. 1997; Choi *et al*. 2000; Scollan *et al*. 2001*a*). Evidence now exists of a strong negative exponential relationship between the amount of intramuscular fat and P:S. Very lean animals such as the double-muscled Belgian Blue have P:S values of 0·5–0·6 (Raes *et al.* 2001) and intramuscular fat less than 1000 mg/100 g muscle. In contrast, with intramuscular fat ranging between 2000 and 4000 mg/100 g muscle, P:S values are typically 0·05–1·1 (Choi *et al*. 2000; Scollan *et al*. 2001*a*). Hence in the present study the increased P:S is the result of two factors : (1) increased deposition of 18 : 2*n*-6 and 18 : 3*n*-3 in intramuscular lipid; (2) a decrease in total intramuscular fat.

The Σn-6:*n*-3 was increased on diets PLS1 and PLS2 compared with Mega, averaging 3·4 and 2·7, respectively. Choi *et al*. (2000) and Scollan *et al*. (2001*a*) reported values around 1·0–1·2 *v*. 2·0–3·1 in animals fed unprotected *n*-3 rich dietary lipids (linseed and fish oil). The higher values in the present study reflect the greater propensity to deposit dietary 18 : 2*n*-6 compared with 18 : 3*n*-3 in intramuscular lipid. Ideally it is preferable to achieve values of less than 4 in line with the Committee on the Medical Aspects of Food recommendations (Department of Health, 1994).

Based on the results presented in Tables 5 and 6 and assuming an average consumption of 100 g beef/d (Enser *et al*. 1996), then it is possible to calculate the contribution of beef produced in this experiment to the consumption of the differing fatty acids. Consumption of 100 g beef muscle/d from animals fed on Mega,

PLS1 and PLS2 would provide 3607, 3389 and 2604 mg total fatty acids/d, of which 1634, 1397 and 1064 mg/d would be SFA (14 : 0, 16 : 0 and 18 : 0), respectively. Intake of *n*-3 PUFA would be 58·0, 75·9 and 76·6 mg/d, including 13·7, 11·6 and 11·8 g/d as 20 : 5*n*-3 and 22 : 6*n*-3. 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). Similarly, intake of *n*-6 PUFA would be 154, 255 and 285 g/d, across the diets, respectively.

The results suggest that the protected lipid used, which was rich in PUFA, had a high degree of protection from the hydrogenating action of rumen micro-organisms. The PLS resulted in meat with a lower content of total fat, decreased SFA and much higher 18 : 2*n*-6 and 18 : 3*n*-3. The net result was a large shift in P:S value, 0·28 *v.* 0·08 on feeding PLS2 compared with Mega, respectively, resulting in beef that is healthier by having a higher content of PUFA and lower saturated fat. Further work is required to increase P:S towards the target level of 0·4 and maintain *n*-6:*n*-3 below 4.

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