Effects of Essential Oils on Digestion, Ruminal Fermentation, Rumen Microbial Populations, Milk Production, and Milk Composition in Dairy Cows Fed Alfalfa Silage or Corn Silage¹

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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 NH3-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 CSbased 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 (C₂₀) 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).

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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 Commit-

Table 1. Ingredients and chemical composition of the TMR

	TMR	
	Alfalfa	Corn
Item	silage	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 , 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

 $^1\mathrm{Megalac}$ calcium salts of palm oil (Church and Dwight Co., Inc., Princeton, NJ).

 $^2\mathrm{Contained}$ 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.

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

tee 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).

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

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