British Journal of Nutrition (2007), **97**, 502–513
DOI: 10.1017/S0007114507381361

The fatty acid composition of muscle and adipose tissue of steers offered unwilted or wilted grass silage supplemented with sunflower oil and fishoil

F. Noci[1,2], F. J. Monahan[2], N. D. Scollan[3] and A. P. Moloney[1]*

[1]*Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland*
[2]*School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin 4, Ireland*
[3]*Institute of Grassland and Environmental Research, Plas Goggerdan, Aberystwyth SY23 3EB, UK*

(Received 20 February 2006 – Revised 9 October 2006 – Accepted 23 October 2006)

The effects of the type of grass silage and dietary inclusion of fish oil (FO) on the fatty acid profile of bovine intramuscular and subcutaneous adipose tissue were investigated. Eighty Friesian steers were assigned (*n* 10) to unwilted or wilted silage, and to one of four rations which contained, per kg, 80 g of sunflower oil and either 0, 10, 20 or 40 g of FO replacing lard. Animals were slaughtered after 108 d and the fatty acid profile of the neutral, polar and total lipid fractions of the *M. longissimus dorsi*, and the total lipid fraction of the subcutaneous adipose tissue were determined. Wilting of grass prior to ensiling increased the concentration of conjugated linoleic acid (CLA) in intramuscular total lipid ($P<0{\cdot}01$), but did not affect the *n*-6:*n*-3 PUFA ratio. Increasing FO supply linearly increased ($P<0{\cdot}05$) the concentration of the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers of CLA and *trans*-11 18 : 1 predominantly in the neutral lipid fraction of intramuscular total lipid, and linearly decreased the *n*-6:*n*-3 PUFA ratio. Wilting of grass prior to ensiling increased the concentration of CLA in subcutaneous adipose tissue ($P<0{\cdot}001$), while increasing FO supply linearly increased the concentration of *cis*-9,*trans*-11 CLA. From a human nutrition perspective, increasing the level of FO in the ration or wilting of grass prior to ensiling appear to modify the fatty acid composition of beef muscle favourably. However, the health implications of associated increases in *trans* fatty acids remain to be elucidated.

Conjugated linoleic acid: Fish oil: Wilted silage: PUFA

In humans, fat of ruminant origin represents a high proportion of the dietary intake of saturated fatty acids (SFA) (Demeyer & Doreau, 1999), which have been implicated in the onset of CVD (Department of Health, 1994). However, ruminant fats also contain higher concentrations of conjugated linoleic acid (CLA) than other food fat sources and are the main sources of CLA in the human diet (Chin *et al.* 1992). CLA refers to a mixture of positional and geometric isomers of linoleic acid. The *cis*-9,*trans*-11 isomer is the most common natural isomer with biological activity, representing 75–90 % of total CLA in meat depending on the diet of the animal, but biological activity has been proposed for other isomers, in particular *trans*-10,*cis*-12 (Pariza *et al.* 2001).

The anticancer effect of CLA was initially discovered in animal models using lipid extracted from beef (Ha *et al.* 1987) and an anticancer effect of beef CLA has been confirmed *in vitro* by De la Torre *et al.* (2006). A range of other positive isomer-specific effects on human health has been proposed, including a reduction in atherosclerosis, decreased inflammation and improved cardiovascular health (e.g. Pariza *et al.* 2001). Consequently, there is considerable interest in increasing the concentration of CLA in ruminant fat.

CLA is produced following incomplete ruminal biohydrogenation of dietary 18 : 2 (Kepler & Tove, 1967) and by tissue desaturation of *trans*-11 18 : 1, another product of incomplete ruminal biohydrogenation of dietary fatty acids (Griinari *et al.* 2000). An increase in the concentration of CLA in ruminant muscle fat has been achieved primarily by dietary addition of plant oils or seeds rich in PUFA (Enser *et al.* 1999; Mir *et al.* 2002; Raes *et al.* 2004; Noci *et al.* 2005*b*). Thus, supplementation with sunflower oil (SFO), a rich source of 18 : 2, has been shown to increase the concentration of CLA and *trans*-11, 18 : 1 by providing substrate for ruminal biohydrogenation (Mir *et al.* 2002; Noci *et al.* 2005*b*). Inclusion of fish oil (FO) has been shown to increase the concentration of CLA in milk (reviewed by Chilliard *et al.* 2001; Offer *et al.* 2001; Shingfield *et al.* 2003), and a combination of a source of 18 : 2 and FO increased the concentration of CLA in milk still further (AbuGhazaleh *et al.* 2002, 2003). The first hypothesis tested in this study was that bovine muscle and subcutaneous adipose tissue concentrations of CLA and long-chain *n*-3 PUFA would respond in a dose-dependent manner to supplementation of diets with FO and that FO could be used to optimise CLA accretion in ruminants fed a PUFA-rich ration.

Grazed and conserved grass are the major sources of *n*-3 PUFA, particularly 18 : 3, in ruminant diets. This makes fat from ruminants fed grass-based diets an important source

Abbreviations: CLA, conjugated linoleic acid; FO, fish oil; GC, gas chromatography; NL, neutral lipid; PL, polar lipid; SFA, saturated fatty acids; SFO, sunflower oil.

* **Corresponding author:** Dr A. P. Moloney, fax +353 469026154, email aidan.moloney@teagasc.ie

of *n*-3 PUFA for humans. On a laboratory scale, wilting grass prior to ensiling decreased the content of total fatty acids and 18 : 3*n*-3 in silage (Dewhurst & King, 1998). While wilting grass prior to ensiling is environmentally beneficial, it may result in animals fed wilted silage having lower concentrations of *n*-3 PUFA in tissue than animals fed unwilted silage. The second hypothesis tested was that the effects of the wilting process are reflected in the fatty acid composition of muscle and subcutaneous adipose tissue of cattle consuming the resulting silage.

Materials and methods

Experimental design and animal management

Eighty Friesian steers (mean initial body weight 565 kg, SD 37·24) were blocked on initial body weight and assigned to one of eight dietary treatments (*n* 10/treatment) in a randomised complete block (initial body weight) design. Animals were assigned at random to pens in a slatted-floor shed that accommodated five or six animals/pen and were fed individually through Calan gates. The initial silage allowances were 15 kg of wilted silage and 30 kg of unwilted silage, and were increased, as the experiment progressed, to 20 and 35 kg, respectively. The silages were offered once daily, and refusals (which were rare) were recorded daily. The silages were prepared from a sward of predominantly perennial ryegrass in mid May. Grass for unwilted silage was ensiled directly with 3 litres of Addsafer (Interchem Ltd., Dublin, Ireland) (48 % formic acid and 16 % ammonium tetraformate)/tonne grass. The remaining grass was wilted for an average of 32 h (maximum temperature 20·2°C, minimum temperature 5·7°C, relative humidity 68·1 %), turned once and then ensiled without an additive. Concentrate rations were formulated to have 120 g fat/kg, which included 80 g of SFO/kg and increasing levels of FO (0, 10, 20 or 40 g/kg concentrate) derived from a mix of mackerel and herring oil (Fish Industries, Killybegs, Co. Donegal, Ireland), balanced with decreasing amounts of lard (Table 1). Animals were offered one of the four concentrate rations, at an initial daily allowance of 4·5 kg/head, which increased with increasing body weight to average 5 kg/head over the duration of the 108 d experiment. The concentrates were offered daily in a separate container, in two equal meals, and no refusals were observed. On the day of slaughter, animals were weighed without fasting and transported 120 km to a commercial facility (Meadow Meats, Rathdowney, Co. Laois, Ireland) within 3 h from Grange Research Centre and slaughtered within 60 min of arrival.

Table 1. Ingredients and formulation of the concentrate rations

	Fish oil inclusion (g/kg)			
Ingredients (g/kg)	0	10	20	40
Barley	345	345	345	345
Sugarbeet pulp	360	360	360	360
Soyabean meal	140	140	140	140
Molasses	10	10	10	10
Mineral/vitamins*	25	25	25	25
Oil				
Sunflower oil	80	80	80	80
Lard	40	30	20	0
Fish oil	0	10	20	40

* The mineral and vitamin mix contained Ca (28·5 %), P (1·6 %), Na (5·6 %), vitamin A (150 mg/kg), vitamin D_3 (3·1 mg/kg), vitamin E (16,7-50), cobalt carbonate (42 mg/kg), cupric sulphate (500 mg/kg), calcium iodate (910 mg/kg), iron sulphate (1000 mg/kg), manganese sulphate (5800 mg/kg), sodium selenite (16 mg/kg) and zinc sulphate (7500 mg/kg) on an as-fed basis.

Post-slaughter measurement and sampling procedure

Carcass and perirenal fat weights were recorded immediately after slaughter. The carcasses were hung from the Achilles tendon and chilled in the abattoir for 24 h. The *M. longissimus dorsi* and associated muscles and adipose tissue were excised from the right side of the carcass and transported to the Teagasc National Food Centre (Castleknock, Dublin, Ireland) where they were held at 4°C for a further 24 h. The procedures for tissue sampling, lipid extraction and fatty acid analysis of the neutral lipid (NL) and polar lipid (PL) fractions of intramuscular fat and total lipid (TL) fraction of subcutaneous adipose tissue were as described by Noci *et al.* (2005*a*).

In brief, extracted lipid was separated into NL and PL fractions using solid-phase extraction cartridges with 500 mg of aminopropyl packing (Bone-Elut 500 mg, 3 ml reservoir; Varian Instruments, Palo Alto, CA). The separated lipid classes were dried, weighed, dissolved in toluene and initially methylated with $NaOCH_3$, which was followed with a 4 % solution of HCl in methanol. Both methylation procedures were carried out at 50°C for 20 min, and tricosanoic acid (C23 : 0) methyl ester was used as an internal standard for fatty acid quantification.

The fatty acid methylesters were separated by gas chromatography (GC) using a Varian 3800 gas chromatograph (Varian Instruments) equipped with a CP-Sil 88 capillary column (100 m × 0·25 mm i.d., 0·2 μm film thickness; Chrompack, The Netherlands) and a Varian 8400 autosampler. The injector and the flame ionization detector were kept at constant temperatures of 250 and 260°C, respectively. The column oven temperature was held at 40°C for 2 min, increased at 20°C/min to 80°C and held for 2 min, increased to 160°C at 20°C/min, to 220°C at 4°C/min, and to 240°C at 2°C/min and held for 8 min. The total run time was 43 min, and the carrier gas used was H_2. For peak identification, a standard mix of 37 fatty acid methylesters (Supelco Inc., Bellefonte, PA) was used, and individual standards from Matreya (Matreya Inc., Pleasant Gap, PA) were used for identification of those fatty acid methylesters not contained in the mix.

Feed chemical analysis

The dry matter content of the concentrates and silages was determined as described by Moloney *et al.* (1996) and Porter & Murray (2001), respectively. Concentrates were analysed for crude protein concentration as described by Association of Analytical Chemists (1990), for ash concentration as described by Moloney *et al.* (1996) and for oil Procedure B (extraction after acid hydrolysis) as described in European Communities (1984). The fatty acid composition of lipid sources and feedstuffs was determined as described by Sukhija & Palmquist (1988). The fatty acid methylesters were

dissolved in toluene and analysed by GC following the GC conditions described above.

Statistical analysis

Data were subjected to the analysis of variance procedures of Genstat (Release 3.2, Lawes Agricultural Trust, Rothamstead Experimental Station, UK). The model used had block, type of silage, level of FO inclusion and the interaction between the main effects as sources of variation. Linear and quadratic effects of increasing levels of inclusion of FO were partitioned using orthogonal polynomials. The concentration of fatty acids in intramuscular TL was calculated from the concentrations of fatty acids in PL and NL.

Results

Feed composition

The fatty acid compositions of the dietary fat sources are summarised in Table 2 and the chemical compositions of the silages and concentrates are summarised in Table 3. Wilted silage had a lower total fat and fatty acid content than unwilted silage mainly as a result of the lower 18 : 3*n*-3 and total PUFA content. The concentrates had similar total oil, crude protein and ash contents. The concentrate containing 40 g FO/kg had the lowest SFA and MUFA contents and the highest *n*-3 PUFA and total PUFA contents.

Animal production

Concentrate intake was similar for all treatments, but consumption of wilted silage was higher than of unwilted silage, resulting in a higher total dry matter intake for the wilted silage-based rations (Table 4). Neither the type of silage nor the level of inclusion of FO in the diet affected average daily gain or pre-slaughter weight of the steers. However, the interaction between the two main effects was significant for carcass weight. Thus, when unwilted silage was offered, steers receiving 0 or 10 g FO/kg concentrate had the highest carcass weight, which decreased for steers fed 20 g FO/kg concentrate, but increased again for those receiving 40 g FO/kg concentrate. With wilted silage, however, steers fed 0 or 10 g FO/kg concentrate had the lowest carcass weight, which increased when animals were fed the concentrates containing 20 and 40 g FO/kg.

Total intramuscular lipids

Feeding wilted instead of unwilted silage led to an increase in the concentration of *trans*-9 18 : 1, *trans*-11 18 : 1 ($P=0{\cdot}07$), *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA and to a decrease in the *n*-6:*n*-3 PUFA ratio ($P=0{\cdot}06$) (Table 5).

Increasing the level of inclusion of FO in the concentrates led to a significant linear increase in the concentration of *trans*-9 18 : 1 (quadratic term also significant), *trans*-11 18 : 1, *trans*-10,*cis*-12 CLA, *cis*-9,*trans*-11 CLA, 20 : 0, 20 : 1, 20 : 2*n*-6, 20 : 5*n*-3, 22 : 0, 22 : 1, 22 : 2*n*-6 and 22 : 6*n*-3, and to a linear decrease in the *n*-6:*n*-3 PUFA ratio (Table 5).

There was an interaction between the effects of type of silage and increasing levels of inclusion of FO on the total concentration of intramuscular fatty acids. As the level of inclusion of FO increased, there was an increasing quadratic response in muscle from steers offered unwilted silage, but a linear decrease in muscle from those offered wilted silage. A similar interaction was found for the concentration of 12 : 0, 16 : 0, 17 : 0, 17 : 1, 18 : 0, *cis*-9 18 : 1 and 18 : 3*n*-3, total SFA and MUFA.

Table 2. Fatty acid composition of oil supplements
(Mean and standard deviation)

	Lard (*n* 6)		Fish oil (*n* 8)		Sunflower oil (*n* 8)	
Fatty acid composition (g/100 g fatty acids)	Mean	SD	Mean	SD	Mean	SD
14 : 0	1·17	0·06	4·19	0·64	0·06	0·00
16 : 0	21·11	0·38	10·55	0·68	5·34	0·11
18 : 0	14·30	0·47	2·26	0·15	3·80	0·24
18 : 1	37·72	0·90	12·70	0·53	26·16	0·24
18 : 2*n*-6	16·80	0·12	1·87	0·09	61·49	0·40
18 : 3*n*-3	1·56	0·30	1·57	0·06	0·16	0·02
20 : 4	0·26	0·03	0·64	0·05	0·03	0·00
20 : 1	0·70	0·62	10·09	0·48	0·20	0·00
20 : 2*n*-6	0·69	0·02	3·28	0·19	0·02	0·00
20 : 5*n*-3	0·03	0·03	6·95	0·45	0·01	0·01
22 : 1	0·04	0·01	18·96	2·10	0·01	0·01
22 : 2*n*-6	0·03	0·01	0·98	0·07	0·00	0·00
22 : 5*n*-3	0·16	0·06	1·45	0·11	0·00	0·00
22 : 6*n*-3	0·07	0·03	11·76	0·60	0·00	0·00
SFA	37·53	0·20	18·02	1·42	11·34	0·44
MUFA	40·65	0·49	46·40	1·48	26·48	0·22
PUFA	20·22	0·28	29·05	1·07	61·76	0·41
Total *n*-6 PUFA†	18·11	0·17	7·06	0·19	61·54	0·39
Total *n*-3 PUFA‡	1·81	0·36	21·95	1·07	0·19	0·03

SFA, saturated fatty acids.
† Total *n*-6 PUFA = sum of 18 : 2, 20 : 4 and 22 : 2.
‡ Total *n*-3 PUFA = sum of 18 : 3*n*-3, 20 : 5, 22 : 5 and 22 : 6.

Table 3. Chemical and fatty acid composition of grass silages and concentrate rations
(Mean and standard deviation)

	Unwilted silage (*n* 8)		Wilted silage (*n* 8)		Fish oil g/kg							
					0 (*n* 8)		10 (*n* 8)		20 (*n* 8)		40 (*n* 8)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM (g/kg)	211	8·42	423	30·77	891	5·60	892	3·37	893	2·69	894	1·65
Crude protein (g/kg DM)	184	10·57	184	7·11	156	6·32	160	11·0	154	6·63	161	10·28
Ash (g/kg DM)	88·5	5·62	96·6	3·80	65	3·52	67	2·20	72	4·08	72	4·53
DM digestibility (g/kg)	753	13·81	784	11·18	–	–	–	–	–	–	–	–
Oil (AH)* (g/kg DM)	40·5	2·66	32·6	1·41	131	9·33	128	10·91	128	7·63	133	9·46
Fatty acid composition (mg fatty acid/g DM)	(*n* 8)		(*n* 8)		(*n* 12)		(*n* 12)		(*n* 12)		(*n* 12)	
16 : 0	3·27	0·22	3·09	0·34	13·20	2·10	12·75	1·56	11·41	2·96	10·24	1·88
18 : 0	0·32	0·03	0·31	0·07	7·17	1·21	6·51	0·86	5·61	1·05	3·88	0·75
18 : 1	0·70	0·05	0·62	0·13	31·74	5·77	30·87	4·88	29·85	6·00	25·25	5·16
18 : 2*n*-6	3·49	0·17	3·42	0·33	48·93	10·62	55·90	7·93	56·66	10·82	51·24	10·41
18 : 3*n*-3	13·86	0·71	11·39	0·86	2·02	0·67	1·88	0·86	1·61	0·29	1·70	0·23
20 : 1	0·04	0·02	0·01	0·01	0·22	0·22	1·04	0·45	2·28	0·66	3·35	1·47
20 : 4*n*-6	–	–	–	–	0·09	0·01	0·11	0·03	0·17	0·07	0·20	0·08
20 : 5*n*-3	–	–	–	–	0·06	0·03	0·55	0·18	1·27	0·41	2·00	0·93
22 : 5*n*-3	–	–	–	–	0·05	0·01	0·15	0·02	0·26	0·08	0·41	0·10
22 : 6*n*-3	–	–	–	–	0·03	0·02	0·87	0·18	1·87	0·60	3·24	0·92
SFA	4·57	0·40	4·20	0·43	22·05	3·52	21·29	2·58	19·31	4·26	16·89	2·28
MUFA	1·30	0·06	1·46	0·62	32·40	5·51	33·54	4·64	34·59	6·88	31·36	4·55
PUFA	17·53	0·84	14·96	1·13	56·39	10·57	61·04	8·65	65·50	13·01	64·52	9·23
Total *n*-6 PUFA†	3·68	0·18	3·57	0·33	54·55	10·53	57·94	8·24	60·49	11·77	57·55	7·58
Total *n*-3 PUFA‡	13·86	0·71	11·39	0·86	1·83	0·39	3·11	0·48	5·01	1·31	6·97	1·94
Total fatty acids	26·30	1·32	24·12	1·41	114·0	19·35	118·9	15·81	121·0	24·12	116·0	2·60

DM, dry matter; SFA, saturated fatty acids.
*Ether extract after acid hydrolysis.
† Total *n*-6 PUFA = sum of 18 : 2 and 20 : 4 (18 : 3*n*-6, 20 : 2, 20 : 3*n*-6 and 22 : 2, present in trace amounts).
‡ Total *n*-3 PUFA = sum of 18 : 3*n*-3, 2 ;5, 22 : 5 and 22 : 6.

Table 4. Daily feed intake and animal performance

Silage (S)	Unwilted				Wilted				SED	S	FO	S × FO
Fish oil (FO; g/kg)	0	10	20	40	0	10	20	40				
Concentrate intake (kg DM)	4·46	4·47	4·47	4·48	4·46	4·47	4·47	4·48	–	–	–	–
Silage intake (kg DM)	6·22	6·33	6·13	6·26	6·64	6·58	6·51	6·63	0·106	***	NS	NS
Total intake (kg DM)	10·68	10·80	10·60	10·73	11·10	11·05	10·98	11·11	0·106	***	NS	NS
Average daily gain (g)	997	1060	820	996	866	908	1080	1029	120·0	NS	NS	NS
Pre-slaughter weight (kg)	679	682	653	674	659	659	676	678	13·89	NS	NS	NS
Carcass weight (kg)	345	344	331	338	333	329	341	344	7·34	NS	NS	*
Kill out (kg carcass/kg liveweight)	0·508	0·505	0·508	0·501	0·505	0·498	0·504	0·507	0·069	NS	NS	NS

DM, dry matter.
* and *** refer to significance levels $P<0.05$ and $P<0.001$, respectively.

Intramuscular neutral lipids

Feeding wilted instead of unwilted silage lead to an increase in the proportion of *trans*-9 18 : 1, *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA and MUFA, and to a decrease in the proportions of 18: 0 ($P=0{\cdot}07$), SFA ($P=0{\cdot}05$) and *n*-6 PUFA ($P=0{\cdot}06$) (Table 6).

Increasing the level of inclusion of FO in the concentrates led to a significant linear increase in the proportion of *trans*-11 18 : 1, *cis*-9,*trans*-11 CLA, 18 : 3*n*-6, 20 : 0, 20 : 1, 22 : 0, 22 : 1 and 22 : 2*n*-6, and to a linear decrease in the proportion of *cis*-9 18 : 1, 18 : 2*n*-6, 18 : 3*n*-3 (quadratic term also significant), 20 : 3*n*-6, MUFA ($P=0{\cdot}07$) and *n*-6 PUFA and in the *n*-6:*n*-3 PUFA ratio (Table 6).

There was an interaction between the effects of type of silage and increasing level of FO inclusion on the proportion of 20 : 4*n*-6 which decreased following a quadratic trend as the level of FO increased when unwilted silage was fed, but was unchanged when wilted silage was offered.

Intramuscular polar lipids

Feeding wilted instead of unwilted silage led to a decrease in the proportion of 20 : 4*n*-6 in muscle PL (Table 7). Increasing the level of inclusion of FO in the concentrates led to a significant linear increase in the proportion of 17 : 1, 20 : 1, 20 : 5*n*-3, 22 : 2*n*-6, 22 : 6*n*-3 and *n*-3 PUFA and to a linear decrease in the proportion of 20 : 4*n*-6, SFA (quadratic) and *n*-6 PUFA and the *n*-6:*n*-3 PUFA ratio (Table 7).

Total subcutaneous adipose tissue lipids

Feeding wilted instead of unwilted silage led to an increase in the concentration of *cis*-9,*trans*-11 CLA and total PUFA and in the PUFA:SFA (P:S) ratio, and to a decrease in the concentration of 18 : 0 ($P=0{\cdot}07$) (Table 8).

Increasing the level of inclusion of FO in the concentrates led to a significant linear increase in the concentration of *cis*-9 18: 1 ($P=0{\cdot}06$), *cis*-9, *trans*-11 CLA, 20 : 1, 20 : 0 ($P=0{\cdot}07$), 20 : 5*n*-3 ($P=0{\cdot}06$) and 22 : 6*n*-3, and a linear decrease in the concentration of 18 : 2*n*-6, 18 : 3 *n*-3 and total *n*-6 PUFA.

Discussion

The myriad putative health benefits of CLA, and in particular the *cis*-9,*trans*-11 isomer, have stimulated interest in increasing its concentration in beef. Since CLA is formed either directly or indirectly during ruminal biohydrogenation of dietary lipids, the most common strategy examined has been to increase/modify the supply of lipids for cattle. Recent studies have indicated an influence of dietary long-chain PUFA on pathways of biohydrogenation in the rumen, either by direct action of the PUFA or via the action of intermediate products of PUFA biohydrogenation (Scollan *et al.* 2001*b*; Shingfield *et al.* 2003). Specifically, these studies showed that dietary long-chain PUFA increased *trans*-11 18 : 1 as a product of rumen biohydrogenation of dietary PUFA. An increase in the outflow of *trans*-11 18 : 1 from the rumen may then induce an increase in the concentration of CLA in tissues, via the action of Δ^9-desaturase (Griinari *et al.* 2000).

Table 5. Fatty acid concentration in the total lipid fraction of intramuscular fat from *M. longissimus dorsi*

Silage (S)	Unwilted				Wilted							
Fish oil (FO g/kg)	0	10	20	40	0	10	20	40	SED	S	FO	S × FO
Fatty acids (mg/100 g 5muscle)												
10 : 0	7·30	7·08	6·04	5·75	6·37	6·76	7·22	6·60	1·131	NS	NS	NS
11 : 0	0·01	0·00	0·02	0·03	0·00	0·01	0·00	0·02	0·015	NS	NS	NS
12 : 0	2·30	1·38	2·03	3·18	2·42	2·85	1·96	1·84	0·579	NS	NS	*L
13 : 0	0·10	0·07	0·16	0·26	0·10	0·20	0·07	0·10	0·108	NS	NS	NS
14 : 0	347·1	276·1	321·7	368·1	340·3	350·3	381·0	323·8	51·14	NS	NS	NS
14 : 1	16·15	10·90	14·63	21·02	16·21	27·17	17·76	11·11	6·397	NS	NS	*
15 : 0	34·72	24·63	31·61	43·28	35·66	37·23	35·29	32·32	5·239	NS	NS	*L
15 : 1	0·08	0·03	0·16	0·59	0·19	0·12	0·39	0·03	0·252	NS	NS	NS
16 : 0	1519	1245	1366	1983	1708	1695	1599	1522	206·5	NS	NS	**L
16 : 1	189·8	128·2	177·4	210·4	193·6	226·8	204·3	166·5	38·45	NS	NS	NS
17 : 0	102·1	80·77	89·91	118·5	112·2	106·9	100·5	98·48	12·17	NS	*Q	*L
17 : 1	38·63	27·36	36·52	46·30	46·70	46·26	39·74	36·88	6·617	NS	NS	*L
18 : 0	899·1	809·4	792·0	1121	1057	921·5	846·2	885·7	116·3	NS	NS	NS
18 : 1*cis*-9	2442	1719	2284	2670	2622	2725	2456	2217	346·5	NS	NS	*L
18 : 1*trans*-9	14·42	11·17	11·09	22·57	18·88	18·72	18·78	20·01	3·534	*	*L,Q	NS
18 : 1*cis*-11	20·59	1·10	3·90	9·34	11·64	16·50	24·48	5·69	11·62	NS	NS	NS
18 : 1*trans*-11	298·8	223·6	276·9	374·7	296·8	321·1	334·4	362·3	39·54	0·07	**L	NS
C18 : 2*n*-6 *cis*	152·7	119·1	142·8	155·5	163·7	160·4	165·9	130·4	18·42	NS	NS	NS
C18 : 2*n*-6 *trans*	1·67	2·02	0·79	2·41	1·27	1·28	0·48	1·11	0·835	NS	NS	NS
CLA *cis*-9,*trans*-11	41·88	32·01	40·38	59·15	49·56	53·31	52·23	55·77	6·785	**	**L	NS
CLA *trans*-10,*cis*-12	1·91	1·33	1·96	2·70	2·60	2·55	2·83	3·46	0·718	*	*L	NS
18 : 3*n*-6	2·15	1·96	2·83	5·75	1·95	3·13	2·88	2·91	1·001	NS	**L	*L
18 : 3*n*-3	28·16	22·04	25·65	33·55	31·13	30·17	29·83	26·15	3·387	NS	NS	*L
20 : 0	5·64	5·62	7·26	11·76	8·14	6·88	6·35	9·04	1·618	NS	*L,Q	NS
20 : 1	14·20	11·06	19·71	37·42	15·87	1·18	23·40	32·56	4·222	NS	***L	NS
20 : 2*n*-6	2·45	1·82	3·04	4·79	2·92	2·58	3·08	3·22	0·995	NS	*L	NS
20 : 3*n*-3	0·43	0·29	0·46	0·74	0·30	0·41	0·90	0·42	0·380	NS	NS	NS
20 : 3*n*-6	10·10	7·27	7·85	8·79	10·90	10·40	8·52	8·23	1·761	NS	NS	NS
20 : 4*n*-6	40·96	29·37	34·34	22·37	27·46	35·00	19·52	22·49	10·36	NS	NS	NS
20 : 5*n*-3	8·43	7·80	10·18	14·66	7·69	10·69	9·37	12·60	2·131	NS	***L	NS
22 : 0	0·71	0·60	1·32	1·82	0·66	0·54	0·66	1·33	0·390	NS	***L	NS
22 : 1*n*-9	4·12	4·68	9·69	18·31	2·31	6·22	10·38	16·29	2·487	NS	***L	NS
22 : 2*n*6	4·35	3·54	6·00	11·12	3·39	4·70	5·72	9·58	1·309	NS	***L	NS
22 : 5*n*-3	16·30	12·49	15·00	18·44	16·30	18·56	14·86	18·19	2·637	NS	NS	NS
22 : 6*n*-3	3·16	1·79	3·46	4·78	2·09	2·23	2·61	3·84	0·833	NS	***L	NS
24 : 0	0·24	0·20	0·18	0·31	0·38	0·19	0·11	0·15	0·123	NS	NS	NS
24 : 1	0·02	0·00	0·06	0·32	0·02	0·00	0·02	0·25	0·108	NS	***L	NS
SFA	2918	2451	2618	3657	3272	3128	2978	2881	351·7	NS	NS	*L
MUFA	3039	2137	2834	3411	3225	3407	3129	2869	425·8	NS	NS	*L
PUFA	314·6	242·8	294·7	344·8	321·3	335·4	318·7	298·3	39·00	NS	NS	NS
P:S ratio	0·11	0·10	0·11	0·10	0·10	0·11	0·11	0·10	0·008	NS	NS	NS
n-6 fatty acids†	214·4	165·0	197·7	210·8	211·7	217·4	206·1	177·9	26·42	NS	NS	NS
n-3 fatty acids‡	56·48	44·41	54·75	72·17	57·51	62·06	57·57	61·20	7·615	NS	*L	NS
n-6:n-3 ratio	3·89	3·77	3·64	2·95	3·72	3·45	3·62	2·95	0·206	0·06	***L	NS
Total fatty acids	6521	5045	6009	7802	7078	7131	6713	6331	809·3	NS	NS	*L

CLA, conjugated linoleic acid; SFA, saturated fatty acids; L and Q, significant ($P<0·05$) linear and quadratic effects of level of inclusion of fish oil; P:S, PUFA:SFA.
*, ** and *** refer to significance levels $P<0·05$, $P<0·01$ and $P<0·001$, respectively.
† n-*6* fatty acids = sum of 18 : 2, 18 : 3*n*-6, 20 : 2, 20 : 3*n*-6, 20 : 4 and 22 : 2.
‡ *n*-3 fatty acids = sum of 18 : 3*n*-3, 20 : 3*n*-3, 20 : 5, 22 : 5 and 22 : 6.

Table 6. The proportion of individual fatty acids in the neutral lipid fraction of intramuscular fat from *M. longissimus dorsi*

Silage (S)	Unwilted				Wilted							
Fish oil (FO g/kg)	0	10	20	40	0	10	20	40	SED	S	FO	S × FO
Fatty acids (proportion × 100)												
10 : 0	0·13	0·16	0·11	0·09	0·10	0·11	0·12	0·12	0·021	NS	NS	*L
12 : 0	0·04	0·03	0·04	0·04	0·04	0·04	0·03	0·03	0·006	NS	NS	NS
13 : 0	–	–	–	–	–	–	–	–	–	–	–	–
14 : 0	5·85	6·04	5·80	5·41	5·08	5·28	6·16	5·79	0·717	NS	NS	NS
14 : 1	0·25	0·23	0·24	0·27	0·24	0·38	0·29	0·21	0·073	NS	NS	NS
15 : 0	0·58	0·53	0·56	0·61	0·56	0·57	0·58	0·58	0·039	NS	NS	NS
15 : 1	–	–	–	–	–	–	–	–	–	–	–	–
16 : 0	23·50	24·66	23·27	25·41	23·69	24·04	23·79	24·14	0·874	NS	NS	NS
16 : 1	3·00	2·66	3·05	2·75	2·86	3·23	3·24	2·82	0·299	NS	NS	NS
17 : 0	1·37	1·41	1·35	1·39	1·38	1·35	1·33	1·36	0·036	NS	NS	NS
17 : 1	0·59	0·52	0·60	0·56	0·66	0·62	0·57	0·56	0·046	NS	NS	NS
18 : 0	14·54	17·02	14·47	15·20	15·16	14·09	13·16	14·44	1·174	0·07	NS	NS
18 : 1*cis*-9	37·97	34·73	38·40	34·40	38·50	38·58	37·34	35·58	1·347	NS	**L	NS
18 : 1*trans*-9	0·24	0·25	0·21	0·31	0·30	0·29	0·30	0·34	0·049	*	NS	NS
18 : 1*cis*-11	0·27	0·03	0·08	0·10	0·14	0·23	0·44	0·12	0·177	NS	NS	NS
18 : 1*trans*-11	4·99	4·75	4·79	5·21	4·65	4·95	5·35	6·11	0·471	NS	**L	NS
18 : 2*n*-6 *cis*	1·33	1·18	1·16	1·00	1·27	1·04	1·16	1·04	0·077	NS	***L	NS
18 : 2*n*-6 *trans*	0·02	0·04	0·01	0·04	0·02	0·02	0·01	0·02	0·014	NS	NS	NS
CLA *cis*-9,*trans*-11	0·66	0·65	0·66	0·78	0·73	0·78	0·80	0·91	0·057	***	***L	NS
CLA *trans*-10,*cis*-12	0·03	0·03	0·03	0·04	0·04	0·04	0·05	0·06	0·010	*	NS	NS
18 : 3*n*-6	0·03	0·04	0·05	0·08	0·03	0·05	0·05	0·05	0·015	NS	**L	NS
18 : 3*n*-3	0·34	0·31	0·29	0·30	0·34	0·29	0·30	0·28	0·021	NS	**LQ	NS
20 : 0	0·08	0·09	0·12	0·14	0·10	0·08	0·08	0·14	0·018	NS	***L	NS
20 : 1	0·23	0·25	0·35	0·52	0·25	0·28	0·39	0·54	0·047	NS	***L	NS
20 : 2n-6	0·04	0·03	0·04	0·05	0·04	0·02	0·04	0·04	0·013	NS	NS	NS
20 : 3n-3	0·01	0·01	0·01	0·01	0·00	0·01	0·01	0·01	0·006	NS	NS	NS
20 : 3*n*-6	0·03	0·03	0·02	0·02	0·03	0·03	0·02	0·01	0·007	NS	***L	NS
20 : 4*n*-6	0·06	0·10	0·06	0·05	0·06	0·02	0·06	0·08	0·014	NS	NS	***
20 : 5*n*-3	0·00	0·00	0·00	0·00	0·01	0·00	0·00	0·00	0·002	NS	NS	NS
22 : 0	0·01	0·01	0·03	0·02	0·01	0·01	0·01	0·02	0·006	NS	**L	NS
22 : 1*n*-9	0·05	0·07	0·12	0·23	0·02	0·05	0·14	0·27	0·034	NS	***L	NS
22 : 2*n*-6	0·03	0·03	0·04	0·05	0·02	0·02	0·03	0·05	0·010	NS	***L	NS
22 : 5*n*-3	0·06	0·06	0·06	0·06	0·06	0·06	0·06	0·07	0·009	NS	NS	NS
22 : 6*n*-3	0·00	0·00	0·00	0·01	0·00	0·00	0·00	0·01	0·003	NS	NS	NS
24 : 0	0·00	0·00	0·00	0·00	0·01	0·00	0·00	0·00	0·002	NS	NS	NS
24 : 1	–	–	–	–	–	–	–	–	–	–	–	–
Total SFA	46·12	49·97	45·76	48·33	46·13	45·59	45·28	46·63	1·645	0·05	NS	NS
Total MUFA	47·58	43·49	47·84	44·36	47·63	48·61	48·07	46·57	1·517	*	0·07	0·07
Total PUFA	2·65	2·50	2·43	2·48	2·65	2·38	2·60	2·63	0·130	NS	NS	NS
n-6 fatty acids†	1·54	1·45	1·37	1·29	1·47	1·21	1·38	1·29	0·084	0·06	**L	0·07
n-3 fatty acids‡	0·41	0·38	0·36	0·38	0·40	0·35	0·37	0·37	0·027	NS	0·09	NS
n-6:*n*-3 ratio	4·00	3·69	3·58	2·71	3·78	3·49	3·59	2·74	0·277	NS	***L	NS

CLA, conjugated linoleic acid; SFA, saturated fatty acids; L and Q, significant ($P<0·05$) linear and quadratic effects of level of inclusion of fish oil.
*, ** and *** refer to significance levels $P<0·05$, $P<0·01$ and $P<0·001$, respectively.
† *n*-6 fatty acids = sum of 18 : 2, 18 : *3n*-6, 20 : 2, 20 : 3*n*-6, 20 : 4 and 22 : 2
‡ *n*-3 fatty acids = sum of 18 : 3*n*-3, 20 : 3*n*-3, 20 : 5, 22 : 5 and 22 : 6.

The demonstration that *trans*-11 18 : 1 is also converted to CLA in humans and augments the CLA potential of ruminant products (Kuhnt *et al.* 2006) has focused attention on increasing the concentration of this fatty acid *per se*. The strategy used in this experiment was, first, to supply significant amounts of 18 : 2*n*-6 to the rumen by feeding SFO, because increased dietary 18 : 2*n*-6 *per se* has been shown to increase the concentration of CLA in intramuscular fat (Mir *et al.* 2002; Noci *et al.* 2005*b*). Secondly, in contrast to other studies in which one concentration of FO was examined (Enser *et al.* 1999; Shingfield *et al.* 2003), increasing levels of dietary FO were used to determine the response pattern of an increase in CLA and in the nutritionally important *n*-6:*n*-3 PUFA ratio.

With the objective of enhancing the nutritional attributes of bovine muscle fat and subcutaneous adipose tissue, the contribution of forage to the diet is important because the lipids of grass-based forages are rich in 18 : 3*n*-3. Since grass silage is the major feed ingredient in housed cattle rations in Western Europe, the concentrates were offered in a conserved grass-based ration.

Effects of type of silage

Dewhurst & King (1998) found that wilting perennial ryegrass before ensiling decreased the total fatty acid concentration, particularly the 18 : 3*n*-3 concentration. A similar observation was made by Boufaïed *et al.* (2003), who reported a significant decrease in fatty acids of increasing chain length from 14 : 0 to 18 : 3*n*-3 when wilted grass was compared with fresh grass. The lower concentrations of 16 : 0, 18 : 0, 18 : 1, 18 : 2, 18 : 3*n*-3 and total fatty acids in wilted silage in the present

Table 7. The proportion of individual fatty acids in the polar lipid faction of intramuscular fat from *M. longissimus dorsi*

Silage (S)	Unwilted				Wilted							
Fish oil (FO g/kg)	0	10	20	40	0	10	20	40	SED	S	FO	S × FO
Fatty acids (proportion × 100)												
14 : 0	1·59	1·06	0·90	0·91	2·01	1·05	1·06	1·47	0·380	NS	*Q	NS
14 : 1	0·00	0·00	0·00	0·01	0·01	0·02	0·00	0·00	0·008	NS	NS	NS
16 : 0	23·47	22·49	22·98	24·54	25·46	22·78	24·36	23·14	1·542	NS	NS	NS
16 : 1	1·11	1·15	0·95	1·35	1·00	1·14	1·15	1·12	0·174	NS	NS	NS
17 : 0	3·59	3·49	3·14	2·82	3·52	3·10	3·06	3·50	0·447	NS	NS	NS
17 : 1	0·44	0·62	0·59	0·90	0·61	0·71	0·68	0·79	0·144	NS	**L	NS
18 : 0	9·59	7·19	7·15	6·83	11·72	7·62	7·34	8·71	2·023	NS	*Q	NS
18 : 1*cis*-9	25·07	26·82	26·96	29·20	23·45	26·94	27·83	25·80	2·423	NS	NS	NS
18: 1*trans*-9	0·04	0·02	0·03	0·02	0·01	0·02	0·02	0·02	0·024	NS	NS	NS
18 : 1*cis*-11	0·30	0·00	0·00	0·25	0·26	0·01	0·17	0·00	0·252	NS	NS	NS
18 : 1*trans*-11	1·68	1·51	1·56	1·97	1·31	1·59	1·60	1·77	0·229	NS	NS	NS
18 : 2n-6 *cis*	13·19	14·20	13·95	11·93	13·42	13·95	15·15	12·32	1·584	NS	NS	NS
18 : 2n-6 *trans*	0·01	0·02	0·03	0·01	0·00	0·00	0·01	0·02	0·015	NS	NS	NS
CLA *cis*-9,*trans*-11	0·51	0·50	0·52	0·55	0·50	0·54	0·56	0·61	0·070	NS	NS	NS
CLA *trans*-***10***,*cis*-12	0·00	0·00	0·02	0·00	0·01	0·00	0·00	0·01	0·006	NS	NS	NS
18 : 3*n*-6	0·02	0·05	0·02	0·03	0·03	0·05	0·01	0·03	0·025	NS	NS	NS
18 : 3*n*-3	1·46	1·76	1·68	1·70	1·53	1·76	1·88	1·73	0·192	NS	NS	NS
20 : 0	0·12	0·32	0·21	0·22	0·24	0·24	0·21	0·18	0·079	NS	NS	NS
20 : 1	0·01	0·01	0·02	0·06	0·00	0·00	0·01	0·09	0·032	NS	***L	NS
20 : 2*n*-6	0·05	0·15	0·10	0·13	0·07	0·14	0·11	0·13	0·041	NS	*	NS
20 : 3*n*-3	0·00	0·00	0·00	0·00	0·01	0·00	0·00	0·00	0·009	NS	NS	NS
20 : 3*n*-6	1·35	1·27	1·14	1·03	1·37	1·27	1·14	1·30	0·202	NS	NS	NS
20 : 4*n*-6	6·22	5·27	5·73	2·64	3·68	4·90	2·48	3·11	1·409	*	*L	NS
20 : 5*n*-3	1·36	1·67	1·75	2·00	1·12	1·62	1·51	2·22	0·286	NS	***L	NS
22 : 0	–	–	–	–	–	–	–	–	–	–	–	–
22 : 1*n*-9	0·22	0·37	0·49	0·31	0·22	0·48	0·33	0·17	0·192	NS	NS	NS
22 : 2*n*-6	0·38	0·50	0·60	1·04	0·32	0·51	0·59	1·16	0·129	NS	***L	NS
22 : 5*n*-3	2·04	2·16	2·14	2·02	2·02	2·33	1·82	2·44	0·328	NS	NS	NS
22 : 6*n*-3	0·49	0·37	0·56	0·60	0·30	0·32	0·41	0·60	0·116	NS	**L	NS
24 : 0	0·00	0·01	0·00	0·00	0·00	0·00	0·00	0·00	0·005	NS	NS	NS
24 : 1	–	–	–	–	–	–	–	–	–	–	–	–
SFA	38·38	34·56	34·39	35·33	42·95	34·80	36·04	37·01	3·147	NS	*Q	NS
MUFA	28·87	30·49	30·59	34·11	26·88	30·90	31·85	29·76	2·581	NS	NS	NS
PUFA	27·09	27·93	28·23	23·68	24·40	27·41	25·68	25·68	2·417	NS	NS	NS
n-6 fatty acids†	21·22	21·46	21·56	16·80	18·90	20·82	19·50	18·06	1·973	NS	*L	NS
n-3 fatty acids‡	5·35	5·96	6·12	6·32	4·99	6·04	5·62	7·00	0·642	NS	**L	NS
n-6 : *n*-3 ratio	4·00	3·69	3·58	2·71	3·78	3·49	3·59	2·74	0·277	NS	***L	NS

CLA, conjugated linoleic acid; SFA, saturated fatty acids; L and Q, significant ($P<0·05$) linear and quadratic effects of level of inclusion of fish oil.
*, ** and *** refer to significance levels $P<0·05$, $P<0·01$ and $P<0·001$, respectively.
† *n*-6 fatty acids = sum of 18 : 2, 18 : *3n*-6, 20 : 2, 20 : 3*n*-6, 20 : 4 and 22:2
‡ *n*-3 fatty acids = sum of 18 : 3*n*-3, 20 : 3*n*-3, 20:5, 22:5 and 22 : 6.

study confirm the loss of fatty acids (mainly 18 : 3*n*-3) due to the wilting process, possibly due to oxidative loss, as suggested by Dewhurst & King (1998).

While there was an interaction between type of silage and level of FO inclusion for the concentration of total and several individual fatty acids in the NL (data not shown) and TL of muscle, the fact that the interaction was generally not detected when data were analysed as proportions of the total fatty acids confirmed that the interaction was mainly due to the small differences in fatness across the eight treatments.

With respect to the silages, daily consumption of 18 : 3*n*-3 averaged 95 g for steers fed unwilted silage compared with the 83 g for steers fed wilted silage. Ruminal biohydrogenation of dietary 18 : 3*n*-3 apparently eliminated this difference in intake since the proportions of intramuscular 18 : 3*n*-3 were 0·37 and 0·36 for unwilted and wilted silage-fed steers, respectively, similar to the proportion reported by Scollan *et al.* (2001*a*) for cattle fed grass silage and concentrates.

The increase in the concentration of *cis*-9,*trans*-11 CLA in both TL and subcutaneous adipose tissue as a result of feeding wilted silage indicates a possible effect of the type of silage on rumen biohydrogenation pathways. Since the muscle concentration of CLA is linked to the outflow of *trans*-11 18: 1 from the rumen (Griinari *et al.* 2000), the results suggest that the conditions created by feeding wilted silage instead of unwilted silage were more effective in modifying microbial flora action towards an incomplete biohydrogenation of the dietary PUFA. The higher proportion of *trans*-9 18 : 1 and *trans*-11 18 : 1 in the NL (and TL) lends support to the hypothesis of an increased outflow of *trans*-11 18 : 1 as a precursor for desaturation in the muscle. An effect of concentration and type of substrate, dilution rate and rumen pH on the production of long-chain fatty acids, including *trans* 18 : 1 isomers, by mixed ruminal bacteria in continuous culture was shown by Martin & Jenkins (2002).

Effect of level of FO inclusion

The muscle and subcutaneous adipose tissue fatty acid data in the present study generally support the findings of Enser *et al.* (1999) and Scollan *et al.* (2001*a*) with regard to FO

Table 8. Fatty acid concentration in subcutaneous adipose tissue

Silage (S)	Unwilted				Wilted							
Fish oil (FO g/kg)	0	10	20	40	0	10	20	40	SED	S	FO	S × FO
Fatty acids (mg/g sample)												
10:0	0·27	0·27	0·20	0·22	0·20	0·22	0·19	0·25	0·054	NS	NS	NS
12:0	0·15	0·15	0·14	0·15	0·12	0·12	0·13	0·15	0·030	NS	NS	NS
13:0	0·03	0·05	0·03	0·07	0·03	0·03	0·03	0·03	0·018	*	NS	NS
14:0	20·60	20·85	19·82	19·96	19·11	18·86	18·96	19·28	2·051	NS	NS	NS
14:1	4·01	3·77	4·15	3·99	4·13	4·65	3·83	4·10	0·585	NS	NS	NS
15:0	4·55	4·30	4·05	4·55	4·34	4·96	4·17	4·27	0·523	NS	NS	NS
15:1	0·06	0·08	0·08	0·13	0·07	0·06	0·08	0·07	0·026	NS	NS	NS
16:0	119·3	113·6	108·0	105·6	102·0	115·3	104·1	109·7	7·736	NS	NS	NS
16:1	28·38	27·23	27·27	26·70	29·35	33·31	28·26	27·72	3·250	NS	NS	NS
17:0	8·96	7·43	8·28	7·50	7·25	7·76	7·15	8·08	1·005	NS	NS	NS
17:1	5·68	5·20	5·34	5·61	5·68	5·93	5·27	5·18	0·463	NS	NS	NS
18:0	52·24	55·18	45·20	45·26	42·58	48·36	43·79	44·80	4·983	0·07	NS	NS
18:1*cis*-9	221·3	217·3	209·3	202·7	208·3	230·5	197·8	202·2	16·23	NS	0·06L	NS
18:1*trans*-9	1·70	0·90	2·93	3·43	1073	1·32	2·42	1046	1·430	NS	NS	NS
18:1*cis*-11	2·03	2·73	3·23	4·43	2·87	1·68	2·36	5·08	1·879	NS	NS	NS
18:1*trans*-11	30·72	28·40	24·68	27·45	20·54	23·97	24·98	32·74	4·672	NS	NS	NS
18:2*n*-6 *cis*	6·79	5·96	5·84	5·61	7·13	6·23	6·13	5·63	0·512	NS	***L	NS
18:2*n*-6 *trans*	0·44	0·17	0·21	0·19	0·78	0·12	0·35	0·22	0·201	NS	**	NS
CLA *cis*-9,*trans*-11	5·47	6·00	5·48	6·78	6·37	7·34	6·62	8·67	0·756	***	**L	NS
CLA *trans*-10,*cis*-12	0·33	0·20	0·16	0·23	0·23	0·32	0·25	0·26	0·068	NS	NS	NS
18:3*n*-6	0·10	0·09	0·09	0·10	0·10	0·10	0·08	0·09	0·021	NS	NS	NS
18:3*n*-3	1·44	1·23	1·10	1·30	1·45	1·23	1·29	1·12	0·145	NS	0·05L	NS
20:0	0·46	0·50	0·52	0·66	0·52	0·36	0·86	0·66	0·162	NS	0·07L	NS
20:1	1·88	2·03	2·25	3·32	1·73	2·10	2·82	3·70	0·385	NS	***L	NS
20:2*n*-6	0·35	0·26	0·33	0·53	0·32	0·32	0·50	0·42	0·111	NS	NS	NS
20:3*n*-3	0·00	0·01	0·00	0·00	0·00	0·01	0·04	0·03	0·024	NS	NS	NS
20:3*n*-6	0·19	0·18	0·12	0·16	0·19	0·16	0·15	0·17	0·037	NS	NS	NS
20:4*n*-6	0·26	0·25	0·23	0·24	0·19	0·20	0·26	0·29	0·072	NS	NS	NS
20:5*n*-3	0·04	0·02	0·04	0·06	0·03	0·03	0·02	0·04	0·012	NS	0·06L	NS
22:0	0·13	0·08	0·11	0·12	0·12	0·11	0·15	0·13	0·041	NS	NS	NS
22:1*n*-9	0·08	0·09	0·11	0·23	0·09	0·19	0·32	0·32	0·109	NS	0·07L	NS
22:2*n*-6	0·25	0·20	0·27	0·44	0·14	0·42	0·23	0·35	0·085	NS	**L	*
22:5*n*-3	0·33	0·20	0·21	0·22	0·14	0·24	0·19	0·25	0·060	NS	NS	*L
22:6*n*-3	0·01	0·01	0·02	0·03	0·01	0·00	0·01	0·02	0·011	NS	**L	NS
24:0	0·03	0·02	0·03	0·02	0·02	0·02	0·04	0·02	0·014	NS	NS	NS
24:1	0·01	0·00	0·01	0·02	0·00	0·01	0·00	0·02	0·006	NS	***L	NS
SFA	206·7	202·5	186·4	184·1	176·3	196·1	179·6	187·4	13·50	NS	NS	NS
MUFA	295·9	287·7	279·3	278·0	274·5	303·7	268·1	282·6	16·54	NS	NS	NS
PUFA	16·00	14·78	14·11	15·90	17·07	16·72	16·13	17·57	1·455	*	NS	NS
P:S ratio	0·08	0·08	0·08	0·09	0·10	0·09	0·09	0·09	0·010	*	NS	NS
n-6 fatty acids†	8·38	7·11	7·10	7·27	8·86	7·55	7·70	7·17	0·706	NS	*L	NS
n-3 fatty acids‡	1·82	1·47	1·37	1·62	1·62	1·51	1·55	1·46	0·175	NS	NS	NS
n-6:*n*-3 ratio	4·89	4·94	5·30	4·61	5·58	5·26	4·99	5·04	0·477	NS	NS	NS
Total fatty acids	546·9	536·3	508·1	509·1	497·3	549·0	492·9	521·2	25·24	NS	NS	NS

CLA, conjugated linoleic acid; SFA, saturated fatty acids; L and Q, significant ($P<0·05$) linear and quadratic effects of level of inclusion of fish oil; P:S, PUFA:SFA.
*, ** and *** refer to significance levels $P<0·05$, $P<0·01$ and $P<0·001$, respectively.
† *n*-6 fatty acids = sum of 18:2, 18:*3n*-6, 20:2, 20:3*n*-6, 20:4 and 22:2
‡ *n*-3 fatty acids = sum of 18:3*n*-3, 20:3*n*-3, 20:5, 22:5 and 22:6.

supplementation of beef cattle. Of particular interest from a human nutrition perspective is the increase in *cis*-9,*trans*-11 CLA (and its precursor *trans*-11 18:1), 20:5*n*-3 and 22:6*n*-3 proportions in muscle and subcutaneous adipose tissue, and the decrease in the *n*-6:*n*-3 PUFA ratio in muscle with FO consumption. It has been established that dietary FO increases production of *trans*-11 18:1 in the rumen, and subsequently its concentration in milk fat or in the intramuscular adipose tissue (Chilliard *et al.* 2001; Scollan *et al.* 2001*a*, *b*). The results of the present study indicate that this effect of FO, via the supply of long-chain PUFA to the rumen (or via intermediates of their biohydrogenation in the rumen), is dose-dependent. This was confirmed in a parallel study in which the concentrates containing 0, 10 or 40 g FO/kg were offered to duodenally fistulated steers (Lee *et al.* 2005), i.e. a linear increase in the outflow of *trans*-11 18:1 from the rumen was observed. In contrast, there was a quadratic increase in *cis*-9,*trans*-11 CLA outflow from the rumen such that the ratio *trans*-11 18:1:*cis*-9,*trans*-11 CLA was greater for the 40 g FO/kg concentrate than for the 10 g FO/kg concentrate. Despite this, the *trans*-11 18:1:*cis*-9,*trans*-11 CLA ratio in muscle and subcutaneous adipose tissue was not influenced by FO inclusion. These data suggest a greater efficiency of absorption of *cis*-9,*trans*-11 CLA and/or a lower efficiency of absorption of *trans*-11 18:1 from the intestine of cattle fed the high FO concentrates or differences in metabolism

post-absorption. The lack of an effect of FO inclusion on the *trans*-11 18 : 1:*cis*-9,*trans*-11 CLA ratio does not support the hypothesis that long-chain PUFA inhibit tissue desaturase activity, at least at the concentration of long-chain PUFA observed in the present study. While there were analytical differences in the present study and that of Lee *et al.* (2005), the influence of FO or long-chain PUFA consumption *per se* on post-ruminal *trans*-11 18 : 1 and *cis*-9,*trans*-11 CLA transformations merit investigation.

Enser *et al.* (1999) reported a *cis*-9,*trans*-11 CLA concentration of 24·3 mg/100 g muscle from Charolais steers fed a 20 g FO/kg concentrate diet compared with an average of 57·5 g/100 g muscle for cattle offered the 40 g FO/kg concentrate in the present study. The higher concentration of this CLA isomer achieved in the present experiment may be attributed to the 3-fold and 1·5-fold higher daily intake of 18 : 2*n*-6 and 18 : 3*n*-3, respectively (steers consuming the 40 g FO/kg concentrate had a total intake from silage and concentrate of 227 g of 18 : 2*n*-6 and 89 g of 18 : 3*n*-3), leading to higher *trans*-11 1 8:1 (and CLA) production through biohydrogenation (Lee *et al.* 2005). It should be noted that the animals in the present experiment had a higher concentration of total fatty acids in the muscle than those reported by Enser *et al.* (1999). Since CLA was found predominantly in the NL fraction, which increases as animals accrete lipid, this will account for some of the difference in CLA concentrations between the studies.

While earlier *in vitro* and laboratory animal studies convincingly demonstrated an anticancer effect of the *cis*-9,*trans*-11 isomer of CLA, this effect has not been demonstrated in humans. This relates at least in part to the aetiology of cancer and the difficulty in conducting studies on the prevention of cancer in humans. Long-term studies with appropriate end points such as incidence of cancer remain to be conducted so an anticancer effect of CLA in humans cannot be excluded. As a range of other positive isomer-specific effects on human health have been proposed, including a reduction in atherosclerosis, decreased inflammation and improved cardiovascular health (e.g. Pariza *et al.* 2001), the efficacy of CLA in the treatment and prevention of several of these conditions has also been examined. The findings from human studies to date have been equivocal (reviewed by Yaqoob *et al.* 2006). This review suggests that much of the variability in the human studies conducted to date is due to the use of mixtures of CLA isomers (e.g. *trans*-10,*cis*-12 CLA appears to have a negative effect on blood lipids while the *cis*-9,*trans*-11 isomer does not) and short duration, to which could be added insufficient statistical power to detect differences of biological significance. Considerably more information is required in this regard.

Epidemiological associations between the risk of coronary heart disease and the consumption of *trans* PUFA has also focused attention on the concentration of these fatty acids in food. The *trans* fatty acid profile in ruminant fat tends be enriched with the *trans*-11 isomer of 18 : 1, as was seen in the present study. Industrially derived oils such as hydrogenated vegetable oils, in contrast, have a broader spectrum of *trans* 18 : 1 isomers and have a considerably higher concentration of *trans*-9 and *trans*-10 18 : 1 isomers (Scollan *et al.* 2006). The relative risk to human health of consuming the individual isomers remains to be elucidated, but current epidemiological evidence suggests that consumption of ruminally derived *trans* PUFA is not a risk factor for heart disease (Jakobsen, 2006).

Data in the literature are unclear with respect to ruminal biohydrogenation of long-chain PUFA of FO. In an *in vitro* study, Gulati *et al.* (1999) suggested that the ability of rumen micro-organisms to hydrogenate long-chain PUFA is dependent on the concentration of those fatty acids in the rumen environment. Scollan *et al.* (2001*b*) reported biohydrogenation of 92 and 91 % for 20 : 5*n*-3 and 22 : 6*n*-3, respectively, in beef cattle fed 30 g FO/kg dry matter intake. In our parallel study, Lee *et al.* (2005) reported corresponding values of 79 and 80 % for beef cattle fed the 40 g FO/kg concentrate. In the present study, the incorporation of long-chain PUFA was relatively low in the NL fraction and in the subcutaneous adipose tissue, in agreement with Ashes *et al.* (1992) and Mitchell *et al.* (1991), respectively, while the greatest effect of dietary treatments was observed in the PL where PUFA are present in higher concentrations. While the highest level of inclusion of FO resulted in the highest incorporation of long chain *n*-3 PUFA in the muscle PL, the proportion of long-chain PUFA was lower than that found by Scollan *et al.* (2001*a*). Differences could be attributed to the different proportions of long-chain *n*-3 PUFA in the FO used, to the different proportion of oil included in the diets used (1·9 and 2·4 % of dietary dry matter in our study and that of Scollan *et al.* (2001*a*), respectively) and to the different levels of fatness of the animals in the two experiments. This may also relate to the higher 18 : 2*n*-6 intake in the present study as *n*-6 PUFA compete for deposition with the *n*-3 PUFA and are preferentially deposited.

In the present study, a decrease in 20 : 4*n*-6 in the PL fraction with a concomitant increase in long-chain *n*-3 PUFA suggests that the latter were incorporated at the expense of the former to maintain the structural integrity of the membrane phospholipid fraction. Such a decrease in long-chain *n*-6 PUFA in the PL has also been observed in other studies with both ruminants and non-ruminants in which fishmeal and FO were included as dietary supplements (Morgan *et al.* 1992; Mandell *et al.* 1997).

The considerable proportion of 20 : 1 and 22 : 1 contained in the FO used in this experiment (Table 2) could explain the linear increase in very-long-chain MUFA and SFA in the NL fraction. Shingfield *et al.* (2003) also found increased amounts of some long-chain fatty acids (20 : 1, 20 : 2, 22 : 0 and 22 : 1) entering the omasal canal and being incorporated into milk fat following the inclusion of FO in the diet. Overall, however, the addition of FO had virtually no impact on the total SFA, MUFA or PUFA content of the intramuscular adipose tissue or of the subcutaneous adipose tissue. Consequently, the P:S ratio was similar across the dietary treatments and it was also considerably lower than the recommended value of 0·45 suggested by the Department of Health (1994). Our results are consistent with those of Mandell *et al.* (1997), Choi *et al.* (2000) and Scollan *et al.* (2001*a*). Scollan *et al.* (2003) suggested the existence of a negative exponential relationship between the amount of intramuscular fat and the P:S ratio. When animals have intramuscular fat concentrations ranging between 2000 and 4000 mg/100 g, P:S ratio values, calculated as $(18:2n\text{-}6 + 18:3n\text{-}3)/(\Sigma(12:0,\ 14:0,\ 16:0,\ 18:0))$, generally range between

0·05–0·11 (Choi *et al.* 2000; Scollan *et al.* 2001*a*). The steers in the present study had a high muscle fat content (6600 g fatty acids/100 g muscle), and so the low P:S ratio results were consistent with this negative exponential relationship.

Replacing lard with FO in the diets of finishing cattle had a beneficial effect on the *n*-6:*n*-3 PUFA ratio of intramuscular fat. The ratio observed was below 4:1, a value regarded as compliant with the guidelines from the Department of Health (1994). In the present study, the addition of FO contributed to a modification of the proportion of long-chain *n*-3 and *n*-6 fatty acids, without affecting 18 : 2*n*-6 and 18 : 3*n*-3, leading to a reduction in the *n*-6:*n*-3 PUFA ratio to 2·95:1 (in steers fed 40 g FO/kg). Based on the data from this study, and assuming an average beef consumption of 100 g/day (Enser *et al.* 1996), beef produced using the 40 g FO/kg concentrate (averaged across the type of silage) would supply 67 mg of *n*-3 PUFA (36 mg of which come from *n*-3 long-chain PUFA), accounting for 20–40 % of the recommended daily intake of *n*-3 fatty acids (Department of Health, 1994).

Conclusions

Notwithstanding the fact that emerging data on the human health benefits of CLA are not as dramatic as those from model systems and concerns about the consumption of *trans* PUFA, the results of this experiment are interpreted as indicating that inclusion of FO in rations used for beef cattle has a positive impact on the fatty acid profile of muscle and subcutaneous adipose tissue lipids from a human health perspective. The positive linear relationship between the level of inclusion of FO in the ration and the level of beneficial fatty acids in tissue offers opportunities for inclusion of higher levels of FO. The study also demonstrated that utilisation of wilted rather than unwilted silage will enhance the CLA content of bovine intramuscular fat and subcutaneous adipose tissue without a negative effect on the incorporation of *n*-3 PUFA.

Acknowledgements

This research was supported through the European Union, Fifth Framework Programme (Project QLRT-2000-31 423, 'Healthy Beef'). The technical assistance of V. McHugh, A. McArthur, N. Blount (Grange Research Centre) and D. Donovan (Moorepark Research Centre) is gratefully acknowledged, as is the cooperation of Meadow Meats, Rathdowney, Ireland.

References

AbuGhazaleh AA, Schingoethe DJ, Hippen AR & Whitlock LA (2002) Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. *J Dairy Sci* **85**, 624–631.

AbuGhazaleh AA, Schingoethe DJ, Hippen AR & Kalscheur KF (2003) Milk conjugated linoleic acid response to fish oil supplementation of diets differing in fatty acid profiles. *J Dairy Sci* **86**, 944–953.

Association of Analytical Chemists (1990) *Official Methods of Analysis*, 15th ed. Washington, DC: Association of Analytical Chemists.

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.

Boufaïed H, Chouinard PY, Tremblay GF, Petit HV, Michaud R & Belanger G (2003) Fatty acids in forages I. Factors affecting concentrations. *Can J Anim Sci* **83**, 501–511.

Chilliard Y, Ferlay A & Doreau M (2001) Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest Prod Sci* **70**, 31–48.

Chin SF, Liu W, Storkson JM, Ha YL & Pariza MW (1992) Dietary sources of conjugated linoleic acid, a newly recognised class of anticarcinogens. *J Food Compos Anal* **5**, 185–197.

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.

De la Torre A, Debiton E, Juaneda P, Durand D, Chardigny JM, Baerthomeuf C, Bauchart D & Gruffat D (2006) Beef conjugated linoleic acid isomers reduce human cancer cell growth even when associated with other beef fatty acids. *Br J Nutr* **95**, 346–352.

Demeyer D & Doreau M (1999) Targets and procedures for altering ruminant milk and meat lipids. *Proc Nutr Soc* **58**, 593–607.

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

Dewhurst RJ & King PJ (1998) Effect of extended wilting, shading and chemical additives on the fatty acids in laboratory grass silages. *Grass Forage Sci* **53**, 219–224.

European Communities (1984) *European Communities (Marketing of Feedstuffs) Regulation*. Statutory Instruments SI No. 200 of 1984.

Enser M, Hallett K, Hewett B, Fursey GAF & Wood JD (1996) Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Sci* **42**, 443–456.

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.

Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV & Bauman DE (2000) Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. *J Nutr* **130**, 2285–2291.

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.

Ha YL, Grimm NL & Pariza MW (1987) Anticarcinogens from fried ground beef: heat altered derivatives of linoleic acid. *Carcinogenesis* **8**, 1881–1887.

Kepler CR & Tove SB (1967) Biohydrogenation of unsaturated fatty acid. *J Biol Chem* **242**, 5686–5692.

Kuhnt K, Kraft J, Moeckel P & Jahreis G (2006) *Trans*-11-18 : 1 is effectively delta9-desaturated in humans. *Br J Nutr* **95**, 752–761.

Jakobsen MU (2006) Intake of ruminant versus industrially produced trans fatty acids and the risk of coronory heart disease – what is the epidemiological evidence? *Proceedings of the 4th European Federation Lipid Congress,* p. 18.

Lee MRF, Tweed JKS, Moloney AP & Scollan ND (2005) The effects of fish oil supplementation on rumen metabolism and the biohydrogenation of unsaturated fatty acids in beef steers given diets containing sunflower oil. *Anim Sci* **80**, 361–367.

Mandell IB, Buchanan-Smith JG, Holub BJ & Campbell CP (1997) Effects 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.

Martin SA & Jenkins TC (2002) Factors affecting conjugated linoleic acid and *trans*-$C_{18:1}$ fatty acid production by mixed ruminal bacteria. *J Anim Sci* **80**, 3347–3352.

Mir PS, Mir Z, Huber PS, *et al.* (2002) Growth, carcass characteristics, muscle conjugated linoleic acid (CLA) content, and response to intravenous glucose challenge in high percentage Wagyu, Wagyu × Limousin and Limousin steers fed sunflower oil-containing diets. *J Anim Sci* **80**, 2996–3004.

Mitchell GE, Reed AW & Rogers SA (1991) Influence of feeding regimen on the sensory qualities and fatty acid composition of beef steaks. *J Food Sci* **56**, 1102–1103.

Moloney AP, Read MP & Keane MG (1996) Effects of ardacin supplementation on rumen fermentation and protein degradability in steers. *Anim Feed Sci Technol* **57**, 97–110.

Morgan CA, Noble RC, Cocchi M & McCartney R (1992) Manipulation of the fatty acid composition of pig meat lipids by dietary means. *J Sci Food Agric* **58**, 357–368.

Noci F, Monahan FJ, French P & Moloney AP (2005*a*) The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture-fed beef heifers: influence of the duration of grazing. *J Anim Sci* **83**, 1167–1178.

Noci F, O'Kiely P, Monahan FJ, Stanton C & Moloney AP (2005*b*) Conjugated linoleic acid concentration in *m. longissimus dorsi* from heifers offered sunflower oil based concentrates and conserved forages. *Meat Sci* **69**, 509–518.

Offer NW, Speake BK, Dixon J & Marsden M (2001) Effect of fish-oil supplementation on levels of (*n*-3) polyunsaturated fatty acids in the lipoprotein fractions of bovine plasma. *Anim Sci* **73**, 523–531.

Pariza MW, Park Y & Cook ME (2001) The biologically active isomers of conjugated linoleic acid. *Prog Lipid Res* **40**, 283–298.

Porter MG & Murray RS (2001) The volatility of components of grass silage on oven drying and the inter-relationship between dry-matter content estimated by different analytical methods. *Grass Forage Sci* **56**, 405–411.

Raes K, De Smet S & Demeyer D (2004) Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. *Anim Feed Sci Technol* **113**, 199–221.

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

Scollan ND, Dhanoa MS, Choi NJ, Maeng WJ, Enser M & Wood JD (2001*b*) Biohydrogenation and digestion of long chain fatty acids in steers fed on different samples of lipid. *J Agric Sci* **136**, 345–355.

Scollan ND, Enser M, Gulati SK, Richardson I & Wood JD (2003) Effects of including a ruminally protected lipid supplement in the diet on the fatty acid composition of beef muscle. *Br J Nutr* **90**, 709–716.

Scollan N, Hocquette JF, Nuernberg K, Dannenberger D, Richardson I & Moloney A (2006) Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Sci* **74**, 17–33.

Shingfield KJ, Ahvenjärvi S, Toivonen V, Ärölä A, Nurmela KVV, Huhtanen P & Griinari JM (2003) Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Anim Sci* **77**, 165–179.

Sukhija PS & Palmquist DL (1988) Rapid method for determination of total fatty acid content and composition of feedstuffs and faeces. *J Agric Food Chem* **36**, 1202–1206.

Yaqoob P, Tricon S, Burdge GC & Calder PC (2006) Conjugated linoleic acids (CLAs) and health. In *Improving the Fat Content of Foods*, pp. 182–209 [C Williams and J Buttriss, editors]. Cambridge, UK: Woodhead Publishing Limited.