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Variation of Milk Citrate with Stage of Lactation and De Novo Fatty Acid Synthesis in Dairy Cows

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ABSTRACT

Citrate is a normal constituent of milk that affects milk-processing characteristics. It is an intermediate in the tricarboxylic acid cycle and plays an indirect role in fat synthesis by providing reducing equivalents in the form of NADPH. The objective of this study was to investigate variation in citrate with stage of lactation and de novo fatty acid synthesis, without confounding dietary effects. Twenty-four cows were fed the same diet, and milk citrate and fatty acids were determined over a 10-d period. Eight cows were in early lactation [13 ± 1.8 d in milk (DIM; mean ± standard error], 8 in midlactation (130 ± 4.6 DIM), and 8 in late lactation (283 ± 3.4 DIM). For cows in early, mid, and late lactation, milk yield was 34.4, 34.4, and 21.4 L/d [standard error of difference (SED) 1.78]; milk fat was 50.4, 40.3, and 41.4 g/L (3.68); milk citrate was 11.3, 9.7, and 10.1 mmol/L (0.64); the ratio of 4–14 C:18–20 C fatty acids was 0.9, 1.3, and 1.2 (0.07). Activity of the fatty acid synthase enzyme system (EC 2.3.1.85) was calculated as acetate used for chain elongation (ACE); ACE (mol/d) for cows in early, mid, and late lactation, was 7.3, 11.1, and 8.1 (SED 1.05). For individual cows, citrate (mmol/L) = 14.3 − 0.44 × ACE (r^2 = 0.58). We propose that ACE provides a more accurate indication of synthase activity than do fatty acid ratios or yields. This study confirms the hypothesis that variation in milk citrate with stage of lactation is related to de novo synthesis of fatty acids and that the relationship is independent of diet and milk yield.
Key words: dairy cow, milk citrate, stage of lactation, fatty acid synthesis

INTRODUCTION

Citrate is a normal constituent of milk and forms one of the main buffer systems that regulates the equilibrium between Ca^{2+} and H^+ ions (Faulkner and Peaker, 1982). Citrate affects milk-processing characteristics because it interacts with other milk constituents to influence coagulation of milk protein and its fermentation products yield distinct aromatic flavors characteristic of fermented milk products (Rosenthal, 1991). The biological role of citrate in milk is unknown, but its main role is thought to be maintenance of fluidity through its effects on structure of casein micelles (Faulkner and Peaker, 1982).

Citrate plays a central role in cellular energy metabolism, being an intermediate in the tricarboxylic acid cycle. It has been proposed as an indicator of energy status in the cow, being correlated with ketones in milk (Baticz et al., 2002). However, mammary epithelium is impermeable to citrate in both directions (Linzell et al., 1976), so milk citrate concentration reflects mammary activity rather than general metabolism.

In ruminants, citrate is not a significant intermediate for fatty acid synthesis. Nevertheless, citrate has an indirect role in fat synthesis by providing reducing equivalents in the form of NADPH, which are required for de novo synthesis of fatty acids (Faulkner and Peaker, 1982). In de novo synthesis, each cycle of chain elongation uses 2 molecules of NADPH, similar amounts of which are produced by the pentose phosphate pathway and by the isocitrate cycle (Moore and Christie, 1981). In the isocitrate cycle, NADPH is produced by conversion of isocitrate to α-ketoglutarate. Isocitrate is an isomer of citrate and the 2 are maintained in equilibrium in the cell (Peaker and Faulkner, 1983). Therefore, if de novo synthesis of fatty acids increases, isocitrate concentration decreases, and citrate concentration decreases. These relationships are supported by the studies of Banks et al. (1984, 1990), which used fat supplements to decrease de novo synthesis of fatty acids in the mammary gland and found proportional increases in milk citrate concentration.

In long-term studies, concentrations of citrate in milk have been found to vary according to season and stage of lactation. In general, citrate concentrations are higher during the grazing season (Holt and Muir, 1979; Mitchell, 1979; Keogh et al., 1982) and during early lactation

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(Braunschweig and Puhan, 1999). These effects might be related to de novo synthesis of fatty acids in the mammary gland, which is reduced when cows are fed fresh pasture (Lock and Garnsworthy, 2003) or when cows are rapidly mobilizing body fat (Peaker et al., 1981). However, in all long-term studies of milk citrate, effects of stage of lactation and season have been confounded not only with each other, but also with changes in diet.

The objectives of this study were to determine variation in milk citrate concentration with stage of lactation independently of dietary or seasonal effects, and to see if variation in milk citrate is related to de novo synthesis of fatty acids in the mammary gland. These objectives were achieved using milk samples from cows at different stages of lactation fed the same diet at the same time.

MATERIALS AND METHODS

Animals, Diets, and Sampling

Twenty-four Holstein cows were selected from the University of Nottingham dairy herd according to DIM, with 8 cows in early lactation (4 to 29 DIM), 8 in midlactation (103 to 156 DIM), and 8 in late lactation (265 to 306 DIM). All cows were fed ad libitum the same TMR, which consisted of corn silage (34% of DM), grass silage (14%), soybean meal (17%), wheat (15%), brewers' grains (9%), palm kernel meal (8%), ruminally inert fat (Megalac, Volac International, Royston, UK; 2%) and a mineral and vitamin supplement (Hi-Phos, Bibby Agriculture, Peterborough, UK; 1%). The composition of the TMR was 459 g of DM/kg, 12.1 MJ of ME/kg of DM, 180 g of CP/kg of DM, 207 g of starch/kg of DM, 49 g of oil/kg of DM, and 347 g of NDF/kg of DM. All cows were fed as one group, so individual feed consumption could not be measured. The TMR had been fed to the herd for more than 1 mo before this study commenced. Cows were milked twice daily between 0500 and 0700 h, and between 1500 and 1700 h. Milk samples were collected daily at both milkings for 10 consecutive days. One 20-mL aliquot of each milk sample was stored at 4°C with preservative (30 mg of potassium dichromate; Lactab MkIII tablet, Thomson and Capper Ltd., Runcorn, Cheshire, UK) until analyzed for fat, protein, lactose, and SCC by infrared analysis at the National Milk Records Laboratory (Harrogate, Yorkshire, UK) using reference method (AOAC, 1990; method no. 972.16). Two additional aliquots were stored without preservative at –20°C for determination of milk citrate and fatty acids. Milk samples from a.m. and p.m. milkings were analyzed separately.

Milk Analysis

Milk citrate was determined by HPLC. Samples were thawed at room temperature and defatted by centrifugation at $1,970 \times g$ for 10 min to separate the fat layer. Deproteinization was carried out by treatment of a 0.4-mL aliquot of skimmed milk with 3.6 mL of cold 3% TCA to precipitate proteins, followed by centrifugation at $17,700 \times g$ for 10 min. A 1.5-mL aliquot of supernatant was placed in an HPLC vial, and stored at –20°C until analysis.

The HPLC apparatus (model Gynkotek, Jaytee Biosciences Ltd, Whitstable, Kent, UK) consisted of a gradient pump (m480G), a Gina 50 autosampler, a UVD340 diode array detector, and an Inertsil C8, 5-μm column (150 × 4.6 mm i.d.). The mobile phase was 98% 0.1 *M* KH_2PO_4 (pH 3.0 with H_3PO_4 + 2% acetonitrile). The flow rate was 1.0 mL/min and the UV detector was set at 218 nm. The column temperature was 40°C. Data were analyzed by Chromeleon software (Dionex, Camberley, UK).

Milk fat was extracted by centrifugation and proportions of individual fatty acids were determined as fatty acid methyl esters (**FAME**) by gas chromatography following the procedures described by Feng et al. (2004). A butter-oil reference standard (CRM 164; Commission of the European Community Bureau of References, Brussels, Belgium) was used as a routine check for recoveries and correction factors for individual fatty acids.

Calculations and Statistical Analysis

Individual yields of milk constituents at morning and afternoon milkings were summed each day and divided by daily milk yield to produce daily mean concentrations. All daily data were averaged for each cow before statistical analysis.

Proportions of individual milk fatty acids determined by gas chromatography were converted to molar proportions and daily yields following the calculations of Schauff et al. (1992). Area percentages for FAME were corrected using the butter-oil correction factors to account for recoveries of individual FAME. Corrected areas (g/100 g of FAME) were then adjusted by removing the mass of the methyl group to produce proportions of fatty acids (g/100 g of fatty acids). Molecular weights of fatty acids were then used to calculate molar proportions (mmol/mol of fatty acids). Unidentified fatty acids were assigned a dummy molecular weight equivalent to 18:1 after examination of their contributions to the total peak area and retention times relative to identified fatty acids. It was assumed that milk fat was 100% triglycerides containing 3 mol of fatty acids/mol of glycerol (Schauff et al., 1992). Therefore, molar proportions