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Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets[1]

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ABSTRACT: The effects of grazed grass, grass silage, or concentrates on fatty acid composition and conjugated linoleic acid (*cis*-9, *trans*-11-18:2; CLA) concentrations of i.m. fat of steers fed to achieve similar carcass growth rates were investigated. Fifty steers were divided into 10 blocks based on body weight and assigned at random from within blocks to one of five dietary treatments. The experimental rations offered daily for 85 d preceding slaughter were 1) grass silage for ad libitum intake plus 4 kg of concentrate, 2) 8 kg of concentrate plus 1 kg of hay, 3) 6 kg of grazed grass DM plus 5 kg of concentrate, 4) 12 kg of grazed grass DM plus 2.5 kg concentrate, or 5) 22 kg of grazed grass DM. The concentration of polyunsaturated fatty acids (PUFA) in i.m. fat was higher ($P < .05$) for steers offered ration 5 than for those given any other ration. Decreasing the proportion of concentrate in the diet, which effectively increased grass intake, caused a linear decrease in the concentration of i.m. saturated fatty acids (SFA) ($P < .01$) and in the *n*-6:*n*-3 PUFA ratio ($P < .001$) and a linear increase in the PUFA:SFA ratio ($P < .01$) and the conjugated linoleic acid concentration ($P < .001$). The data indicate that i.m. fatty acid composition of beef can be improved from a human health perspective by inclusion of grass in the diet.

Key Words: Conjugated Linoleic Acid, Fatty Acids, Grasses, Steers

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Introduction

Consumption of saturated fatty acids (**SFA**) 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**) are antithrombogenic (Ulbricht and Southgate, 1991). Ruminant fat has a higher SFA and a lower PUFA:SFA ratio than nonruminant fat, due to hydrogenation of dietary unsaturated fatty acids in the rumen. Strategies that lead to an increase in the PUFA:SFA ratio in i.m. fat would improve the healthiness of beef from a consumer perspective. Although there is evidence that consumption of grass increases the ratio of *n*-3 to *n*-6 PUFA, many studies are confounded by differences in carcass weight and(or) fatness (Marmer et al., 1984; Enser et al., 1998). The first objective of this study was to determine the impact on i.m. fatty acid composition of grazed grass, grass silage, and concentrates in the diet of steers with similar carcass growth rates.

Ruminant fats are among the richest natural sources of conjugated linoleic acid (**CLA**) isomers, in particular the *cis*-9, *trans*-11 isomer (Chin et al., 1992), which arises from microbial hydrogenation of dietary linoleic acid in the rumen (Kepler and Tove, 1967). Conjugated linoleic acid is reported to be anticarcinogenic (Schultz et al., 1992; Parodi, 1994; Belury, 1995) and antiatherogenic (Nicolosi and Laitinen, 1996), to enhance growth (Chin et al., 1994), and to decrease body fat in rats (Pariza et al., 1996). Including grass in the diet of dairy cows increased CLA concentration in milk (Stanton et al., 1997; Kelly et al. 1998; Lawless et al. 1998), but the CLA content of ruminant tissue fat has been studied to a much lesser extent. The second objective of this study was to determine the impact of the inclusion of grazed grass in the diet, on the concentration of CLA in i.m. fat.

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Materials and Methods

Experimental Design and Animal Management

Fifty continental crossbred steers (mean live weight of 504 [SD = 38.4] kg) were ranked on descending live weight into blocks of five and within block assigned at random to one of five dietary treatments. The experimental rations offered daily were 1) grass silage for ad libitum intake plus 4 kg of concentrate (**SC**), 2) 8 kg of concentrate plus 1 kg of hay (**CO**), 3) 6 kg of grazed grass dry matter (DM) plus 5 kg of concentrate (**CG**), 4) 12 kg of grazed grass DM plus 2.5 kg of concentrate (**GC**), and 5) 22 kg of grazed grass DM (**GO**).

The groups of steers offered grass each rotationally grazed 2 ha of grassland. Pregrazing herbage mass was estimated by cutting three strips (each 5 × 1.2 m; 4-cm stubble height) from the areas to be grazed by each treatment group. Daily herbage allowances were achieved by varying the size of the allocated grazing area. Postgrazing herbage mass for each treatment was estimated by cutting four strips, (each 5 × 0.55 m) to a 4-cm stubble height from the area last grazed by the steers. Herbage intake was estimated as the difference between pre- and postgrazing mass. Animals offered SC and CO were accommodated in a slatted-floor shed and offered their rations individually using electronic gates. The grass silage was a primary growth of perennial ryegrass cut with a rotary mower, harvested within 15 min with a precision-chop harvester and ensiled without an additive; it was offered daily in sufficient amounts to ensure 10% refusals. The pelleted concentrate, which was a mixture of ground barley (46%), unmolassed sugar beet pulp (42%), soybean meal (8%), tallow (1%), and a proprietary mineral/vitamin mix (3%), was offered individually to all animals. Animals receiving concentrates along with grass were constrained individually in a specially built mobile feeder in the field until concentrates were consumed (typically 15 min). Animals offered GC were offered their concentrate allowance once daily, and animals offered CG were offered their allowance in two equal feedings. The duration of the feeding period was 85 d, beginning August 22. On the day of slaughter, animals were weighed, transported 15 km to a commercial slaughter facility, and slaughtered within 2 h of removal from the Grange Research Centre. After slaughter, cold carcass weight (hot carcass weight × .98) was recorded. Carcass weight gain was estimated as the difference between final carcass weight and 55% initial live weight.

Fatty Acid Analysis

Immediately after slaughter all carcasses were hung by the Achilles tendon for 48 h at 4°C. The longissmus muscle was then excised, and a steak weighing approximately 250 g was removed from the interior of each muscle and trimmed of intermuscular and s.c. fat and ground using a food processor (Robot Coupe, R301 Ultra). Intramuscular fat was extracted from the ground muscle using a modification of the Folch wash method as described by Ways and Hanrahan (1964). A ground sample (1 g) was homogenized in 30 mL of dichloromethane and methanol (2:1, vol:vol), using an Ultra Turrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik) and filtered through Whatman paper. The residue was re-extracted twice as described above, and the filtrates were combined, mixed with .12 M KCl at a ratio of filtrate:KCl of 2:1 in a separating funnel, and allowed to stand for phase separation. The lower layer containing the lipid was decanted and mixed with methanol:water (1:1, vol:vol) at a ratio of 4:1 and allowed to settle into two phases. The lower layer containing the lipid in dichloromethane was decanted, filtered through 10 g of anhydrous sodium sulphate, and dried in a rotary evaporator at 40°C.

Intramuscular fatty acids were quantified as their fatty acid methyl esters (**FAME**), prepared by acid-catalyzed methanolysis (Stanton et al, 1997). The extracted fat was dissolved by heating to 60°C. To the melted fat was added 12 mL of methanolic HCl (4%), and the samples were methylated by incubation at 60°C for 20 min. After cooling to room temperature, 2 mL of water was added and the samples were extracted with 5 mL of hexane, washed twice with water, and dried over anhydrous sodium sulphate for 2 h. The FAME in the hexane layer were quantified using a Varian 3500 GLC (Varian, Harbor City, CA) fitted with a flame ionization detector. All samples were methylated in duplicate and each sample was injected twice onto the GLC column, using a splitless injector and a Varian 8035 auto-sampler. Separation of the FAME was performed on a Supelcowax-10 capillary GLC column (Supelco, Bellefonte, PA) (60 m × .32 mm i.d; .25 mm film thickness), using He as the carrier gas. The GLC conditions have been described previously (Lawless et al., 1998). Data were recorded and analyzed on a Minichrom PC system (VG Data System, Manchester, U.K.). Individual fatty acids were identified by retention time with reference to fatty acid standards, and the *cis*-9, *trans*-11 CLA isomer was identified with reference to the CLA standard mix generously provided by M. Pariza (The Food Research Institute, University of Wisconsin, Madison). The response factors of the individual fatty acids were calculated relative to the area of 16:0 (palmitic acid), which was assigned a response factor of 1.00. Fat was extracted from the grass, grass silage, 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).

Statistical Analyses

All variables were subjected to analysis of variance using a model that included block and treatment as

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Table 1. The chemical composition of grass, grass silage, and concentrates

Composition	Grass	Concentrate	Grass silage
Crude protein, g/kg DM	224	125	149
Ash, g/kg DM	123	75	91
Dry matter, g/kg	181	880	224
Oil, g/kg DM	29	26	28
Fatty acids			
(g/100 g fatty acid methylesters)			
14:0	4.64	2.45	5.44
16:0	20.81	32.70	24.00
16:1	2.37	.80	.58
18:0	3.29	20.59	2.90
18:1	5.74	25.07	6.32
18:2	14.00	16.53	14.53
18:3	49.15	1.86	46.23

main effects. When a significant effect of treatment was detected, differences between means were examined using a multiple range test. A significance level of $P \leq .05$ was used. The relationships between concentrate intake from grass-based rations and selected variables were determined using regression analysis.

Results and Discussion

The objectives of this experiment were to determine the fatty acid composition and the CLA concentration in i.m. fat of steers with a similar rate of carcass growth offered rations of varying proportions of grass and concentrates. A grass silage plus concentrate ration was also examined, because this is a common preslaughter ration for beef cattle in Western Europe. The chemical composition of the dietary components offered to the five groups of steers is shown in Table 1. The composition of the grass was typical of autumn grass with low DM and high crude protein concentrations relative to early- or mid-season grass (Munro and Walters, 1987). The oil content of the grass, concentrate, and silage was similar, and no fatty acids with ≤ 14 carbons were detected in any of the feeds used. Although the grass and grass silage exhibited similar fatty acid profiles, grass contained a lower proportion of SFA and a higher proportion of unsaturated fatty acids than grass silage. The dominant SFA in both grass and grass silage was palmitic acid, at 20.8 and 24.0 g/100 g FAME, respectively, and the dominant unsaturated fatty acid was linolenic acid, at 49.2 and 46.2 g/100 g FAME in grass and grass silage, respectively. The concentrate ration contained more SFA, with higher levels of 16:0 and 18:0 (stearic acid), and lower levels of 14:0 (myristic acid) relative to the forage, and more MUFA, mainly 18:1 (oleic acid), and less PUFA than the grass or grass silage. The linoleic acid (18:2) concentration of the concentrate (16.5 g/100 g FAME) was higher than that of grass or grass silage (both contained 14.0 g/100 g FAME).

The estimated forage DM intake was .82, 4.71, 7.51, or 12.6 kg/d for rations CO, CG, GC, and GO, respectively. Concentrates represented 900, 490, 230, or 0 g/kg DM, respectively, for rations CO, CG, GC, and GO. The daily intake of steers offered ration SC was 5.8 kg grass silage DM and 3.6 kg concentrate DM.

Of the studies that have examined the effect of inclusion of forage in the ration on fatty acid profiles of beef fat, most have compared grass with ad libitum concentrates (Hidiroglou et al., 1987; Mandell et al., 1997). In such studies, because of the higher energy intake associated with the concentrate rations, concentrate-fed animals were heavier and had fatter carcasses when grown over a predetermined time period. As animals become fatter an increasing proportion of the fat deposited is in the form of MUFA (Leat, 1978). Thus, changes in fatness due to differences in energy intake can confound effects of ration type on fatty acid composition in intramuscular fat. In the present study, carcass weight and carcass weight gains were similar for all rations, as planned (Table 2), and there was no effect of ration type on i.m. fat, ash, protein, or moisture concentrations in the longissimus muscle. A possible confounding effect of the effects of ration type on fatty acid composition due to differences in the amount of fat deposited was therefore avoided. Because i.m. fat content was low for all rations (less than 4.5%), the steaks from all steers can be classified as low-fat foods (NRC, 1988).

With respect to 14:0 (myristic acid), 16:0, 18:0, 18:1 and 18:2, the concentrations for CO (Table 3) were in the range of those reported by Rule et al. (1995) for longissimus muscle. Decreasing the proportion of concentrate in the diet, which effectively increased grass intake, caused a linear ($P < .01$) decrease in i.m. SFA concentration. The relationship is best described by this equation: SFA concentration (g/100g FAME) = .59 concentrate intake (kg) + 42.98, (r = .69). The i.m. SFA concentration of SC did not differ ($P > .05$) from that of CO but was lower ($P < .05$) than that of all other treatments. The decline in SFA in i.m. fat was principally due to a decline in 16:0 concentrations, which reflected the lower concentration of 16:0 in grass than in grass silage and concentrate (Table 1), and thus lower 16:0 intake. With the exception of 10:0, which represented a minor component of total SFA, there was no effect of treatment on any other i.m. SFA or on the total concentrations of MUFA in i.m. fat (Table 3). The concentration of PUFA in i.m. fat was higher ($P < .05$) for animals offered GO than for those offered any other ration, among which no significant differences were noted.

Decreasing concentrate intake in grass-based diets resulted in a linear increase in the PUFA:SFA ratio in i.m. fat, which was best described by the equation PUFA:SFA ratio = .0044 concentrate intake (kg) + .12, (r = .48). The mean PUFA:SFA ratio of .102 observed in this study is similar to that of .11 previously re-

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Table 2. The effect of diet on carcass weight and chemical composition of the longissimus muscle

	Treatment[a]						
Item	SC	CO	CG	GC	GO	SE	SIG
Carcass weight, kg	330	330	326	330	324	5.0	NS
Carcass gain, kg/d	.63	.60	.60	.62	.59	.026	NS
Longissimus muscle							
Ash, g/kg	10.7	10.9	10.7	11.4	13.5	3.36	NS
Protein, g/kg	222	239	226	235	233	21.0	NS
Moisture, g/kg	719	716	711	717	711	19.4	NS
Lipid, g/kg	40.8	34.1	44.9	40.2	43.6	4.6	NS

[a]The treatments were grass silage for ad libitum intake plus 4 kg of concentrate (SC), 8 kg of concentrate plus 1 kg of hay (CO), 6 kg of grass dry matter (DM) plus 5 kg of concentrate (CG), 12 kg of grass DM plus 2.5 kg of concentrate (GC), and 22 kg of grass DM (GO).

ported for beef by Enser et al. (1998). Duckett et al. (1993) reported a higher PUFA:SFA ratio (.26) for muscle from grass-finished steers than for that from concentrate-finished animals (.07). However, in that study, concentrate-fed animals were much fatter (21.08 vs 3.05 mm backfat) than grass-finished animals. 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 15:0, 18:0, 18:3, 20:3 and 20:4

Table 3. The effect of diet on intramuscular fatty acid composition (g/100 g fatty acid methylesters)

	Treatment[a]						
Fatty acid	SC	CO	CG	GC	GO	SE	*P*-value[b]
10:0	.25[wx]	.13[y]	.17[xy]	.31[w]	.12[y]	.021	*
12:0	.09	.08	.08	.08	.09	.003	NS
14:0	2.76	2.34	2.52	2.61	2.71	.049	NS
14:1	.63	.60	.59	.66	.66	.022	NS
15:0	.58	.59	.61	.62	.66	.16	NS
16:0	26.55[w]	27.4[w]	24.72[x]	24.07[x]	22.84[y]	.291	***
16:1	3.73	3.98	3.86	3.82	3.88	.057	NS
17:0	1.20	1.22	1.17	1.19	1.20	.018	NS
17:1	.97	1.19	1.05	.99	1.05	.026	0.06
18:0	16.04	15.95	16.13	15.51	14.72	.273	NS
18:1	39.47	38.64	38.62	39.61	40.58	.255	NS
18:2	2.60	2.96	2.60	2.32	2.11	.105	NS
18:2 (conjugated linoleic acid)	.47[yz]	.37[z]	.54[xy]	.66[x]	1.08[w]	.040	***
18:3	.71[z]	.72[z]	.87[y]	1.01[x]	1.13[w]	.031	***
20:0	.05	.23	.04	.23	.09	.032	NS
20:1	.07[y]	.04[y]	.06[y]	.28[w]	.12[x]	.028	*
20:2	.09[xy]	.07[y]	.14[xy]	.17[x]	.34[w]	.027	*
20:5	.20	.12	.27	.24	.23	.023	NS
20:3	.14[yz]	.09[z]	.17[y]	.26[x]	.38[w]	.024	***
20:4	.14	.10	.21	.30	.32	.029	NS
SFA[c]	47.72[w]	48.07[w]	45.71[x]	44.86[x]	42.82[y]	.415	***
MUFA[c]	41.83	41.48	40.90	42.31	43.07	.249	.08
PUFA[c]	4.14[w]	4.93[w]	4.53[w]	4.71[w]	5.35[x]	.29	.05
n-6 Fatty acids	2.96	3.21	3.12	3.04	3.14	.106	NS
n-3 Fatty acids	.91[y]	.84[y]	1.13[x]	1.25[wx]	1.36[w]	.042	***
n-6:*n*-3 ratio	3.61[w]	4.15[w]	2.86[x]	2.47[x]	2.33[x]	.197	**
PUFA:SFA	.09[w]	.09[w]	.10[w]	.11[wx]	.13[x]	.007	**

[a]The treatments were grass silage for ad-libitum intake plus 4 kg of concentrate (SC), 8 kg of concentrate plus 1 kg of hay (CO), 6 kg grass of dry matter (DM) plus 5 kg of concentrate (CG), 12 kg of grass DM plus 2.5 kg of concentrate (GC), and 22 kg of grass DM (GO).

[b]NS = not statistically significant.

[c]SFA = total saturated fatty acids, MUFA = total monounsaturated fatty acids, PUFA = total polyunsaturated fatty acids.

[w,x,y,z]Means within rows with common superscripts are not significantly different ($P > .05$).

*$P < .05$.

**$P < .01$.

***$P < .001$.

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and less of 16:0 and 17:0 (Brown et al., 1979; Melton et al., 1982) than beef from concentrate-fed animals.

There was no effect of treatment on *n*-6 fatty acid concentration in i.m. fat (Table 3). Decreasing concentrate intake increased the *n*-3 fatty acid concentration and linearly decreased the *n*-6:*n*-3 ratio. The latter relationship was best described by this equation: *n*-6:*n*-3 ratio = .3008 concentrate intake (kg) + .21 (r = .79). Animals offered grass had higher intakes of *n*-3 PUFA because of the higher concentration of 18:3 in grass than in concentrate (approximately 30 times higher) (Table 1). The *n*-6:*n*-3 PUFA ratio of SC did not differ ($P > .05$) from that of CO but was higher ($P < .05$) than that of any other treatment. 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). In this study, muscle from all groups of steers had an i.m. fat *n*-6:*n*-3 PUFA ratio < 4.5:1. However, the *n*-6:*n*-3 PUFA ratio in the i.m. 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.

Chromatograms of the FAME in i.m. fat of a steer offered 22 kg of grass DM daily, of a steer fed 8 kg of concentrate plus 1 kg of hay daily, and of the standard CLA mixture are shown in Figure 1. The CLA isomer, *cis*-9, *trans*-11 18:2, was detected in all samples and constituted approximately .65 g/100 g FAME, whereas the CLA isomer, *trans*-10, *cis*-12 18:2, was not detected in i.m. fat. It should be noted that other isomers such as *cis*-8, *trans*-10 CLA may co-elute with *cis*-9, *trans*-11 18:2, but the latter has been shown to be the major CLA isomer in beef fat (Chin et al., 1992). Discussion of the effects of treatment on CLA, therefore, refers predominantly to the *cis*-9, *trans*-11 18:2 isomer only. Decreasing the proportion of concentrate in the diet caused a linear ($P < .001$) increase in i.m. CLA concentration. The relationship is best described by this equation: CLA concentration (g/100g FAME) = -.079 concentrate intake (kg) + .98, (r = .83). Mean i.m. CLA concentration for SC did not differ ($P > .05$) from that of CO or CG but was lower ($P < .05$) than that of the other two treatments. Shanta et al. (1994) reported CLA concentrations ranging from .31 to .85 g/100 g fat in raw steaks. Chin et al. (1992) reported beef fat CLA concentrations from .29 to .38 g/100 g fat, whereas a survey of German foods (Fritsche and Steinhart, 1998) reported that CLA content of beef fat ranged from .12 to 1.20 g/100 g. The concentration of CLA in lean Australian beef was reported to range from .23 to 1.25 g/100 g fat (Fogerty et al., 1988).

There are several reports of an increase in milk fat CLA concentration associated with increased grass intake (Dhiman et al., 1996; Stanton et al., 1997; Kelly

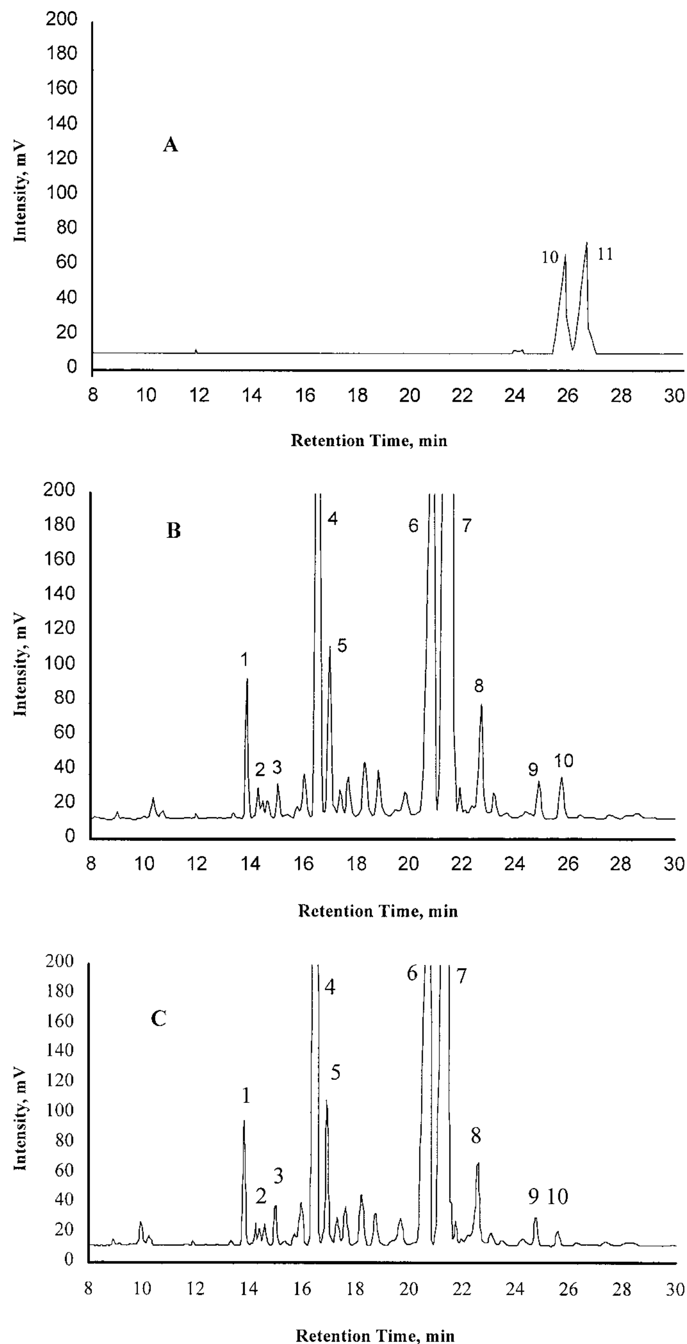

Figure 1. Partial chromatogram of fatty acid methylesters following separation by capillary gas liquid chromatography of (A) standard mixture of conjugated linoleic acid (CLA) isomers, (B) intramuscular fat of an animal offered 22 kg of grass dry matter (DM) daily for 85 d before slaughter, and (C) intramuscular fat of an animal offered 7.2 kg of concentrate DM and 0.82 kg of hay DM daily for 85 d before slaughter. The peaks indicated are 1, myristic acid; 2, myristoleic acid; 3, pentadecanoic acid; 4, palmitic acid; 5, palmitoleic acid; 6, stearic acid; 7, oleic acid; 8, linoleic acid; 9, linolenic acid; 10, *cis*-9, *trans*-11 CLA isomer, and 11, *trans*-10, *cis*-12 CLA isomer.

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et al., 1998). Kelly et al. (1998) observed a greater than twofold increase (to 10.9 mg/g of milk fat) in CLA concentration when comparing a grass silage and a concentrate ration with a grass ration. The pathway of CLA synthesis from linoleic acid (18:2) in the rumen involves an initial isomerization step, resulting in the formation of a conjugated *cis*-9, *trans*-11-octadecanoic acid. This undergoes hydrogenation to *trans*-11-octadecanoic acid and further hydrogenation to stearic acid (Kepler and Tove, 1967). This reaction is catalyzed by linoleic acid isomerase produced by the ruminal bacterium *Butyrivibrio fibrisolvens* (Kepler and Tove, 1967). The factors that determine the amount of CLA that is available for absorption from the gastrointestinal tract are the dietary intake of 18:2 and rumen conditions that may affect the growth and activity of *Butyrivibrio fibrisolvens*. In the present study, the dietary intake of 18:2 was similar across all treatments. This suggests that grass in the diet favored the growth of *Butyrivibrio fibrisolvens*. The high concentrations of rapidly fermentable sugar and soluble fiber that are found in grass create an environment that promotes a greater production or a decreased utilization of CLA by the rumen (Kelly et al., 1998). Dhiman et al. (1996) postulated that the lower concentration of CLA found in milk fat of cows fed grass silage in comparison to grazed grass was because the concentrations of sugars and soluble fiber were decreased during the ensiling process. This altered the ruminal environment in animals consuming the silage either to the detriment of the CLA-producing bacteria or to the benefit of bacteria utilizing CLA. The results of this study support that hypothesis.

Implications

The data obtained from this study demonstrate that high grass intake resulted in a higher polyunsaturated fatty acid (PUFA):saturated fatty acid ratio and a lower n-6:n-3 PUFA ratio in intramuscular fat of steers than in that of similar steers fed concentrates. Moreover, a higher concentration of conjugated linoleic acid was observed for grass-fed steers than for steers fed silage and(or) concentrates, when grown at similar carcass growth rates. These data imply that the fatty acid profile of intramuscular fat in beef can be improved from a human nutrition perspective by the inclusion of grass in the diet.

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