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## Effect of lipid supplementation on milk odd- and branched-chain fatty acids in dairy cows

E. Baumann, P. Y. Chouinard, Y. Lebeuf, D. E. Rico, and R. Gervais<sup>1</sup>

Département des Sciences Animales, Université Laval, 2425 rue de l'Agriculture, Québec, Canada, G1V 0A6

### ABSTRACT

Eight ruminally fistulated, multiparous Holstein cows were arranged in a double 4 × 4 Latin square with 14-d periods to investigate the effects of lipid supplementation on performance, rumen parameters, the milk odd- and branched-chain fatty acid (OBCFA) profile, and the relationships between milk OBCFA and rumen parameters. Lipid supplementation is known to inhibit microbial growth in the rumen, decrease de novo microbial fatty acid synthesis, and increase the uptake of circulating fatty acids by the mammary gland; treatments were selected to isolate these effects on the milk OBCFA profile. The 4 treatments were (1) a lipid-free emulsion medium infused in the rumen (CTL), (2) soybean oil as a source of polyunsaturated fatty acids infused in the rumen (RSO), (3) saturated fatty acids (38% 16:0, 40% 18:0) infused in the rumen (RSF), and (4) saturated fatty acids infused in the abomasum (ASF). Fat supplements were provided continuously as emulsions at a rate of 450 g/d. Preplanned contrasts compared CTL to RSO, RSO to RSF, and RSF to ASF. Infusing RSO slightly decreased ruminal pH, but did not affect volatile fatty acids profile and milk fat concentration as compared with CTL. The yields of energy-corrected milk, fat, and protein were greater with RSF compared with RSO. The concentration of odd-chain fatty acids was decreased by RSO, whereas even-chain *iso* fatty acids were not affected. Milk fat concentration of 17:0 + *cis*-9 17:1 was higher for RSF than for RSO, due to the saturated fatty acids supplement containing 2% 17:0 + *cis*-9 17:1. Limited differences were observed in the milk OBCFA profile between RSF and ASF. A multiple regression analysis yielded the following equation for predicting rumen pH based on milk fatty acids:  $\text{pH} = 6.24 - (0.56 \times 4:0) + (1.67 \times \textit{iso} 14:0) + (4.22 \times \textit{iso} 15:0) + (9.41 \times 22:0)$ . Rumen propionate concentration was negatively correlated with milk fat concentration of *iso* 14:0 and positively correlated with milk 15:0, whereas the acetate-to-propionate ratio gave the op-

posite correlations with milk *iso* 14:0 and 15:0. Milk fat concentration of 17:0 + *cis*-9 17:1 was not related to rumen propionate or to acetate-to-propionate ratio, due to the presence of 17:0 and *cis*-9 17:1 in the saturated fatty acids supplement. The results suggest that although lipid supplementation can affect the profile of milk OBCFA, the promise remains of using these milk fatty acids to evaluate rumen function.

**Key words:** odd- and branched-chain fatty acids, soybean oil, prilled fat, rumen fermentation, milk fat

### INTRODUCTION

Fat supplementation in the nutrition of lactating dairy cows is an approach used to increase the energy density of the ration. In early-lactation cows, this feeding practice limits the extent of the negative energy balance which may support milk yield (Palmquist and Jenkins, 1980). However, lipid supplementation may affect various metabolic processes which determine the fatty acid (FA) profile of milk. It is well documented that the inclusion of supplemental lipids in the diet can alter the rumen environment, affecting microbial fermentation and VFA production (Ferguson et al., 1990; Jenkins and Jenny, 1992; Harvatine and Allen, 2006b). Further, PUFA, such as *cis*-9,*cis*-12 18:2, a predominant FA in soybean oil (approximately 50%), have been shown to be toxic to rumen microbiota, particularly cellulolytic bacteria (Maia et al., 2007), and thereby, inclusion of such lipid supplements may decrease the levels of FA of microbial origin in milk (mechanism 1). Lipid supplementation may also change the milk FA profile by increasing uptake of dietary FA by rumen microbes, thereby decreasing the proportion of de novo synthesized microbial FA, as reported by Emmanuel (1978), and Weisbjerg et al. (1992; mechanism 2). Finally, lipid supplementation may also directly affect FA synthesis by the mammary gland, either by increasing uptake of preformed FA (Palmquist et al., 1993; mechanism 3), or by inhibiting mammary gland lipogenesis through rumen synthesis of the CLA isomer *trans*-10,*cis*-12 18:2 (Bauman and Griinari, 2001; mechanism 4).

Odd- and branched-chain FA (OBCFA) found in ruminant tissues are predominantly produced by ruminal

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<sup>1</sup>Corresponding author: rachel.gervais@fsaa.ulaval.ca

**Table 1.** Feed and chemical composition of basal diet

Item	Content
Ingredient, % of DM	
Grass silage	39.7
Corn silage	24.1
Dry ground corn	25.8
Corn gluten meal	5.5
Soybean meal	2.4
Vitamin and mineral mix <sup>1</sup>	1.7
Sodium bicarbonate	0.8
Chemical composition	
DM, % as fed	44.8
OM, % of DM	92.8
NDF, % of DM	26.4
ADF, % of DM	20.5
CP, % of DM	18.0
Fatty acids, mg/g of DM	
16:0	4.7
18:0	1.1
<i>cis</i> -9 18:1	6.5
<i>cis</i> -11 18:1	0.3
<i>cis</i> -9, <i>cis</i> -12 18:2	14.1
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	4.4
Others <sup>2</sup>	0.4
Total	31.5

<sup>1</sup>Contained, on a DM basis, 18.00% Ca, 5.00% P, 6.00% Mg, 0.06% K, 9.50% Na, 15.00% Cl, 1.00% S, 3,620 mg/kg of Fe, 45 mg/kg of I, 600 mg/kg of Cu, 2,000 mg/kg of Mn, 3,000 mg/kg of Zn, 25 mg/kg of Se, 20 mg/kg of Co, 300 kIU of vitamin A, 100 kIU of vitamin D, and 1,500 IU of vitamin E.

<sup>2</sup>Other fatty acids included minor concentrations of 12:0, 14:0, 15:0, 16:1, and 17:0.

microorganisms (Keeney et al., 1962; Harfoot, 1981), although a small proportion could come from postruminal synthesis (Massart-Leën et al., 1986; Vlaeminck et al., 2015). Different groups of rumen microbial species have unique OBCFA profiles (Vlaeminck et al., 2006a). Cellulolytic bacteria synthesize more *iso* FA, whereas amylolytic bacteria produce elevated levels of *anteiso* and linear odd-chain FA and relatively low levels of *iso* FA (Fievez et al., 2012). The intestinal absorption of microbially produced OBCFA and their uptake by the mammary gland lead to the appearance of OBCFA in milk fat of lactating dairy cows. Given that lipid supplementation can affect the various steps which determine milk FA profile, it is of interest to evaluate how the OBCFA profile of milk is affected by the presence of supplementary lipids in the diet.

The review by Fievez et al. (2012) of milk OBCFA and their relationship to rumen function indicates that *iso* 14:0 and *iso* 16:0 in milk are positively related to rumen acetate production, whereas milk 15:0 and 17:0 are negatively related to rumen acetate production and positively related to propionate production. Therefore, the OBCFA profile of the milk could potentially be used as a noninvasive method to predict certain aspects of rumen function. The first objective of the present experiment was to investigate the effects of lipid supple-

mentation on the aforementioned mechanisms of milk fat synthesis as well as on animal performance, rumen parameters, and milk OBCFA. An additional objective was to estimate the effect of lipid supplementation on the relationship between milk OBCFA and rumen function.

## MATERIALS AND METHODS

### Cows, Feeding, and Treatments

All procedures involving animals were conducted according to the regulations of the Canadian Council on Animal Care (1993), and were approved by the Université Laval Animal Care Committee. Eight ruminally fistulated, multiparous Holstein cows (BW: 698 ± 56 kg) in midlactation (101 ± 11 DIM) were housed in a tiestall facility at the Centre de Recherche en Sciences Animales de Deschambault (Deschambault, QC, Canada). Cows received a TMR (Table 1) formulated to meet predicted nutrient requirements (NRC, 2001). Cows were fed once per day at 1000 h, and always had free access to water. Samples of silages used in the TMR were taken once a week and oven-dried at 55°C for 48 h to determine DM content. The TMR was subsequently recalculated to maintain similar proportions of feed ingredients on a DM basis. Feed refused was weighed daily before feeding, and the amount of feed offered was adjusted to maintain 10% refusals.

Cows were arranged in a double 4 × 4 Latin square design with 14-d periods. For the duration of each period, cows received 1 of the following 4 treatments, which were all prepared as emulsions: (1) a ruminally infused lipid-free emulsion medium used as the control (**CTL**); (2) 450 g/d of a ruminally infused PUFA supplement [soybean oil, Soya Excel, Beloeil, QC, Canada, (**RSO**)] with putative effects on mechanisms 1 to 4; (3) 450 g/d of a ruminally infused SFA supplement [Energy Booster 100, Milk Specialties Global, Eden Prairie, MN, (**RSF**)] with putative effect on mechanisms 2 and 3; or (4) 450 g/d of an abomasally infused SFA supplement (**ASF**) with putative effect on mechanism 3. The FA profile of the experimental fat supplements is presented in Table 2. At 0900 h on d 14 of each period, 5 L of rumen content were transferred from cows who had received a specific treatment during the previous 14 d to cows that would receive that treatment for the next 14 d to decrease the time required for rumen adaptation to the new treatment.

### Preparation of Emulsions and Infusion Procedure

Emulsions were prepared following the procedure described by Drackley et al. (1992) with modifications

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