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Effects of specific essential oil compounds on the ruminal environment, milk production and milk composition of lactating dairy cows at pasture

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ABSTRACT

Sixty multiparous, lactating Holstein cows (57 ± 23.1 d in milk at the start of the experiment) were used in a completely randomized design to examine effects of adding incremental levels of dietary essential oil compounds (EO; 0, 200, 400 and 600 mg/d) on milk production and composition. Cows were allowed to graze on winter oats for 8 h/d with a daily herbage allowance of 15 kg dry matter (DM)/cow, and then received supplemental corn silage and sunflower meal in confinement for the remainder of the day. The EO were fed individually at milking times (0600 and 1600 h), mixed with 0.86 kg DM of dry rolled corn grain. In addition, 4 ruminally cannulated lactating Holstein cows in mid lactation were used in a 4×4 Latin Square design with 14 d periods to study effects of EO on ruminal fermentation characteristics and ruminal *in sacco* DM, crude protein (CP), and neutral detergent fiber (aNDF) degradability. Milk production, which ranged from 18.8 to 20.2 kg/d, and milk composition were not affected by EO. In general, ruminal fermentation characteristics were not affected by EO addition at any level, except for a 13% increase in butyrate concentrations with all EO levels compared to the control. Ruminal ammonia N concentration was high in all treatments (51.5 ± 5.75 mg/100 ml) and tended (P=0.09) to increase when 200 and 400 mg/d of EO were added. In addition, 200 mg/d of EO marginally decreased the potentially ruminally degradable fraction of the CP of the complete ration. Results using dairy cows in mid lactation that grazed 8 h/d on lush pasture showed limited effects of this EO complex on ruminal fermentation, milk production and milk composition.

Abbreviations: *a*, rapidly ruminally degradable fraction; aNDF, neutral detergent fiber; *b*, slowly ruminally degradable fraction; BCS, body condition score; *c*, rate of degradation of *b*; CP, crude protein; DM, dry matter; EEA, Estación Experimental Agropecuaria; EO, essential oils; ERD, effective ruminal degradability; INTA, Instituto Nacional de Tecnología Agropecuaria; kp, passage rate; MEO, milk energy output; MUN, milk urea N; NH_3-N, ammonia N; OM, organic matter; PMR, partial mixed ration; TMR, total mixed ration; VFA, volatile fatty acids.

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1. Introduction

Ionophore antibiotics, such as monensin and lasalocid, have been used for several decades as additives to modify ruminal fermentation. However, some groups have criticized ionophore addition on the grounds that use of antibiotics in livestock production may create cross-resistance to antibiotics used in human medicine. Although available scientific data has concluded that milk from monensin-fed animals is safe for human consumption (Donoho, 1984), the European Union banned the use of monensin as a feed additive inside its territory as of January 2006 (EC, 2003, Regulation 1831/2003/EC). This ban has created the need for suitable alternatives to these antibiotics (Newbold, 2007).

One such alternative is use of herbal extracts, and their active principles the essential oils (EO; Greathead, 2003). Numerous research and review articles have been published on the potential of EO as ruminal modifiers (*e.g.*, McIntosh et al., 2003; Castillejos et al., 2006; Calsamiglia et al., 2007; Benchaar et al., 2008). However, most studies were conducted using *in vitro* systems, mainly dual-flow continuous culture fermenters or with ruminal *in sacco* techniques. In contrast, information on the effects of EO on milk production and milk composition of dairy cows is less available (*e.g.*, Benchaar et al., 2007; Yang et al., 2007; Spanghero et al., 2009; Tassoul and Shaver, 2009; Santos et al., 2010). However, no study has included grazed pasture as a dietary ingredient.

The objective of this study, which is a follow up to an *in vitro* study from our laboratory (Colombatto, Dario, unpublished observations), was to examine effects of a specific blend of EO compounds on the ruminal environment, milk production and milk composition of grazing dairy cows in mid lactation. Our hypothesis was that feeding EO would reduce the rate of CP degradation in the rumen, and that the acetate to propionate ratio would be reduced, resulting in increased milk yield.

2. Materials and methods

2.1. Experimental location and animal care

The study was completed at the Estación Experimental Agropecuaria (EEA) Balcarce of the Instituto Nacional de Tecnología Agropecuaria (INTA), Balcarce, Buenos Aires Province, Argentina (37°45′10″ S; 58°17′34″ W), from April to June 2004. Cows used were cared for in accordance to the EEA INTA Balcarce Guide for Animal Care.

2.2. Essential oil compound and treatments

A specific blend (XTract® 6965) of EO compounds manufactured by Pancosma Bioactives (Geneva, Switzerland) was used in this experiment. XTract® 6965 was composed of 280 g/kg of eugenol and 170 g/kg of cinnamaldehyde, with the balance being excipients. Experimental treatments consisted of an untreated Control (0 mg/d) and three incremental levels (200, 400 and 600 mg/cow/d) of the EO blend. The doses were chosen on the basis of 0.5, 1 and 1.5 times the manufacturer's recommended feeding level. The EO blend was thoroughly mixed with ground corn grain and fed individually to each cow twice a day (0.86 kg corn grain DM/cow each time), at the milking times of 0600 and 1600 h.

2.3. Milk performance study

2.3.1. Milk production and composition

Sixty multiparous dairy cows (568 ± 68.5 kg; 57 ± 23.1 d in milk at the start of the experiment) were blocked according to parity and production level, and randomly assigned to one of the four dietary treatments. The first 3 weeks of the experiment were used for adaptation to the dietary regimen and general management, with the following 6 weeks used for experimental determinations. During the week prior to adaptation, milk production and composition were determined to be used as covariates.

2.3.2. Pasture management

After the morning milking at 0600 h, cows were allowed to graze on an annual oat pasture (*Avena sativa* L.) in a paddock divided into daily strips. The pasture consisted of 980 g/kg oats and 20 g/kg weeds (DM basis) and the harvest efficiency index was set at 600 g/kg as-is biomass to calculate pasture allowance per cow. Pasture availability was measured every 15 d with a pasture meter developed at INTA. Cows belonging to each treatment grazed separate pasture strips until the afternoon milking at 1600 h. Thereafter the whole group of cows was kept in confinement with free access to an *ad libitum* mixture of corn silage and pelleted sunflower meal until the following morning. In addition, the cows received 200 g/d of a mineral mixture (Raciones Argentinas SRL, Pilar, Buenos Aires, Argentina), which contained 185 g/kg Ca, 85 g/kg Mg, 195 g/kg Na, and 1840, 140, 40, 8 and 52 mg/kg of Zn, Cu, Se, Co and I, respectively.

2.3.3. Measurements and chemical analyses

Dry matter intake at pasture was determined over two consecutive days each week as the difference between the final and initial pasture availability. Pasture availability was adjusted weekly in order to prepare a new daily forage strip using the relationship between pasture height and available biomass. Equations were fitted for both initial and final biomass availability. Daily forage area was adjusted to ensure a minimum availability of 15 kg DM/cow/d.

Dry matter intake in the milking parlor, and of the total ration, was determined by difference on the same days that pasture intake was determined. It was not possible to separate each group of cows into sub-groups to obtain replications, and therefore the intake data are descriptive.

Hand plucked pasture (Meijs et al., 1982) and supplement samples were collected every 15 d and analyzed for DM and organic matter (OM) (methods 930.04 and 930.05, respectively of the Association of Official Analytical Chemists (AOAC, 1997). Total N was determined by thermal conductivity (LECO FP-528 Nitrogen Determinator, LECO Corp., Saint Joseph, MI, USA) according to Horneck and Miller (1998). Neutral detergent fiber (aNDF) was determined as described by Van Soest et al. (1991), with a heat-stable amylase included, but sodium sulfite omitted from the ND solution. Acid detergent fiber (ADF) was determined according to AOAC (1997, Method 973.18). Both aNDF and ADF procedures were adapted for use in an ANKOM^{200} fiber analyzer (ANKOM Corp., Macedon, NY, USA). The aNDF and ADF values are expressed ash inclusive. Starch concentrations were determined according to McRae and Armstrong (1968).

Cows were weighed at the beginning and at the end of the experiment. Milk production and composition from each cow was determined at each milking time, on two consecutive days of each measurement week. Milk composition was determined using two sub-samples (one from each milking time) collected from each cow and pooled as a weighted sample. Each pooled sample was analyzed for milk fat, lactose and CP concentration by infrared spectroscopy (Foss 300, MilkoScan, Foss Electric, Hillerød, Denmark). Milk urea N (MUN) was determined using an enzymatic kit (Wiener Laboratory, Rosario, Argentina).

2.4. Ruminal fermentation and in sacco degradation kinetics

Four multiparous Holstein cows (average initial body weight 557 ± 27.1 kg) in mid lactation (>100 d in milk) fitted with permanent ruminal cannulas were used in a 4 × 4 Latin square design. Management of this group of cows was identical to that described for the milk performance study in Section 2.3. Each experimental period lasted 14 d, with the first 10 d for adaptation and the last 4 d for sampling.

During the first day of the sampling period (*i.e.*, day 11 of each period), a pooled sample of ruminal fluid was collected from the rumen of each cow. Samples were collected from the anterior dorsal, anterior ventral, posterior dorsal and posterior ventral sacs. Times of sampling were 1000, 1300, 2000 and 2300 h, which were at equidistant hours from each milking, and hence from each EO addition time. Ruminal fluid samples were filtered through two layers of cheesecloth, and pH was immediately measured using a portable, digital pH-meter (Model 200, VWR Scientific, West Chester, PA, USA). Additional 100 ml samples were added to plastic bottles containing 1 ml of sulphuric acid (500 ml/l), and stored at −20 °C for determination of volatile fatty acid (VFA) and NH_3 N. For NH_3 N determination, samples were first thawed and centrifuged at 10,000 × *g* for 10 min at 4 °C. Ammonia N concentrations were determined in a 1:10 (vol/vol) dilution of the original ruminal fluid using a Tecator Autoanalyzer (Kjeltec 1030, Höganäs, Sweden). Volatile fatty acids were determined by gas chromatography (Shimadzu GC-14, Kyoto, Japan).

Ruminal kinetics parameters for DM, CP, and aNDF, were determined using an *in sacco* dacron bag technique (Mehrez and Ørskov, 1977; Ørskov and McDonald, 1979). Approximately 5 g of a total mixed ration (TMR; on DM basis, 0.33 grazing oats, 0.33 corn silage, 0.22 ground corn grain, and 0.12 pelleted sunflower meal), pre-dried at 60 °C and milled to pass a 2 mm screen (Wiley mill, standard model 4, Arthur M. Thomas, Philadelphia, PA, USA), was weighed into dacron bags, which were then tied and inserted into the rumen. Bags were removed in triplicate from each cow after incubation for 0, 3, 6, 13, 18, 24, 36, 48 and 72 h. After removal, bags were immediately frozen (−20 °C) until completion of the experiment. Bags were later thawed, washed in a domestic washing machine, and dried at 60 °C for at least 48 h.

Residues remaining in the bags were weighed, and DM disappearance was determined by difference. Residues were then milled to pass a 1 mm screen (Wiley mill) and analyzed for aNDF and total N concentrations using the same procedures as described below. Data were fitted to an exponential equation (Ørskov and McDonald, 1979) to obtain parameters of potential degradation and rate of degradation:

$$p = a + b(1 - e^{(-c*t)})$$

where, p is the proportion degraded at time t; a is the intercept at zero time (or soluble fraction for protein); $a+b$ is the potentially degradable fraction, c is the degradation rate of b, and t is the incubation time (h). Effective ruminal degradabilities (ERD) of DM, aNDF, and CP were calculated as:

$$\text{ERD} = a + \frac{bc}{c + kp}$$

where, kp is the ruminal outflow rate, assumed to be 0.05 h^{-1} for the incubated feed.

2.5. Statistical analysis

Average and change in BW were analyzed as a completely randomized design, with treatment as a fixed effect, using the Proc. Mixed of SAS (SAS, 2009). The statistical model was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Table 1
Chemical composition of feeds.[a]

	DM (g/kg)[b]	OM	CP	aNDF (g/kg) DM	ADF	Starch	pH
Diet ingredients							
Grazing oats	197 ± 1.4	889 ± 3.3	228 ± 4.5	354 ± 2.5	198 ± 1.2	nd[c]	nd
Corn silage	362 ± 1.5	939 ± 2.1	67.5 ± 0.72	391 ± 3.6	235 ± 0.8	274 ± 4.1	3.74 ± 0.080
Corn grain	866 ± 0.5	970 ± 3.2	59.8 ± 0.63	114 ± 2.7	37 ± 0.6	708 ± 3.9	nd
Pelleted sunflower meal	908 ± 0.5	898 ± 4.7	378 ± 1.3	274 ± 2.2	212 ± 2.0	nd	nd
Total ration[d]	381 ± 4.0	917 ± 0.4	162 ± 1.1	339 ± 0.4	198 ± 0.1	172 ± 2.5	nd

[a] Mean ± standard error; and $n = 4$.
[b] DM, dry matter; OM, organic matter; CP, crude protein; aNDF, neutral detergent fiber; and ADF, acid detergent fiber.
[c] nd, not determined.
[d] Values estimated from the proportions of each ingredient actually consumed by the cows during the experiment.

where Y_{ij}, observation of the response variable corresponding to the ith treatment; μ, the overall mean; T_i, effect of treatment i; and e_{ij}, random error.

The milk production study was analyzed according to a completely randomized design with the repeated measures (weeks) command of the Proc. Mixed of SAS (SAS, 2009). The model included treatment, experimental week, and their interaction as fixed effects. The within-treatment animal effect was included as a random effect. Determinations of milk production and composition measured the week before the beginning of the experiment were used as covariates. The statistical model was:

$$Y_{ijk} = \mu + T_i + \delta_{ij} + S_k + (T * S)_{ik} + e_{ijk}$$

where Y_{ijk}, observation of the response variable corresponding to the ith treatment in the kth week; μ, the overall mean; T_i, effect of treatment i; δ_{ij}, random error, the variance among animals (subjects) within treatments; S_k, effect of kth week; $(T^*S)_{ik}$, interaction between ith treatment with kth week; and e_{ijk}, random error, the variance between measurements within animals. Unless stated otherwise, significance level was established at P<0.05, whereas trends are discussed at P<0.10.

The DM, aNDF, and CP degradation kinetics in the *in sacco* study were analyzed according to a Latin square design using the Mixed option of SAS (Littell et al., 1998). Ruminal fermentation characteristics (*i.e.*, pH, NH_3 N, VFA) were analyzed as a Latin square design using a mixed model with sampling time as a repeated measure.

3. Results

Average chemical composition of the pastures and other feeds used throughout the experiment is in Table 1. The DM intake data are descriptive as no pasture replication was possible. However, pasture DM intakes (mean ± SD, $n = 4$) were 8.8 ± 2.80, 8.7 ± 4.02, 8.3 ± 3.54 and 9.0 ± 2.73 kg DM for the 0, 200, 400, and 600 mg EO/d treatments, respectively. Corn silage and sunflower meal intakes were 7.7 ± 0.84 and 1.9 ± 0.06 kg DM, respectively. Finally, corn grain DM intakes in the milking parlor were 1.7 ± 0.16, 1.6 ± 0.15, 1.6 ± 0.30, and 1.5 ± 0.32 kg DM for the 0, 200, 400, and 600 mg EO/d treatments, respectively. Thus, total DM intake was 19.7 ± 2.53, 19.5 ± 3.44, 19.1 ± 2.99 and 19.7 ± 2.29 kg DM for the 0, 200, 400, and 600 mg EO/d treatments, respectively.

Milk production ranged from 18.9 to 20.2 kg/d but did not differ among treatments (Table 2). Furthermore, milk components were not affected by EO addition. Milk urea N was relatively high but not affected by treatments.

No differences in ruminal pH occurred among treatments (Table 3), whereas NH_3 N concentrations were highest at the intermediate EO addition levels of 200 and 400 mg/d (Q:P=0.02). These ruminal NH_3 N levels are extremely high, although this is not unusual for lactating cows grazing lush pasture (Di Marco and Aello, 2002). Total VFA concentrations had a trend (Q:P=0.08) maximum at 400 mg EO/d and a minimum at 600 mg EO/d. Addition of EO influenced (Q:P=0.03) butyrate concentrations with the highest level at 400 mg/d. There were no differences in the A:P or A:(P+B) ratios due to EO addition.

Ruminal degradation kinetics for DM, aNDF and CP are in Table 4. Overall, there were no meaningful differences among treatments.

4. Discussion

The study was conducted using lactating cows grazing a lush oat (*Avena sativa*) pasture for at least 8 h/d, and receiving the rest of the diet as a corn silage-based partial mixed ration (PMR) plus some dry rolled grain at milking. These routine management procedures are representative of many dairy farms in Argentina, and other parts of the world where grazing is a management tool for lactating dairy cows. To our knowledge, this is the first published report on supplementing grazing animals with this EO complex, thus it was not possible to make direct comparisons to similar studies. Consequently, comparisons were made with studies that used non-grazing cows or cows receiving other EO compounds, which may be of only limited relevance.

Milk production yields were lower than expected based on estimated DM intake and diet chemical composition. In addition, there was a quadratic change in body weight, which is difficult to interpret with the available evidence. However,

Table 2
Effects of essential oil (EO) compounds on milk production and milk composition of lactating dairy cows in mid lactation.[a]

	Level of EO addition (mg/cow/d)				SEM	p[b]	
	0	200	400	600		*L*	*Q*
Milk production (kg/d)	20.22	19.97	19.76	18.82	0.661	0.31	0.73
Milk energy[c]							
MJ/kg	2.94	2.98	2.98	2.96	0.056	0.84	0.67
MJ/d	59.4	59.4	58.2	55.1	2.77	0.26	0.57
Milk fat							
g/kg	33.0	33.7	33.1	34.3	0.96	0.85	0.90
kg/d	0.70	0.69	0.68	0.64	0.034	0.30	0.71
Milk CP							
g/kg	35.7	36.5	36.7	37.1	0.66	0.38	0.53
kg/d	0.73	0.75	0.74	0.70	0.035	0.78	0.43
Milk lactose							
g/kg	49.7	49.1	49.6	48.5	0.38	0.41	0.16
kg/d	1.02	1.01	1.03	0.93	0.052	0.30	0.40
MUN (mg/100 ml)[d]	18.7	19.1	18.5	18.7	0.52	0.76	0.82
Average body weight (kg)	558	585	583	569	24.3	0.79	0.45
Body weight change (kg)	1.7	−1.6	−6.8	6.7	2.51	0.36	0.05

[a] $n = 15$.
[b] Probability levels of the linear (*L*) and quadratic (*Q*) effects of increasing EO dose.
[c] Milk energy: *E* (Kcal/lb milk) = 41.63 (g fat/dl milk) + 24.13 (g CP/dl milk) + 21.60 (g lactose/dl milk) – 11.72 (Tyrrell and Reid, 1965). Results were then converted from the calculated Kcal/lb to MJ/kg milk.
[d] MUN, milk urea N.

the lack of a milk production response is consistent with the ruminal data. Furthermore, this finding concurs with data from dairy cows fed TMR diets supplemented with EO containing eugenol among its components, reported by Benchaar et al. (2006, 2007) and Santos et al. (2010). No effects on milk production and composition were found when dairy cows were fed 2 g/d (Benchaar et al., 2006) Crina Ruminants which contained eugenol as part of its formulation, or 1 g/d of a mixture of eugenol, geranyl acetate and coriander oil (Santos et al., 2010). In contrast, Kung et al. (2008) fed Crina Ruminants at 1.2 g/d and found increased milk yield (41.7 *versus* 39.8 kg/d), with no changes in milk composition, but an increase in DM intake from 26.4 to 28.3 kg/d.

Regarding milk composition, an increase in milk fat proportion (339 *versus* 333 g/kg) was reported by Santos et al. (2010), who speculated that eugenol might have been implicated in this change. Overall, although EO compounds, application rates and diets differ, there appears to be general agreement with our results that EO compounds have limited effects on milk production and milk composition of dairy cows in mid lactation.

Mean ruminal NH_3 N concentrations were very high, but not unusual for typical grazing situations in Argentina (Di Marco and Aello, 2002). In addition, NH_3 N concentrations were only slightly affected by EO addition, but the effect is unlikely to be of biological importance. Several authors (Cardozo et al., 2005; Busquet et al., 2006) have reported reductions in ruminal NH_3 N concentrations due to addition of EO containing cinnamaldehyde and eugenol, but the research was conducted in batch cultures (Cardozo et al., 2005) or continuous culture fermenters (Busquet et al., 2006), maintained under constant pH conditions.

Table 3
Effects of addition of essential oil (EO) compounds on ruminal fermentation characteristics.[a]

	Level of EO addition (mg/cow/d)				SEM	p[b]	
	0	200	400	600		*L*	*Q*
pH	5.72	5.71	5.70	5.77	0.123	0.64	0.56
NH_3 N (mg/dl)	46.8	56.2	56.7	46.2	7.37	0.93	0.02
Total VFA (mM)	138.0	136.4	152.7	125.7	14.88	0.45	0.08
VFA (mol/100 mol of VFA)							
Acetate (A)	56.1	57.6	57.1	57.2	1.99	0.64	0.61
Propionate (P)	25.0	21.3	23.1	23.1	1.19	0.36	0.08
Butyrate (B)	18.9	21.0	19.8	19.7	1.22	0.59	0.03
A:P	2.33	2.74	2.50	2.51	0.194	0.64	0.20
A:(P+B)	1.33	1.39	1.36	1.38	0.114	0.78	0.82

[a] $n = 4$.
[b] Probability levels of the linear (*L*) and quadratic (*Q*) effects of increasing EO dose.

Table 4
Effects of essential oil (EO) compounds on the *in sacco* ruminal disappearance kinetics of a total mixed ration in lactating dairy cows in mid lactation.[a,b,c]

	Level of EO addition (mg/cow/d)				SEM	p[d]	
	0	200	400	600		*L*	*Q*
DM							
a	0.37	0.37	0.38	0.38	0.118	0.61	0.99
b	0.49	0.47	0.48	0.50	0.183	0.83	0.27
a + *b*	0.87	0.84	0.86	0.87	0.179	0.53	0.17
c	0.06	0.08	0.05	0.07	0.011	0.75	0.64
ERD[e]	0.63	0.65	0.63	0.64	0.013	0.84	0.89
aNDF							
a	0.07	0.09	0.08	0.05	0.019	0.41	0.17
B	0.81	0.59	0.79	0.75	1.099	0.92	0.23
a + *b*	0.87	0.69	0.82	0.79	1.129	0.73	0.33
C	0.02	0.04	0.02	0.06	0.022	0.20	0.43
ERD	0.29	0.31	0.28	0.31	0.030	0.73	0.90
CP							
a	0.49	0.45	0.46	0.48	0.259	0.85	0.29
B	0.44	0.46	0.47	0.46	0.249	0.52	0.64
a + *b*	0.93	0.91	0.93	0.94	0.069	0.05	0.01
C	0.08	0.13	0.09	0.10	0.014	0.91	0.12
ERD[e]	0.77	0.79	0.76	0.77	0.015	0.80	0.79

[a] $n = 4$.
[b] Equation model was $p = a + b\,(1 - e^{-ct})$, where p, proportion degraded at time t; a, rapidly ruminally degradable fraction; b, slowly ruminally degradable fraction; and c, rate of degradation of b.
[c] On DM basis, 0.33 grazing oats, 0.33 corn silage, 0.22 ground corn grain and 0.12 pelleted sunflower meal.
[d] Probability levels of the linear (L) and quadratic (Q) effects of increasing EO dose.
[e] ERD, effective ruminal degradability (kp assumed to be 0.05 h^{-1}).

The apparent contradiction between *in vitro* and *in vivo* work may indicate possible adaptation of ruminal microbes to EO addition, as suggested by Benchaar et al. (2008) or a difficulty in extrapolating from the rate used *in vitro* to an effective dose rate *in vivo*. For instance, eugenol has been found to decrease the ruminal NH_3 N concentrations when added *in vitro* at 5 g/L but not at lower doses (Castillejos et al., 2006). Macheboeuf et al. (2008) used cinnamaldehyde in a dose response *in vitro* study and found cubic responses in ruminal NH_3 N concentrations, such that at a concentration of 2 mM, cinnamaldehyde decreased NH_3 N by 39%, but at 3 mM ammonia concentrations were higher and not different from the controls. From these findings, one must be cautious when extrapolating data obtained *in vitro* to *in vivo* situations.

Feeding EO compounds to lactating cows only marginally affected total VFA concentrations, especially with an EO dose of 400 mg/d. However, the VFA profile (*i.e.*, butyrate concentration) was moderately changed. Effects of EO on ruminal fermentation in other studies have been inconsistent (Benchaar et al., 2006; Yang et al., 2007). Busquet et al. (2006) reported decreases in total VFA concentrations when high doses (up to 3000 mg/L) of cinnamaldehyde and eugenol were added to dual flow continuous culture, maintained under constant pH. In addition, Cardozo et al. (2005) suggested a pH-mediated effect of EO, because at pH 7.0, cinnamaldehyde increased acetate to propionate ratio, whereas at pH 5.5 (closer to our study), the same EO decreased the acetate to propionate ratio compared with the control. However, the increase in butyrate production (13% for 400 mg/d compared with the control) found in the present study suggests that, despite no effect on total VFA concentration, microbial populations were affected.

The effects of EO on increasing butyrate concentration suggest that the mode of action of these EO differ to that of monensin (Benchaar et al., 2008), as monensin generally decreases butyrate concentration in the rumen (Duffield et al., 2008). Castillejos et al. (2005) added eugenol to batch culture incubations at 5, 50, 500 and 5000 mg/L, finding an increase in molar proportions of propionate and butyrate at the highest eugenol rate. Busquet et al. (2006) found similar results when 3000 mg/L of cinnamaldehyde or eugenol were added to continuous culture. However, these levels are much higher than those typically fed *in vivo* (Benchaar et al., 2006).

Ruminal degradation kinetics of DM and aNDF in the TMR were not affected by EO addition, which is in line with the lack of overall effects. However, 200 mg/d decreased the $a + b$ (potentially degradable fraction) of CP and there was a trend toward higher CP rate of disappearance. These findings agree with Macheboeuf et al. (2008), who reported a dose-related response of protein degradation (measured by ruminal NH_3 N production) to cinnamaldehyde. However, in our study we did not find a decrease in NH_3 N concentration; rather we found a trend toward an increase with a dose of 200 and 400 mg/d. Benchaar et al. (2006) also found a trend toward an increase in the degradation rate of soybean meal CP with EO supplementation.

5. Conclusions

Addition of a specific blend of EO compounds, consisting of a mixture of cinnamaldehyde and eugenol, slightly affected the ruminal environment of dairy cows that were allowed to graze for 8 h/d. These small changes were not reflected in changes in milk production or milk composition.

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