Effect of Supplementing Myristic Acid in Dairy Cow Rations on Ruminal Methanogenesis and Fatty Acid Profile in Milk

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ABSTRACT

The objective of this study was to evaluate the effects of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and the fatty acid profile in milk. Twelve multiparous Holstein dairy cows (710 ± 17.3 kg of live weight; 290 ± 41.9 d in milk) housed in a tie-stall facility were used in the study. The cows were paired by parity and days in milk and allocated to 1 of 2 treatments: 1) the regular milking cow total mixed ration (control diet), and 2) the regular milking cow total mixed ration supplemented with 5% myristic acid on a dry matter basis (MA diet). The cows were fed and milked twice daily (feeding, 0830 and 1300 h; milking, 0500 and 1500 h). The experiment was conducted as a completely randomized design and consisted of a 7-d pretrial period when cows were fed the control diet to obtain baseline measurements, a 10-d dietary adaptation period, and a 1-d, 8-h measurement period. The MA diet reduced methane (CH_4) production by 36% (608.2 vs. 390.6 ± 56.46 L/d, control vs. MA diet, respectively) and milk fat percentage by 2.4% (4.2 vs. $4.1 \pm 0.006\%$, control vs. MA diet, respectively). The MA diet increased 14:0 in milk by 139% and *cis*-9 14:1 by 195%. There was a correlation ($r = -0.58$) between the 14:0 content in milk and CH_4 production and *cis*-9 14:1 and CH_4 production ($r = -0.47$). Myristic acid had no effect on the contents of CLA or *trans*-10 18:1 and *trans*-11 18:1 isomers in milk. These results suggest that MA could be used to inhibit the activities of methanogens in ruminant animals without altering the conjugated linoleic acid and *trans*-18:1 fatty acid profile in milk.

Key words: dietary myristic acid, ruminal methanogenesis, milk fatty acid profile

INTRODUCTION

Methane is produced as an unavoidable by-product of OM fermentation in the rumen and represents a 2 to

12% loss of gross energy intake (Johnson and Johnson, 1995). It has been estimated that an adult dairy cow produces 80 to 100 kg of methane (CH_4) from enteric fermentation per year (EPA, 1993). On a global basis, ruminants (sheep, goats, camels, cattle, and buffalo) produce about 80 Tg of CH_4 annually (1 Tg = 1 million metric tons), accounting for about 22% of CH_4 emissions from human-related activities (NRC, 2002). Because CH_4 is a potent greenhouse gas and contributes to global warming (NRC, 1992), it has a global warming potential 23 times that of CO_2 (IPCC, 2001); mitigating CH_4 emission from dairy cattle will have long-term environmental benefits in terms of reducing the contribution of animal agriculture to greenhouse gas emissions.

However, CH_4 production in ruminants is influenced by many factors. One option to alter CH_4 production is the addition of dietary fats in the ration (Czerkawski et al., 1966; Van Nevel and Demeyer, 1981; Moss et al., 2000), which are normally added to increase energy density, enhance milk production, or modify the fatty acid (FA) composition of milk fat (Murphy et al., 1995; Ashes et al., 1997). Among the saturated fatty acids (SFA), medium-chain fatty acids (MCFA; C_8 to C_{16}) are known to have the greatest potential to suppress rumen methanogenesis (Blaxter and Czerkawski, 1966) and methanogens (Henderson, 1973; Soliva et al., 2004). In *in vitro* studies, addition of palm kernel oil, coconut oil, and canola oil at 5% (on a DM basis) reduced CH_4 production by 34, 21, and 20%, respectively (Dohme et al., 2000). When coconut oil was fed to sheep at 3.5 and 7%, daily CH_4 production was reduced by 28 and 73%, respectively (Machmüller and Kreuzer, 1999). However, so far no reports exist on whether supplementing dairy cow rations with MCFA such as myristic acid (MA) would alter the ruminal environment markedly enough to cause milk fat depression or change the conjugated linoleic acid (CLA) and *trans*-18:1 FA profile in milk in addition to inhibiting ruminal methanogenesis and methanogens. We hypothesized that supplementing dairy cow rations with 5% (on a DM basis) MA in the TMR would not alter the FA profile in milk. The objective of this study was therefore to evaluate the effects of supplementing MA in dairy cow rations on ruminal methanogenesis and the FA profile in milk.

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MATERIALS AND METHODS

Animals and Experimental Design

Twelve multiparous Holstein dairy cows (710 ± 17.3 kg of live weight; 290 ± 41.9 DIM) housed in a tie-stall facility at the Elora Dairy Research Centre, University of Guelph (Guelph, ON, Canada) were used in the study. Animals were cared for and handled in accordance with the Canadian Council on Animal Care regulations, and the University of Guelph Animal Care Committee reviewed and approved the experiment and all procedures carried out in the study. The cows were paired by parity and DIM and allocated to 1 of 2 treatments: 1) the regular milking cow TMR (control diet), and 2) the regular milking cow TMR supplemented with 5% MA on a DM basis (MA diet). The experiment was conducted as a completely randomized block design and consisted of a 7-d pretrial period when cows were fed the control diet to obtain baseline measurements, a 10-d dietary adaptation period, and a 1-d, 8-h CH₄ measurement period. The ingredient and chemical composition of the diet is presented in Table 1.

Cows were fed ad libitum (allowing for a 5 to 10% refusal) twice daily at 0830 and 1300 h and had unlimited access to fresh water. The cows were milked twice daily at 0500 and 1500 h in their stalls, and milk samples were collected daily from morning and afternoon milkings and preserved with 2-bromo-2-nitropropane-1,2-diol. The morning and afternoon milk samples were pooled daily based on milk yield, and the pooled samples were immediately submitted to the Central Milk Testing Laboratory (Laboratory Services Division, University of Guelph) for compositional analysis. A second set of milk samples without preservative was also collected daily from morning and afternoon milkings and stored at -20°C for milk FA analysis.

Measurements of CH₄ Production

Methane production from the cows was determined using a mobile, open-circuit indirect calorimetry system as described by Kelly et al. (1994) and updated by Odongo et al. (2007). Briefly, the calorimetry unit was based on 2 separate but linked sampling lines attached to 2 airtight ventilated head-hoods. The hoods were large enough to allow the animals to move their heads without restriction such that the animals could stand or lie down in the stanchions with their heads remaining in the hoods. The animal's head was enclosed in the hood using a sleeve around the animal's neck to minimize air leakage. The cows had free access to feed and water in the hood. Outside air was circulated around the animal's head, mouth, and nose through the sleeve, and a subsample of the expired air was

Table 1. Ingredients and chemical composition (DM basis) of the diets

Item	Control	Myristic acid
Ingredient composition, % (DM basis)		
Hay	5.5	5.2
Haylage	22.1	21.1
Corn silage	33.2	31.7
High-moisture corn	20.2	19.3
Custom TMR dairy supplement ¹	18.9	22.8 ²
Chemical composition, % (DM basis)		
CP, % (N $\times$ 6.25)	17.3	17.3
Soluble protein, %	6.15	4.82
Soluble protein, % of CP	35.3	28.1
Undegradable protein, % of CP	32.4	35.9
ADF, %	26.4	25.0
NDF, %	40.8	37.8
NFC ³	31.4	34.7
Lignin, % NDF	8.0	10.8
Calcium, %	0.83	0.79
Phosphorus, %	0.46	0.45
Potassium, %	1.50	1.41
Magnesium, %	0.25	0.25
Sodium, %	0.45	0.46
NE _L , Mcal/kg	1.46	1.48
Ether extract, %	3.02	3.22
Ash, %	7.50	7.01

¹Provided as a mash supplement. Ingredients (%): corn gluten meal, 210; soybean meal, 250; beet pulp, 50; herring meal, 50; canola meal, 100; roasted soybeans, 100; soybean hulls, 41; white salt, 23; dicalcium phosphate, 29; limestone, 45; bicarbonate, 35; magnesium oxide (56% Mg), 5; Fibrozyme (Alltech Inc., Nicholasville, KY), 3; Organic Ruminant Micro Premix (Floradale Feed Mill Limited, Floradale, ON, Canada), 5; sulfur 99.5%, 2.1; Yea-Sacc¹⁰²⁶ (Alltech Inc.), 2; Rovimix H-2 (DSM Nutritional Products, Inc., Parsippany, NJ), 0.1; DCAD+, 159; Golden Flake (Floradale Feed Mill), 20; molasses, 20.

²Myristic acid in supplement (%): myristic acid, 194; corn gluten meal, 170; soybean meal, 202; beet pulp, 50; herring meal, 40; canola meal, 86; roasted soybeans, 86; soybean hulls, 15; white salt, 18; dicalcium phosphate, 23; limestone, 36; bicarbonate, 28; magnesium oxide (56% Mg), 4; Fibrozyme (Alltech Inc.), 2.4; Organic Ruminant Micro Premix (Floradale Feed Mill Limited), 4.7; sulfur 99.5%, 1.9; Yea-Sacc¹⁰²⁶ (Alltech Inc.), 1.6; Rovimix H-2 (DSM Nutritional Products, Inc.), 0.1; DCAD+, 127; Golden Flake (Floradale Feed Mill Ltd.), 15; molasses, 15.

³Nonfiber carbohydrates were calculated as $\text{NFC} = 100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ fat} + \% \text{ ash})$.

passed through Drierite (W. A. Hammond Drierite Co., Ltd., Xenia, OH) to remove water vapor and delivered at positive pressure to inline analyzers. Methane and CO₂ concentrations in samples of inspired and expired air were measured using a nondispersive infrared CH₄/CO₂ analyzer (Servomex Xentra 4100 gas purity analyzer; Servomex Group Ltd., Crowborough, East Sussex, UK), whereas the concentration of O₂ was measured using a paramagnetic O₂ analyzer (Servomex Xentra 4100 gas purity analyzer; Servomex Group Ltd.) with a response time of <12 s for the O₂ analyzer and <20 s for the CH₄/CO₂ analyzer. A Foxboro 823 IFO integral flow orifice with cell transmitter (Invensys Systems, Inc., Foxboro, MA) was used to measure the air-

flow rate. The analyzers were calibrated each week as described by McLean and Tobin (1987).

Chemical Analysis

Feed intakes were monitored daily throughout the experiment. Orts from individual cows were weighed each morning prior to feeding, and representative feed and ort samples were collected 3 times per week and stored at -20°C until analysis. The feed and Orts were analyzed for DM by oven-drying at 60°C for 48 h (method 930.15; AOAC, 1990). Dried feed and ort samples were ground to pass through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA) for chemical composition determination in duplicate at a commercial laboratory (Agri-Food Laboratories, Guelph, ON, Canada). Analytical DM content was determined by oven-drying at 135°C for 2 h (method 3.002; AOAC, 1990), OM by ashing at 500°C for 16 h (method 942.05; AOAC, 1990), and CP using a Leco FP 428 nitrogen analyzer (Leco Corporation, St. Joseph, MI; method 4.2.08; AOAC, 1990). The samples were also analyzed for ether extract (method 920.39; AOAC, 1990), ADF (method 973.18c; AOAC, 1990), NDF (Van Soest et al., 1991) using α -amylase (Sigma no. A3306; Sigma Chemical Co., St. Louis, MO), sodium sulfite corrected for ash concentration adapted for an Ankom 200 fiber analyzer (Ankom Technology, Fairport, NY), and Ca and P by inductively coupled plasma spectroscopy (method 945.46; AOAC, 1990). Milk samples were analyzed for CP and fat using a near-infrared analyzer (Foss System 4000; Foss Electric, Hillerød, Denmark).

Analysis of FA Composition

Frozen milk samples were thawed in a 38°C water bath and mixed according to AOAC method 925.21 (AOAC, 1990). Total milk fat was extracted with chloroform, methanol, and water in the ratio of 1:1:0.9 as described by Bligh and Dyer (1959). The total lipids were methylated using NaOCH_3 as catalyst (Cruz-Hernandez et al., 2004) and analyzed directly by GLC. Fatty acid methyl ester analysis was performed using an Agilent 6890N GLC (Agilent Technologies, Palo Alto, CA) equipped with a split-splitless injector at 250°C , a flame-ionization detector at 250°C , and a CP Sil 88 column (100 m, 0.25 mm, 0.2 μm film thickness; Varian Inc., Mississauga, ON, Canada). 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 $13^{\circ}\text{C}/\text{min}$ to 175°C , and held for 27 min, increased at the rate of $4^{\circ}\text{C}/\text{min}$ to a final temperature of 215°C , and held for 35 min. Agilent Technologies

Chemstation software (Rev. B.01.01) was used for data analysis. A 1- μL sample was injected at splitless mode. Peaks were routinely identified by comparison of retention times with FA methyl ester standards (GLC #463, #UC-59-M, 21:0, 23:0, and 26:0; NuCheck 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 GLC oven was maintained at 45°C for 4 min, increased to 167°C at a rate of $13^{\circ}\text{C}/\text{min}$ and held for 40 min, and again increased at the rate of $4^{\circ}\text{C}/\text{min}$ 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; Looor et al., 2004) using *trans*-11 18:1 as a landmark isomer. Fatty acid composition was expressed as grams/100 grams of total FA.

Statistical Analysis

Data on the response variables were analyzed using PROC MIXED of SAS (v. 9.1; SAS Institute, Cary, NC) using the model

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},$$

where Y_{ij} is the dependent variable, μ is the overall mean, α_i is the effect of treatment ($i = 1, 2$), β_j is the effect of pair ($j = 1, 2, \dots, 6$), and ε_i is the random residual error. Baseline DMI was used as covariate. The correlations between CH_4 production and 14:0 and CH_4 production and *cis*-9 14:1 were determined using the Corr procedure of SAS. Effects were considered significant at a probability of $P < 0.05$.

RESULTS AND DISCUSSION

DMI and CH_4 Production

Dry matter intake and CH_4 production are summarized in Table 2. All animals adapted easily to confinement in the hoods during CH_4 measurement, and there were no indications of discomfort or stress to the animals. Myristic acid reduced ($P < 0.05$) CH_4 production by 36% (608.2 vs. 390.6 ± 56.46 L/d, control vs. MA diet,

Table 2. Effects of a control vs. myristic acid-treated TMR on DMI, milk yield, milk protein, milk fat, and methane production from lactating dairy cows¹

Response	Control	Myristic acid	SEM
DMI, kg/d	15.2	14.2	2.84
Milk yield, kg/d	14.9	13.4	1.53
Protein, %	3.5	3.6	0.16
Fat, %	4.2 ^a	4.1 ^b	0.006
Methane production, L/d	608.2 ^a	390.6 ^b	56.46
Methane production, MJ/d	23.0 ^a	14.8 ^b	2.14

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Means are based on 6 cows per treatment.

respectively) consistent with previous studies. Diets containing MCFA (e.g., coconut oil) have been shown in in vitro (Dong et al., 1997; Machmüller et al., 1998) and in vivo studies (Machmüller and Kreuzer, 1999) to significantly suppress methane production in ruminants. Dohme et al. (2000) observed from in vitro studies that addition of 5.3% palm kernel oil reduced CH₄ production by 34%, similar to the reduction in CH₄ production obtained in the current study. When coconut oil was fed to sheep at inclusion rates of 3.5 and 7%, daily CH₄ production was reduced by 28 and 73%, respectively (Machmüller and Kreuzer, 1999). The predominant FA in coconut oil are lauric acid (average proportion approximately 47%) and MA (18%; Dohme et al., 2000). Machmüller et al. (2002) reported a 58% reduction in methane production in sheep supplemented with 5% MA.

There was a tendency ($P = 0.09$) for the MA diet to reduce DMI. This tendency to reduce DMI was not surprising because lauric acid and MA have been shown to interact with dietary fiber in the rumen to depress fiber degradability (Dohme et al., 2001; Machmüller et al., 2001). In general, adding >5 to 6% of fats in the ration has been shown to depress fiber degradation in the rumen (Dong et al., 1997; Mathison et al., 1998) and reduce acetate production and milk fat content (Jenkins et al., 1996; Ashes et al., 1997). These MCFA have the potential to adversely affect ruminal ciliate protozoa, several bacterial species, and methanogens (Henderson, 1973; Dohme et al., 2001; Soliva et al., 2003). Soliva et al. (2004) suggested that the initial reduction in methane production was associated with decreased fiber degradation, which Miller (1995) suggested was due to H₂ accumulation; however, these microbes adapted to lauric acid and MA, in contrast to other microbes such as the methanogens and ciliate protozoa (Soliva et al., 2004).

The MA diet appeared to have no adverse effect on *Butyrivibrio fibrisolvens*, a cellulolytic bacteria that synthesizes mainly *cis*-9, *trans*-11 CLA from linoleic

acid (Kepler et al., 1966; Kepler and Tove, 1967; Kim et al., 2000) and, as a result, did not shift the high production of *trans*-10 18:1 from linoleic acid via *trans*-10, *cis*-12 CLA, as shown in Table 4. Methanogenesis has also been inhibited as a direct toxic effect of unsaturated FA on methanogens (e.g., Prins et al., 1972; Henderson, 1973). Unsaturated FA have no effect on gram-negative bacteria (e.g., *Megasphaera elsdenii*, *Selenomonas ruminantium*, or *Prevotella ruminicola*) but inhibit gram-positive or gram-positive-type cell wall bacteria (e.g., *B. fibrisolvens*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*; Henderson, 1973; Maczulak et al., 1981). Schonhusen et al. (2003) showed that methanogenesis in the rumen, and not in the hindgut, was associated with the development of protozoal populations. Because virtually all bacteria attached to protozoa are methanogens (Vogels et al., 1980), by removing the protozoal population through defaunation, the ruminal bacterial population is modified, VFA production is shifted from acetate and butyrate toward propionate, and CH₄ production is reduced (Hegarty, 1999).

Milk Yield and Components

Milk yield and milk components are presented in Table 2. The MA diet reduced ($P < 0.05$) milk fat percentage by 2.4% but had no effect ($P > 0.05$) on milk yield. Myristic acid had no ($P > 0.05$) effect on milk protein percentage, although milk protein depression (lower protein percentage but not necessarily protein production) has been associated with the feeding of supplemental dietary fat to dairy cows (DePeters and Cant, 1992; Wu and Huber, 1994). The total yields of milk fat and protein were 638.6 vs. 485.0 ± 73.08 g/d and 520.9 vs. 414.4 ± 45.85 g/d, control vs. MA diet, respectively.

Because the cows used in the current study were in late lactation (290 ± 41.9 DIM), this raised the question of how much the benefit of lower CH₄ would be compromised by the need to maintain more animals to obtain equal yield. Kirchgeßner et al. (1995) estimated that although increasing milk production from 5,000 to 10,000 L/yr would increase total CH₄ production per cow per year by 23% (i.e., from 110 to 135 kg/yr), CH₄ production per kilogram of milk would be reduced by 40% (i.e., from 0.022 to 0.014 kg of CH₄/kg of milk). Therefore, overall CH₄ production could be decreased by reducing animal numbers while maintaining milk production.

Milk FA Composition

Milk FA including 18:1 isomers are summarized in Tables 3 and 4. The MA diet increased ($P < 0.05$) 14:0

in milk by 139% and *cis*-9 14:1 by 195% compared with the control diet (Table 3). Palmitic acid (16:0) was 23% higher ($P < 0.05$) in the control group than in the MA group, whereas stearic acid (18:0) was 43% lower ($P < 0.05$) in the MA group compared with the control group. The increased proportion of MA in milk fat decreased the proportions of palmitic and stearic acids in the MA group compared with the control group. However, if excessive amounts of long-chain SFA were incorporated in milk fat, it would cause milk fat to solidify. Because the melting points of short-chain SFA and MCFA are lower than those of long-chain SFA, the synthesis and supply of short-chain SFA and MCFA in the mammary tissue to modify milk fat fluidity is therefore critical. The increased level of MA in milk fat of the MA group in the current study would therefore give better fluidity than the control diet. Although total SFA were not ($P > 0.05$) affected by dietary treatment, the concentration of long-chain SFA (16:0 to 26:0) in the MA group were 2-fold lower ($P < 0.05$) than in the control group.

The contents of odd-chain SFA 19:0 and 21:0 were lower ($P < 0.05$) in the MA group compared with the control group (Table 3). The concentration of branched-chain SFA iso-14:0, anteiso-15:0, and iso-16:0 were lower ($P < 0.05$) in the MA group compared with the control group. Odd- and branched-chain FA in milk are synthesized by microbes in the rumen and are generally rare or absent in feeds (Diedrich and Henschel, 1990). These odd- and branched-chain FA can make up to 1.0 to 3.0% of milk and carcass lipids (Alonso et al., 1999; Bas and Morand-Fehr, 2000). The contents of these FA in a ruminant animal's milk or meat is therefore considered a reflection of microbial activities in the rumen. The difference in FA content (i.e., odd- and branched-chain FA) between the 2 groups suggests that MA supplementation must have altered the activities of some groups of microbes in the rumen. Bas et al. (2003) and Vlaeminck et al. (2006) have also reported that odd- and branched-chain FA contents in mixed rumen bacteria were changed with dietary differences.

Linoleic acid was the highest polyunsaturated FA in both the control and MA-treated group, followed by linolenic acid (Table 3). Myristic acid-supplemented cows had 22% lower ($P < 0.05$) linoleic acid concentrations in their milk than did the control group. The concentration of *trans*-9, *cis*-12 18:2 and *trans*-11, *cis*-15 18:2 were not ($P > 0.05$) affected by dietary treatment. The milk of the MA-treated cows had 56% lower ($P < 0.05$) nervonic acid (*cis*-15 24:1) than did the control group, although the concentration was very low in both groups. The concentration of highly unsaturated FA such as arachidonic acid (20:4), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3) were not

Table 3. Effects of a control vs. myristic acid-treated TMR on milk fatty acid contents (g/100 g of fatty acid) in lactating dairy cows

Fatty acid ¹	Control	Myristic acid	SEM
6:0	1.74 ^a	1.35 ^b	0.066
8:0	0.920 ^a	0.659 ^b	0.0768
10:0	2.88	2.35	0.222
11:0	0.312	0.292	0.0244
12:0	3.59	3.17	0.338
ai-13:0	0.090	0.086	0.0093
12:1	0.092	0.095	0.0066
13:0	0.101	0.108	0.0131
iso-14:0	0.211 ^a	0.068 ^b	0.0175
14:0	12.8 ^a	30.6 ^b	0.73
iso-15:0	0.229	0.197	0.0204
ai-15:0	0.501 ^a	0.321 ^b	0.0249
<i>Cis</i> -9 14:1	0.97 ^a	2.86 ^b	0.135
15:0	1.28	1.25	0.055
iso-16:0	0.500 ^a	0.227 ^b	0.0357
16:0	31.3 ^a	25.4 ^b	0.02
iso-17:0	0.281	0.265	0.0195
<i>Trans</i> -9 16:1	0.065	0.068	0.0090
ai-17:0	0.608	0.548	0.0232
<i>Cis</i> -9 16:1	1.45	1.39	0.080
17:0	0.452	0.319	0.0202
18:0	12.0 ^a	6.8 ^b	0.66
19:0	0.038 ^a	0.016 ^b	0.0026
<i>Trans</i> -9, <i>cis</i> -12 18:2	0.042	0.066	0.0129
<i>Trans</i> -11, <i>cis</i> -15 18:2	0.039	0.037	0.0037
18:2n-6	2.32 ^a	1.81 ^b	0.144
20:0	0.208 ^a	0.097 ^b	0.0071
18:3n-6	0.027	0.027	0.0029
<i>Cis</i> -9 20:1	0.156 ^a	0.087 ^b	0.0062
<i>Cis</i> -11 20:1	0.084	0.065	0.0062
18:3n-3	0.399 ^a	0.259 ^b	0.0258
21:0	0.035 ^a	0.020 ^b	0.0021
20:2n-6	0.030	0.024	0.0035
22:0	0.089 ^a	0.030 ^b	0.0041
20:3n-6	0.100	0.095	0.0088
<i>Cis</i> -13 22:1	0.162	0.144	0.0013
20:3n-3	0.006	0.007	0.0007
20:4n-6	0.127	0.149	0.0088
23:0	0.029	0.016	0.0041
20:4n-3	0.033	0.024	0.0038
22:2n-6	0.011	0.008	0.0018
24:0	0.043 ^a	0.014 ^b	0.0043
20:5n-3	0.051	0.046	0.0059
<i>Cis</i> -15 24:1	0.009 ^a	0.004 ^b	0.0011
22:4n-6	0.025	0.020	0.0015
22:4n-3	0.006	0.004	0.0006
26:0	0.029	0.017	0.0059
22:5n-3	0.068	0.081	0.0063
22:6n-3	0.058	0.051	0.0075
Total CLA ²	0.49	0.47	0.135
Total SFA ³	68.8	72.5	1.23
MCSFA ⁴	51.9 ^a	62.6 ^b	0.91
LCSFA ⁵	14.1 ^a	7.9 ^b	0.65
Total MUFA ⁶	27.4	24.0	0.86
Total n-6 PUFA ⁷	3.33	2.96	0.484
Total n-3 PUFA ⁸	0.891	0.771	0.0793
Total <i>trans</i> -18:1	2.75	2.64	0.333
Total <i>cis</i> -18:1	21.9 ^a	16.7 ^b	0.65

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Means are based on 6 cows per treatment.

²Total conjugated linoleic acid (CLA): *cis*-9, *trans*-11 18:2; *cis*-10, *trans*-12 18:2; *trans*-9, *cis*-11 18:2; *trans*-10, *cis*-12 18:2; *trans*-11, *trans*-13 18:2; *trans*-9, *trans*-11 18:2; and *trans*-10, *trans*-12 18:2.

³Total saturated fatty acids (SFA): all fatty acids without any double bond (9:0 to 26:0).

⁴MCSFA: medium-chain SFA (9:0 to 16:0).

⁵LCSFA: long-chain SFA (17:0 to 26:0).

⁶Total monounsaturated fatty acids (MUFA): all fatty acids with a single double bond (16:1 to 24:1).

⁷Total n-6 polyunsaturated fatty acids (PUFA): *trans*-9, *trans*-12 18:2; *cis*-9, *trans*-12 18:2; *trans*-9, *cis*-12 18:2; 18:2n-6; 18:3n-6; 20:2n-6; 20:3n-6; 22:2n-6; and 22:5n-6.

⁸Total n-3 PUFA: 18:3n-3; 20:3n-3; 20:4n-3; and 22:4n-3.

Table 4. Effects of a control vs. myristic acid-treated TMR on milk 18:1 isomers (g/100 g of fatty acid) in lactating dairy cows

Fatty acid ¹	Control	Myristic acid	SEM
<i>Trans</i> -4 18:1	0.015 ^a	0.009 ^b	0.0012
<i>Trans</i> -5 18:1	0.011 ^a	0.008 ^b	0.0007
<i>Trans</i> -6-8 18:1	0.206 ^a	0.153 ^b	0.0122
<i>Trans</i> -9 18:1	0.319 ^a	0.242 ^b	0.0169
<i>Trans</i> -10 18:1	0.338	0.312	0.0452
<i>Trans</i> -11 18:1	0.972	0.867	0.1262
<i>Trans</i> -12 18:1	0.409 ^a	0.315 ^b	0.0244
<i>Trans</i> -13-14 18:1	0.300	0.324	0.0222
<i>Cis</i> -9 18:1	18.8 ^a	14.3 ^b	0.94
<i>Cis</i> -11 18:1	1.24	1.16	0.078
<i>Cis</i> -12 18:1	0.329 ^a	0.252 ^b	0.0231
<i>Cis</i> -13 18:1	0.025 ^a	0.063 ^b	0.0058
<i>Trans</i> -16 18:1	0.165 ^a	0.096 ^b	0.0099

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Means are based on 6 cows per treatment.

($P > 0.05$) affected by diet. The 18:1 isomers in milk are presented in Table 4. The concentration of *cis*-9 18:1 was highest among the monounsaturated FA in both groups. Total *cis*-18:1 was lower ($P < 0.05$) in the MA group than in the control group. The concentration of *cis*-9 18:1 in the MA group was 24% lower ($P < 0.05$) than in the control group. Other *cis*-18:1 FA, such as *cis*-12, *cis*-13, were also lower ($P < 0.05$) in the MA group, except for *cis*-11 (Table 4). Dietary supplementation with MA did not ($P > 0.05$) alter the concentration of *trans*-11 18:1 or *trans*-10 18:1 in the milk. The concentration of *trans*-10 18:1 was 3 times lower than the concentration of *trans*-11 18:1 in both groups. Myristic acid supplementation did not shift the ruminal microbial population significantly to produce more *trans*-10 18:1 from linoleic acid via *trans*-10, *cis*-12 CLA. Vaccenic acid was the main *trans*-18:1 isomer in both groups (Table 4). The milk FA profiles of cows fed diets high in unsaturated FA oils are characterized by higher levels of *trans*-10 18:1 compared with *trans*-11 18:1 (Pottier et al., 2006) because the oils induce a shift in the ruminal biohydrogenation process, resulting in the production of a higher amount of *trans*-10 18:1 in the mammary gland, thus causing milk fat depression. Bauman and Grinari (2003) showed that marked milk fat depression was associated with an increase in the milk fat content of the *trans*-10 18:1 isomer.

Total CLA contents in the milk did not ($P > 0.05$) differ between treatments (Table 3). The most abundant CLA was *cis*-9, *trans*-11 CLA, which constituted 83.3 to 85% of the total CLA content (data not shown). *Trans*-10, *cis*-12 CLA, which is known to be a potent inhibitor of milk fat synthesis (Baumgard et al., 2000), accounted for only 2 to 2.3% of total CLA. The concentrations of both *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in milk were not affected by dietary treatment.

CONCLUSIONS

Dietary supplementation with MA reduced methane production from dairy cows but had no effect on DMI, milk yield, total CLA, or *cis*-9, *trans*-11 CLA compared with the control group. Generally, supplementation with unsaturated FA from oil shifts the production of vaccenic acid (*trans*-11 18:1) in the rumen toward *trans*-10 18:1, resulting in a higher level of *trans*-10 18:1 in milk; however, this phenomenon was not observed with MA in the current study. These results suggest that MA could be used to inhibit the activities of methanogens in ruminant animals without altering the CLA and *trans*-18:1 FA profiles in milk.

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