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Fatty acid composition of intra-muscular triacylglycerols of steers fed autumn grass and concentrates

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Abstract

The objective of this study was to determine the impact of increasing the rate of carcass growth of steers grazing autumn grass by either supplementing with concentrates or by increasing grass supply on fatty acid composition of triacylglycerols within the *M. longissmus thoracis*. One hundred and ten steers (567 kg) were assigned to ten dietary treatments in a 3 (daily grass allowances: 6, 12 and 18 kg dry matter (DM)/animal) by 3 (daily concentrate allowances: 0, 2.5 and 5 kg/animal) factorial experiment with a control group offered concentrates ad libitum. Animals were slaughtered after 100 days and lipid extracted from samples of the *M. longissmus thoracis* for fatty acid analysis. There was an interaction ($P < 0.001$) between grass and concentrate allowance for the proportions of major fatty acid classes which reflected the patterns observed for individual fatty acids. Thus, supplementary concentrates had little effect on total fatty acids when offered with the high grass allowance, but high concentrate supplementation of the low grass allowance decreased the saturated fatty acid (SFA) and increased the monounsaturated fatty acid (MUFA) proportions. The high increment of concentrates at the medium grass allowance decreased the SFA and increased the MUFA proportions compared to the low increment of concentrates. In the absence of concentrates the high grass allowance decreased the proportion of SFA ($P < 0.001$) and increased MUFA proportion ($P < 0.001$). Intra-muscular fat from the positive control group had lower ($P < 0.05$) SFA and higher ($P < 0.05$) MUFA proportions than that of animals offered the low grass allowance alone or with 2.5 kg concentrates or the medium grass allowance alone or with 5 kg concentrates. There was an interaction between grass and concentrate allowance for polyunsaturated fatty acid (PUFA): SFA ratio whereby this ratio was higher ($P < 0.05$) for the first concentrate increment on the medium grass allowance than this grass allowance either unsupplemented or supplemented with the high concentrate allowance. There was no effect of grass or concentrate allowance on the n-3: n-6 PUFA ratio but this ratio tended ($P < 0.1$) to be higher for all grass-based treatments compared to the control group. It is concluded that similar carcass growth rate can be achieved from an allowance of 18 kg autumn grass DM/day and 5 kg concentrates or from ad libitum concentrates and that muscle from the former ration has a lower fat concentration and a more favorable n-3 and n-6 PUFA profile, from a human health perspective.

Keywords: Beef; Grass; Concentrates; Fatty acids; Cattle

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1. Introduction

Consumption of saturated fatty acids (SFA) with 12–16 carbon atoms has been associated with increased serum low-density lipoprotein cholesterol concentrations, a risk factor for coronary heart disease (Keys, 1970). Monounsaturated fatty acids (MUFA) and some polyunsaturated fatty acids (PUFA), particularly those of the fish-oil n-3 series are, however, antithrombogenic (Ulbricht and Southgate, 1991). Animal fat is a major dietary source of SFA and in ruminant fat the ratio of PUFA to SFA is lower than in non-ruminant fat, due to hydrogenation of dietary unsaturated fatty acids by rumen microorganisms. A proportion of dietary unsaturated fatty acids bypasses the rumen intact and is absorbed and deposited in body fat (Wood and Enser, 1997). Strategies that lead to an increase in the PUFA to SFA ratio in intra-muscular fat would improve the healthiness of beef from a consumer perspective.

French et al. (2000) demonstrated that when grown at the same rate, muscle from cattle which had a high grass intake had a higher PUFA: SFA ratio and a lower n-6: n-3 PUFA ratio than muscle from cattle fed concentrates. However, in commercial grass-based beef production systems the objective is to maximise carcass growth while optimizing the inclusion of grass. In autumn, nutrient supply from grass may be insufficient to support the rate of carcass growth achievable early in the grazing season. This may be related to the quantity and/or quality of grass supplied relative to the requirements of grazing cattle. Two strategies could potentially increase animal performance from autumn grass: (a) an increase in the supply of grass or (b) supplementation of grass with high-energy concentrates. Little information is available on the effect of such production strategies on the fatty acid composition of bovine intra-muscular fat.

The objective of this study was to determine the impact of increasing carcass growth of steers grazing autumn grass by supplementing with concentrates, and/or by increasing grass supply, on the profile of intra-muscular fatty acids.

2. Materials and methods

2.1. Experimental design and animal management

The experimental details were described previously (French et al., 2001). In brief, one hundred and ten continental crossbred steers with a mean initial liveweight of 567 (S.D. 32.3) kg, were ranked on descending liveweight into 11 blocks of 10 animals each. They were then assigned at random, from within blocks, to one of ten treatments. Nine treatments were arranged in a 3 (daily grass allowance: 6, 12 or 18 kg dry matter (DM) by 3 (daily concentrate allowance: 0, 2.5 and 5 kg fresh weight/head) factorial design. The tenth treatment group (positive control) was fed concentrates ad libitum. The duration of the experiment was 100 days from 22 August.

Daily grass allowances were achieved by varying the size of the grazing area. Animals were offered a fresh allowance daily and did not have access to the previous day's allowance. On 3 days per week pre-grazing grass mass was estimated by cutting four strips (each 1.2 m $\times$ 5 m) to 4 cm stubble height from the areas to be grazed by each group. Post-grazing grass yield was estimated three times weekly by cutting four strips (each 0.55 m $\times$ 5 m) to a 4 cm stubble height, from the area grazed by the steers on each treatment.

A pelleted concentrate, of (per kg) ground barley (290 g), unmolassed beet pulp (290 g), maize gluten (290 g), soya-bean meal (50 g), molasses (50 g) and a mineral/vitamin mix (30 g), was offered individually to all animals receiving supplementary concentrates. The appropriate animals were restrained individually in a purpose built mobile feeder in the field and offered 2.5 kg concentrates per head at 08:00 h, before being allocated a fresh daily grass allowance. Animals remained restrained until the concentrates were consumed (approximately 20 min). Animals receiving 5 kg concentrates daily were restrained again and offered a further 2.5 kg concentrates at 16:00 h. The control group of animals was offered concentrates ad libitum individually and 1 kg straw indoors, through electronically controlled gates.

All animals were slaughtered at a commercial facility 150 km from the research centre. After slaughter, cold carcass weight (hot carcass weight $\times$ 0.98) was recorded. The carcasses were classified for fat score (1 = leanest and 5 = fattest) using the EU Beef Carcass Classification Scheme (Commission of the European Communities, 1982). The weight of kidney and channel fat (KCF) on each carcass was recorded immediately post-slaughter.

2.2. Extraction of intra-muscular fat and quantification of fatty acid methyl esters

A steak weighing approximately 250 g was removed from the right side *M. longissimus thoracis* (LD) of each carcass at the 10th rib after 2 days ageing at 4 °C, trimmed of visible subcutaneous and intermuscular fat and ground using a food processor (Robot Coupe Inc., Jackson, MS, USA) for 1.5 min. Crude fat was measured as described by AOAC (1992). For fatty acid analysis, fat was extracted in a Soxhlet apparatus, using petroleum ether (boiling point range 40–60 °C) as the extraction solvent. The solvent was evaporated off at 70 °C for 18 h, fat was saponified by refluxing with 0.5 M sodium hydroxide for 10 min and fatty acids were then esterified by refluxing for 2 min with 14% boron trifluoride in methanol. After cooling to room temperature, saturated sodium chloride was added and the samples were extracted with *n*-heptane. Anhydrous sodium sulphate was added to the heptane solution which was filtered prior to analysis. Fatty acid methylesters (FAME) were measured using a Varian 3400 CX Chromatograph (Varian, Harbor City, CA, USA) fitted with a flame ionization detector. Separation of FAME was performed on a WCOT fused silica capillary GLC column (50 m × 0.25 mm i.d., CP-SIL 88 coating), using N_2 (28.3 ml/min) and He (1.7 ml/min) as carrier gases. The initial column temperature of 50 °C was increased at 30 °C/min to 120 °C and then at 10 °C/min to a final temperature of 225 °C at which it was held for 13 min. Data were recorded and analyzed on a Minichrom PC system (VG Data System, Manchester, England). Individual fatty acids were identified by comparison of their retention times with fatty acid standards.

2.3. Feed analyses

Fat was extracted from the grass and concentrates using the method of Folch et al. (1957) and methylated according to the method described by Slover and Lanza (1979). Other feed analyses were as previously described by Moloney and O'Kiely (1995).

2.4. Statistical analyses

All variables for the nine treatments that included grass were subjected to analysis of variance using a model that included block, concentrate allowance, grass allowance and the concentrate by grass interaction. Data for the ten treatments were subjected to analysis of variance using a model that included block and treatment. All treatment means were compared to the positive control using a Dunnetts test (Steel and Torrie, 1960). For both sets of analysis, the effects of inclusion of intra-muscular fat concentration as a co-variate was also examined. The relationship between intra-muscular fat concentration and fatty acid composition was examined for the three unsupplemented grass treatments by linear regression.

3. Results

3.1. Composition of dietary ingredients

The chemical composition and fatty acid profile of the grass and concentrates is shown in Table 1. The predominant SFA in grass was $C_{16:0}$ (palmitic acid) and the predominant unsaturated fatty acid was $C_{18:3}$ (linolenic acid), both present at 35 g/100 g FAME. The concentrate ration contained a substantially higher proportion of MUFA ($C_{18:1}$) and a lower proportion of PUFA than grass.

Table 1
The chemical composition and fatty acid profiles of grass and concentrates

	Grass	Concentrate
Composition		
Dry matter (DM) (g/kg)	198	872
Crude protein (g/kg DM)	225	143
Ash (g/kg DM)	125	48
Dry matter digestibility (g/kg)	738	843
Crude fibre (g/kg DM)	287	101
Oil (g/kg DM)	29	24
Fatty acids (g/100 g FAME)[a]		
$C_{12:0}$	0.00	0.93
$C_{14:0}$	3.42	1.25
$C_{16:0}$	35.24	32.65
$C_{16:1}$	3.53	0.00
$C_{18:0}$	5.66	6.69
$C_{18:1}$	8.91	39.09
$C_{18:2}$	7.98	16.59
$C_{18:3}$	35.25	2.80

[a] Fatty acid methylesters.

Table 2
The effect of grass dry matter (DM) and concentrate allowances on intake and carcass characteristics

Daily grass DM allowance (kg/head) (G)	6			12			18			Control	EMS[a]	Significance[b]		
Daily concentrate allowance (kg/head) (C)	0	2.5	5	0	2.5	5	0	2.5	5	14.4		G	C	G×C
Number of animals	11	11	11	11	11	11	11	11	11	11				
Grass DM intake (kg/day)	5.59	5.16	5.13	9.37	9.16	8.44	12.46	11.87	10.76					
Carcass weight (kg)	304^{z}	332^{z}	352^{z}	323^{z}	348^{z}	361	330^{z}	355^{z}	363	371	135.9	***	***	N.S.
KCF (kg)[c]	5.4^{z}	7.4^{z}	8.8	6.8^{z}	7.6^{z}	8.9	7.9	9.2	10.3	10.7	5.90	**	***	N.S.
KCF (g/kg carcass)	17^{z}	22	25	21	22	25	24	26	28	29	1.0	*	**	N.S.
Intramuscular fat (g/kg muscle)	28.5^{z}	28.2^{z}	23.2^{z}	22.0^{z}	25.4^{z}	27.7^{z}	23.2^{z}	23.7^{z}	29.0^{z}	44.2	1.39	N.S.	N.S.	N.S.

[a] EMS = error mean square (10 treatments).
[b] Means with superscript 'z' differ ($P < 0.05$) from the ad libitum concentrate (control) group.
[c] KCF = kidney plus channel fat.

3.2. Feed intake and carcass characteristics

The trend for grass intake (Table 2) was that at the low grass allowance, there was little apparent effect of increasing concentrates on grass intake, while at the medium and high grass allowances, increasing concentrate allowance led to a reduction in grass intake. Offering animals an increased concentrate or grass allowance increased ($P < 0.001$) final carcass weight. The positive control group of animals had higher ($P < 0.05$) carcass weight than all other groups except the groups offered the medium or high grass allowance and 5 kg concentrate. Both KCF weight and KCF expressed as a proportion of carcass weight increased with either increasing concentrate ($P < 0.01$) or increasing grass ($P < 0.05$) allowance. When expressed as a proportion of carcass weight, KCF was lower for the group offered the low grass allowance without concentrates than all other treatment groups.

3.3. Intra-muscular fat concentration and fatty acid composition

3.3.1. The interaction between grass and concentrate allowance

There was no effect of grass or concentrate allowance on intra-muscular fat concentration. The proportion of $C_{20:4}$ was the only fatty acid for which intra-muscular fat concentration was a significant co-variate. Accordingly, unadjusted data are presented (except for $C_{20:4}$) in the Tables and discussed in the text.

There was no effect of grass or concentrate allowance on $C_{15:0}$, $C_{17:0}$, $C_{20:1}$, $C_{20:0}$, $C_{20:3}$, $C_{22:0}$, $C_{24:0}$ or $C_{23:0}$ proportions (Table 3). Increasing concentrate allowance increased ($P < 0.01$) the proportion of $C_{14:0}$, and decreased ($P < 0.1$) the proportion of $C_{18:3}$, but increasing grass allowance had no effect. There was an interaction ($P < 0.01$) between grass and concentrate allowance for the intra-muscular proportions of $C_{14:1}$, $C_{16:0}$, $C_{16:1}$, $C_{17:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{20:2}$, $C_{20:4}$ and $C_{20:5}$. The interactions reflect inconsistencies in the effects of concentrate allowance at each grass allowance. Of the predominant fatty acids, there was little effect of the first increment of concentrates at the lower grass allowance while supplementary concentrates general-

Table 3

The effect of grass dry matter (DM) and concentrate allowances on individual fatty acid proportions in intra-muscular triacylglycerols

Daily grass DM allowance (kg/head) (G)	6			12			18			Control	EMS[a]	Significance[b]		
Daily concentrate allowance (kg/head) (C)	0	2.5	5	0	2.5	5	0	2.5	5	14.4		G	C	G × C
Number of animals	11	11	11	11	11	11	11	11	11	11				
Fatty acids (g/kg FAME)[c]														
$C_{14:0}$	23.4	24.1	24.7	22.5	23.6	29.0	23.5	23.8	27.2	27.4	2.06	N.S.	**	N.S.
$C_{14:1}$	2.7[z]	2.8[z]	4.9	2.0[z]	4.9	3.5	4.4	4.6	5.8	5.1	0.32	**	**	*
$C_{15:0}$	5.8	5.6	5.2	5.4	5.7	6.3	5.7	5.7	5.5	5.0	0.24	N.S.	N.S.	N.S.
$C_{16:0}$	259.8	273.1	262.4	265.2	259.7	278.9	256.4	263.3	269.5	265.3	25.94	N.S.	*	*
$C_{16:1}$	36.4[z]	33.3[z]	41.6	32.2[z]	41.2	34.9[z]	42.6	40.3	44.3	43.5	3.90	***	N.S.	**
$C_{17:0}$	16.6	15.6	15.7	15.9	16.1	15.0	16.5	16.1	15.5	16.9	0.27	N.S.	N.S.	N.S.
$C_{17:1}$	7.6[z]	6.9[z]	7.8[z]	6.1[z]	7.7[z]	6.2[z]	8.1[z]	7.6[z]	7.7[z]	9.7	0.18	**	N.S.	*
$C_{18:0}$	190.5[z]	201.0[z]	158.9	208.9[z]	160.8	196.5[z]	159.4	156.8	155.5	136.9	55.84	***	*	***
$C_{18:1}$	421.1[z]	407.3[z]	446.9	412.2[z]	440.3	398.7[z]	450.9	445.6	441.9	456.5	60.24	***	N.S.	***
$C_{18:2}$	18.2	14.1[z]	18.2	15.2[z]	19.4	12.8[z]	16.0[z]	18.0	16.2[z]	22.5	2.15	N.S.	N.S.	***
$C_{18:3}$	8.7[z]	5.6	4.7	7.1	8.9[z]	6.3	6.6	7.6	5.6	3.8	1.60	N.S.	N.S.	N.S.
$C_{20:0}$	0.2	0.2	0.2	0.1	0.2	0.6	0.6	0.1	0.3	0.2	0.03	N.S.	N.S.	N.S.
$C_{20:1}$	1.1	1.6	1.6	1.1	1.5	1.6	1.6	1.6	1.6	1.6	0.12	N.S.	N.S.	N.S.
$C_{20:2}$	0.6	7.7[z]	2.6	2.3	1.5	5.6[z]	1.3	1.6	0.5	1.0	1.12	*	*	***
$C_{22:0}$	0.4	0.2	ND[d]	0.4	ND	ND	0.6	0.3	0.2	0.1	0.06	N.S.	N.S.	N.S.
$C_{20:3}$	0.3	0.1	0.1	ND	ND	0.2	0.1	0.2	0.1	ND	0.01	N.S.	N.S.	N.S.
$C_{20:4}$[e]	2.4	0.6	2.4	1.7	3.9	1.2	3.1	3.1	2.1	1.9	0.35	N.S.	N.S.	***
$C_{24:0}$	0.4	0.1	ND	0.3	ND	0.1	0.1	0.3	ND	0.2	0.03	N.S.	N.S.	N.S.
$C_{20:5}$	1.3	0.1	1.0	0.8	2.3[z]	0.6	1.6	1.5	0.9	0.5	0.13	N.S.	N.S.	**
$C_{23:0}$	0.6	0.2	0.5	0.4	1.3	0.6	0.9	1.0	0.6	0.4	0.07	N.S.	N.S.	N.S.

[a] EMS = error mean square (10 treatments).

[b] Means with superscript ‘z’ differ ($P < 0.05$) from the ad libitum concentrate (control) group.

[c] Fatty acid methyl esters.

[d] ND = not detected.

[e] Adjusted for differences in intra-muscular fat concentration by covariance analysis.

ly had little effects on the fatty acid composition when offered with the high grass allowance. High concentrate supplementation of the low grass allowance decreased the proportion of $C_{16:0}$ ($P<0.1$) and $C_{18:0}$ ($P<0.05$) and increased the proportion of $C_{16:1}$ ($P<0.05$), $C_{18:1}$ ($P<0.05$) and $C_{18:2}$ ($P<0.1$) compared to low concentrate supplementation. The first increment of concentrates at the medium grass allowance increased the proportion of $C_{16:1}$ ($P<0.05$), $C_{18:1}$ ($P<0.1$) and $C_{18:2}$ ($P<0.1$) and decreased the proportion of $C_{18:0}$ ($P<0.05$). Relative to the first increment, the second increment of concentrates decreased the proportion of $C_{18:2}$ ($P<0.05$), $C_{18:1}$ ($P<0.05$) and $C_{16:1}$ ($P<0.05$) and increased the proportion of $C_{18:0}$ ($P<0.05$) and $C_{16:0}$ ($P<0.05$). In the absence of concentrates, the high grass allowance decreased ($P<0.05$) the $C_{18:0}$ proportion and increased ($P<0.05$) the $C_{16:1}$ and $C_{18:1}$ proportions compared to the low grass allowance.

There was an interaction ($P<0.001$) between grass and concentrate allowance for SFA and MUFA concentrations which reflected the patterns observed for individual fatty acids (Table 4). Thus, supplementary concentrates had little effect on SFA and MUFA proportions when offered with the high grass allowance, but high concentrate supplementation of the low grass allowance decreased the SFA and increased the MUFA proportions. The high increment of concentrates at the medium grass allowance increased the SFA and decreased the MUFA proportions compared to the low increment of concentrates. In the absence of concentrates the high grass allowance decreased the proportion of SFA ($P<0.001$) and increased MUFA proportion ($P<0.001$). There were few effects of diet on the total PUFA proportion which tended to increase with the first increment of concentrates and to decrease ($P<0.05$) with the second increment (29.1, 32.1 and 27.0 g/kg respectively). There was an interaction between grass and concentrate allowance for the PUFA: SFA ratio whereby this ratio was higher ($P<0.05$) for the first concentrate increment on the medium grass allowance than either this grass allowance unsupplemented or supplemented with the high concentrate allowance. There was no effect of grass or concentrate allowance on the n-6 PUFA proportion but the n-3 PUFA proportion tended ($P<0.1$) to be decreased

Table 4
The effect of grass dry matter (DM) and concentrate allowances on total fatty acid proportions in intra-muscular triacylglycerols

Daily grass DM allowance (kg/head) (G)	6			12			18			Control	EMS[a]	Significance[b]		
Daily concentrate allowance (kg/head) (C)	0	2.5	5	0	2.5	5	0	2.5	5	14.4		G	C	G × C
Number of animals	11	11	11	11	11	11	11	11	11	11				
Saturated fatty acids (g/kg FAME)[c]	498^z	520^z	468	512^z	468	527^z	463	467	473	452	73.5	***	N.S.	***
Monounsaturated fatty acids (g/kg FAME)	468^z	452^z	503	454^z	496	445^z	507	500	501	516	77.8	***	N.S.	***
Polyunsaturated fatty acids (g/kg FAME)	32	28	29	27	36	27	29	32	25	30	8.5	N.S.	+	N.S.
Polyunsaturated: Saturated ratio	0.064	0.054	0.062	0.052	0.077	0.052	0.062	0.068	0.054	0.067	0.0004	N.S.	+	*
n-6 fatty acids (g/kg FAME)	21.6	22.6	23.1	19.0^y	24.7	19.8	20.4	22.8	18.8^y	25.6	3.98	N.S.	N.S.	N.S.
n-3 fatty acids (g/kg FAME)	10.0^z	5.6	5.7	7.9	11.2^z	6.9	8.2	9.1	6.5	4.3	2.21	N.S.	+	N.S.
n-3: n-6 ratio	0.45^z	0.26	0.27	0.40^y	0.47^z	0.39	0.39^y	0.41^y	0.34	0.18	0.048	N.S.	N.S.	N.S.

[a] EMS = error mean square.
[b] Means with superscript 'y' and 'z' differ ($P<0.1$ and $P<0.05$, respectively) from the ad libitum concentrate (control) treatment.
[c] Fatty acid methylesters.

by concentrate supplementation. There was no effect of grass or concentrate allowance on the n-3: n-6 ratio.

3.3.2. Comparison of grass-based treatments with positive control

The positive control group of animals had a higher ($P < 0.05$) concentrations of intra-muscular fat than all other groups (Table 2). Compared to the positive control group, intra-muscular triacyglycerols from animals offered the high grass allowance had lower ($P < 0.05$) $C_{17:1}$ and $C_{18:2}$ proportions; grass-based diets generally had higher $C_{18:3}$ proportions, low and medium grass-based diets supplemented with 0 or 2.5 kg concentrates had lower ($P < 0.05$) $C_{18:1}$ and higher $C_{18:0}$ proportions (Table 3). Intra-muscular triacylglycerols from the positive control group had lower ($P < 0.05$) SFA and higher ($P < 0.05$) MUFA proportions than that of animals offered the low grass allowance alone or with 2.5 kg concentrates or the medium grass allowance alone or with 5 kg concentrates (Table 4). The total PUFA: SFA ratio for the positive control group did not differ from the other treatment groups. Compared to the positive control group, the n-6 PUFA proportion tended to be lower on the high grass/high concentrate allowance whereas the n-3 PUFA proportion was higher ($P < 0.05$) for the unsupplemented low grass allowance and the medium concentrate/medium grass allowance. The n-3: n-6 ratio tended ($P < 0.1$) to be higher for all grass only treatments compared to the control group.

The relationships between intra-muscular fat concentration and the proportions of selected fatty acids is shown in Table 5 for animals offered unsupplemented grass. The negative relationship with SFA approached significance ($P = 0.06$) but no other significant relationships were detected.

4. Discussion

The composition of the grass was typical of grass harvested in autumn with low digestibility and high crude protein concentrations relative to early or mid-season grass (Munro and Walters, 1987). The fatty acid profile of the grass was similar to that reported by French et al. (2000) for an earlier study with the grass having higher $C_{18:3}$ and lower $C_{18:1}$ and $C_{18:2}$ proportions relative to the concentrates. French et al. (2001) reported the production data for the present study and concluded that, as a strategy for increasing carcass growth on autumn grass, offering supplementary concentrates offers more score than altering grass allowance. However French et al. (2000) showed that increasing concentrate supplementation led to a decrease in the concentration of beneficial fatty acids in muscle when animals had a similar carcass weight. The preferred strategy of French et al. (2001) may have a deleterious effect on beef quality. The objective of this study therefore was to document the effects of the production strategies examined by French et al. (2001) on the fatty acid composition of beef muscle adipose tissue.

Fatty acids were extracted in a commercial laboratory using petroleum ether. Relative to more polar solvents, the extracted lipid therefore probably consisted of triacylglycerol and free fatty acids (Schmidt et al., 1995). Petroleum ether was chosen as solvent, since this is a standard method for measurement of

Table 5
Relationships between intra-muscular fat concentration and fatty acid composition (Y) in steers offered unsupplemented grass

Y	B	Intercept	R.S.D.	R^2 (%)	Significance
$C_{18:1}$	0.669	41.17	2.63	7.7	N.S.
$C_{18:2}$	0.106	1.39	0.44	6.9	N.S.
$C_{18:3}$	0.076	0.565	0.006	3.7	N.S.
n-3 PUFA[a]	0.091	0.651	0.527	3.7	N.S.
n-6 PUFA	0.103	1.781	0.632	3.3	N.S.
SFA[a]	−0.979	51.70	3.162	11.0	$P = 0.06$
MUFA[a]	0.760	45.81	3.188	6.8	N.S.
PUFA	0.207	2.414	1.040	4.8	N.S.

[a] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

fat concentration of meat (AOAC, 1992). In addition, some nutritional labelling schemes define total fat as 'total lipid fatty acids expressed as triglycerides' (USDHHS/FDA, 1993) and it is implied that AOAC methods should be used where practicable. It is recognised that this lipid extraction procedure extracts little or none of the phospholipid fraction (Schmidt et al., 1995). The exclusion of this fraction likely contributes comparatively little to the estimate of caloric value of the muscle, (5.9% vs. 6.9% intramuscular lipid in LD measured using ether or chloroform/methanol, respectively (Marchello et al., 1968). Similarly, Duckett et al. (1993) reported that mean LD crude fat concentrations of 2.62 and 4.09% represented 86% and 98% respectively of total lipid. This fraction however can contribute to the profile of fatty acids since PUFA are located predominantly in the muscle membranes (Duckett et al., 1993). Thus Marchello et al. (1968) reported 1.91% and 2.60% linoleic acid for ether and chloroform–methanol extracts of beef muscle, respectively. The fatty acid profiles reported in the present study are therefore more typical of the fatty acid composition of subcutaneous adipose tissue than total intramuscular lipid since the concentration of phospholipids in the former depot is low (Christie, 1981). Zembayashi et al. (1995) and Choi et al. (2000) reported similar fatty acid profiles for neutral lipids extracted from subcutaneous adipose tissue or LD. However, using a petroleum ether extraction, Westerling and Hedrick (1979) observed that subcutaneous fat contained more palmitic and oleic and less linoleic, linolenic, 11-eicosenoic and arachidonic acids than intramuscular fat. However, no significant difference existed between the two fat depots for total saturated or total unsaturated fatty acids. Roberts (1966) also observed lower linoleic and linolenic acid in external rib fat compared to intramuscular rib fat.

Despite the increases in carcass weight and internal fat deposition due to increases in the allowance of grass and concentrates, within the grass-fed treatments there were no differences in intra-muscular fat concentration. Moreover, intra-muscular fat concentration was not a significant co-variate in the examination of dietary effects on the majority of fatty acids measured. Dietary effects on fatty acid composition within these groups can therefore be examined independent of differences in fatness per se which is known to alter fatty acid composition (Leat, 1978). Within these treatments, the increase in fatty acid unsaturation due to high concentrate supplementation of the low grass allowance reflects, in part, fatty acid consumption, i.e. concentrates represented 46% of total dry matter intake (DMI) for this treatment. It may also reflect an increase in outflow of fatty acids from the rumen, with decreased opportunity for saturation by ruminal microorganisms since DMI was 30% higher for high concentrate compared to low concentrate supplementation. The increase in unsaturation due to low concentrate supplementation of the medium grass allowance could also reflect substitution of grass with concentrate and increased DMI (21%). Why fatty acid saturation was greater for high concentrate supplementation of the medium grass allowance compared to low concentrate supplementation is unclear. It was expected that since the difference in DMI between the two diets is relatively small (13%) the increase in intake of unsaturated fats, together with the likely depression in rumen pH due to the higher proportion of concentrates in the diet would inhibit saturation (Sutton et al., 1987; Doreau and Ferlay, 1994).

Previous work comparing grass and concentrates for finishing steers has shown that beef from steers grazed on pasture contained higher concentrations of the fatty acids $C_{15:0}$, $C_{18:0}$, $C_{18:3}$, $C_{20:3}$ and $C_{20:4}$ and less of $C_{16:0}$ and $C_{17:0}$ than beef from concentrate-fed animals (Brown et al., 1979; Melton et al., 1982). When the fatty acid profile of animals offered the ad libitum concentrate diet is compared with that from the animals offered the grass-based diets, the findings of the present study are largely consistent with the above studies. Our findings also support the observations of Leat (1978) and Itoh et al. (1999) that the oleic acid ($C_{18:1}$) proportion of fatty acids increases and the stearic acid ($C_{18:0}$) proportion decreases as the intra-muscular fat concentration increases leading to a general increase in unsaturation. Itoh et al. (1999) reported that the triacylglycerol faction of LD from steers grazing perennial pasture had lower $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$ and higher $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ proportions than LD from steers finished on a grain-based ration. For grass and the medium concentrate allowance, compared to ad libitum concentrates in the present study similar effects were seen for $C_{14:0}$, $C_{16:1}$, $C_{18:3}$. Differences between the two studies may reflect differences in the fatty acid composition of the feedstuffs examined together with different rates of intra-muscular fat deposition (below). The effect of intra-muscular fat concentration on fatty acid composition was examined within the grass-only treatments to avoid the possible confounding effect of differences in diet composition. That there were few significant relationships probably reflects the narrow range in intra-muscular fat concentration in these samples. The trend ($P = 0.12$) towards an increase in the oleic acid proportion and a decrease in the SFA proportion also supports Itoh et al. (1999). The positive slope for $C_{18:3}$ and $C_{18:2}$, in contrast to the negative slopes reported by Itoh et al. (1999) in a similar analysis may also reflect a relatively greater increase in PUFA consumption than intra-muscular fat accretion since Itoh et al. (1999) observed a range in intra-muscular fat concentration from 50 to 110 g/kg for LD for cattle that consumed annual or perennial pasture whereas the range in this study was 6 to 47 g/kg LD.

French et al. (2000) reported a higher intra-muscular PUFA: SFA ratio in grass-fed animals than concentrate-fed animals when grown to similar carcass fatness. A lower PUFA: SFA ratio in meat from cattle fed grass than concentrates were reported by Enser et al. (1998). However, in that study, the concentrate-fed animals were 1 year old bulls of fat class 2.5 (1 = leanest to 5 = fattest) and the grass fed animals were 1.5 year old steers of fat class 4.33. Fatness affects the PUFA: SFA ratio of total lipid, because the triacyglycerols, which increase with increasing fatness, contain less PUFA than the more constant phospholipids in muscle membranes. In the present study, the trend was for a decrease in PUFA proportion with increased concentrate supplementation. However the lack of effect on the PUFA: SFA ratio also reflects alterations in the SFA proportion. The negative effect of concentrate supplementation on the myristic acid ($C_{14:0}$) proportion is noteworthy, since this SFA is considered to have a greater hypercholesterolaemic effect on humans than palmitic acid ($C_{16:0}$) (Hays et al., 1991), which was also increased by concentrate supplementation. The trend towards a decrease in the stearic acid ($C_{18:0}$) proportion due to concentrate supplementation is of no consequence for human health since it is considered to be neutral with respect to serum cholesterol (Bonanome and Grundy, 1988).

An increase in the consumption of n-3 PUFA has been recommended (American Heart Association, 1986) to overcome the perceived imbalance in the ratio of n-6: n-3 PUFA in current diets (10:1) compared with those of primitive humans (1:1). The general trend in the data in this study was for concentrate supplementation (or ad libitum concentrates) to decrease the n-3 PUFA proportion of fatty acids. A similar finding was reported by French et al. (2000). In the present study, the proportion of n-3 PUFA was generally low and in some samples, not detectable, reflecting in part the solvent used to extract the lipid. The n-3: n-6 ratio was therefore statistically analysed rather than the more common n-6: n-3 ratio. If the ratio of the n-6 and n-3 PUFA treatment means is considered, the ratio for animals offered the grass-based diets was less than 3.8 in contrast to a ratio of 5.6 for animals offered the ad libitum concentrate group. In the study of French et al. (2000), muscle from all groups of steers had an intra-muscular fat n-6: n-3 PUFA ratio $< 4.5:1$. However, the n-6: n-3 PUFA ratio in the intra-muscular fat of steers offered grass only (mean 2.33) was approximately half that of the treatment group offered the highest concentrate allowance (mean 4.15). Marmer et al. (1984) and Wood and Enser (1997) similarly observed a lower n-6: n-3 PUFA ratio in grass-fed cattle than in concentrate-fed cattle.

It is concluded that increasing grass and concentrate allowance increased carcass growth rate and fatness. As a strategy for optimising carcass growth, similar carcass growth can be achieved from an allowance of 18 kg autumn grass DM and 5 kg concentrates as from ad libitum concentrates. However, meat from the former ration has a lower fat concentration and a more favourable n-3 and n-6 PUFA profile from a human health perspective.

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