Animal Feed Science and Technology 176 (2012) 123–130

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Plant extracts containing cinnamaldehyde, eugenol and capsicum oleoresin added to feedlot cattle diets: Ruminal environment, short term intake pattern and animal performance☆

José I. Geraci[a], Alberto D. Garciarena[a], Gerardo A. Gagliostro[a], Karen A. Beauchemin[b], Dario Colombatto[c,d,*]

[a] Instituto Nacional de Tecnología Agropecuaria, Km 73.5 Ruta Nacional 226, 7620 Balcarce, Argentina
[b] Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada T1J 4B1
[c] Departamento de Producción Animal, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSQ Buenos Aires, Argentina
[d] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Keywords:
Feedlot
Plant extracts
Performance
Intake pattern

ABSTRACT

The objective was to evaluate effects of adding a blend of essential oil compounds on ruminal fermentation and animal performance of feedlot cattle in comparison to sodium monensin. In Exp. 1, 24 angus steers (initial weight 141 ± 6.6 kg) were blocked by weight into 4 groups and randomly allocated to 8 pens of 3 steers. Treatments were monensin (46.7 mg/kg dietary dry matter (DM)), or plant extracts (PE; 266 mg/steer/d of cinnamaldehyde and eugenol + 133 mg/steer/d of capsicum oleoresin) added to a mineral mixture. The experiment lasted 84 d and was divided in 2 periods of 0–44 and 45–84 d. Diets were fed once daily and consisted of a corn grain based concentrate fed *ad libitum*, plus 200 g alfalfa hay/steer/d as fed. The DM intake, average daily gain (ADG), feed conversion ratio (FCR) and rate of backfat deposition (BFD) were determined throughout the study. Short term intake patterns were evaluated by visual appraisal. In Exp. 2, two ruminally fistulated steers were used in a crossover design to determine how the ruminal fermentation variables pH, NH_3-N and volatile fatty acids (VFA) were affected by PE or monensin. Compared to monensin, PE did not alter overall DM intake (0.124 kg/$BW^{0.75}$ *versus* 0.123 kg/$BW^{0.75}$), FCR (0.21 *versus* 0.20), BFD (1.87 mm/mo *versus* 1.76 mm/mo), or *longissimus dorsi* muscle (LM) area (6.56 cm^2/mo *versus* 6.69 cm^2/mo) for PE and monensin, respectively. However, a treatment × period interaction occurred (P=0.02) for ADG, with steers fed PE having a higher (P=0.01) ADG in the second period (1.43 kg/d *versus* 1.23 kg/d for PE and monensin, respectively). Short term intake patterns were not altered by PE compared to monensin, as steers visited the feeders a similar number of times and the

Abbreviations: ADF, acid detergent fiber; ADG, average daily gain; aNDF, neutral detergent fiber; BFD, rate of backfat deposition; BW, body weight; DM, dry matter; FCR, feed conversion ratio; LM, *longissimus dorsi*; MO, monensin; PE, plant extracts; VFA, volatile fatty acids.

☆ This paper is part of the special issue entitled: Plant Bioactive Compounds in Ruminant Agriculture – Impacts and Opportunities, Guest Edited by A.Z.M. Salem and S. López, and Editor for Animal Feed Science and Technology, P.H. Robinson.

* Corresponding author. Tel.: +54 11 4524 8000x4003; fax: +54 11 4524 8000.
E-mail address: colombat@agro.uba.ar (D. Colombatto).

0377-8401/$ – see front matter
http://dx.doi.org/10.1016/j.anifeedsci.2012.07.015

length of each visit was also similar (11.5 min *versus* 10.6 min and 8.28 min *versus* 9.57 min for PE and monensin, respectively). Although ruminal pH was not affected (5.55 *versus* 6.05 for PE and monensin, respectively), ruminal NH_3-N was lowered by PE (10.78 mg/dl *versus* 20.05 mg/dl, P=0.02). Ruminal total VFA concentrations did not differ between treatments (80.7 mM *versus* 62.5 mM), and feeding PE did not alter ruminal acetate (48.5 mol/100 mol *versus* 58.2 mol/100 mol), or propionate (32.8 mol/100 mol *versus* 25.2 mol/100 mol, P=0.65) proportions. Results show that steers fed PE performed equivalently to those fed monensin in a high concentrate diet, and that some productive variables were improved with PE feeding.

1. Introduction

Antibiotic growth promoters such as ionophores have been successfully used for decades to improve animal performance (Nagaraja, 1995). Typically, ionophores are included in beef diets to reduce disease, increase average daily gain (ADG) and improve the feed conversion ratio (FCR; Tedeschi et al., 2003). However, use of antibiotics in diets is facing increased scrutiny from consumers in some parts of the world. This has led to their prohibition inside the European Union from 1st January 2006 (Official Journal of the European Union, 2003). As a result, research programs have been prompted to seek alternative feed additives that improve animal performance. One such alternative is use of plant extracts (PE) and their constitutive elements, essential oils, which are naturally occurring secondary plant metabolites obtained through steam distillation or by extraction using organic solvents. Recent literature reviews have outlined the potential of PE to increase animal production (Calsamiglia et al., 2007; Benchaar et al., 2008).

Most research with PE has been conducted *in vitro*, and data on animal performance are much less available. Meyer et al. (2009) reported that a mixture of PE fed to finishing beef cattle was similar to monensin in terms of the resultant feed efficiency and ADG, whereas Benchaar et al. (2006) found a dose response in FCR of growing cattle, with lower levels of PE being more effective. However, differences in the essential oil compound(s) make direct comparisons among studies difficult. Among essential oil compounds, cinnamaldehyde has been examined *in vitro* and *in vivo* (Calsamiglia et al., 2007; Chaves et al., 2011), whereas a mixture of cinnamaldehyde and eugenol has shown promise in decreasing acetate and propionate ratio and ruminal ammonia production (Cardozo et al., 2005, 2006).

Our study was completed to determine effects of a mixture of cinnamaldehyde, eugenol and capsicum oleoresin (a capsaicin source) on performance of feedlot cattle, compared to monensin as the control. A second objective was to examine the influence of this PE on ruminal fermentation variables and short term intake patterns.

2. Materials and methods

The study was carried out at the Estación Experimental Agropecuaria Balcarce of the Instituto Nacional de Tecnología Agropecuaria (EEA INTA), Balcarce, Buenos Aires Province, Argentina (37°45′10″S; 58°17′34″W), from March to June 2007. The study received approval from the Institutional Animal Care Committee and animals were cared for in accordance to the EEA INTA Balcarce Guide for Animal Care.

2.1. Experiment 1

2.1.1. Animals and treatments

Twenty four angus steers (initial weight 141 ± 6.6 kg) were weaned, castrated and dewormed at least 2 wks before the beginning of the experiment and then adapted to a high concentrate diet (Table 1) by gradually increasing the concentrate level in the diet. According to their initial body weight (BW), steers were stratified into 4 blocks of 6, and 3 steers from each block were randomly assigned to one of the two treatments and placed in groups of 3 in 3 m × 15 m pens. Each pen was fitted with a feeder and animals had free access to clean fresh water at all times. Thus there were 4 pens with 3 steers/pen/treatment. Treatments were monensin (MO; included at 46.7 mg/kg dietary DM; Rumensin, Elanco Animal Health, Indianapolis, IN, USA) and plant extracts (PE; 266 mg/animal/d of Xtract 6965 + Xtract 6933 at 133 mg/animal/d). The PE compounds were provided by Pancosma SA (Geneva, Switzerland), and Xtract 6965 contained 170 g/kg cinnamaldehyde and 280 g/kg eugenol encapsulated in a hydrogenated oil matrix. The Xtract 6933 is a natural capsicum oleoresin containing 12 g/kg capsaicin. The application rates were based on preliminary *in vitro* studies and recommendations of the manufacturers. The MO and PE additives were thoroughly mixed into the mineral-vitamin supplement, plus a minimum amount of dry ground corn, and fed once daily with the concentrate.

2.1.2. Diet and feeding

During the first 44 d of the study, steers were fed *ad libitum* a diet of (g/g DM) 0.7 dry rolled corn, 0.28 pelleted sunflower meal, and 0.02 mineral vitamin supplement. From d45 to the end of the study, steers were fed a diet of (g/g DM) 0.748 dry rolled corn, 0.225 pelleted sunflower meal, 0.019 mineral vitamin supplement and 0.008 urea. In addition, 200 g (as fed)

Table 1
Composition of basal diets with differing feed additive treatments included in the mineral–vitamin supplement (Exp. 1). Diet used in period 2 of Exp. 1 was also used for Exp. 2.

	Period 1 (0–44 d)	Period 2 (45–84 d)
Composition, g/kg DM		
Concentrate		
Dry rolled corn grain	700	748
Pelleted sunflower meal	280	225
Urea	0	8
Mineral–vitamin mixture[a]	20	19
Forage, g as fed/steer/d		
Alfalfa hay	200	200
Additives		
Plant extracts, mg/steer/d[b]	0 or 400	0 or 400
Monensin premix, mg/kg DM[c]	0 or 46.7	0 or 46.7
Nutrient composition, g/kg DM[d]		
CP	138	150
aNDF	181	164
ADF	106	92
Calcium	7.5	6.9
Phosphorus	4.9	4.5

[a] Mineral–vitamin mixture (Raciones Argentinas SRL, Pilar, Argentina) contained (DM basis) 297 g/kg Ca, 100 g/kg NaCl, 7.5 g/kg Mg, 3000 mg/kg S, 1163 mg/kg Fe, 1500 mg/kg Zn, 450 mg/kg Mn, 510 mg/kg Cu, 24 mg/kg I, 5 mg/kg Co, 5 mg/kg Se, 127,500 IU vitamin A, 25,500 IU vitamin D, 225 IU vitamin E, and 3 g/kg antioxidant (BHT).
[b] Plant extract mixture (Pancosma SA, Geneva, Switzerland), contained 266 mg of Xtract 6965 plus 133 mg of Xtract 6933. This mixture was included into the mineral–vitamin mixture and mixed thoroughly with the concentrate prior to feeding.
[c] Formulated to provide monensin (Elanco Animal Health, Indianapolis, IN, USA) at 0 or 46.7 mg/kg DM. Monensin was included into the mineral–vitamin mixture and mixed thoroughly with the concentrate prior to feeding.
[d] Based on analysis, with the exception of calcium and phosphorus, which were estimated from tabular values (NRC, 1996).

animal/d of coarsely ground alfalfa hay was mixed with the concentrate and provided in both diets (Table 1). The mineral vitamin supplement contained the appropriate additive for each treatment. Diets were fed once daily at 0730 h. To ensure total daily intake of the targeted amounts of PE of 400 mg/steer, the required daily amounts of PE and forage was supplied in 10 kg feed DM/pen and, once consumed, more feed (with no PE or added forage) was provided until *ad libitum* intake was reached. The experiment lasted 84 d achieving an approximate slaughter BW of 280 kg.

2.1.3. Measurements and sample collection

Quantities of feed offered and refused were determined 5 d/wk throughout the 84 d study, and intake was calculated/pen as the DM offered minus DM refused. Total DM intake/pen was divided by the number of steers/pen to obtain mean DM intake/steer. Samples of dietary ingredients, diets and refusals were collected 4 times throughout the study for DM assay. Samples were dried at 60 °C for 48 h, ground to pass a 1 mm screen (Wiley mill, standard model 4, Arthur M. Thomas, PA, USA) and stored for chemical analysis.

Steer BW was determined weekly by weighing the steers at 0800 h to minimize gut fill effects. Final ADG was calculated from the best fit slope of the regression between BW evolution and experimental days. The FCR was determined as the ratio of DM intake to ADG expressed as kg/d.

Backfat was determined 4 times throughout the study on d23, 44, 68 and 84, using a portable ultrasound instrument (Pie Medical 200, Genoa, Italy), equipped with a 18 cm long, 3.5 MHz linear transducer array. Ultrasound measurements were made in the region of the 12th and 13th ribs. Monthly rates of backfat deposition (BFD) were obtained through linear regression. In addition, *longissimus dorsi* muscle (LM) area and its monthly growth rate were also determined by ultrasound.

Short-term intake patterns were measured during the second half of the finishing period (*i.e.*, d45–84). Visual appraisal was conducted every 5 min from 0800 to 1700 h, and repeated 5 times on non-consecutive days. Located in an isolated cubicle, two independent operators quantified the number of times the steers visited the feeders/d, and total intake time. Average time spent at the feeders was calculated by dividing the total time spent eating/d by the number of visits/d. The variability in the frequency of visits to the feeders was determined by averaging the number of visits to the feeders recorded for each steer in both treatments every hour from 0800 to 1200 h.

2.1.4. Chemical analysis

Dry matter contents were determined by drying samples at 60 °C in a forced air oven for 48 h, whereas organic matter was determined as weight lost upon heating at 550 °C for 3 h (AOAC, 1990; #942.05). Total N content was determined by thermal conductivity (LECO FP-528 Nitrogen Determinator, LECO Corp., Saint Joseph, MO, 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. Acid detergent fiber content (ADF) was determined according to AOAC (1997; #973.18). Both aNDF and ADF procedures were adapted for use in an ANKOM^{200} fiber analyzer (ANKOM Corp., Macedon, NY,

USA) and results are expressed ash-inclusive. Starch concentrations were determined according to McRae and Armstrong (1968).

2.1.5. Statistical analysis

All data were analyzed using the MIXED procedure of SAS (2001) and, unless stated otherwise, pen was the experimental unit. The DM intake, ADG and FCR values were analyzed as a split plot experimental design in time (period) with fixed effects of treatment. Differences between means were tested using Tukey's test. The regression slopes of the backfat, LM area and short term intake pattern data as a function of days were analyzed as a completely randomized block design for the two periods (*i.e.*, diet) simultaneously, with fixed effects of period (diet) and treatment.

Analysis of the variability of the frequency in visiting the feeders was completed using individual steers as experimental units. Variances from both treatments (populations) were estimated and then subjected to a Snedecor's *F* test, as:

$$\text{Ho}: \sigma_1^2 = \sigma_2^2$$

$$\text{Ho}: \sigma_1^2 \neq \sigma_2^2$$

where: σ_1^2 is the variance of the average number of times that steers from PE treatment visited the feeders, σ_2^2 is the variance of the average number of times that steers from MO treatment visited the feeders.

In all cases, statistical differences were declared if $P<0.05$, and trends were accepted when $P<0.10$.

2.2. Experiment 2

2.2.1. Animals, diets and measurements

Two angus steers (average BW 425 kg), permanently fitted with ruminal cannulas, were used in a cross-over design with two periods of 14 d with the first 10 d for adaptation and the last 4 d for sampling. The steers were fed 10 kg DM/d of finishing diet, consisting (DM basis) of 0.748 dry ground corn, 0.225 pelleted sunflower meal, 0.019 mineral–vitamin supplement and 0.008 urea, plus 200 g (as fed) of coarsely chopped alfalfa hay/steer/d (*i.e.*, the same diet fed to steers in Exp. 1 during period 2). The diet was fed once daily at 0700 h. In period 1, one steer was fed the diet with added PE, and the other the diet with added MO, changing over in the second period.

During the first day of determinations (*i.e.*, day 11 of each period), a pooled sample of ruminal fluid was collected from multiple sites of the rumen of each steer at 0800, 1100, 1400, 1700, and 2000 h to determine pH, VFA and NH_3-N concentrations. Ruminal fluid samples were filtered through 4 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 sulfuric acid (0.5 v/v), and stored at −24 °C for determination of VFA and NH_3-N. For NH_3-N determination, samples were thawed and centrifuged at 10,000 × *g* for 10 min at 4 °C, and then concentrations were determined as described by Chaney and Marbach (1962). The VFA as described by Jouany (1982).

2.2.2. Statistical analysis

The experimental unit in Exp. 2 was the steer and the ruminal fermentation variables were analyzed as a crossover design, with repeated measures, with the model:

$$Y_{ikl} = \mu + T_i + P_k + H_l + (T * H)_{il} + A(T)_l + e2_{ikl}$$

where: Y_{ikl} is the observation of *i* treatment in the *k* period and *l* sampling time; μ is the general mean; T_i is the treatment effect; P_k is the period effect; H_l is the sampling time effect; $(T^*H)_{il}$ is the treatment by sampling time interaction effect; $A(T)_l$ is the random effect of animal nested within treatment; and $e2_{ikl}$ is the random error.

In all cases, statistical differences were accepted if $P<0.05$ and trends were accepted when $P<0.10$.

3. Results

3.1. Experiment 1

Given the concentration of monensin (46.7 mg/kg dietary DM) provided, steers on the MO treatment consumed an average of 280 ± 38.2 mg monensin/d in period 1 and 343 ± 43.9 mg monensin/d in period 2.

Steers fed the PE mixture ate similar amounts of DM as those fed MO (Table 2). Regardless of treatments, all steers ate more ($P=0.03$) feed in the later stages of the finishing period. A treatment × period interaction occurred ($P=0.02$) for ADG as feeding PE increased ($P=0.01$) ADG in the second period, but not in the first. A treatment × period interaction effect also occurred ($P=0.04$) for FCR, with the steers being more efficient in the first period than in the second ($P<0.01$). However, there was no overall treatment effect on feed conversion and no differences in BFD or LM area growth rate.

Overall, no differences were detected in time spent at the feeders (Table 3), and the number of times the steers visited the feeders or average time per visit. However, when the variance associated with the number of visits was evaluated by time

Table 2
Dry matter intake and animal performance of steers fed a plant extract mixture or monensin for a 84-d finishing period (Exp. 1).

	Treatment[b]		SEM	Effects (*P*)[a]		
	PE	MO		Trt	Per	Trt × Per
Initial BW, kg	143.4	140.9	4.58	0.48		
Final BW, kg	256.3	252.3	10.34	0.44		
ADG, kg/d						
d1–44	1.27	1.27	0.032			
d45–84	1.43a	1.23b	0.027			
Effects				0.01	0.39	0.02
DM intake, kg/BW$^{0.75}$						
d1–44	0.11	0.11	0.002			
d45–84	0.14	0.13	0.006			
Effects				0.83	0.03	0.22
FCR						
d1–44	4.19	3.94	0.184			
d45–84	5.46	6.49	0.351			
Effects				0.42	<0.0001	0.04
BFD, mm/mo[c]	1.87	1.76	0.195	0.69		
LM area, cm^2/mo[d]	6.56	6.60	1.053	0.97		

[a,b]Within a row, means without a common letter differ ($P<0.05$).
[a] Trt: treatment effect; Per: period effect; Trt × Per: treatment × period interaction effect.
[b] PE: plant extract mixture (Pancosma SA, Geneva, Switzerland), contained 266 mg of Xtract 6965 plus 133 mg of Xtract 6933; MO: monensin (Elanco Animal Health, Indianapolis, IN, USA), added at 46.7 mg/kg dietary DM.
[c] Rate of backfat deposition, obtained through best-fit slope of linear regression of 4 determinations throughout the study.
[d] Rate of LM area growth, obtained through best-fit slope of linear regression of 4 determinations throughout the study.

interval (from 0800 to 1200 h), feeding PE was associated with less variance ($P<0.01$) in the 0800–0900 h period, whereas a trend ($P=0.07$) occurred in the 0900–1000 h period, with no differences thereafter (Fig. 1).

3.2. Experiment 2

Steers fed PE had lower ($P=0.02$) ruminal NH_3-N concentrations than those fed MO (Table 4). Moreover, there was a treatment × time interaction for pH values ($P=0.03$). At 0800 and 1100 h (*i.e.*, 1 and 3 h post feeding) pH values were 5.23

Table 3
Effects of plant extracts (PE) or monensin (MO) on intake patterns (Exp. 1).

Intake patterns[b]	Treatment[a]		SEM	*P*
	PE	MO		
Time spent at the feeders (min/9 h)	92.2	93.4	6.10	0.88
Visits to the feeders, n	11.5	10.6	0.96	0.47
Average time spent at the feeders, min/visit	8.3	9.6	0.66	0.17

[a] PE: plant extract mixture (Pancosma SA, Geneva, Switzerland), contained 266 mg of Xtract 6965 plus 133 mg of Xtract 6933; MO = monensin (Elanco Animal Health, Indianapolis, IN, USA), added at 46.7 mg/kg dietary DM.
[b] Intake patterns were obtained through visual appraisal from an isolated cubicle. Individual steers were used as the experimental unit.

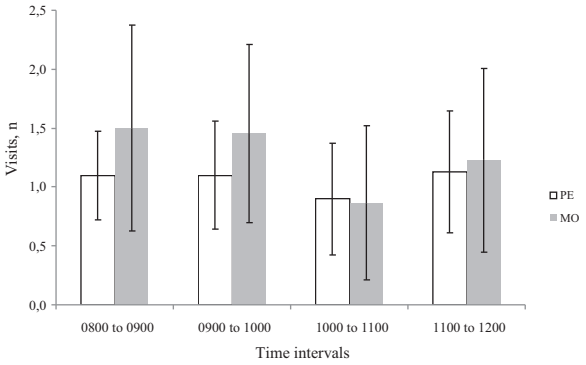

Fig. 1. Effects of feeding a plant extract mixture (PE) or monensin (MO) on the number of visits to the feeders and associated variances at each time interval of feedlot steers. While number of visits did not differ at any time interval, variance means differed ($P<0.01$) from 0800 to 0900 h; tended to differ ($P=0.07$) from 0900 to 1000 h, and did not differ from 1000 to 1100 h and 1100 to 1200 h (Exp. 1).

Table 4
Ruminal fermentation parameters of feedlot steers fed a plant extract mixture or monensin (Exp. 2).

	Treatment[a]		SEM	*P*		
	PE	MO		Trt	Time	Trt × Time
pH	5.55	6.05	0.157	0.27	0.03	0.03
NH_3-N, mg/dl	10.78a	20.05b	0.235	0.03	0.16	0.35
VFA concentration						
Total VFA, mM	78.2	62.5	5.10	0.30	0.15	0.19
Acetate, mol/100 mol	48.5	58.2	2.59	0.21	0.82	0.64
Propionate, mol/100 mol	32.8	25.2	8.68	0.65	0.004	<0.01
Butyrate, mol/100 mol	13.8	12.0	4.54	0.83	0.49	0.51
Isovalerate, mol/100 mol	1.44	2.51	0.201	0.18	0.84	0.75
Valerate, mol/100 mol	1.62	1.12	0.147	0.29	0.52	0.36
Acetate:propionate	1.79	2.48	0.862	0.67	0.45	0.58

a, b: Within a row, means without a common letter differ ($P < 0.05$).
[a] PE: plant extract mixture (Pancosma SA, Geneva, Switzerland), contained 266 mg of Xtract 6965 plus 133 mg of Xtract 6933; MO: monensin (Elanco Animal Health, Indianapolis, IN, USA), added at 46.7 mg/kg dietary DM.

and 5.15 in the PE treatment, compared to 6.03 and 6.16 in MO. Total VFA concentrations did not differ with no differences in VFA profiles or the acetate:propionate ratio between treatments.

4. Discussion

4.1. Experiment 1

Beef farmers in Argentina and neighboring countries make extensive use of monensin in their feedlot diets because of its proven efficacy, making it rare that feedlots do not use ionophores. Thus monensin is considered to be a benchmark against which another rumen modifier should be compared. The blend of PE used contained cinnamaldehyde, eugenol and capsicum oleoresin, and was developed after indications that it favorably modified ruminal fermentation parameters (Calsamiglia et al., 2007) and may alter DM intake (Fandiño et al., 2008). However no published paper was found in which this blend of PE were used, making direct comparisons with published literature difficult. Since different PE may have unlike modes of action (Benchaar et al., 2008), making comparison of results among studies in which other blends of PE were used is of little value, we focused comparison of PE with monensin.

The lack of difference between treatments in DM intake was somewhat unexpected given that monensin usually decreases DM intake (Stock et al., 1995), while it has been suggested that capsicum feeding may increase DM intake compared to controls or monensin fed ruminants (Fandiño et al., 2008). Effects of PE on DM intake will depend on PE source, application rates and diet interactions, and direct comparisons of DM intake between monensin and PE fed beef cattle are limited. In a production study, Meyer et al. (2009) reported a reduction in DM intake (11.4 kg DM/d *versus* 12.0 kg DM/d) when steers were fed monensin at 300 mg/d plus tylosin, compared to those fed a blend of thymol, eugenol, vanillin, guaiacol and limonene (DSM Nutritional Products, Inc., Parsippany, NJ, USA). However, Meyer et al. (2009) also reported an accompanying digestibility and an intake pattern study where no differences in DM intake between the PE blend and monensin occurred.

The steers fed PE considerably increased ADG (+16%) during the last part of the finishing period (*i.e.*, d45–84). Generally, there have been no differences in ADG between monensin and PE fed cattle, and thus the FCR has not been altered (Benchaar et al., 2006; Devant et al., 2007; Meyer et al., 2009; Yang et al., 2010).

That intake patterns showed no differences in total time spent at the feeder, number of visits and average time spent at the feeder agrees with Meyer et al. (2009) who reported no differences in number of meals, total time spent eating, meal size or meal length between PE and monensin fed steers. However, the large differences in the variances of number of visits to the feeders are noteworthy as it suggests a more consistent intake pattern. In agreement with Rotger et al. (2006), our steers ate most of their diet during the first 4 h after feeding. Perhaps the lower variability in the frequency of visits to the feeders might be related to reduced stress. Overall, data suggest that PE is equivalent to monensin for feedlot cattle in terms of animal performance and intake.

4.2. Experiment 2

Ruminal pH was not altered with PE compared to MO, although a large numerical difference occurred. However the treatment × time interaction indicates that PE had lower pH values during the first hours post feeding, which might reflect higher diet fermentability. The pH values were typical of steers fed high concentrate diets, and the average pH values agree with Meyer et al. (2009) who reported 5.64 for PE and monensin fed steers. Furthermore, Meyer et al. (2009) did not find differences on overall ruminal pH between an essential oil and monensin in their study, but reported that the time spent with a pH below 5.0 was less for the essential oil treatment than monensin. Devant et al. (2007) found that steers fed monensin or a PE mixture showed no differences in ruminal pH, but both were lower than the control diet. Overall, our study seems to

concur with this previous research, although it is difficult to extrapolate from other research due to differences in the type of diets, PE fed and feeding management.

Ruminal NH_3-N concentrations decreased by almost 50% with PE supplementation compared to MO, which may be a partial reflection of the numerically lower pH in the PE treatment (Lana et al., 1998; Colombatto et al., 2003). No differences occurred in either total VFA or the VFA profile, although numerical trends coincided. For example, the numerical reduction in isovalerate concentrations in the rumen of PE fed steers could provide a partial explanation for the decreased NH_3-N concentrations, as isovalerate production in the rumen is the result of the deamination of branched chain amino acids, which suggests that deamination could have been decreased in the PE fed steers. Compared to an unsupplemented control, a mixture of cinnamaldehyde and eugenol reduced ruminal NH_3-N and tended to reduce branched chain VFA concentrations when ruminal pH was not altered (Cardozo et al., 2006). When cinnamaldehyde, eugenol and capsicum were individually examined *in vitro*, Cardozo et al. (2005) found that at pH 5.5 (*i.e.*, the average pH), cinnamaldehyde and capsicum reduced NH_3-N concentration, while eugenol and capsicum decreased branched chain VFA concentrations. It appears that there was a combined effect of pH and PE to reduce NH_3-N concentrations.

Although results are inconclusive, VFA concentrations were favorably altered by PE compared to monensin feeding, suggesting that the mode of action of PE in the rumen might differ from that of monensin, which agrees with Benchaar et al. (2008). When direct comparisons between PE and monensin have been examined *in vivo* with beef cattle, no differences in VFA profiles occurred (Devant et al., 2007; Meyer et al., 2009). However, none of these studies included cinnamaldehyde and capsicum, although eugenol was a constituent of the product used by Meyer et al. (2009). Fandiño et al. (2008) compared capsicum to monensin *in vivo* and found that monensin increased propionate, but decreased butyrate, proportion in the rumen.

There is a growing body of evidence supporting use of mixtures of cinnamaldehyde, eugenol and capsicum as ruminal modifiers (Calsamiglia et al., 2007). Capsicum has been added mainly because of its reported positive effects on DM intake and intake patterns (Cardozo et al., 2006; Fandiño et al., 2008), whereas cinnamaldehyde and eugenol have been included to alter the VFA profile and reduce protein degradation (Cardozo et al., 2006).

5. Conclusions

Steers fed a plant extract mixture of cinnamaldehyde, eugenol and capsicum oleoresin performed at least equivalent to those fed monensin, and there were indications that some ruminal fermentation variables were favorably altered thereby suggesting that the mode of action of these additives are different.

Acknowledgements

The authors gratefully acknowledge INTA for awarding an MSc Scholarship (J.I. Geraci). Pancosma SA (Geneva, Switzerland) is also acknowledged for providing partial financial support. Mrs. Claudia Faverin and Mrs. Adriana Cano are gratefully acknowledged for help with statistical analysis.

References

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists International, Arlington, VA, USA.
AOAC, 1997. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists International, Arlington, VA, USA.
Benchaar, C., Duynisveld, J.L., Charmley, E., 2006. Effects of monensin and increasing dose levels of a mixture of essential oil compounds and intake, digestion and growth performance of beef cattle. Can. J. Anim. Sci. 86, 91–96.
Benchaar, C., Calsamiglia, S.C., Chaves, A.V., Fraser, G.R., Colombatto, D., McAllister, T.A., Beauchemin, K.A., 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145, 209–228.
Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A., 2007. Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90, 2580–2595.
Cardozo, P.W., Calsamiglia, S., Ferret, A., Kamel, C., 2005. Screening for the effects of natural plant extracts at different pH on in vitro rumen microbial fermentation of a high-concentrate diet for beef cattle. J. Anim. Sci. 83, 2572–2579.
Cardozo, P.W., Calsamiglia, S., Ferret, A., Kamel, C., 2006. Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. J. Anim. Sci. 84, 2801–2808.
Chaney, A.L., Marbach, E.P., 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8, 130–132.
Chaves, A.V., Dugan, M.E.R., Stanford, K., Gibson, L.L., Brystom, J.M., McAllister, T.A., Van Herk, F., Benchaar, C., 2011. A dose–response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. Livest. Sci. 141, 213–220.
Colombatto, D., Hervás, G., Yang, W.Z., Beauchemin, K.A., 2003. Effects of enzyme supplementation of a total mixed ration on microbial fermentation in continuous culture, maintained at high and low pH. J. Anim. Sci. 81, 2617–2627.
Devant, M., Anglada, A., Bach, A., 2007. Effects of plant extract supplementation on rumen fermentation and metabolism in young Holstein bulls consuming high levels of concentrate. Anim. Feed Sci. Technol. 137, 46–57.
EC, 2003. Regulation EC No 1831/2003 of the European Parliament and Council of 22 September 2003 on Additives for Use in Animal Nutrition. Off. J. Eur. Commun. L268, 29–43.
Fandiño, I., Calsamiglia, S., Ferret, A., Blanch, M., 2008. Anise and capsicum as alternatives to monensin to modify rumen fermentation in beef heifers fed a high concentrate diet. Anim. Feed Sci. Technol. 145, 409–417.
Horneck, D.A., Miller, R.O., 1998. Determination of total nitrogen in plant tissue. In: Kalra, Y.P. (Ed.), Handbook of Reference Methods for Plant Analysis. Soil and Plant Analysis Council, Inc., CRC Press, Boca Raton, FL, USA, pp. 75–84.
Jouany, J.P., 1982. Volatile fatty acids and alcohol determination in digestive contents, silage juice, bacterial cultures and anaerobic fermenter contents. Sci. Aliments 2, 131–144.

Lana, R.P., Russell, J.B., van Amburgh, M.E., 1998. The role of pH in regulating ruminal methane and ammonia production. J. Anim. Sci. 76, 2190–2196.
McRae, J.C., Armstrong, D.G., 1968. Enzyme method for determination of alpha-linked glucose polymers in biological materials. J. Sci. Food Agric. 19, 578–581.
Meyer, N.F., Erickson, G.E., Klopfenstein, T.J., Greenquist, M.A., Luebbe, M.K., Williams, P., Engstrom, M.A., 2009. Effect of essential oils, tylosin, and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation, and digestibility. J. Anim. Sci. 87, 2346–2354.
Nagaraja, T.G., 1995. Ionophores and antibiotics in ruminants. In: Wallace, R.J., Chesson, A. (Eds.), Biotechnology in Animal Feeds and Animal Feeding. VCH, Weinheim, Germany, pp. 173–204.
NRC, 1996. Nutrient Requirements of Beef Cattle, 7th rev. ed. Natl. Acad. Press, Washington, DC, USA.
Rotger, A., Ferret, A., Manteca, X., De la Torre, J.L.R., Calsamiglia, S., 2006. Effects of dietary nonstructural carbohydrates and protein sources on feeding behavior of tethered heifers fed high-concentrate diets. J. Anim. Sci. 84, 1197–1204.
SAS, 2001. SAS User's Guide: Statistics Version 8, 2nd ed. SAS Institute Cary, NC, USA.
Stock, R.A., Laudert, S.B., Stroup, W.W., Larson, E.M., Parrott, J.C., Britton, R.A., 1995. Effect of monensin and monensin and tylosin combination on feed intake variation of feedlot steers. J. Anim. Sci. 73, 39–44.
Tedeschi, L.O., Fox, D.G., Tylutki, T.P., 2003. Potential environmental benefits of ionophores in ruminant diets. J. Environ. Qual. 32, 1591–1602.
Van Soest, P.J., Robertson, J.B., Lewis, B.I., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharide in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Yang, W.Z., Ametaj, B.N., Benchaar, C., He, M.L., Beauchemin, K.A., 2010. Cinnamaldehyde in feedlot cattle diets: intake, growth performance, carcass performance, and blood metabolites. J. Anim. Sci. 88, 1082–1092.