Occurrence of Conjugated Linoleic Acid Isomers in Beef

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ABSTRACT: The amounts of Δ9,Δ11-conjugated linoleic acid (CLA) isomers were determined in loin-associated fat samples of bulls ($n = 6$) and steers ($n = 7$) by capillary gas chromatography of fatty acid methyl ester (FAME) derivatives. The main CLA-isomer—18:2 *c*9,*t*11—provided approximately 0.76 ± 0.15% and 0.86 ± 0.15% of total FAME in bulls and steers, respectively. No differences ($P > 0.05$) were observed between the CLA isomer distribution of bulls (*t*9,*c*11, 0.026 ± 0.014%; *c*9,*c*11, 0.015 ± 0.008%; and *t*9,*t*11, 0.029 ± 0.003%) and steers (*t*9,*c*11, 0.027 ± 0.014%; *c*9,*c*11, 0.015 ± 0.005%; and *t*9,*t*11, 0.030 ± 0.007%).
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KEY WORDS: Beef, bulls, conjugated linoleic acid isomers, gas chromatography, steers.

Conjugated linoleic acid (CLA) describes a mixture of octadecadienoic fatty acid moieties that contain two conjugated double bonds. Increasing interest in CLA was sparked by the anticarcinogenic activity in animal model systems and human cell cultures (1). Additionally, CLA has been reported to reduce atherosclerosis and total cholesterol and to alter the low-density lipoprotein/high-density lipoprotein cholesterol ratio in rabbits (2).

The dietary sources of CLA are mainly food products derived from ruminants, such as beef, lamb, and milk products (3,4). So far, little is known about the natural CLA isomer distribution in foodstuffs. Previous studies focused mainly on the principal CLA isomer, 18:2 *c*9,*t*11. However, it is unknown if the main isomer is the biologically active CLA compound, and earlier studies indicated the occurrence of additional CLA isomers (5,6). Recently, Fritsche *et al.* (7) identified three CLA minor isomers—18:2 *t*9,*c*11, 18:2 *t*9,*t*11, and 18:2 *c*9,*c*11—in human adipose tissue. The aim of this study was to examine the occurrence of these minor isomers in beef samples.

MATERIALS AND METHODS

Subcutaneous and intermuscular fat samples were obtained from musculus longissimus dorsi of bulls and steers (German Simmental). Animals were fed maize silage and concentrate and slaughtered at live weights of 500–650 kg. The feeding regimen and growth parameters are described elsewhere (8).

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All reagents and solvents were of analytical grade and supplied by Merck (Darmstadt, Germany). Conjugated linoleic acid standards—18:2 *c*9,*c*11 [81% purity, determined by gas chromatography (GC)] and 18:2 *t*9,*t*11 (96% purity, determined by GC) were purchased from Matreya (Pleasant Gap, PA). A CLA methyl ester mixture (containing 37.9% 18:2 *c*9,*t*11, 27.6% 18:2 *t*10,*c*12, 1.0% 18:2 *c*9,*c*11, and 3.2% 18:2 *t*9,*t*11) was obtained from Sigma (Deisenhofen, Germany). AOAC method 969.33 was used to convert the standard fatty acids to the fatty acid methyl ester (FAME) derivatives (9).

The analytical methods for lipid extraction, FAME preparation, and GC analysis have been described previously (4). Identity of Δ9,Δ11-CLA isomers was confirmed by GC–mass spectrometry and GC–direct deposition–Fourier transform infrared spectrometry (7). Statistical comparisons between bulls and steers were performed with student's *t*-test (level of significance: $P < 0.05$).

RESULTS AND DISCUSSION

No differences were observed between the amount of CLA isomers in intermuscular and subcutaneous fat. Data were therefore summarized. Values expressed as percentages of total FAME are presented in Table 1 (including 18:2 *c*9,*c*12 for comparison). The results of the 18:2 *c*9,*t*11 amounts are in good agreement with previous studies that showed 0.17–0.65 g 18:2 *c*9,*t*11/100 g fat in beef (10) and 0.65% 18:2 *c*9,*t*11 of total FAME in beef fillet (4).

Besides the CLA main isomer—18:2 *c*9,*t*11—three minor CLA isomers could be determined in fat samples of bulls and steers (Fig. 1). The ratio of 18:2 *c*9,*c*11 to 18:2 *c*9,*t*11 (1:50–1:60) was in accordance with results of Werner *et al.* (6), who found a ratio of 1:50–1:100 in cheese. The ratio of 18:2 *t*9,*t*11 to 18:2 *c*9,*t*11 reported by these authors (≈ 1:10)

TABLE 1
Amounts of Δ9,Δ11-Conjugated Linoleic Acid Isomers and Linoleic Acid in Fatty Tissue of Bulls and Steers (mean ± standard deviation)

	Steers ($n = 7$) (% of total FAME[a])	Bulls ($n = 6$) (% of total FAME)
18:2 *c*9,*t*11	0.859 ± 0.146	0.762 ± 0.151
18:2 *t*9,*c*11	0.027 ± 0.014	0.026 ± 0.014
18:2 *c*9,*c*11	0.015 ± 0.005	0.015 ± 0.008
18:2 *t*9,*t*11	0.030 ± 0.007	0.029 ± 0.003
18:2 *c*9,*c*12	1.113 ± 0.126	1.363 ± 0.309

[a]FAME, fatty acid methyl ester.

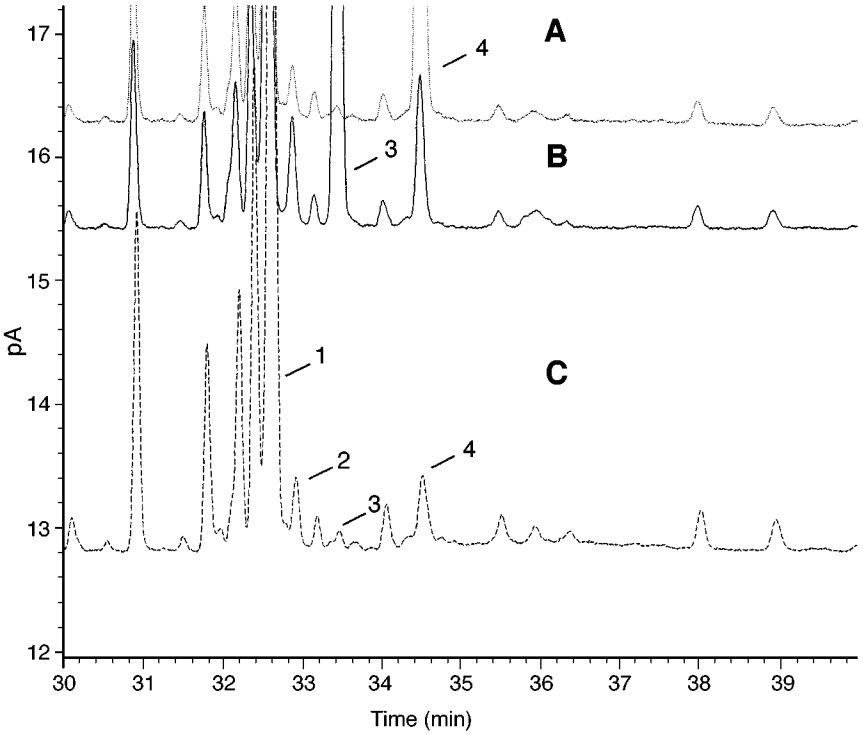

FIG. 1. Conjugated linoleic acid region of gas chromatograms obtained on a CP Sil 88 column (50 m × 0.25 mm) of fatty acid methyl esters (FAME). Steer fat sample (A) spiked with 18:2 *t*9,*t*11, (B) spiked with 18:2 *c*9,*c*11, (C) unspiked sample. Peak identification: (1) 18:2 *c*9,*c*11; (2) 18:2 *t*9,*c*11; (3) 18:2 *c*9,*c*11; and (4) 18:2 *t*9,*t*11.

exceeded the results of the present study (≈ 1:30), and may be due to a coelution with 18:2 *t*10,*t*12. Some authors have claimed that 18:2 *t*10,*c*12 occurs naturally, for example in dairy products (5,11). This isomer could not be detected in the beef samples analyzed in the present study, however. Probable reasons for the occurrence of 18:2 *t*10,*c*12 may be the transesterification procedures used (12) or the conversion of allylic hydroxy oleate to CLA (13).

No difference in CLA amounts or isomer distribution could be observed between fat samples from bulls and steers (Table 1). The CLA profile does not seem to be influenced by the different hormonal status and resulting differences in the growth performance of the animals. Consistently, Shantha *et al.* (14) could not detect an effect of administered zeranol (estrogenic anabolic implant) on the concentrations of 18:2 *c*9,*t*11 in beef. Recent results obtained with silver-ion high-performance liquid chromatography indicate that the gas chromatographically separated geometrical CLA peaks consist of different positional isomers.

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