J. Dairy Sci. 90:886–897

Effects of Essential Oils on Digestion, Ruminal Fermentation, Rumen Microbial Populations, Milk Production, and Milk Composition in Dairy Cows Fed Alfalfa Silage or Corn Silage[1]

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ABSTRACT

Four Holstein cows fitted with ruminal cannulas were used in a 4 × 4 Latin square design (28-d periods) with a 2 × 2 factorial arrangement of treatments to investigate the effects of addition of a specific mixture of essential oil compounds (MEO; 0 vs. 750 mg/d) and silage source [alfalfa silage (AS) vs. corn silage (CS)] on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition. Total mixed rations containing either AS or CS as the sole forage source were balanced to be isocaloric and isonitrogenous. In general, no interactions between MEO addition and silage source were observed. Except for ruminal pH and milk lactose content, which were increased by MEO supplementation, no changes attributable to the administration of MEO were observed for feed intake, nutrient digestibility, end-products of ruminal fermentation, microbial counts, and milk performance. Dry matter intake and milk production were not affected by replacing AS with CS in the diet. However, cows fed CS-based diets produced milk with lower fat and higher protein and urea N concentrations than cows fed AS-based diets. Replacing AS with CS increased the concentration of NH_3-N and reduced the acetate-to-propionate ratio in ruminal fluid. Total viable bacteria, cellulolytic bacteria, and protozoa were not influenced by MEO supplementation, but the total viable bacteria count was higher with CS- than with AS-based diets. The apparent digestibility of crude protein did not differ between the AS and CS treatments, but digestibilities of neutral detergent fiber and acid detergent fiber were lower when cows were fed CS-based diets than when they were fed AS-based diets. Duodenal bacterial N flow, estimated using urinary purine derivatives and the amount of N retained, increased in cows fed CS-based diets compared with those fed AS-based diets. Feeding cows AS increased the milk fat contents of *cis*-9, *trans*-11 18:2 (conjugated linoleic acid) and 18:3 (n-3 fatty acid) compared with feeding cows CS. Results from this study showed limited effects of MEO supplementation on nutrient utilization, ruminal fermentation, and milk performance when cows were fed diets containing either AS or CS as the sole forage source.

Key words: dairy cow, essential oil, alfalfa silage, corn silage

INTRODUCTION

In the last few years, a number of studies have been devoted to investigating the potential use of plants and plant extracts as alternatives to in-feed antibiotics in ruminant nutrition. Plant extracts, such as saponins, have been evaluated for their antimicrobial effects and for their ability to favorably alter ruminal fermentation and improve nutrient utilization in ruminants (Hristov et al., 1999; Wang et al., 2000). More recently, essential oils have attracted attention for their potential as alternatives to feed antibiotics and growth promoters in livestock (Wallace, 2004). Essential oils are naturally occurring volatile components that can be extracted from plants by distillation methods, in particular steam distillation (Greathead, 2003). Chemically, essential oils are variable mixtures of principally terpenoids, especially monoterpenes (C_{10}) and sesquiterpenes (C_{15}), although diterpenes (C_{20}) may also be present. Essential oils may also include a variety of low-molecular-weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, or lactones and, exceptionally, nitrogen- and sulfur-containing compounds, coumarins, and homologs of phenylpropanoids (Dorman and Deans, 2000). Essential oils from a variety of sources have been shown to alter the bacterial growth and metabolism of several types of bacteria, including rumen bacteria (Wallace, 2004). Many of the investigations conducted to date on essential oils have been laboratory based (i.e., in vitro incubations) and of a short-term nature (McIntosh et al., 2003; Newbold et al., 2004; Castillejos et al., 2005).

Received May 4, 2006.
Accepted September 27, 2006.
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Furthermore, few in vivo studies have been carried out to evaluate the effectiveness of essential oils to manipulate ruminal fermentation and improve nutrient utilization and performance by dairy cows (Benchaar et al., 2005a,b, 2006).

Alfalfa silage (**AS**) and corn silage (**CS**) are the 2 most common forages fed to dairy cows in North America. A number of studies have compared the effects of feeding CS vs. AS or a combination of both forages on the digestion, milk production, and milk composition of dairy cows (Broderick, 1985; Charmley et al., 1993; Onetti et al., 2002; Ruppert et al., 2003; Wattiaux and Karg, 2004a,b). However, few studies to date have compared CS and AS when they represent the sole forage source of the diet (Broderick, 1985; Hristov and Broderick, 1996). The objective of the present study was to investigate the effects of dietary addition of a specific mixture of essential oil compounds (**MEO**) on digestion, ruminal fermentation characteristics, ruminal microbial populations, milk production, and milk composition, including fatty acid (**FA**) composition, of dairy cows fed a TMR containing either AS or CS as the sole forage source.

MATERIALS AND METHODS

Cows, Experimental Design, and Diets

Four lactating Holstein cows fitted with ruminal cannulas (10 cm; Bar Diamond Inc., Parma, ID) were used in a 4 × 4 Latin square design over four 28-d periods. The cows averaged 61 ± 12 DIM at the start of the experiment, with an average BW of 551 ± 43 kg. They were housed in individual tie stalls and had free access to water during the experiment. Cows were fed ad libitum (10% orts, on an as-fed basis) a TMR containing either AS or CS as the sole forage source (Table 1) without supplementation (0 mg/d) or supplemented with 750 mg/d of MEO (Crina ruminants; CRINA S.A., Gland, Switzerland). The Crina ruminants supplement consisted of a mixture of natural and nature-identical essential oil compounds, including thymol, eugenol, vanillin, guaiacol, and limonene (McIntosh et al., 2003; Castillejos et al., 2005).

The amount of 750 mg/d was chosen based on the recommended dose of MEO for an adult lactating dairy cow (Innovation Développement en Nutrition Animale, Sautron, France). Diets were formulated to be isonitrogenous and isocaloric. Treatments were arranged as a 2 × 2 factorial to evaluate the main effects of MEO addition (0 vs. 750 mg/cow per d), silage source (CS vs. AS), and their interaction. Adaptation to experimental treatments was from d 1 to 14, ruminal sampling on d 21, and milk yield and sampling as well as total fecal and urine collection from d 21 to 28. All experimental procedures were approved by the Animal Care Committee of the Dairy and Swine Research Center (Agriculture and Agri-Food Canada; Sherbrooke, Quebec, Canada), and cows were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Table 1. Ingredients and chemical composition of the TMR

Item	TMR	
	Alfalfa silage	Corn silage
Ingredient, % of DM		
Alfalfa silage	49.2	—
Corn silage	—	50.1
Corn grain, ground	43.3	9.7
Barley grain, ground	—	19.5
Corn gluten meal	—	3.0
Soybean meal, 48% of CP	4.0	7.4
Soybean hulls	—	5.1
Megalac[1]	1.0	1.0
Urea	—	0.5
Limestone	—	1.2
Dicalcium phosphate	0.7	0.7
Magnesium oxide	0.1	0.1
Minerals and vitamins[2]	1.7	1.7
Chemical composition		
DM, %	59.0	51.5
OM, % of DM	92.8	94.4
CP, % of DM	16.4	15.5
NDF, % of DM	39.3	37.5
ADF, % of DM	26.1	20.5
Starch, % of DM	17.6	25.0
Ether extract, % of DM	4.1	3.0
NE_L,[3] Mcal/kg of DM	1.64	1.65
Fatty acid, g/100 g of total FA		
12:0	0.29	0.26
14:0	0.56	0.47
16:0	20.35	17.32
16:1	0.98	0.29
18:0	3.95	3.72
18:1	21.78	29.20
18:2	35.95	46.69
18:3	16.14	2.05

[1]Megalac calcium salts of palm oil (Church and Dwight Co., Inc., Princeton, NJ).

[2]Contained 10% Ca, 10% P, 11% Na, 4% Mg, 0.2% K, 0.3% S, 870 mg/kg of Cu, 2,900 mg/kg of Mn, 4,355 mg/kg of Zn, 4,800 mg/kg of Fe, 32 mg/kg of Co, 87 mg/kg of I, 650 mg/kg of F, 17.1 mg/kg of Se, 391,000 IU of vitamin A/kg, 86,000 IU of vitamin D/kg, and 1,320 IU of vitamin E/kg.

[3]Calculated using published values of feed ingredients (NRC, 2001).

Feed Intake, Apparent Total Tract Digestibility, and N Balance

Diets were offered in 2 equal amounts twice daily (0800 and 1600 h). Feed consumption was recorded daily by weighing feeds offered to and refused by the cows, and data from d 14 to 28 were included in the statistical analysis. Samples of TMR, feed ingredients, and orts were collected daily and kept frozen. Samples were composited by period, dried at 55°C for 48 h, ground through a 1-mm screen Wiley mill (standard

model 4; Arthur M. Thomas, Philadelphia, PA) and analyzed for DM, OM, total N, NDF, ADF, starch, lipids, and FA composition. Cows were weighed at the beginning and at the end of each experimental period after the a.m. milking on 2 consecutive days. For 7 consecutive days, feces were weighed and mixed daily, and a representative sample (2%) was taken, stored at –20°C, and subsequently thawed, dried at 55°C for 48 h, and ground though a 1-mm screen (Wiley mill) for chemical analysis. Total urine was collected daily into stainless-steel containers and acidified with H_2SO_4 (50% vol/vol) to maintain pH <2.0. A representative sample (2%) was taken and kept frozen at –20°C until analysis.

Ruminal Fermentation Characteristics

Ruminal fluid was collected from multiple sites (anterior dorsal, anterior ventral, medium ventral, posterior dorsal, and posterior ventral) within the rumen at 0, 1, 2, 4, 6, and 8 h after the 0800 h feeding. Samples (250 mL/site) were withdrawn using a syringe screwed to a stainless-steel tube ending with a probe covered by a 50-μm metal mesh (RT Rumen Fluid Collection Tube; Bar Diamond Inc.). Ruminal fluid pH was measured immediately after sampling (Accumet pH meter; Fisher Scientific, Montreal, Quebec, Canada), and samples were acidified to pH 2 with 50% H_2SO_4 and frozen at –20°C for later determination of VFA and ammonia N (NH_3-N) concentrations.

Microbial Counts

Microbial counts were carried out on ruminal fluid samples collected 2 h after the a.m. feeding. Ruminal fluid (1-L) and solid digesta (500-g) samples were collected from different sites of the rumen of each cow. Samples were mixed thoroughly, and subsamples of 500 mL of ruminal fluid and 250 g of solid digesta were blended anaerobically under oxygen-free CO_2 and strained through 2 layers of cheesecloth. A 3-mL portion of the strained ruminal fluid was preserved using 3 mL of methyl green formalin-saline solution for protozoa enumeration (Ogimoto and Imai, 1981). Protozoa samples were stored at room temperature in the dark until counting. Protozoa were enumerated microscopically in a Levy-Hausser counting chamber (Hausser Scientific, Horsham, PA). Each sample was counted twice, and if the average of the duplicates differed by more than 10%, the counts were repeated.

Serial 10-fold dilutions of strained ruminal fluid were prepared under 95% CO_2-5% H_2 in an anaerobic chamber and used as inoculum for microbial counts (Bryant and Burkey, 1953). Total viable counts were enumerated on triplicate layered plates (Koch, 1994) containing ruminal fluid-starch-agar medium (Grubb and Dehority, 1976). Cellulolytic bacteria were counted by the most probable number method based on the degradation of a filter paper strip (Mann, 1968).

Milk Production and Milk Composition

Cows were milked twice daily in their stalls at 0500 and 1700 h, and milk yield was recorded at each milking. During the last week of each 28-d period, milk samples were taken from each cow at each milking, pooled on a yield basis, and stored at 4°C with a preservative (bronopol-B2) until analyzed for fat, protein, and lactose. Composite milk samples without preservative were frozen at –20°C until analyzed for the milk FA profile. Milk FA were prepared and methylated after each experimental period of the Latin square. The methylated samples were stored at –80°C until analyzed by GLC.

Chemical Analyses

Analytical DM contents of TMR, silages, orts, and feces were determined by oven-drying at 105°C for 48 h (AOAC, 1990; method 930.15). Ash contents of TMR, silages, orts, and feces were determined by incineration at 550°C overnight, and the OM content was calculated as the difference between 100 and the percentage of ash (AOAC, 1990; method 942.05). Total N contents of TMR, silages, orts, and feces were determined by combustion assay (Leco model FP-428 Nitrogen Determinator; Leco, St. Joseph, MI). Crude protein was calculated as N × 6.25. The concentrations of NDF in TMR, silages, orts, and feces were determined as described by Van Soest et al. (1991) without the use of sodium sulfite and with the inclusion of heat-stable α-amylase. The ADF contents in TMR, silages, orts, and feces were determined according to AOAC (AOAC, 1990; method 973.18). The NDF and ADF procedures were adapted for use in an Ankom^{200} Fiber Analyzer (Ankom Technology Corp., Fairport, NY). Starch concentrations in TMR, silages, orts, and feces were determined colorimetrically (Keppler and Decker, 1974) using a commercial kit (#10 207 748 035; Boehinger Mannheim, Burgessville, Ontario, Canada). Ether extract contents of TMR, silages, and orts were determined using a Soxtec system HT6 apparatus (Tecator; Fisher Scientific, Montreal, Quebec, Canada) according to AOAC procedure 920.39 (AOAC, 1990). The concentration of N in acidified urine samples was determined by micro-Kjeldahl analysis (AOAC, 1990). Purine derivatives in urine samples were analyzed by HPLC (Shingfield and Offer, 1999) and bacterial N entering the duodenum (g/d) was calculated as described by Chen and Gomez (1992). Con-

centrations of NH_3-N and VFA in ruminal fluid were analyzed by colorimetry (Weatherburn, 1967) and by GLC (Varian 3700; Varian Specialities Ltd., Brockville, Ontario, Canada), respectively. Protein, fat, and lactose concentrations in milk samples were analyzed (AOAC, 1990) by infrared spectrophotometry (System 4000 MilkoScan; Foss Electric, Hillerød, Denmark). Milk concentrations of urea N were determined with an automatic analyzer (Technicon Autoanalyser II; Technicon Instruments Corporation, Tarrytown, NY) as described by Huntington (1984). For the analysis of milk FA, methyl esters were prepared by base-catalyzed transmethylation according to the method of Chouinard et al. (1997) and FA methyl esters were analyzed by GLC (5890 Series II gas chromatograph; Hewlett-Packard, Palo Alto, CA) as described by Destaillats et al. (2005). Composition of FA in feed samples was analyzed according to the procedure of Sukhija and Palmquist (1988).

Statistical Analysis

Data were analyzed using PROC MIXED of SAS (SAS Institute, 2000) according to the model

$$Y_{ijk} = \mu + a_i + \beta_j + \tau_k + e_{ijk},$$

where Y_{ijk} is the response variable, μ is the overall mean, a_i is the random effect of cow i, β_j is the effect of period j, τ_k is the effect of treatment k, and e_{ijk} is the random residual error. For the statistical analysis of ruminal fermentation characteristics (pH, VFA, and NH_3-N), sampling time and sampling time × treatment were added to the model and analyzed using repeated measures of PROC MIXED. The compound symmetry was used as the covariance structure. Factorial contrasts were used to test the main effects of MEO (0 vs. 750 mg/d), silage source (AS vs. CS), and their interaction (MEO × silage). Results are reported as least squares means ± standard errors of the mean. Significance was declared at $P < 0.05$ and a trend at $0.05 \leq P < 0.10$ unless otherwise stated.

RESULTS AND DISCUSSION

Feed Intake, Milk Production, and Milk Composition

There was no interaction between MEO addition and silage for daily DMI, milk production, 4% FCM yield, milk composition, and yield of milk constituents (Table 2). Intake of DM, expressed in kilograms per day or as a percentage of BW, was not affected by MEO addition to the TMR. Little information is available on the effect of essential oils on feed intake in ruminants. Benchaar et al. (2006) observed no change in DMI when lactating dairy cows were supplemented with the same MEO (Crina ruminants) at a dose of 2 g/d.

Intake of DM averaged 17.4 kg/d, and was similar between cows fed AS-based diets and those fed CS-based diets (Table 2). Similarly, Wattiaux and Karg (2004a) observed no change in DMI when cows were fed 55% of forage in TMR containing 14% AS and 41% CS vs. 41% AS and 14% CS. In contrast, Ruppert et al. (2003) reported a higher DMI for cows consuming AS-based diets, as compared with cows fed CS-based diets when forage was added at 40% of the DM.

Milk and 4% FCM yields averaged, respectively, 28.4 and 23.4 kg/d, and were not affected by the addition of MEO to the diet (Table 2). This agreed with the results of Benchaar et al. (2006), who found no change in the milk production and milk composition of cows fed 2 g/d of Crina ruminants supplement. Except for milk lactose concentration, which was higher for cows fed MEO than for those fed no MEO (4.78 vs. 4.58%), MEO supplementation had no effect on milk concentrations of fat, protein, urea N, and milk yields of fat, protein, and lactose.

Feeding cows either CS or AS had no influence on the production of either milk or 4% FCM (Table 2). These results were expected because the DMI was similar for cows fed CS and AS, and diets were balanced to provide equal amounts of energy and MP. Results of the present study corroborate the lack of difference in milk yield response of lactating cows reported in previous studies when AS-based diets were compared with CS-based diets (Broderick, 1985; Ruppert et al., 2003; Wattiaux and Karg, 2004b). In contrast, Wattiaux and Karg (2004a) observed higher milk production for cows fed CS-based diets than for those fed AS-based diets, but the yield of 3.5% FCM was similar between AS and CS.

The milk fat content tended ($P = 0.07$) to be lower for cows fed CS-based diets as compared with those fed AS-based diets (2.57 vs. 3.04%). This observation is consistent with the tendency ($P = 0.12$) for a higher milk fat content of *trans*-10 18:1 and the lower ($P < 0.05$) ruminal acetate-to-propionate ratio observed when cows were fed CS-based diets than when fed AS-based diets (see subsequent discussion; Tables 3 and 4). The fat-depressing effect of diets based on CS compared with AS has been reported previously (Onetti et al., 2002; Ruppert et al., 2003; Wattiaux and Karg, 2004a,b). Other studies, however, have reported no difference in milk fat content when cows were fed diets based on either AS or CS (Broderick, 1985; Charmley et al., 1993).

The milk protein concentration was higher and the milk protein yield tended ($P = 0.05$) to be higher for cows fed CS-based diets than for those fed AS-based diets (Table 2). The milk protein content was similar

Table 2. Dry matter intake, milk production, and milk composition of cows fed a TMR based on either alfalfa silage (AS) or corn silage (CS) without supplementation (0 mg/d) or supplemented with a mixture of essential oil compounds (MEO; 750 mg/d)

	AS		CS			Effect (P =)[1]		
Item	0	750	0	750	SEM	MEO	Silage	MEO × silage
DMI, kg/d	17.3	17.2	17.7	17.5	1.5	0.83	0.55	0.95
DMI, % of BW	2.96	3.00	3.06	3.03	0.23	1.00	0.55	0.77
Milk production, kg/d	29.3	27.8	28.4	28.1	2.3	0.13	0.54	0.32
4% FCM,[2] kg/d	24.5	24.2	22.9	21.8	2.5	0.56	0.13	0.74
Milk composition, %								
Fat	2.95	3.13	2.69	2.45	0.35	0.89	0.07	0.38
Protein	3.18	3.24	3.52	3.48	0.11	0.89	<0.01	0.47
Lactose	4.58	4.77	4.58	4.79	0.05	<0.01	0.97	0.81
MUN, m*M*	4.76	4.28	8.70	8.53	0.34	0.26	<0.01	0.63
Milk yield, kg/d								
Fat	0.85	0.87	0.77	0.70	0.12	0.74	0.11	0.56
Protein	0.93	0.90	1.00	0.96	0.06	0.34	0.05	0.98
Lactose	1.34	1.33	1.30	1.34	0.11	0.64	0.68	0.40

[1]*P*-value for the effect of MEO (0 vs. 750 mg/d), silage source (AS vs. CS), and the interaction between essential oils and silage source (MEO × silage).

[2]4% FCM = 0.4 (kilograms of milk) + 15.0 (kilograms of fat).

(Broderick, 1985) or higher (Broderick, 1985) when cows were fed CS compared with when they were fed AS. No differences in milk protein content and yield were observed when lactating cows were fed diets varying in proportions of AS and CS (Dhiman and Satter, 1997; Onetti et al., 2002).

Under the experimental conditions of the current study, the urea N concentration was higher in milk of cows fed CS-based diets compared with those fed AS-based diets (8.62 vs. 4.52 m*M*; Table 2). This result agreed with that of Broderick (1985), who reported a higher MUN concentration when cows were fed CS as

Table 3. Fatty acid (FA) profile (g/100 g of total FA) of milk fat of cows fed a TMR based on either alfalfa silage (AS) or corn silage (CS) without supplementation (0 mg/d) or supplemented with a mixture of essential oil compounds (MEO; 750 mg/d)

	AS		CS			Effect,[1] P =		
Fatty acid	0	750	0	750	SEM	MEO	Silage	MEO × silage
4:0	3.15	3.44	2.41	2.46	0.34	0.64	0.05	0.73
6:0	1.86	1.96	1.51	1.47	0.20	0.87	0.08	0.75
8:0	1.25	1.30	1.08	1.07	0.14	0.89	0.20	0.86
10:0	3.03	3.07	2.90	2.87	0.37	0.99	0.67	0.92
12:0	3.57	3.60	3.85	3.86	0.35	0.97	0.47	0.98
14:0	11.47	11.49	11.89	11.88	0.55	0.99	0.49	0.98
14:1	1.27	1.23	1.87	1.95	0.22	0.93	0.02	0.79
15:0	1.02	0.97	1.50	1.57	0.11	0.94	<0.01	0.61
16:0	29.19	29.19	32.56	33.03	1.26	0.86	0.03	0.87
16:1	1.74	1.69	2.85	2.91	0.45	0.99	0.04	0.91
17:0	0.52	0.51	0.59	0.59	0.03	0.87	0.04	0.87
17:1	0.16	0.17	0.29	0.29	0.04	0.85	0.03	0.89
18:0	9.77	9.92	7.58	7.06	0.47	0.70	<0.01	0.50
Trans-10 18:1	0.57	0.54	1.45	1.60	0.53	0.92	0.12	0.86
Trans-11 18:1	1.48	1.38	0.50	0.68	0.08	0.61	<0.01	0.13
Cis-9 18:1	20.87	20.72	18.49	17.74	0.069	0.53	<0.01	0.68
Other 18:1	3.33	3.11	3.01	3.25	0.29	0.97	0.76	0.47
18:2	2.48	2.51	2.73	2.73	0.20	0.95	0.28	0.95
18:3	0.63	0.62	0.23	0.21	0.04	0.72	<0.01	0.95
CLA[2]	0.69	0.64	0.37	0.39	0.04	0.92	<0.01	0.39
Unknown	2.00	1.98	2.34	2.41	0.08	0.72	<0.01	0.61

[1]*P*-value for the effect of MEO (0 vs. 750 mg/d), silage source (AS vs. CS), and the interaction between MEO and silage source (MEO × silage).

[2]*Cis*-9, *trans*-11 18:2.

Table 4. Ruminal fermentation characteristics and rumen microbial counts in cows fed a TMR based on either alfalfa silage (AS) or corn silage (CS) without supplementation (0 mg/d) or supplemented with a mixture of essential oil compounds (MEO; 750 mg/d)

	AS		CS			Effect,[1] (P =)		
Item	0	750	0	750	SEM	MEO	Silage	MEO × silage
pH	6.36	6.39	6.26	6.41	0.10	0.07	0.31	0.19
NH_3-N, mg/100 mL	7.18	6.03	8.72	8.94	1.19	0.67	0.07	0.53
Total VFA, m*M*	88.3	92.8	89.2	80.0	3.4	0.09	<0.01	<0.01
VFA, mol/100 mol								
Acetate (A)	65.6	66.9	60.1	58.6	1.5	0.97	<0.01	0.33
Propionate (P)	20.5	18.3	27.3	27.7	1.6	0.55	<0.01	0.39
Butyrate	10.9	11.6	8.5	9.7	1.1	0.11	<0.01	0.72
Isobutyrate	0.78	0.82	0.85	0.81	0.09	0.99	0.55	0.49
Valerate	1.23	1.21	1.79	1.94	0.24	0.76	0.02	0.71
Isovalerate	1.10	1.14	1.47	1.25	0.20	0.53	0.11	0.33
A:P	3.26	3.72	2.22	2.20	0.24	0.32	<0.01	0.29
Microbial counts								
Total viable bacteria, × 10^9/mL	1.40	1.53	4.90	3.38	0.69	0.32	<0.01	0.25
Cellulolytic bacteria, × 10^7/mL	3.83	6.62	5.31	5.61	2.43	0.47	0.91	0.56
Protozoa, × 10^5/mL	4.04	5.38	4.76	5.37	1.02	0.20	0.62	0.61

[1]*P*-value for the effect of MEO (0 vs. 750 mg/d), silage source (AS vs. CS), and the interaction between MEO and silage source (MEO × silage).

compared with AS as the sole source of forage at a proportion of 60% of the total diet DM. However, the reverse was observed by Dhiman and Satter (1997), who reported a decrease in MUN concentration with an increased proportion of CS in the diet at the expense of that of AS. On the other hand, Wattiaux and Karg (2004a,b) found no difference in MUN concentrations when cows were fed 55% of forage in a TMR containing either 14% AS and 41% CS or 41% AS and 14% CS.

Essential oils have antimicrobial activity against a wide range of microorganisms, including gram-negative and gram-positive bacteria (Helander et al., 1998). Many ruminal bacteria possess the capacity to biohydrogenate unsaturated FA (Harfoot and Hazlewood, 1988). Supplementation of dairy cow diets with essential oils could decrease FA biohydrogenation in the rumen by inhibiting the growth of some bacteria involved in that process. Accordingly, we were interested in evaluating whether the milk FA composition could be altered by feeding essential oils to dairy cows. However, the results presented in Table 3 indicate that there was no effect of dietary addition of MEO on the milk FA profile, suggesting that the mixture and amount of MEO supplied in the current study did not significantly affect overall lipid metabolism in the rumen.

The milk of cows fed AS-based diets, compared with those fed CS-based diets, was higher in concentrations of 4:0 (3.3 vs. 2.4%), 6:0 (1.9 vs. 1.5%; P = 0.08), 18:0 (9.8 vs. 7.3%), *trans*-11 18:1 (1.4 vs. 0.6%), *cis*-9 18:1 (20.8 vs. 18.1%), *cis*-9, *trans*-11 18:2 (CLA; 0.7 vs. 0.4%), and 18:3 (n-3 FA; 0.6 vs. 0.2%). Replacing AS with CS in the diet increased the milk fat contents of 14:1 (1.9 vs. 1.2%), 15:0 (1.5 vs. 1.0%), 16:0 (32.8 vs. 29.2%), 16:1 (2.9 vs. 1.7%), 17:0 (0.6 vs. 0.65%), and 17:1 (0.17 vs. 0.29%). Milk fat concentrations of 8:0, 10:0, 12:0, 14:0, other 18:1, and 18:2 were not affected by silage source. The milk fat content of *trans*-10 18:1 was numerically higher for cows fed CS-based diets compared with those fed AS-based diets (1.5 vs. 0.6%, P = 0.12). Onetti et al. (2002) also reported similar effects on milk FA composition with higher 18:0 and 18:3, and lower *trans*-10 18:1 concentrations when the proportion of AS increased in the diet at the expense of the proportion of CS. In the current study, the concentration of 18:3 was higher in AS-based diets (Table 1), which explains the increased level of this FA in the milk fat of cows fed AS-based diets, as compared with those fed CS-based diets. On the other hand, feeding AS seemed to favor a more stable rumen environment, leading to the production of *trans*-11 18:1 as a biohydrogenation intermediate at the expense of *trans*-10 18:1. An increased concentration of *trans*-10 18:1 in milk has been associated with a decreased milk fat concentration (Griinari et al., 1998), thus explaining the tendency for the lower milk fat concentration observed for cows fed CS-based diets (Table 2).

Ruminal Fermentation Characteristics and Microbial Counts

There was no interaction between MEO addition and silage source for ruminal fermentation parameters, except for total VFA concentration (Table 4). The mean pH value tended (P = 0.07) to be higher in the ruminal fluid of cows fed diets supplemented with MEO as compared with those fed diets without MEO (6.40 vs. 6.30).

This would agree with the results of Benchaar et al. (2006), who reported higher ruminal pH in cows fed diets supplemented with 2 g/d of Crina ruminants than in cows fed diets without supplementation. Evans and Martin (2000) also reported that the addition of 400 mg/L of thymol, a common essential oil derived from *Thymus* and *Origanum* plants, increased the pH in 24-h in vitro batch cultures of mixed rumen bacteria, but no effects were reported at lower doses (i.e., 50, 100, and 200 mg/L). More recently, Castillejos et al. (2006) evaluated the effects of increasing doses (0, 5, 50, 500, and 5,000 mg/L) of eugenol, thymol, guaiacol, limonene, and vanillin on ruminal fermentation in a 24-h in vitro batch culture of ruminal bacteria. At the highest dose (i.e., 5,000 mg/L), all these compounds increased ruminal pH, but no effects were observed at lower doses.

Replacing AS with CS in the diet had no influence on ruminal pH, which averaged 6.36 (Table 4). Similarly, no difference in ruminal pH was observed when diets with different proportions of AS and CS were fed to cows (Dhiman and Satter, 1997; Onetti et al., 2002). However, Broderick (1985) and Ruppert et al. (2003) reported lower ruminal pH for cows fed CS-based diets compared with those fed AS-based diets.

Supplementation of the diet with MEO had no effect on the ruminal fluid concentration of NH_3-N (Table 4). This disagrees with the short-term in vitro results of McIntosh et al. (2003) and Newbold et al. (2004), who observed a reduction in the rate of NH_3-N production when CN acid hydrolysate (i.e., free AA) was incubated for 24 to 48 h in strained ruminal fluid collected from cows or sheep fed the same MEO as the one used in the current study (1 or 110 mg/d, respectively; Crina ruminants). On the other hand, longer term in vitro (i.e., continuous-culture system) incubations (Castillejos et al., 2005) and in vivo (Newbold et al., 2004; Benchaar et al., 2006) studies have shown no inhibiting effect of Crina ruminants MEO on protein degradation in the rumen. Similarly, Castillejos et al. (2006) reported no change in the NH_3-N concentration when thymol and eugenol were added at doses of 5, 50, and 500 mg/L in a continuous-culture fermenter. This discrepancy among studies could be due to the experimental procedure used (batch vs. continuous culture). The greater exposure time of ruminal bacteria to essential oils (24 to 48 h in batch cultures vs. 9 d in the continuous-culture system) may allow ruminal microbes to adapt to the essential oils, as shown by Cardozo et al. (2004) and Busquet et al. (2005), who observed that some of the effects of essential oils on ruminal fermentation disappeared after 6 to 7 d of fermentation in a dual-flow continuous-culture system, indicating that rumen microorganisms are able to adapt to the essential oils. Based on these observations, those authors concluded that data from short-term in vitro fermentation may lead to erroneous conclusions and must therefore be interpreted with caution. More research is required to understand the mechanisms of rumen microorganisms' adaptation to essential oil compounds. The inconsistencies among studies could be also explained by the different doses of essential oils used. For instance, Castillejos et al. (2006) observed that eugenol decreased the NH_3-N concentration when added at a dose of 5,000 mg/L in 24-h in vitro batch-culture fermentations, but no effects were reported at doses of 5, 50, and 500 mg/L. The results suggest that the effects of essential oils on ruminal microbial fermentation are dose-dependent and that these compounds are more effective when administered at high doses than at low doses. However, a concentration of 5,000 mg/L would be much higher than the doses generally fed to lactating dairy cows (Benchaar et al., 2005a,b, 2006).

Cows fed CS-based diets tended ($P = 0.07$) to have a higher ruminal concentration of NH_3-N than those fed AS-based diets (8.83 vs. 6.61 mg/100 mL; Table 4), which would contribute to explaining the higher MUN content observed for cows fed CS-based diets compared with those fed AS-based diets (Table 2). In previous studies, replacing CS with AS in dairy cow diets increased (Broderick, 1985; Ruppert et al., 2003), reduced (Charmley et al., 1993), or had no effect on the ruminal concentration of NH_3-N (Broderick, 1985; Onetti et al., 2002).

The ruminal total VFA concentration decreased with the addition of MEO in the CS-based diet, whereas it increased slightly when MEO was added in the AS-based diet, resulting in an interaction ($P < 0.01$) between MEO and the silage source (Table 4). The decrease in ruminal total VFA concentration observed when MEO was added in the CS-based diet is consistent with the higher ruminal pH. For the AS-based diet, addition of MEO slightly increased the total VFA concentration, but this change was too small (+0.03 percentage units) to affect the ruminal pH. Molar proportions of individual VFA and the acetate-to-propionate ratio were unaffected by MEO supplementation. Newbold et al. (2004) reported no change in the ruminal total VFA concentration and molar percentages of individual VFA in sheep fed 100 mg/d of the same MEO. Castillejos et al. (2005) observed an increase in the total VFA concentration but no change in molar proportions of individual VFA when the Crina ruminants MEO was added at a dose of 1.5 mg/L of ruminal fluid culture in continuous-culture fermenters. More recently, using a dual-flow continuous-culture fermenter, Castillejos et al. (2006) reported that at 500 mg/L, thymol decreased the diet fermentability, total VFA concentration, and molar proportion of acetate, and increased the molar propor-

tion of propionate, but no effects were reported at 5 and 50 mg/L. More interesting, Busquet et al. (2006) observed that both clove bud oil and its main active compound, eugenol, decreased the total VFA concentration when added at 3,000 mg/L in batch cultures. However, the molar proportion of propionate was increased only by eugenol, whereas the acetate molar proportion was decreased only by clove bud oil. Based on these results, Busquet et al. (2006) suggested that other minor compounds in clove bud oil interacted with eugenol and exerted additional effects.

The ruminal concentration of total VFA was lower in cows fed CS than in cows fed AS when diets were supplemented with MEO, whereas it was similar among unsupplemented diets (interaction; $P < 0.01$). Onetti et al. (2002) observed a linear decrease in the ruminal total VFA concentration as the proportion of CS increased in the diet at the expense of the proportion of AS. In previous experiments, no difference was observed in the ruminal total VFA concentration when cows were fed diets with different AS:CS ratios (Broderick, 1985; Dhiman and Satter, 1997; Ruppert et al., 2003).

In the present study, the molar proportion of acetate decreased, whereas that of propionate increased when CS replaced AS in the diet (59.4 vs. 66.3% and 27.5 vs. 19.4%, respectively). As a result, the acetate-to-propionate ratio was lower in cows fed CS-based diets compared with those fed AS-based diets (2.21 vs. 3.49). These results agree with those of Ruppert et al. (2003), who reported a lower acetate-to-propionate ratio in cows fed diets containing 40% AS and 10% CS (DM basis) compared with the reverse proportions in 50% forage diets. Such differences in the ruminal VFA pattern are typical of diets providing different amounts of fermentable starch and fiber (Ruppert et al., 2003), which may contribute to explaining the lower milk fat and the higher milk protein concentrations for cows fed CS-based diets than for those fed AS-based diets (Table 2). However, when Broderick (1985) compared AS with CS (60% of total diet DM) in 2 trials, the milk fat concentration was depressed without any change in VFA concentrations in one trial, whereas it was not different between silages despite a lower ruminal pH and acetate-to-propionate ratio in the second trial.

Cows fed CS-based diets had a lower ruminal molar proportion of butyrate and a higher proportion of valerate than cows consuming AS-based diets (9.1 vs. 11.3% and 1.86 vs. 1.22%, respectively; Table 4). The molar proportion of butyrate increased (Broderick, 1985; Hristov and Broderick, 1996), whereas that of valerate decreased (Broderick, 1985) or increased (Hristov and Broderick, 1996) when feeding CS compared with AS. No differences in the molar proportions of butyrate and valerate were observed by Dhiman and Satter (1997) when lactating cows were fed diets containing different ratios of CS and AS. The molar proportions of isobutyrate and isovalerate averaged, respectively, 0.82 and 1.24%, and they did not differ between silages, suggesting that under the experimental conditions of the present study, no change occurred in the ruminal microbial catabolism of branched-chain AA when cows consumed either CS or AS as the main forage source.

Data on the effects of essential oils on ruminal microbial populations are scarce. In the present study, total viable bacteria, cellulolytic bacteria, and protozoa counts were not changed by addition of MEO in the diet (Table 4). Wallace et al. (2002) observed no change in total viable bacteria counts in sheep fed high- or low-protein diets supplemented with 100 mg/d of Crina ruminants MEO. In agreement with our findings, Newbold et al. (2004) reported no effect of the same MEO (110 mg/d) on the total number of protozoa in the ruminal fluid of sheep.

When compared with cows fed AS-based diets, those fed CS-based diets had higher ruminal counts of total viable bacteria (4.14 vs. 2.93×10^9/mL); however, cellulolytic bacteria and protozoa counts were unaffected by the source of silage.

Apparent Total Tract Nutrient Digestibilities and N Balance

There was no interaction between MEO addition and source of silage for the apparent total tract digestibility of nutrients, N output, N retained, and duodenal bacterial N flow (Table 5). Apparent digestibilities of DM, CP, NDF, and ADF were not influenced by MEO supplementation, which agrees with the results of Benchaar et al. (2006), who reported no change in apparent total tract digestibilities of DM, CP, and NDF in lactating cows supplemented with 2 g/d of Crina ruminants MEO. This lack of effect of Crina ruminants MEO on diet digestibility corroborates the results reported by Castillejos et al. (2005) with a continuous-culture system (1.5 mg/L of MEO). Recently, Castillejos et al. (2006) observed that addition of 5, 50, and 500 mg/L of eugenol in a continuous-culture fermenter did not affect DM, NDF, and ADF digestion. In the same study, thymol at 500 mg/L reduced the digestion of DM, NDF, and ADF, but no effects were observed at lower doses (5 and 50 mg/L). These results suggest that the effects of essential oil compounds on rumen microbial activity may vary depending on the dose and the type of essential oil compound used.

In the present study, MEO supplementation had no effect on the apparent digestibility of starch. Very little information is available in the literature on the effect

Table 5. Nutrient digestibility, N balance, and duodenal bacterial N flow in cows fed a TMR based on either alfalfa silage (AS) or corn silage (CS) without supplementation (0 mg/d) or supplemented with a mixture of essential oil compounds (MEO; 750 mg/d)

	AS		CS			Effect,[1] P =		
Item	0	750	0	750	SEM	MEO	Silage	MEO × silage
Digestibility, %								
DM	67.0	66.2	66.1	66.1	0.7	0.48	0.38	0.45
CP	59.8	60.3	61.7	63.4	2.5	0.57	0.22	0.78
ADF	57.6	55.0	48.2	47.8	2.5	0.48	<0.01	0.65
NDF	60.6	57.2	45.2	45.1	3.4	0.60	<0.01	0.63
Starch	97.3	95.8	98.2	98.3	1.0	0.48	0.11	0.41
N balance, g/d								
Intake	452	450	437	434	36	0.83	0.23	0.97
Feces	182	180	168	157	17	0.44	0.06	0.57
Urine	94	86	156	153	9	0.32	<0.01	0.59
Milk	146	141	156	152	11	0.34	0.05	0.98
Retained	30	43	–43	–28	9	0.12	<0.01	0.86
Duodenal bacterial N flow[2]	238	232	347	319	26	0.26	<0.01	0.47

[1]*P*-value for the effect of MEO (0 vs. 750 mg/d), silage source (AS vs. CS), and the interaction between MEO and silage source (MEO × silage).

[2]Estimated from urinary purine derivatives (allantoin and uric acid) excretion.

of essential oils on starch digestion. Benchaar et al. (2006) observed no change in ruminal degradability of starch when corn grain was incubated in the rumen of lactating cows fed 2 g/d of the same MEO.

In the current study, apparent digestibilities of DM and CP averaged, respectively, 66.4 and 61.3%, and they were not affected by the source of silage. In previous studies, digestibilities of DM and CP were not different among diets based on either AS or CS (Charmley et al., 1993; Ruppert et al., 2003). Conversely, Hristov and Broderick (1996) reported higher digestibilities of DM and CP when cows were fed CS than when they were fed AS in a 100% forage diet (DM basis). Similarly, Wattiaux and Karg (2004b) observed higher CP digestibility for cows fed a mixture of 41% CS and 14% AS, as compared with those fed 41% AS and 14% CS in 55% forage diets.

The apparent digestibilities of NDF and ADF were higher in cows fed AS that in cows fed CS (58.9 vs. 45.2% and 56.3 vs. 48.0%, respectively). This would agree with the results reported by Ruppert et al. (2003) but disagree with those of Hristov and Broderick (1996), who reported that the digestibility of ADF was unchanged and that the digestibility of NDF increased when cows were fed CS as compared with cows fed AS in 100% forage diets. On the other hand, Broderick (1985) observed no difference in NDF digestibility between cows fed a TMR based on either AS or CS. Diets with higher concentrations of starch may decrease fiber digestibility as a result of lower ruminal pH and ruminal passage rates and changes in ruminal microbial populations (Mould and Ørskov, 1983; Allen and Mertens, 1988).

Although the starch concentration was higher in CS-based diets than in AS-based diets (25.0 vs. 17.6%; Table 1), the apparent total tract digestibility of starch was similar between diets (Table 5). However, a larger quantity of starch was probably fermented in the rumen of cows fed CS-based diets, which would contribute to explaining the lower acetate-to-propionate ratio and fiber digestibility observed in cows fed CS-based diets compared with those fed AS-based diets (Table 4). Ruppert et al. (2003) also observed no change in total tract digestibility of starch when cows were fed the AS-based diet compared with when they were fed the CS-based diet.

Outputs of N in feces, urine, and milk were not different between cows fed no MEO and those fed MEO, resulting in a similar retention of N between MEO-supplemented diets and diets containing no MEO. Similarly, Benchaar et al. (2006) observed no change in N retention when cows were fed 2 g/d of the Crina ruminants supplement.

Intake of N was not affected by silage source, as a result of the similar DMI between cows fed AS diets and those fed CS diets. The fecal N output tended to be lower (163 vs. 181 g/d, $P = 0.06$), whereas the outputs of urinary N and milk N were greater in cows fed CS-based diets as compared with those fed AS-based diets (155 vs. 90 and 154 vs. 144 g/d, respectively). The increased urinary N output could be related to the higher ruminal NH_3-N concentration observed in cows fed CS-based diets compared with those fed AS-based diets (Table 4). Ruppert et al. (2003) and Wattiaux and Karg (2004b) reported no difference in urinary N output be-

tween cows fed AS-based diets and those fed CS-based diets.

Nitrogen retention was higher in cows fed AS-based diets than in cows fed CS-based diets (36 vs. –37 g/d). The retention of N was higher (Ruppert et al., 2003) or tended to be higher (Wattiaux and Karg, 2004b) in lactating cows fed AS-based diets as compared with those fed CS-based diets. In the present study, the negative N balance observed for cows fed CS-based diets might indicate that cows had mobilized tissue protein to meet nutrient requirements. Assuming that body tissue contains 17% protein (NRC, 2001), an N balance of –37 and 36 g of N per day would result in a loss and accretion of –1.4 and 1.3 kg/d, respectively, which contrasts with the small BW change recorded in the present experiment (0.43 and 0.38 kg/d for the AS-based diet and the CS-based diet, respectively; data not shown). Therefore, it is likely that N retention was overestimated in the present study, which would agree with the fact that balance trials tend to underestimate N excretion (MacRae et al., 1993; Spanghero and Kowalski, 1997).

Duodenal bacterial N flow estimated using purine derivatives was not affected by MEO supplementation, which corroborates the results of Newbold et al. (2004), who reported no change in bacterial N flow, estimated from excretion of urinary purine derivatives, when sheep were supplemented daily with 100 mg of the same MEO as used in the current study.

The bacterial duodenal N flow was higher for cows fed CS-based diets compared with those fed AS-based diets (332 vs. 235 g/d), which agrees with the results of Hristov and Broderick (1996), who observed a greater duodenal flow of microbial N when cows were fed CS, compared with AS, as the sole forage source. The greater duodenal bacterial N flow when feeding CS could be explained by the higher starch content in corn than in alfalfa, which may have provided more energy to rumen microbes and enhanced the efficiency of ruminal microbial protein synthesis. This is in agreement with the greater total viable bacterial count observed when cows were fed CS-based diets compared with when they were fed AS-based diets (Table 4). The increased amount of microbial protein reaching the small intestine and the eventual use of AA for gluconeogenesis might have increased AA deamination and the concentration of blood urea N for cows fed CS, which would contribute to the increased milk urea N concentration (Table 2).

CONCLUSIONS

Results from the present study indicate that addition of a specific mixture of essential oil compounds at a dose of 750 mg/d in a diet containing either AS or CS as the sole forage source had limited effects on the digestion, ruminal fermentation, rumen microbes, milk production, and milk composition of early lactation dairy cows. The lack of effect of essential oils was attributed to a possible adaptation of rumen microbes to these compounds.

Feeding either AS or CS had no influence on milk yield. However, the milk fat content tended to be lower and the milk urea N concentration was higher when CS replaced AS in the diet, as a result of the lower acetate-to-propionate ratio and higher NH_3-N concentration in ruminal fluid when feeding CS-based TMR. The digestibilities of NDF and ADF were lower for cows fed the CS-based TMR than for cows fed the AS-based TMR. Under the experimental conditions of the present study, the digestibility of N was not affected by silage source in the diet, but nitrogen retention was higher when CS replaced AS in the diet. Compared with milk from cows fed CS, milk from cows fed AS was higher in concentrations of 18:3, an n-3 FA, and in *cis*-9, *trans*-11 18:2, a conjugated linoleic acid. Feeding dairy cows a diet based on AS may therefore improve the nutritive quality of milk fat as compared with a diet based on CS.

Recent in vitro studies have shown that high doses of essential oils (as a mixture or as individual compounds) could favorably alter ruminal fermentation and therefore potentially improve the feed efficiency of ruminants. However, the results of the current study showed that when used at more normal feeding doses, essential oils had no effect on rumen microbial fermentation, digestion, and dairy cow performance. Further research is required to validate in vitro results under long-term in vivo experimental conditions.

ACKNOWLEDGMENTS

The authors thank D. Bournival, L. Veilleux, S. Provencher, and F. Markwell for technical assistance and S. Méthot for his help with statistics. The authors are pleased to acknowledge the financial contribution of Innovation Développement en Nutrition Animale (IDENA, Sautron, France) and the Matching Investment Initiative of Agriculture and Agri-Food Canada.

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