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Incremental effect of a calcium salt of *cis*-monounsaturated fatty acids supplement on milk fatty acid composition in cows fed maize silage-based diets

K. E. Kliem,*[1] C. K. Reynolds,* D. J. Humphries,* R. M. Kirkland,† C. E. S. Barratt,* K. M. Livingstone,* and D. I. Givens*
*Division of Food Production and Quality, School of Agriculture, Policy and Development, University of Reading, Reading RG6 6AR, United Kingdom
†Volac International Ltd., Royston, Hertfordshire SG8 5QX, United Kingdom

ABSTRACT

In most Western countries, saturated fatty acid (SFA) intake exceeds recommended levels, which is considered a risk factor for cardiovascular disease (CVD). As milk and dairy products are major contributors to SFA intake in many countries, recent research has focused on sustainable methods of producing milk with a lower saturated fat concentration by altering dairy cow diets. Human intervention studies have shown that CVD risk can be reduced by consuming dairy products with reduced SFA and increased *cis*-monounsaturated fatty acid (MUFA) concentrations. This milk fatty acid profile can be achieved by supplementing dairy cow diets with *cis*-MUFA-rich unsaturated oils. However, rumen exposure of unsaturated oils also leads to enhanced milk *trans* fatty acid (TFA) concentrations. Because of concerns about the effects of TFA consumption on CVD, feeding strategies that increase MUFA concentrations in milk without concomitant increases in TFA concentration are preferred by milk processors. In an attempt to limit TFA production and increase the replacement of SFA by *cis*-MUFA, a preparation of rumen-protected unsaturated oils was developed using saponification with calcium salts. Four multiparous Holstein-Friesian cows in mid-late lactation were used in a 4 × 4 Latin square design with 21-d periods to investigate the effect of incremental dietary inclusion of a calcium salt of *cis*-MUFA product (Ca-MUFA; 20, 40, and 60 g/kg of dry matter of a maize silage-based diet), on milk production, composition, and fatty acid concentration. Increasing Ca-MUFA inclusion reduced dry matter intake linearly, but no change was observed in estimated ME intake. No change in milk yield was noted, but milk fat and protein concentrations were linearly reduced. Supplementation with Ca-MUFA resulted in a linear reduction in total SFA (from 71 to 52 g/100 g of fatty acids for control and 60 g/kg of dry matter diets, respectively). In addition, concentrations of both *cis*- and *trans*-MUFA were increased with Ca-MUFA inclusion, and increases in other biohydrogenation intermediates in milk fat were also observed. The Ca-MUFA supplement was very effective at reducing milk SFA concentration and increasing *cis*-MUFA concentrations without incurring any negative effects on milk and milk component yields. However, reduced milk fat and protein concentrations, together with increases in milk TFA concentrations, suggest partial dissociation of the calcium salts in the rumen.
Key words: milk fatty acid, calcium salt, monounsaturated fatty acid, *trans* fatty acid

INTRODUCTION

Dietary fat content and composition is considered one of the most important modifiable determinants of cardiovascular disease (**CVD**) risk, with a 1% reduction in intake of energy from SFA predicted to result in a 3% decrease in CVD risk, based on its effect on low density lipoprotein-cholesterol (Givens, 2009). The United Kingdom exceeds its dietary target for SFA intake, by around 10% on average (Bates et al., 2010). In the United Kingdom, milk and dairy products contribute about 27% of total SFA consumed (Bates et al., 2010), with higher contributions in other EU countries (Hulshof et al., 1999). Intervention studies have shown that replacing SFA with *cis*-MUFA in dairy products has favorable effects on serum cholesterol levels, which may lead to a reduction in CVD risk over a longer period (Wood et al., 1993; Tholstrup et al., 1998; Seidel et al., 2005).

Numerous studies have shown the potential of dairy cow nutrition to produce milk with reduced SFA and increased MUFA (e.g., Kirchgessner et al., 1967; Ashes et al., 1992; Jenkins, 1998). A series of studies at the University of Reading demonstrated that the inclusion

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[1]Corresponding author: k.e.kliem@reading.ac.uk

of crushed rapeseeds in the diet of the dairy cow can reduce SFA from 70 to 55 or 60% of total FA while increasing *cis*-MUFA from 20 to 33% of total FA (Givens et al., 2009). However, the increase in *cis*-MUFA concentration in response to feeding full-fat rapeseed was accompanied by an increase in *trans*-18:1 concentration, which is a consequence of rumen biohydrogenation of the rapeseed oil fed. An alternative approach is to feed rumen-protected sources of *cis*-MUFA, which should minimize the development of *trans*-monoenes and maximize the increase in *cis*-MUFA concentration in milk. In previous studies, a variety of approaches have been used to reduce the rumen biohydrogenation of rapeseed or canola oil FA with varying success (e.g., Chouinard et al., 1997; Enjalbert et al., 1997). Calcium salts of canola oil FA have been effective in elevating *cis*-MUFA concentration of milk (Ferlay et al., 1992), but not without an increase in *trans*-isomer concentrations (Enjalbert et al., 1997; Chouinard et al., 1997, 1998), suggesting that Ca-salts of FA from rapeseed or canola oil are at least partially susceptible to rumen biohydrogenation. This may be due in part to the differences in the dissociation constant of the Ca-salts formed, which vary with the degree of their unsaturation (Chouinard et al., 1997 and 1998).

The objectives of the present study were to measure the effect of feeding increasing amounts of a Ca-salt of high *cis*-MUFA on feed intake, milk yield and composition, and milk FA composition in lactating Holstein cows.

MATERIALS AND METHODS

Design, Animals, and Management

The effect of incremental doses of a high-C18:1 Ca-salt product was measured using a balanced 4 × 4 Latin square design experiment with 21-d experimental periods. Four multiparous, mid- to late-lactation Holstein-Friesian cows were used with (mean ± SE) liveweight of 727 ± 23.0 kg, parity of 5.8 ± 0.75, milk yield of 29 ± 2.9 kg/d, and 244 ± 25.1 DIM at the start of the study. Cows were house in individual tiestalls equipped with a rubber mattress and bedded with wood shavings. Clean water and trace mineralized blocks (Rockies, Tithebarn Ltd., Cheshire, UK) were available ad libitum. Cows were milked in situ at 0530 h and 1530 h.

Experimental Diets

Diets were offered as a TMR using computerized feeders (Insentec, Marknesse, the Netherlands; forage:concentrate ratio 50:50 on a DM basis) with the forage component consisting of maize silage and grass silage (750 and 250 g/kg of forage DM, respectively). Treatments consisted of a control diet containing no supplemental lipid (control) or the same basal diet with Ca-salts containing a high proportion of *cis*-MUFA (**Ca-MUFA**; Volac International Ltd., Royston, UK) fed at incremental inclusion rates of 20, 40, and 60 g/kg of ration DM (CS2, CS4, and CS6, respectively), to provide supplement intakes of 440, 880, and 1320 g/day at a predicted mean daily DM intake of 22 kg. The supplement was produced by industrial saponification of an 18:1-rich oil, resulting in a vigorous reaction leading to a product rich in fine particles. The supplement was incrementally included in rations over the first 4 d of each experimental period. Ingredients and chemical composition of the experimental diets are shown in Table 1. The supplements were added to the basal TMR, rather than replacing specific components, and thus diluted the other ingredients proportionally. Feed refusals were removed and weighed before the morning milking.

Experimental Measurements, Sample Collection, and Chemical Analysis

Feed intake was recorded daily. Representative samples of the 4 TMR, individual forages (maize silage, grass silage) and concentrates [concentrates, Sopralin (Trouw Nutrition, Belfast, UK), Ca-MUFA] were taken on d 16 to 20 of each experimental period, bulked and stored in sealed bags at −20°C. A representative sample of refused feed was taken during the last 5 d of each experimental period and analyzed for DM content (100°C) to determine individual DM intakes. Samples of forages and concentrates were stored frozen until analyzed for NDF, ADF, OM, CP, water-soluble carbohydrates, starch, and estimated ME concentrations according to reference procedures as outlined elsewhere (Kliem et al., 2008).

Milk yields were recorded daily throughout the study. Samples of milk preserved with potassium dichromate (1 mg/mL, Broad Spectrum Microtabs, D&F Control Systems Inc., Dublin, CA) for the determination of fat, CP, and lactose by infrared spectroscopy (Milkoscan FT6000 infrared analyzer, Foss Ltd., Warrington, UK) were collected during the last 3 d of each experimental period. Additional samples of unpreserved milk were also collected on the last day of each period and stored at −20°C until composited according to yield, and submitted for FA analysis.

Lipids in 1 mL of milk and appropriate sample weights of forage, concentrate, and lipid supplement samples was extracted and transesterified to fatty acid methyl esters (**FAME**), and then FAME were separated using standard methods (Kliem et al., 2008).