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Short-term effects of dietary *trans* fatty acids compared with saturated fatty acids on selected measures of inflammation, fatty acid profiles, and production in early lactating Holstein dairy cows

Jason S. Watts,* Pedram Rezamand,*[1] Dallace L. Sevier,† William Price,† and Mark A. McGuire*
*Department of Animal and Veterinary Science, and
†College of Agricultural and Life Sciences, University of Idaho, Moscow 83844

ABSTRACT

Feeding rations supplemented with fats may provide an opportunity to manipulate the health and performance of dairy cows; however, the relative effects of specific fats, such as *trans* fatty acids (TFA), are poorly understood. The objective of this study was to investigate the effects of a ration supplemented with TFA on the fatty acid (FA) profile of peripheral blood mononuclear cells (PBMC), plasma lipids, and milk; the gene expression of inflammatory markers; production of acute phase proteins; and production performance in early lactating dairy cows. *Trans* fat was fed at 0, 1.5, and 3% of dry matter, replacing (1:1 wt:wt) saturated fatty acids (SFA). Multiparous lactating Holstein cows at 7 d in milk (n = 12) were randomly assigned to a treatment sequence in a 3 × 3 balanced Latin square design; each period lasted 14 d. Milk and heparinized blood were collected on d 0 (pretreatment) and on d 10 and 14 of each period. Plasma was collected and solid-phase extraction was used to isolate plasma phospholipids and nonesterified fatty acids. Additionally, PBMC were isolated for FA analysis and gene expression analysis by reverse transcription-PCR using bovine *RPS9* as the endogenous control. The FA composition of PBMC, plasma lipid fractions, and milk were analyzed by gas chromatography. Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC). As dietary TFA increased, the percentage of some 18:1 *trans* isomers increased in PBMC, plasma lipids, and milk. Dietary TFA had no detectable effect on mRNA expression of proinflammatory *TNFα* or *IL6*. Expression of *IL1β* and *ICAM1* decreased with increasing TFA. In addition, supplementation of TFA did not affect percentages of milk fat, protein, lactose, or solids-not-fat, or somatic cell count. Overall, dietary TFA increased the *trans* FA present in PBMC, plasma lipids, and milk; however, dietary TFA decreased PBMC expression of some of the proinflammatory markers tested at the mRNA level compared with SFA in early lactating dairy cows. Together, these findings provide evidence that over short period of times, dietary TFA might be slightly less immune-stimulatory than dietary SFA.

Key words: *trans* fat, inflammation, fatty acid, gene expression

INTRODUCTION

Acute inflammation contributes to the occurrence and severity of economically important diseases such as mastitis (Contreras and Rodríguez, 2011). In addition to providing energy and serving as critical structural components of all cells, dietary fats play a critical role in both the precipitation and resolution of inflammation (Calder, 2008). Furthermore, the various structural properties of FA correspond to different functional properties within a cell. Saturated fats and *trans* fats are 2 distinct groups of FA that have garnered a great deal of attention for their potential negative effects on health. The presence of *trans* fats in the human diet, in particular, has come under intense public scrutiny in recent years. Consumption of *trans* fats is associated with induction of systemic inflammation and represents a greater dietary risk factor for cardiovascular disease than saturated fat (Mozaffarian, 2006). The *trans* double bond results in FA more structurally similar to saturated fats than to naturally occurring *cis* unsaturated fatty acids (**UFA**), leading to reduced cell membrane fluidity and possibly altered cell signaling (Roach et al., 2004). Naturally occurring *trans* FA arise during biohydrogenation of UFA in the rumen; however, the main source of *trans* fats in the human diet is from industrial hydrogenation of vegetable oils (Steinhart et al., 2003). Dietary *trans* FA (**TFA**) are associated with induction of an inflammatory state via production of proinflammatory cytokines by cells of the immune system and the endothelium (Han et al., 2002; Harvey et al., 2008). During the periparturient period, when cows are particularly susceptible to infection and

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[1]Corresponding author: rezamand@uidaho.edu

inflammation, shifts in the composition of plasma lipids are coupled with changes in the FA composition of immune cells (Contreras et al., 2010), and if fed, TFA have the potential to integrate into cell membranes and alter immune cell function through various processes, including alteration in expression of genes encoding for proinflammatory mediators (Calder, 2008). We have recently demonstrated that in an in vitro model of bovine mammary epithelial cells, TFA, compared with no *trans* FA, increased mRNA expression of both *IL-1β* and intracellular adhesion molecule (***ICAM***)-1 (Rezamand and McGuire, 2011), although mammary epithelial cells (in vitro) and mononuclear cells (ex vivo) may behave differently in response to TFA treatment. Changes in gene expression at the epithelium or in circulating immune cells have the potential to systemically alter the inflammatory state of an animal. In response to an immune stressor, cytokines produced locally by monocytes, macrophages, or epithelial cells act systemically by signaling the liver to produce acute phase proteins (Petersen et al., 2004). Concentration of the acute phase protein haptoglobin usually peaks in healthy cows shortly after parturition at >0.5 mg / mL and then typically returns to <0.3 mg/mL by the second week postpartum (Humblet et al., 2006; Huzzey et al., 2009). Haptoglobin concentration was previously shown to be modified by dietary safflower oil during the periparturient period due to alterations in the ratio of n-6 to n-3 PUFA (Silvestre et al., 2011).

Therefore, we hypothesized that increasing dietary intake of *trans* fat, compared with increasing dietary SFA, would increase TFA incorporation into plasma lipid fractions and peripheral blood mononuclear cells (**PBMC**; as circulating immune cells, which includes both monocytes and lymphocytes, responsive to dietary treatment), and thus upregulate gene expression of inflammatory mediators in PBMC and increase the plasma concentration of acute phase proteins.

The objective of the present research was to investigate the ability of calcium salts of TFA fed to periparturient Holstein cows to be incorporated into plasma lipid fractions and PBMC compared with a diet providing SFA. A specific aim of the study was to determine if dietary TFA would alter the FA composition of PBMC and affect basal expression of proinflammatory cytokines and adhesion molecules in PBMC compared with dietary SFA.

MATERIALS AND METHODS

Animals, Treatments, and Experimental Design

Twelve multiparous Holstein cows at 7 DIM were randomly assigned to a treatment sequence in replicated paired 3 × 3 balanced Latin squares. Cows were fed 3 diets containing a graded amount of calcium salt of TFA (EnerGTR; Virtus Nutrition, Corcoran, CA) and SFA (EnerGII; Virtus Nutrition). The FA composition of the supplements is shown in Table 1. Mixtures of TFA and SFA were completely mixed into a lactation ration at 3% of diet DM to contain 3% SFA (**SAT**), 1.5% SFA:1.5% TFA (**MIX**), or 3% TFA (**TRANS**). The basal lactation ration (50.2% DM) consisted of (on a DM basis) dairy alfalfa (11.65%), triticale silage (45.32%), canola meal (9.71%), dried distillers grain (9.71%), rolled corn (9.71%), rolled barley (11.65%), sodium bicarbonate (0.33%), and a premix of minerals and vitamins (1.94%) that contained Ca (17.5%), P (0.17%), S (0.3%), Mg (3.1%), Fe (812 mg/kg), Zn (2,822 mg/kg), Mn (1,741 mg/kg), Se (13.5 mg/kg), vitamin A (275,000 IU/kg), vitamin D (77,000 IU/kg), and vitamin E (1320 IU/kg). The basal ration to which supplemental fats were added contained (on a DM basis) 18 ± 1.00% CP (34.7% soluble protein as a % of CP), 25.8 ± 1.77% ADF, 39.3 ± 2.65% NDF, 5.43 ± 0.13% lignin, 3.63 ± 0.12% ether extract, 8.39 ± 0.09% ash, 0.72 ± 0.08% Ca, 0.46 ± 0.02% P, 1.55 ± 0.06% K, 0.30 ± 0.01% Mg, and 1.52 ± 0.02 Mcal of NE_L/kg. Cows were housed in individual box stalls and milked twice daily at 0700 and 1900 h. Diets were fed ad libitum twice daily to ensure 5 to 10% orts. Feed was provided for each cow individually, and intake and milk yield were recorded daily. After each 14-d period, cows were moved to the next treatment without any washout period. All animal procedures were approved by the University of Idaho Animal Care and Use Committee (protocol 2010-29).

Sample Collection

Pretreatment samples (d 0) were taken when each cow reached 7 DIM, and blood and milk samples were taken on d 10 and 14 of each period. Blood was collected via coccygeal venipuncture using 10-mL BD Vacutainer tubes containing 143 USP units of sodium heparin (BD Diagnostics, Franklin Lakes, NJ). Peripheral blood mononucleocytes were isolated from 15 mL of blood by gradient centrifugation using Histopaque 1077 (Sigma Aldrich, St. Louis, MO). Blood was mixed 1:1 with PBS-citrate dextrose solution (80:20 vol:vol). The mix was layered on Histopaque 1077. After centrifugation at 456 × *g* for 1 h at room temperature, plasma was collected and stored at −80°C. The buffy coat was collected and washed 3× with 50 mL of PBS:acid citrate dextrose. To lyse remaining red blood cells, 2 mL of water was briefly added to the cell pellet, and then the cells were resuspended in PBS, aliquoted, and stored at −80°C for RNA and FA analysis. Cells collected for