Effects of Monensin and Dietary Soybean Oil on Milk Fat Percentage and Milk Fatty Acid Profile in Lactating Dairy Cows

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ABSTRACT

The objective of this study was to investigate the effect of monensin (MN) and dietary soybean oil (SBO) on milk fat percentage and milk fatty acid (FA) profile. The study was conducted as a randomized complete block design with a 2×3 factorial treatment arrangement using 72 lactating multiparous Holstein dairy cows (138 ± 24 d in milk). Treatments were [dry matter (DM) basis] as follows: 1) control total mixed ration (TMR, no MN) with no supplemental SBO; 2) MN-treated TMR (22 g of MN/kg of DM) with no supplemental SBO; 3) control TMR including 1.7% SBO; 4) MN-treated TMR including 1.7% SBO; 5) control TMR including 3.4% SBO; and 6) MN-treated TMR including 3.4% SBO. The TMR (% of DM; corn silage, 31.6%; haylage, 21.2%; hay, 4.2%; high-moisture corn, 18.8%; soy hulls, 3.3%; and protein supplement, 20.9%) was offered ad libitum. The experiment consisted of a 2-wk baseline, a 3-wk adaptation, and a 2-wk collection period. Monensin, SBO, and their interaction linearly reduced milk fat percentage. Cows receiving SBO with no added MN (treatments 3 and 5) had 4.5 and 14.2% decreases in milk fat percentage, respectively. Cows receiving SBO with added MN (treatments 4 and 6) had 16.5 and 35.1% decreases in milk fat percentage, respectively. However, the interaction effect of MN and SBO on fat yield was not significant. Monensin reduced milk fat yield by 6.6%. Soybean oil linearly reduced milk fat yield and protein percentage and linearly increased milk yield and milk protein yield. Monensin and SBO reduced 4% fat-corrected milk and had no effect on DM intake. Monensin interacted with SBO to linearly increase milk fat concentration (g/100 g of FA) of total *trans*-18:1 in milk fat including *trans*-6 to 8, *trans*-9, *trans*-10, *trans*-11, *trans*-12 18:1 and the concentration of total conjugated linoleic acid isomers including *cis*-

9, *trans*-11 18:2; *trans*-9, *cis*-11 18:2; and *trans*-10, *cis*-12 18:2. Also, the interaction increased milk concentration of polyunsaturated fatty acids. Monensin and SBO linearly reduced, with no significant interaction, milk concentration (g/100 g of FA) of short- and medium-chain fatty acids (<C16). Soybean oil reduced total saturated FA and increased total monounsaturated FA. These results suggest that monensin reduces milk fat percentage and this effect is accentuated when SBO is added to the ration.

Key words: dairy cow, monensin, soybean oil, milk fat percentage

INTRODUCTION

Monensin (MN) is a carboxylic polyether ionophore antibiotic produced by fermentation of *Streptomyces cinnamonensis* (Russell, 2002). The benefits of feeding MN to dairy cattle in early lactation include increased milk production and improved energy balance associated with reduced incidence of subclinical ketosis, clinical acidosis, and displaced abomasums (Duffield and Bagg, 2000). Various studies have reported depressed milk fat percentage with MN (Phipps et al., 2000; Odongo et al., 2007a), whereas others (Lean et al., 1994; Duffield et al., 1999) reported no effect. The source of inconsistency of the fat-depressing effect of MN in the literature may be due to interaction with dietary factors such as MN dose level (Symanowski et al., 1999), method of administration of MN, stage of lactation (Duffield and Bagg, 2000), and feeding system (TMR vs. component-fed; Duffield et al., 2003).

Feeding unsaturated oil impaired ruminal biohydrogenation (BH) of unsaturated fatty acids (FA; Bauman and Griinari, 2001, 2003) and increased ruminal outflow of BH intermediates that were considered potent inhibitors of milk fat synthesis such as *trans*-10, *cis*-12 conjugated linoleic fatty acid (CLA; Baumgard et al., 2000), *cis*-10, *trans*-12 CLA (Sæbø et al., 2005), and *trans*-9, *cis*-11 CLA (Perfield et al., 2007). Similar to unsaturated oil, ionophores inhibited ruminal BH (Jen-

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kins et al., 2003). Fellner et al. (1997) successfully maintained high concentrations of linoleic acid, *trans* 18:1, and CLA in continuous cultures of ruminal bacteria when different ionophores such as MN, nigericin, and tetroneasin were added. Furthermore, Jenkins et al. (2003) found that combining MN with soybean oil (SBO) increased the level of *trans* 18:1 in bacterial culture, especially *trans*-10 18:1.

The objective of this study was to investigate the effect of MN and dietary SBO on milk fat percentage and milk FA profile in lactating Holstein cows during mid lactation.

MATERIALS AND METHODS

Animals and Experimental Design

Seventy-two lactating Holstein dairy cows (1.8 ± 1.3 parity, 138 ± 24 DIM) housed in a tie-stall facility at the Elora Dairy Research Centre, University of Guelph (Guelph, Ontario, Canada) were used in this study. Two animals were removed from the experiment due to health problems. Animals were cared for and handled in accordance with the Canadian Council on Animal Care regulations. The University of Guelph Animal Care Committee approved their use for this experiment. The study was conducted as a 2×3 factorial arrangement with 2 levels of MN (0 or 22 mg/kg of DM) and 3 levels of supplemental SBO (0, 1.7, and 3.4% of DM). The experiment was conducted using 12 cows at a given time (6 blocks). Within each block the cows were randomly assigned to 1 of the 6 treatments. Each block consisted of a 2-wk baseline period, a 3-wk adaptation period, and a 2-wk collection period.

Experimental Measures and Samples Analyses

Diets were formulated according to the NRC (2001) guidelines to meet the requirement for a dairy cow (BW = 680 kg) during mid lactation (DIM = 90) and producing 35 kg/d milk, 4.0% milk fat, and 3.5% true protein. The diet was fed as a TMR twice daily at 0700 and 1500 h and water was offered at all times. The offered amount of TMR was adjusted weekly for each individual animal based on the average feed intake from the previous week to maintain approximately 5 kg of orts.

Ingredients, chemical composition, and the FA profile of experimental TMR and protein supplements are presented in Tables 1, 2, and 3, respectively.

During adaptation and collection periods, animals were fed 1 of 3 TMR [low (L), moderate (M), or high (H)] with either no monensin (control) or with supplemental MN (DM basis, 22 g/kg Rumensin Premix, Elanco Animal Health, Division Eli Lilly Canada Inc., Guelph,

Table 1. Ingredients and chemical composition of low, medium, and high polyunsaturated fatty acid TMR

Ingredient	% of DM		
Corn silage	31.6		
Mixed haylage	21.2		
High-moisture corn	18.8		
Mixed hay	4.2		
Soybean hulls	3.3		
Protein supplement ¹	20.9		
Chemical composition	Low	Medium	High
DM, %	50.5	50.5	50.7
	% of DM		
CP	17.6	17.7	17.7
Soluble protein, % of CP	37.0	38.0	37.1
RUP, % of CP	31.5	31.0	31.4
ADP, % of CP	7.6	7.7	8.0
NDP, % of CP	25.7	26.7	27.5
ADF	23.2	22.6	22.4
NDF	36.2	35.5	34.8
Lignin, % of NDF	8.8	9.2	11.5
Ca	1.1	1.0	1.0
P	0.5	0.5	0.5
K	1.4	1.4	1.5
Mg	0.3	0.3	0.3
Na	0.5	0.5	0.4
NE _L , ² Mcal/kg	1.5	1.5	1.5
NFC ³	39.1	39.3	39.8
Crude fat	3.3	4.4	4.9
Ash	8.0	7.8	7.8

¹Ingredients and chemical composition of supplements are presented in Table 2.

²Estimated using equations and values according to NRC (2001).

³NFC = $100 - [\text{NDF} + (\text{CP} - \text{neutral detergent insoluble protein}) + \text{ether extract} + \text{ash}]$.

Ontario, Canada). The L, M, and H TMR included (% of DM) 0, 1.7, or 3.4% supplemental SBO, respectively. The SBO was added to the protein supplement where applicable. The MN premix was incorporated into soybean hulls, which acted as the carrier, and mixed with the TMR. The control TMR was mixed with a placebo premix consisting of only soybean hulls without MN. Cows were offered the experimental diets for 3 wk (adaptation period) to allow the ruminal microflora to adjust to the supplemental MN and SBO. Therefore, during the collection period, the cows were assumed to have reached a steady state. During the baseline period, animals were fed the L TMR with no supplemental MN.

Feed intake and milk yield were monitored daily throughout the experiment. Feed samples and individual cow orts samples were collected 3 times per week and frozen at -20°C for analysis. Feed samples were pooled by treatment each week, whereas orts samples were pooled weekly by cow in proportion to the amount of orts. Pooled feed and ort samples were dried at 100°C for 48 h in a forced-air oven to determine the DM content. Dried samples were ground (1-mm screen; Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and

Table 2. Ingredients and chemical composition of low, moderate, and high polyunsaturated fatty acid supplements

Ingredient	Supplement, % of DM		
	Low	Medium	High
Soyhulls	13.6	4.4	0.6
Soybean meal 48%	20.5	21.2	26.9
Canola meal	10.1	9.6	3.3
Corn gluten meal	16.3	17.3	18.9
Roasted whole soybeans	10.0	10.2	9.5
Beet pulp	4.1	4.1	1.4
Blood meal	2.5	2.5	2.5
Herring fish meal	3.7	3.8	3.4
Golden flake	2.0	2.0	2.0
Molasses	1.6	1.6	0.8
Soybean oil	0.0	8.1	16.3
Salt	2.5	2.5	2.5
Calcium carbonate	4.3	4.4	4.6
Dicalcium phosphate	3.3	3.3	3.5
Sodium bicarbonate	3.3	3.4	3.4
Micro premix ¹	0.63	0.63	0.63
Magnesium oxide	0.60	0.60	0.68
Sulfur flour (99.5)	0.30	0.30	0.33
Other ingredients ²	0.55	0.76	0.87
Chemical composition			
DM, %	91.4	91.9	92.6
	% of DM		
CP	37.5	38.1	38.1
Soluble protein, % of CP	6.3	11.4	7.1
RUP, % of CP	46.8	44.3	46.5
ADF	13.4	10.5	9.9
NDF	20.2	17.0	13.6
Lignin, % of NDF	10.0	12.0	23.0
Ca	2.83	2.63	2.68
P	1.22	1.20	1.21
K	1.23	1.19	1.26
Mg	0.55	0.55	0.55
Na	2.00	2.01	1.90
NE _L , ³ Mcal/kg	1.68	1.73	1.74
NFC ⁴	27.3	28.0	30.3
Crude fat	5.4	10.3	12.7
Ash	17.6	16.4	16.6

¹Contained (DM basis): 0.70% S, 1.56% Cl, 660 mg of Fe/kg, 496 mg of Zn/kg, 116.05 mg of Cu/kg, 495 mg of Mn/kg, 1.16 mg of Se/kg, 8.4 mg of Co/kg, 5.72 mg of I/kg, 48,290 IU of vitamin A/kg, 12,080 IU of vitamin D/kg, and 179 IU vitamin E/kg.

²Other ingredients included Fibrozyme (Alltech Inc., Lexington, KY), Yea-Sacc 1026 (Alltech Inc.), potassium chloride, and Rovimix H2 (DSM, Ayr, Ontario, Canada).

³Estimated using equations and values according to NRC (2001).

⁴NFC = 100 – [NDF + (CP – neutral detergent insoluble protein) + ether extract + ash].

pooled by period. Representative samples of each supplement were taken from each batch during the adaptation and collection periods and pooled for analysis. Samples were analyzed at Agri-Food Laboratory, Guelph (Ontario, Canada) for DM, CP, Ca, P, Mg, K, Na, ash, ether extract, lignin, ADF (AOAC, 1996), and NDF (Mertens, 2002). Soluble CP, NDF-CP, and neutral detergent insoluble protein were determined by methods described by Licitra et al. (1996).

Table 3. Fatty acid profile (g/100 g of total fatty acids) of low, moderate, and high soybean oil TMR

Item	TMR		
	Low	Moderate	High
10:0	0.051	0.047	0.045
12:0	0.22	0.19	0.18
14:0	0.53	0.49	0.46
15:0	0.106	0.099	0.097
16:0	15.2	14.9	14.8
16:1 <i>cis</i> -9	0.42	0.38	0.34
17:0	0.31	0.30	0.30
17:0 <i>anteiso</i>	0.147	0.148	0.146
18:0	3.17	3.10	2.96
18:1 <i>cis</i> -9	0.34	0.29	0.26
18:1 <i>cis</i> -11	20.2	20.3	20.1
18:1 <i>cis</i> -12	2.1	2.0	2.0
18:1 <i>cis</i> -13	0.434	0.467	0.467
18:1 <i>cis</i> -14/ <i>trans</i> -16	0.055	0.057	0.059
18:1 <i>trans</i>	0.527	0.527	0.527
18:2n-6	42.8	43.4	44.0
18:3n-3	11.9	12.0	12.1
20:0	0.400	0.398	0.395
20:1 <i>cis</i> -11	0.030	0.027	0.026
20:2n-6	0.31	0.23	0.18
20:4n-6	0.064	0.061	0.058
20:5n-3	0.08	0.04	0.02
22:0	0.268	0.266	0.268
22:1 <i>cis</i> -13	0.04	0.02	0.01
22:6n-3	0.09	0.04	0.03
23:0	0.042	0.043	0.043
24:0	0.170	0.168	0.165

Cows were milked twice daily at 0500 and 1500 h, and milk weights were recorded. Milk samples were collected in duplicate 3 times per week during wk 1 and 2 (baseline) and wk 6 and 7 (treatment) from a.m. and p.m. milkings. Samples for milk components analysis were preserved with 2-bromo-2-nitropropane-1,2-diol and stored at 4°C until analysis. Milk samples were pooled daily based on a constant proportion of 60:40 (a.m.:p.m.) and then weekly using equal proportions and submitted to the DHI testing laboratory (Ontario Dairy Herd Improvement Corp., Guelph, Ontario, Canada) for analysis. Milk samples were analyzed for milk protein, fat, and lactose using a near-infrared analyzer (Foss System 4000, Foss Electric, Hillerød, Denmark). Milk samples for FA analysis were frozen immediately at –20°C until analysis.

Milk FA Analysis

Frozen milk samples were thawed at 37°C and pooled per cow daily as explained above and then pooled by period; that is, baseline (n = 70) and treatment (n = 70). Lipids for FA analysis were extracted from pooled milk samples according to Bligh and Dyer (1959) and as modified by Odongo et al. (2007b).

Fatty acids methyl ester (FAME) analysis was performed using an Agilent 6890N GLC (Agilent Techno-

logies, Palo Alto, CA) equipped with a split/splitless injector at 250°C, a flame-ionization detector at 250°C (Agilent Technologies), and a CP Sil 88 column (100 m, 0.25 mm, 0.2-μm film thickness, Varian, Mississauga, Ontario, Canada). Agilent Chemstation software (Rev. B.01.01) was used for data analysis. Hydrogen was used as carrier gas at a constant flow rate of 1 mL/min. The temperature of the GLC oven was set to 45°C for 4 min, increased at 13°C/min to 175°C, held for 27 min, increased at 4°C/min to a final temperature of 215°C, and held for 35 min. A 1-μL sample containing 1 to 2 μg was injected at splitless mode to determine FAME presence at low concentration. Peaks were identified by comparison of retention times with a GLC reference FAME standard (GLC #463, #UC-59-M, 21:0, 23:0, and 26:0; Nu-Chek Prep Inc., Elysian, MN). The *trans*-11, *cis*-15 18:2 was identified by cross-referencing with previously published isomeric profiles reported for milk fat using *cis*-9, *cis*-12 18:2 as a landmark isomer (Ulberth and Henninger, 1994; Precht and Molkentin, 1997). Individual isomers of 18:1 FA were determined as follows: The temperature of the GLC oven was maintained at 45°C for 4 min, increased to 167°C at a rate of 13°C/min and held for 40 min, and again increased at the rate of 4°C/min to a final temperature of 218°C and held for 23 min. Separation of *trans* and *cis* octadecenoic acids was incomplete, but the chromatography allowed the major isomers of interest to be resolved. The *trans*-6, *trans*-7, and *trans*-8 18:1 isomers and the *trans*-13 and *trans*-14 18:1 isomers remained unresolved as single peaks. Individual *trans* isomers were identified by cross-referencing with previously published isomeric profiles reported for milk fat (Precht and Molkentin, 1997; Shingfield et al., 2003; Loor et al., 2004) using *trans*-11 18:1 as a landmark isomer. Fatty acid composition was expressed in grams per one hundred grams of total FA. Feed FA analysis was conducted on dried, ground, and pooled samples of L, M, and H TMR as mentioned above.

Statistical Analysis

Dry matter intake, milk yield, and milk component averages for the collection period and milk FA concentrations of composite samples were analyzed using Proc Mixed (SAS Institute, 2004) using the model

$$Y_{ijk} = \mu + M_i + S_j + (M \times S)_{ij} + B_k + e_{ijk}$$

where Y_{ijk} = the dependent variable, μ = overall mean, M_i = effect of monensin ($i = 1, 2$), S_j = effect of soybean oil ($j = 1, 2, 3$), $(M \times S)_{ij}$ = effect of monensin $\times$ soybean oil ($ij = 1, 2, 3, 4, 5, 6$), B_k = effect of block ($k = 1, 2, 3, 4, 5, 6$), and e_{ijk} = random residual error.

The model included the average of the baseline period as a covariate and the effects of MN, SBO, and block were considered as fixed effects. Orthogonal polynomial contrasts were used to describe the linear and quadratic terms of SBO effect and their interactions with MN.

RESULTS

Intake, Milk Yield, and Milk Components

Dry matter intake, milk yield, milk fat percentage, milk fat yield, milk protein percentage, and milk protein yield are presented in Table 4. Monensin had no effect on DMI, milk yield (kg/d), milk protein percentage, or milk protein yield (kg/d), and reduced milk fat percentage, fat yield, and 4% FCM (Table 4).

Soybean oil supplementation had no effect on DMI, linearly increased ($P < 0.05$) milk yield and milk protein yield, and linearly reduced ($P < 0.05$) milk protein percentage, milk fat percentage, and 4% FCM (Table 4). Soybean oil supplementation interacted with MN to linearly further reduce milk fat percentage. Cows receiving the H TMR with MN had the lowest milk fat percentage (2.43%) compared with cows receiving H TMR without MN (3.14%).

Milk FA Composition

The effect of MN and SBO on milk fat concentration (g/100 g of FA) during the treatment period is presented in Tables 5, 6, and 7.

Monensin, SBO, and their interaction increased *cis*-9 14:1 (Table 5); *trans*-6 to 8; *trans*-9; *trans*-10; *trans*-11; *trans*-12 18:1 (Table 7); total *trans* 18:1 (Table 5); *cis*-9, *trans*-11 CLA, *trans*-9, *cis*-11 CLA, *trans*-10, *cis*-12 CLA; and total CLA (Table 6).

Monensin reduced ($P < 0.05$) 6:0, 8:0, 10:0, 14:0 *iso*, 15:0 *iso*, 15:0 *anteiso*, 16:0 *iso*, 18:0, 18:3n-3, 18:3n-6, 20:0, 20:2n-6, 20:3n-6, 20:4n-3, 20:4n-6, 20:5n-3, 22:0, 22:4n-6, 22:5n-3, and 22:6n-3 FA. Monensin reduced total de novo FA (<C16). Monensin numerically ($P < 0.10$) reduced total saturated FA by 2% and increased numerically total monounsaturated FA (MUFA) by 5% (Table 5). Monensin increased ($P < 0.05$) *cis*-9 16:1 (Table 5).

Soybean oil had a significant linear effect on most FA analyzed in this study (Tables 5, 6, and 7). Soybean oil reduced total de novo FA (<C16), total 16:0 + 16:1, and total saturates (Table 5). On the other hand, SBO increased total preformed FA (>C16) and total MUFA.

Soybean oil interacted with MN (main effect of MN was not significant) to increase *trans*-4 18:1; 18:2n-6 (linoleic acid); *trans*-11, *cis*-15 18:2; *cis*-11 20:1; and total polyunsaturated FA (Tables 6 and 7).

Table 4. Mean effects of 3 levels of soybean oil (SBO; 0, 1.7, or 3.4% of DM) and monensin (MN; 0 vs. 22 g/kg of DM) in the diet on DMI, milk yield, milk fat percentage, milk fat yield, milk protein percentage, and milk protein yield in lactating dairy cows

Item	Control (no MN)			MN (22 g/kg of DM)			SEM	P-value ¹		
	0	1.7	3.4	0	1.7	3.4		MN	SBO	MN × SBO
DMI, kg/d	21.3	20.7	20.9	20.6	21.0	21.5	0.50	NS	NS	NS
Milk yield, kg/d	27.5	29.5	29.4	26.9	28.9	30.1	0.75	NS	**	NS
Milk fat, %	3.76	3.59	3.14	3.74	3.21	2.43	0.15	**	***	*
Milk fat, kg/d	1.03	1.05	0.92	0.99	0.92	0.74	0.04	**	***	NS
Milk protein, %	3.35	3.26	3.27	3.31	3.24	3.23	0.04	NS	*	NS
Milk protein, kg/d	0.93	0.96	0.95	0.88	0.93	0.97	0.02	NS	*	NS
4% FCM, kg/d	26.4	27.6	25.5	25.6	25.2	23.1	0.65	**	*	NS

¹Probability of significant effects of MN, SBO (linear), and their interaction (MN × SBO, linear); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

The potential of MN to improve the performance of lactating dairy cows has received considerable attention over the last few years. In several studies, MN has been shown to reduce DMI in dairy cows (Johnson et al., 1988; Sauer et al., 1989; Green et al., 1999) and increase milk production (Hayes et al., 1996; van der Werf et al., 1998; Duffield et al., 1999). In the current study, MN had no effect on DMI and milk yield (Table 4) in agreement with Dye et al. (1988), Ramanzin et al. (1997), and Phipps et al. (2000). Monensin also had no effect on milk protein percentage or milk protein yield, in agreement with several previous studies (van der Werf et al., 1998; Duffield et al., 1999) although other studies have reported a reduction in milk protein percentage with MN (Hayes et al., 1996; Green et al., 1999; Phipps et al., 2000).

Feeding unsaturated oil often caused depressed DMI and milk production (Lock and Shingfield, 2004) because of impaired rumen function (Jenkins, 1993). In the current study, SBO had no effect on DMI and significantly increased milk yield (Table 4). The increase in milk production could be related to an increased energy intake with greater SBO level. Soybean oil reduced milk protein percentage and increased ($P < 0.05$) milk protein yield (Table 4). Reduction in milk protein percentage was almost always associated with an increase in milk yield; therefore, it is suggested that milk protein reduction is often caused by a dilution effect rather than a reduction in protein synthesis (DePeters and Cant, 1992; Ipharraguerre and Clark, 2003).

Soybean oil, MN, and their interaction reduced milk fat percentage. Milk fat percentage dropped by 4.5 and 14.2% when 1.7% SBO was supplemented and by 16.5 and 35.1% when 3.4% of SBO was supplemented, no MN vs. MN, respectively, suggesting that the depression of milk fat percentage was dependent on the level of fat in the diet. The depression in milk fat percentage

caused by SBO is in agreement with previous studies that used a combination of extruded soybeans and fish oil (Whitlock et al., 2002; AbuGhazaleh et al., 2004).

In the current study, milk fat depression was associated with a significant increase in total *trans* 18:1 including *trans*-10 18:1 (Table 5) and total CLA including *cis*-9, *trans*-11 CLA, *trans*-9, *cis*-11 CLA, *trans*-10, *cis*-12 CLA (Table 6).

Trans-10, *cis*-12 CLA (Baumgard et al., 2000) is considered a potent inhibitor of milk fat synthesis (Bauman and Griinari, 2001, 2003), and the relationship between this CLA isomer and milk fat depression is well established (de Veth et al., 2004; Shingfield et al., 2006). In the current study, *trans*-10, *cis*-12 CLA had a strong relationship with milk fat percentage [milk fat percentage (g/kg) = $20.09 \pm 3.09 + 25.01 \pm 2.87 \exp(-59.53 \pm 22.25 \text{ } trans\text{-}10, \text{ } cis\text{-}12 \text{ CLA})$, $n = 70$, $R^2 = 0.53$, $P < 0.001$].

However, in several diet-induced milk fat depressions, changes in *trans*-10, *cis*-12 CLA were not adequate to completely explain the reduction in milk fat, suggesting that additional BH intermediates might be involved in the regulation of milk fat synthesis (Bauman and Griinari, 2001). Recent studies demonstrated that *cis*-10, *trans*-12 CLA (Sæbø et al., 2005) and *trans*-9, *cis*-11 CLA (Perfield et al., 2007) are also potent inhibitors of milk fat synthesis in dairy cows. The current study showed, however, a weak relationship between milk fat percentage and *trans*-9, *cis*-11 CLA ($R^2 = 0.28$). Other CLA such as *trans*-10, *trans*-12 CLA (Perfield et al., 2006) and *trans*-9, *trans*-11 CLA (Perfield et al., 2007) had no effect on milk fat depression, but caused a shift in Δ^9 -desaturation indices.

Impaired ruminal BH yields many *trans* 18:1 intermediates, mainly *trans*-10 18:1. The relationship between this isomer in milk fat and milk fat depression has been well established (Bauman and Griinari, 2003). Recently, Lock et al. (2007) investigated the direct role of *trans*-10 18:1 on milk fat synthesis. They used 3 mid-lactating cows in a 3×3 Latin square design. Cows

Table 5. Mean effects of 3 levels of soybean oil (SBO; 0, 1.7, or 3.4% of DM) and monensin (MN, 0 vs. 22 g/kg DM) in the diet on milk fatty acid (FA) concentrations (g/100 g of FA)

FA	Control (no MN)			MN (22 g/kg of DM)			SEM	P-value ¹		
	0	1.7	3.4	0	1.7	3.4		MN	SBO	MN × SBO
6:0	1.82	1.62	1.24	1.74	1.35	0.97	0.105	*	***	NS
8:0	1.21	1.06	0.78	1.07	0.86	0.59	0.068	**	***	NS
10:0	2.99	2.50	1.82	2.70	2.18	1.48	0.158	*	***	NS
11:0	0.35	0.28	0.21	0.30	0.28	0.18	0.026	NS	***	NS
12:0	3.90	3.24	2.50	3.59	3.06	2.25	0.160	NS	***	NS
14:0	13.23	12.28	10.86	12.72	12.09	10.52	0.306	NS	***	NS
14:0 <i>iso</i>	0.16	0.13	0.12	0.11	0.10	0.08	0.010	***	**	NS
14:1 <i>cis</i> -9	0.98	0.98	0.99	0.97	1.22	1.27	0.071	*	*	*
15:0	1.38	1.27	1.16	1.47	1.23	1.16	0.040	NS	***	NS
15:0 <i>iso</i>	0.27	0.24	0.19	0.23	0.20	0.17	0.012	**	***	NS
15:0 <i>anteiso</i>	0.54	0.48	0.43	0.51	0.45	0.40	0.012	**	***	NS
16:0	35.00	33.31	30.94	37.57	33.13	31.19	0.683	NS	***	NS
16:0 <i>iso</i>	0.40	0.37	0.33	0.31	0.30	0.27	0.019	***	**	NS
16:1 <i>cis</i> -9	1.36	1.34	1.51	1.49	1.57	1.95	0.122	**	*	NS
17:0	0.55	0.50	0.48	0.58	0.50	0.47	0.015	NS	***	NS
17:0 <i>anteiso</i>	0.69	0.66	0.63	0.69	0.65	0.62	0.018	NS	***	NS
18:0	10.38	11.05	11.45	9.87	10.40	9.84	0.404	**	NS	NS
18:1 <i>cis</i>	18.36	20.12	23.02	17.69	21.27	22.45	0.678	NS	***	NS
18:1 <i>trans</i>	2.90	4.32	6.41	2.81	4.64	8.73	0.348	**	***	**
18:1 total	21.08	24.44	29.46	20.47	26.04	31.20	0.884	NS	***	NS
18:2 ²	2.02	2.40	2.57	1.87	2.33	2.84	0.080	NS	***	*
CLA ³	0.49	0.75	1.09	0.48	0.87	1.47	0.073	**	***	*
18:3n-3	0.38	0.42	0.40	0.35	0.37	0.41	0.013	*	**	NS
18:3n-6	0.028	0.022	0.017	0.024	0.021	0.013	0.002	*	***	NS
19:0	0.071	0.077	0.082	0.075	0.074	0.088	0.004	NS	**	NS
20:0	0.165	0.158	0.146	0.152	0.140	0.117	0.007	**	***	NS
20:1 <i>cis</i> -11	0.073	0.069	0.074	0.069	0.071	0.088	0.004	NS	*	*
20:2n-3	0.006	0.007	0.009	0.006	0.009	0.008	0.001	NS	*	NS
20:2n-6	0.024	0.023	0.021	0.022	0.021	0.017	0.001	**	**	NS
20:3n-3	0.006	0.005	0.007	0.006	0.006	0.006	0.001	NS	NS	NS
20:3n-6	0.101	0.094	0.078	0.084	0.085	0.066	0.006	**	***	NS
20:4n-3	0.026	0.024	0.021	0.021	0.023	0.018	0.002	*	*	NS
20:4n-6	0.118	0.110	0.090	0.113	0.096	0.070	0.006	*	***	NS
20:5n-3	0.041	0.037	0.029	0.040	0.034	0.023	0.002	*	***	NS
22:0	0.063	0.054	0.048	0.048	0.048	0.042	0.004	*	*	NS
22:1 <i>cis</i> -13	0.009	0.007	0.009	0.009	0.009	0.010	0.001	NS	NS	NS
22:2n-6	0.007	0.006	0.008	0.007	0.008	0.009	0.000	NS	*	NS
22:4n-3	0.005	0.006	0.005	0.005	0.005	0.006	0.001	NS	NS	NS
22:4n-6	0.014	0.013	0.011	0.013	0.011	0.008	0.001	*	***	NS
22:5n-3	0.061	0.054	0.048	0.054	0.047	0.037	0.004	*	***	NS
22:5n-6	0.007	0.008	0.007	0.006	0.006	0.006	0.001	NS	NS	NS
22:6n-3	0.038	0.029	0.023	0.035	0.026	0.017	0.002	*	***	NS
23:0	0.020	0.016	0.014	0.019	0.015	0.011	0.001	NS	***	NS
24:0	0.021	0.017	0.017	0.021	0.017	0.014	0.002	NS	**	NS
Summation by source ⁴										
<C16	26.88	24.08	20.32	25.38	23.00	19.03	0.772	*	***	NS
16:0 and 16:1	36.77	35.02	32.76	39.37	35.00	33.41	0.712	NS	***	NS
>C16	36.56	41.13	46.84	35.08	41.90	47.51	1.067	NS	***	NS
Total saturated FA	73.14	69.16	63.48	73.87	67.11	60.46	1.025	NS	***	NS
Total MUFA ⁵	23.51	26.84	32.07	23.00	28.91	34.51	0.959	NS	***	NS
Total PUFA ⁶	3.37	4.00	4.45	3.13	3.98	5.02	0.133	NS	***	**

¹Probability of significant effects of MN, SBO (linear), and their interaction (MN × SBO, linear); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

²Sum of 18:2 FA excluding isomers of conjugated linoleic acid.

³Total conjugated linoleic acid.

⁴Fatty acids <C16 originated from de novo synthesis, FA >C16 were preformed FA taken up by the mammary gland, and 16:0 and 16:1 FA came from both de novo and preformed sources.

⁵Monounsaturated FA.

⁶Polyunsaturated FA.

Table 6. Mean effects of 3 levels of soybean oil (SBO; 0, 1.7, or 3.4% of DM) and monensin (MN, 0 vs. 22 g/kg DM) in the diet on milk 18:2 concentration (g/100 g of fatty acids)

Item	Control (no MN)			MN (22 g/kg of DM)			SEM	P-value ¹		
	0	1.7	3.4	0	1.7	3.4		MN	SBO	MN × SBO
Nonconjugated										
<i>Cis</i> -9, <i>cis</i> -12	1.92	2.24	2.35	1.78	2.19	2.57	0.075	NS	***	*
<i>Trans</i> -11, <i>cis</i> -15	0.11	0.16	0.22	0.09	0.15	0.27	0.013	NS	***	*
Conjugated										
<i>Cis</i> -9, <i>trans</i> -11	0.41	0.65	0.96	0.40	0.76	1.28	0.073	*	***	*
<i>Trans</i> -9, <i>cis</i> -11	0.036	0.045	0.059	0.038	0.051	0.09	0.005	**	***	**
<i>Trans</i> -10, <i>cis</i> -12	0.006	0.009	0.016	0.007	0.013	0.037	0.003	***	***	***
<i>Trans</i> -11, <i>trans</i> -13	0.015	0.016	0.021	0.016	0.017	0.022	0.002	NS	**	NS
<i>Trans</i> , <i>trans</i> ²	0.021	0.030	0.038	0.019	0.029	0.042	0.002	NS	***	NS

¹Probability of significant effects of MN, SBO (linear), and their interaction (MN × SBO, linear); **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

²Sum of unresolved *trans*-9, *trans*-11, and *trans*-10, *trans*-12.

were infused abomasally with ethanol as a control treatment, 43 g/d of *trans*-10 18:2, or 4.3 g/d of *trans*-10, *cis*-12 CLA. The experiment confirmed the role of the *trans*-10, *cis*-12 CLA isomer in inducing milk fat depression. However, they demonstrated that *trans*-10 had no role in milk fat depression, but it was considered an important biological marker of impaired ruminal BH due to its high incorporation in milk. Nonetheless, the milk *trans*-10 18:1 concentration reported by Lock et al. (2007) in the *trans*-10 18:1 infusion (1.11 g/100 g of FA) was lower than the values reported in the current study for the 3.4% supplemental SBO treatment (1.30 and 2.64 g/100 g of FA, without and with added MN, respectively; Table 7). In the current study, milk fat percentage had the greatest correlation with *trans*-10 18:1 [milk fat percentage (g/kg) = 22.47 ± 1.27 + 31.97 ± 5.11 exp (−1.99 ± 0.48 *trans*-10 18:1), *n* = 70, *R*² = 0.67, *P* < 0.001].

Jenkins et al. (2003) studied the interaction between MN and unsaturated plant oils on *trans* FA in bacterial cultures grown in continuous fermentors and fed either corn or barley. They reported that the addition of SBO and MN to the fermentors individually increased total *trans* 18:1 by 100 and 53%, respectively, with the majority of the increase being in a single isomer, *trans*-10 18:1. Additionally, they found that feeding a combination of MN and SBO had an additive effect on *trans*-10 18:1 when barley was fed, and no additive effect was measured when corn was fed. Therefore, the additive effect of *trans*-10 with barley was not attributed to starch level or to continuous culture pH. High starch digestibility has been shown to reduce the rates of lipolysis and BH (Gerson and King, 1985). They concluded that greater starch digestibility could intensify the ability of MN and unsaturated oil to disrupt BH. In the current study, a corn silage and high-moisture corn

Table 7. Mean effects of 3 levels of soybean oil (SBO; 0, 1.7, or 3.4% of DM) and monensin (MN, 0 vs. 22 g/kg of DM) in the diet on milk 18:1 concentration (g/100 g of fatty acids)

Item	Control (no MN)			MN (22 g/kg of DM)			SEM	P-value ¹		
	0	1.7	3.4	0	1.7	3.4		MN	SBO	MN × SBO
<i>Cis</i> -9	17.34	18.78	21.56	16.64	19.96	20.87	0.653	NS	***	NS
<i>Cis</i> -11	0.70	0.77	0.82	0.74	0.79	0.99	0.061	NS	**	NS
<i>Cis</i> -12	0.27	0.49	0.57	0.26	0.44	0.49	0.034	NS	***	NS
<i>Cis</i> -13	0.055	0.073	0.078	0.051	0.072	0.091	0.007	NS	***	NS
<i>Trans</i> -4	0.014	0.021	0.026	0.014	0.019	0.031	0.001	NS	***	NS
<i>Trans</i> -5	0.012	0.015	0.023	0.011	0.017	0.028	0.002	NS	***	NS
<i>Trans</i> -6+7+8	0.21	0.31	0.43	0.20	0.33	0.59	0.031	*	***	**
<i>Trans</i> -9	0.25	0.35	0.46	0.24	0.38	0.59	0.024	*	***	**
<i>Trans</i> -10	0.41	0.59	1.30	0.45	0.78	2.64	0.249	*	***	*
<i>Trans</i> -11	0.90	1.47	2.12	0.91	1.64	2.74	0.133	*	***	*
<i>Trans</i> -12	0.34	0.55	0.71	0.34	0.54	0.82	0.021	*	***	*
<i>Trans</i> -13+14	0.42	0.65	0.89	0.41	0.63	0.93	0.045	NS	***	NS
<i>Trans</i> -16+ <i>cis</i> -14	0.27	0.35	0.41	0.27	0.36	0.39	0.019	NS	***	NS

¹Probability of significant effects of MN, SBO (linear), and their interaction (MN × SBO, linear); **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

based diet was fed (Table 1). Estimated NFC (% of DM) for experimental diets ranged from 39 to 40%, which is considered typical for high-producing cows in this region of Ontario. Nonetheless, the ration provided sufficient NDF (35 to 36%) with sufficient particle size to maintain a healthy rumen. More studies are needed to investigate the interaction effect of MN, SBO, and grain level (production level) in the diet on milk fat.

The mechanism by which MN depresses milk fat is not well understood. It was suggested that MN interferes with the BH of long-chain FA. In an in vitro study, Fellner et al. (1997) demonstrated that ionophores disrupt ruminal BH by depressing the rate of 18:0 production and increasing the concentration of MUFA. In the rumen, the BH of linoleic acid to linoleic acid is performed by gram-positive bacteria, which are sensitive to MN, whereas the reduction of oleic acid to stearic acid is performed by gram-negative bacteria (Harfoot and Hazelwood, 1988). Additionally, Van Nevel and Demeyer (1995) reported that MN inhibited lipolysis and subsequently reduced the formation of free carboxyl groups necessary for the hydrogenation of double bonds. However, the accumulation of 18:1 isomers cannot be explained by the effect of MN on gram-positive bacteria (Fellner et al., 1997).

In the current study, evidence of ruminal BH disruption included a reduction in ruminal milk concentration of stearic acid, and an increase in CLA and vaccenic acid. The changes in milk FA profile also suggest a shift in the BH pathways represented by an increase in the ratio of *trans*-10 18:1 to total *trans* 18:1 ($P = 0.022$).

Monensin reduced milk fat yield (kg/d) likely by altering ruminal BH. However, the reduction in de novo synthesized short- and medium-chain FA (<C16) including 6:0, 8:0, 10:0, 14:0 *iso*, 15:0 *iso*, and 15:0 *anteiso* suggests an indirect role of MN in reducing fat synthesis.

In conclusion, our results showed that MN reduced milk fat percentage. However, the extent of this reduction was dependent on the dietary level of SBO. Milk fat depression was associated with impaired ruminal BH, as indicated by an increase in total *trans*-10 18:1, and was associated with an increase in CLA isomers believed to have a direct role in inhibiting milk fat synthesis, namely *trans*-10, *cis*-12 CLA and *trans*-9, *cis*-11 CLA. However, more research is needed to investigate other dietary factors that may depresses milk fat such as grain level and its interaction with known dietary factors such as unsaturated fat and MN and to examine mechanism(s) involved in milk fat depression.

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