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Effect of unsaturated fatty acids and triglycerides from soybeans on milk fat synthesis and biohydrogenation intermediates in dairy cattle

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ABSTRACT

Increased rumen unsaturated fatty acid (FA) load is a risk factor for milk fat depression. This study evaluated if increasing the amount of unsaturated FA in the diet as triglycerides or free FA affected feed intake, yield of milk and milk components, and feed efficiency. Eighteen Holstein cows (132 ± 75 d in milk) were used in a replicated 3 × 3 Latin square design. Treatments were a control (CON) diet, or 1 of 2 unsaturated FA (UFA) treatments supplemented with either soybean oil (FA present as triglycerides; TAG treatment) or soybean FA distillate (FA present as free FA; FFA treatment). The soybean oil contained a higher concentration of *cis*-9 C18:1 (26.0 vs. 11.8 g/100 g of FA) and lower concentrations of C16:0 (9.6 vs. 15.0 g/100 g of FA) and *cis*-9,*cis*-12 C18:2 (50.5 vs. 59.1 g/100 g of FA) than the soybean FA distillate. The soybean oil and soybean FA distillate were included in the diet at 2% dry matter (DM) to replace soyhulls in the CON diet. Treatment periods were 21 d, with the final 4 d used for sample and data collection. The corn silage- and alfalfa silage-based diets contained 23% forage neutral detergent fiber and 17% crude protein. Total dietary FA were 2.6, 4.2, and 4.3% of diet DM for CON, FFA, and TAG treatments, respectively. Total FA intake was increased 57% for UFA treatments and was similar between FFA and TAG. The intakes of individual FA were similar, with the exception of a 24 g/d lower intake of C16:0 and a 64 g/d greater intake of *cis*-9 C18:1 for the TAG compared with the FFA treatment. Compared with CON, the UFA treatments decreased DM intake (1.0 kg/d) but increased milk yield (2.2 kg/d) and milk lactose concentration and yield. The UFA treatments reduced milk fat concentration, averaging 3.30, 3.18, and 3.11% for CON, FFA, and TAG treatments, respectively. Yield of milk fat, milk protein, and 3.5% fat-corrected milk remained unchanged when comparing CON with the UFA treatments. No differences existed in the yield of milk or milk components between the FFA and TAG treatments. The UFA treatments increased feed efficiency (energy-corrected milk/DM intake), averaging 1.42, 1.53, and 1.48 for CON, FFA, and TAG treatments, respectively. Although milk fat yield was not affected, the UFA treatments decreased the yield of de novo (<16-carbon) synthesized FA (40 g/d) and increased the yield of preformed (>16-carbon) FA (134 g/d). Yield of FA from both sources (16-carbon FA) was reduced by the UFA treatments but to a different extent for FFA versus TAG (72 vs. 100 g/d). An increase was detected in the concentration of *trans*-10 C18:1 and a trend for an increase in *trans*-10,*cis*-12 C18:2 and *trans*-9,*cis*-11 C18:2 for the UFA treatments compared with CON. Under the dietary conditions tested, UFA treatments supplemented at 2% diet DM as either soybean FA distillate or soybean oil increased milk yield but did not effectively cause a reduction in milk fat yield, with preformed FA replacing de novo synthesized FA in milk fat. Further research is required to determine if the response to changes in dietary free and esterified FA concentrations is different in diets that differ in their risk for milk fat depression.

Key words: dairy cow, biohydrogenation, milk fat, unsaturated fatty acid

INTRODUCTION

Feed ingredients vary in the amount and composition of FA that they contribute to dietary FA intake. In a recent study conducted in the Netherlands, approximately 100 samples of corn silage and grass silage were analyzed for FA concentration, with both types of silages varying from approximately 1 to 3% total FA (DM basis; Khan et al., 2012). Grains and by-products also vary in FA concentration, depending on hybrid, processing, and growing conditions (Boufaïed et al., 2003). Notably, distillers grains products vary considerably based on the amount of solubles added back to the grains, with the ratio of grains to solubles being a major contributor to the variation in FA content of the end product (Cao et al., 2009). Limited information exists regarding the total concentration of free FA, and the proportion of total FA present as free FA, in dairy cow diets. However, it has been reported previously that

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harvesting and ensiling of forages can increase the proportion of free FA due to hydrolytic cleavage of esterified FA (Yang and Fujita, 1997; Elgersma et al., 2003; Vanhatalo et al., 2007; Halmemies-Beauchet-Filleau et al., 2013a), whereas suboptimal storage of by-products in humid conditions can also increase the proportion of free FA (Cooke et al., 2007). Dietary ingredients, therefore, not only vary in the amount of total FA that they contribute to the diet but also in their relative proportion of free to esterified FA.

Unsaturated FA are toxic to specific rumen bacteria because they alter cell integrity (Maia et al., 2007). Therefore, rumen bacteria convert (biohydrogenate) unsaturated FA to SFA as a protection mechanism and, consequently, SFA are often considered to be rumen inert. A free carboxyl group is required for the initial isomerase reaction of biohydrogenation to occur (Jenkins, 1993). However, the majority of dietary FA are esterified, and are typically present either as triglycerides or glycolipids in feed ingredients. Upon entering the rumen, esterified FA must first go through the process of hydrolysis, in which the ester bond connecting the FA to the glycerol backbone is cleaved, exposing the carboxyl group and allowing biohydrogenation to proceed. If FA enter the rumen as free FA, they can undergo biohydrogenation immediately without having to go through hydrolysis.

Limited research exists examining whether the amount or proportion of free to esterified FA has an effect on biohydrogenation rates and pathways and whether this could affect milk fat synthesis in the dairy cow. Cooke et al. (2007) reported that increasing the proportion of free FA in cottonseed, while keeping the total FA concentration the same, decreased milk fat concentration when a greater proportion of free FA were fed. Additionally, in vitro work comparing free versus esterified FA found an increase in the amount of unsaturated biohydrogenation intermediates remaining when free FA were added to the culture (Moore et al., 1969; Noble et al., 1974), potentially due to an accumulation of FA that inhibit or change the pathways of biohydrogenation.

Altered rumen fermentation can result in shifts from normal biohydrogenation to alternative pathways, producing specific FA intermediates that reduce fat synthesis in the mammary gland resulting in milk fat depression (**MFD**; Bauman et al., 2011). It is well documented that increasing the amount of PUFA contained in the diet can reduce milk fat yield through the production of specific biohydrogenation intermediates (e.g., Griinari et al., 1998; Leonardi et al., 2005). However, it is unclear if a difference exists in the risk for MFD depending on if the increased dietary unsaturated FA are esterified or free FA. Our objective, therefore, was to examine if altering the amount of unsaturated FA in the diet as esterified or free FA affected feed intake and production responses of dairy cows. We hypothesized that free FA fed to lactating dairy cows would be more detrimental than esterified FA for milk fat production because free FA are more rapidly available in the rumen and this could cause a greater shift in biohydrogenation toward pathways that produce FA intermediates that cause MFD.

MATERIALS AND METHODS

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Eighteen mid-lactation (132 ± 75 DIM) Holstein cows (6 primiparous and 12 multiparous) from the Michigan State University Dairy Field Laboratory (East Lansing) were blocked by parity and 3.5% FCM yield and then randomly assigned to treatment sequence in a replicated 3 × 3 Latin square design experiment with 21-d periods.

Treatments consisted of a control (**CON**) diet, and 2 unsaturated FA (**UFA**) treatments supplemented with either soybean oil (FA present as triglycerides; **TAG** treatment) or soybean FA distillate (FA present as free FA; **FFA** treatment). The soybean oil and soybean FA distillate supplements were included in the diet at 2% DM to replace soyhulls in the CON diet. Addition of supplements to diets was based upon weight of lipid, not total FA content, and the supplements were premixed with dried ground corn before inclusion in TMR. The soybean oil (West Central Cooperative, Ralston, IA) contained approximately 84% FA, of which 99% were present as triglycerides, as the source of esterified FA. The soybean FA distillate (Arm & Hammer Animal Nutrition, Ewing, NJ) contained approximately 97% FA, of which 95% were present as free FA, as the source of free FA (Table 1). Although both supplements contained comparable concentrations of total unsaturated FA, differences existed in the concentration of individual FA: soybean oil contained a higher concentration of *cis*-9 C18:1 (26.0 vs. 11.8 g/100 g of FA) and a lower concentration of C16:0 (9.6 vs. 15.0 g/100 g of FA) and *cis*-9,*cis*-12 C18:2 (50.5 vs. 59.1 g/100 g of FA) than the soybean FA distillate (Table 1). The ingredient and nutrient composition of the diets fed as TMR are described in Table 2. All treatment diets contained equal amounts of forages in an approximate 2:1 ratio of corn silage to alfalfa silage and diets were formulated to contain 23% forage NDF and 17% CP, and mineral and vitamins were formulated according to NRC (2001) recommendations. The DM concentration