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Short Communication: The Nature of Heptadecenoic Acid in Ruminant Fats

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ABSTRACT

Heptadecenoic acid (17:1) is a minor constituent of ruminant fats and its isomeric definition remains undefined in most reports on ruminant milk and intramuscular fat. Samples of milk and intramuscular fat of bovine, ovine, and caprine origin were analyzed by gas chromatography (GC) using 3 capillary columns with and without addition of 17:1 *cis*-10. Additionally, *cis* isomers of ovine milk fat samples were isolated as methyl esters by preparative thin-layer chromatography and analyzed by GC. The structural analysis of 17:1 present in samples was achieved by chemical ionization tandem mass spectrometry techniques. The isomer 17:1 *cis*-9 is the overwhelming heptadecenoic isomer in ruminant milk and intramuscular fat; 17:1 *cis*-10 is virtually absent. Moreover, current GC methods were able to resolve *cis*-9 from *cis*-10 and *cis*-8 isomers, so reports on 17:1 contents in ruminant fat should define its isomeric composition.

Key words: gas chromatography-tandem mass spectrometry, heptadecenoic acid, milk fat, muscle

Heptadecenoic acid (17:1) has long been recognized as minor constituent of ruminant fats (Shorland and Jessop, 1955). Recently it was proposed, along with other odd-chain fatty acids, as a potential marker of microbial biomass (Vlaeminck et al., 2005). The odd-chain fatty acids are assumed to be mainly of rumen microbial origin and, after intestinal absorption, they are deposited in tissues or exported to milk fat. However, the origin of 17:1 is not clear. Recently Fievez et al. (2003) suggested that it could be an endogenous product of Δ^9-desaturation of heptadecanoic acid (17:0). This would imply that the main 17:1 isomer in ruminant fats is 17:1 *cis*-9. However, in spite of the earliest reports indicating that 17:1 *cis*-9 is the main isomer in ruminant fat (Hansen et al., 1960; Hay and Morrison, 1970, 1973), most of the recent reports are ambiguous. In fact, most reports either do not present 17:1 or leave it undefined, whereas others call it 17:1 *cis*-10 (Franklin et al., 1999; Marks et al., 2004; Loor et al., 2005). The fact that the methyl ester standard available from commercial chemical companies is the *cis*-10 isomer, which probably coelutes with *cis*-9 isomer in some gas chromatography (**GC**) systems certainly contributes to the ambiguity. Our objective was to clarify which 17:1 isomers are predominant in ruminant fats through GC chemical ionization-tandem mass spectrometry techniques and to determine if common GC conditions can resolve 17:1 isomers.

Samples tested were chosen from several experimental sets analyzed for fatty acid composition in our laboratory, and included milk fat from bovine, ovine, and caprine, and intramuscular fat of lambs, kids, and beef. Lipid extraction in samples was conducted according to Folch et al. (1957) and methyl esters were prepared using a base-catalyzed transesterification procedure described by Christie (1993). The *cis* isomers of ovine milk fat were separated by preparative TLC on plates impregnated with 20% $AgNO_3$ and were located by spraying with 2′,7′-dichlorofluorescein solution in isopropanol. Samples were also spiked with 17:1 *cis*-10 standard obtained from Sigma Chemical Co. (St. Louis, MO). Fatty acid methyl esters were resolved in 3 GC systems: 1) Varian Saturn 2000 (Varian Inc., Walnut Creek, CA) equipped with ion-trap mass detector and a BPX-70 (SGE Chromatography Supplies, Austin, TX) capillary column (60 m, 0.25 mm i.d., 0.25-μm film thickness); 2) Agilent HP6890 (Agilent Tech. Inc., Palo Alto, CA) equipped with a flame-ionization detector and a CP-Sil 88 (Chrompack CP 7489, Varian Inc.) capillary column (100 m, 0.25 mm i.d., 0.20-μm film thickness); and 3) Varian 3800 GC equipped with a flame-ionization detector and an OmegaWax 250 (Supelco, Bellefont, CA) capillary column (30 m, 0.25 mm i.d., 0.25-μm film thickness). Helium was the carrier gas in all systems.

Structural analysis of 17:1 methyl ester isomers was conducted in the chemical ionization-tandem mass spectrometry mode of the Varian Saturn 2000 system. Ion-trap parameters used in all analyses presented included: trap temperature, 170°C; manifold temperature, 80°C; transfer line temperature, 200°C; and axial

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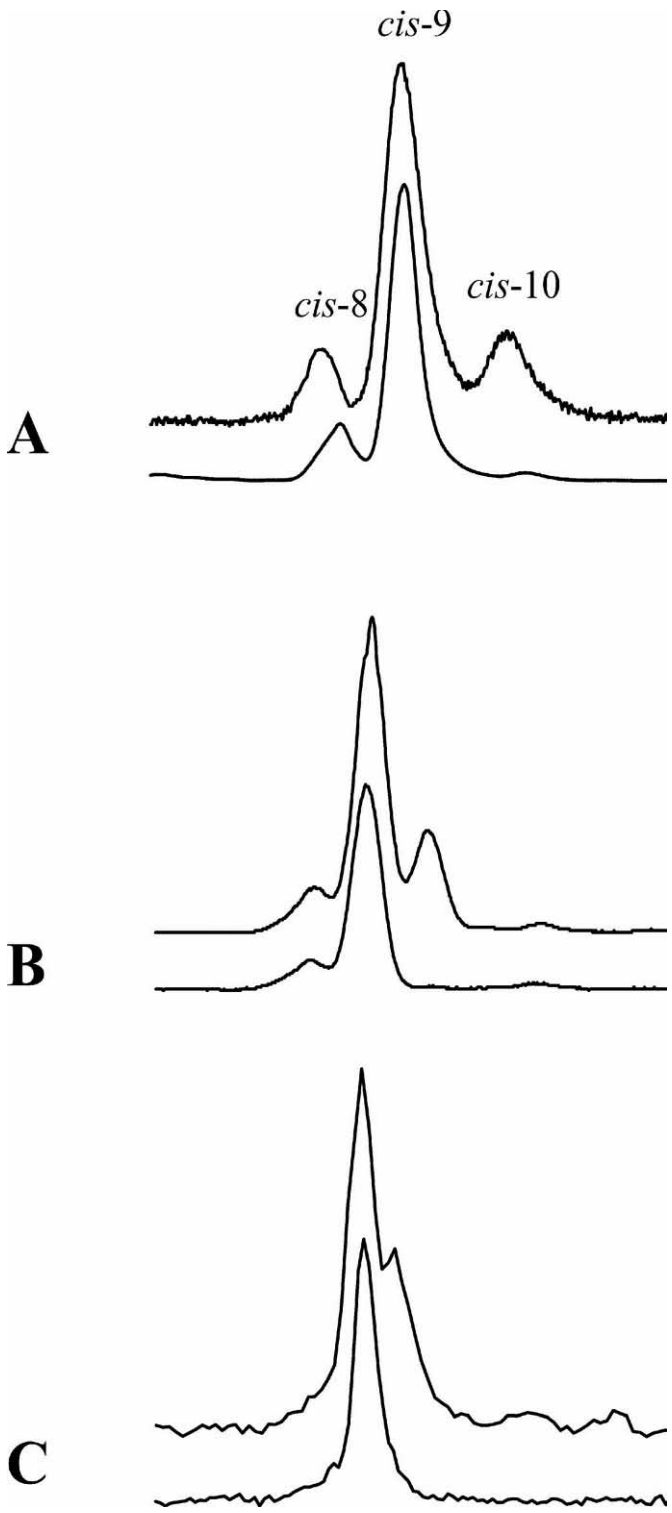

Figure 1. Partial chromatogram of the 17:1 region of the *cis* fraction of ovine milk fat sample analyzed using 3 different columns: 100-m CP-Sil 88 (A), 30-m OmegaWax (B), and 60-m BPX-70 (C). The lower curve in each panel represents the sample, and the upper curve represents the sample spiked with 17:1 *cis*-10 standard.

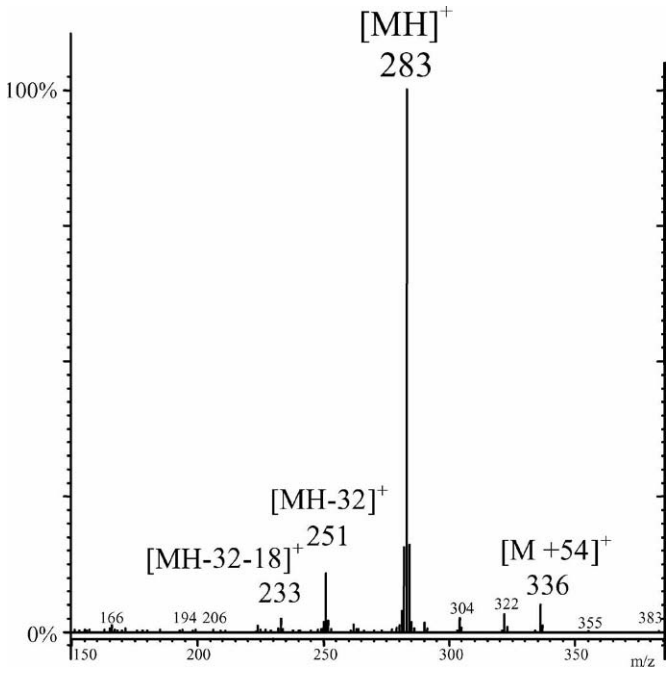

Figure 2. Acetonitrile chemical ionization-mass spectrum of 17:1 *cis*-9 methyl ester of ovine milk fat sample, showing the ions $[MH-32-18]^+$, $[MH-32]^+$, MH^+, and $[M + 54]^+$.

modulation amplitude, 3.8 V. Acetonitrile was used as chemical ionization (**CI**) reagent gas with the following CI parameters: CI storage level, 19 m/z; ejection amplitude, 15 m/z; maximum ionization time, 2,000 μsec; maximum reaction time, 40 ms; prescan ionization time, 200 μsec; and target total ion current, 5,000 counts. Additional tandem mass spectrometry parameters were as follows: emission current, 40 μA; scan time, 0.34 s; mass isolation window, 3 m/z; and excitation storage level, 85 m/z. The resonant excitation amplitudes used to collisionally dissociate the $(M + 54)^+$ ions varied from 1.60 to 2.0 V.

Samples of ovine, bovine, and caprine origin were resolved in the 3 GC systems with and without addition of 17:1 *cis*-10 methyl ester standard (Figure 1). The presence of *cis*-10 isomer was not detected in all samples studied. All samples spiked with 17:1 *cis*-10 showed a well-defined peak (presumably 17:1 *cis*-9) and occasionally other minor peaks (presumably 17:1 *cis*-8) preceding the 17:1 *cis*-10 peak. Thin-layer chromatography isolation of the *cis* fraction of ovine milk fat samples allowed the evidence of the *cis*-8 isomer peak. Precht and Molkentin (2000), using a 100-m CP-Sil 88 column, reported good resolution between *cis*-8 and *cis*-9 17:1 isomers in human milk fat. Hay and Morrison (1970) reported that *cis*-8 was the second major 17:1 isomer in cow milk fat, comprising 20.1% of total heptadecenoates. We found in the *cis* fraction of ovine milk that

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