

Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture¹

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ABSTRACT: Eight dual-flow continuous culture fermenters were used in four consecutive periods of 10 d to study the effects of six natural plant extracts on ruminal protein degradation and fermentation profiles. Fermenters were fed a diet with a 52:48 forage:concentrate ratio (DM basis). Treatments were no extract (CTR), 15 mg/kg DM of a mixture of equal proportions of all extracts (MIX), and 7.5 mg/kg DM of extracts of garlic (GAR), cinnamon (CIN), yucca (YUC), anise (ANI), oregano (ORE), or pepper (PEP). During the adaptation period (d 1 through 8), samples for ammonia N and VFA concentrations were taken 2 h after feeding. On d 9 and 10, samples for VFA (2 h after feeding), and peptide, AA, and ammonia N concentrations (0, 2, 4, 6, and 8 h after feeding) were also taken. Differences were declared at $P < 0.05$. During the adaptation period, total VFA and ammonia N concentrations were not affected by treatments. The acetate proportion was higher from d 2 to 6 in CIN, GAR, ANI, and ORE, and the propionate proportion was lower from d 2 to 4 in CIN and GAR, and from d 2 to 5 in ANI and ORE, compared with CTR. However, the proportion of individual VFA (mol/100 mol) was similar in all treatments after d 6, except for

valerate in d 9 and 10, which was lower in PEP (2.8 ± 0.27) compared with CTR (3.5 ± 0.27). The average peptide N concentration was 31% higher in MIX, and 26% higher in CIN and YUC compared with CTR (6.5 ± 1.07 mg/100 mL). The average AA N concentration was 17 and 15% higher in GAR and ANI, respectively, compared with CTR (7.2 ± 0.77 mg/100 mL). The average ammonia N concentration was 31% higher in ANI and 25.5% lower in GAR compared with CTR (5.5 ± 0.51 mg/100 mL). The accumulation of AA and ammonia N in ANI suggested that peptidolysis and deamination were stimulated. The accumulation of AA N and the decrease in ammonia N in GAR suggests that deamination was inhibited. The accumulation of peptide N and the numerical decrease in AA N in CIN suggest that peptidolysis was inhibited. Results indicate that plant extracts modified ruminal fermentation, but microbes were adapted to some extracts after 6 d of fermentation. Therefore, data from short-term in vitro fermentation studies may lead to erroneous conclusions, and should be interpreted with caution. Careful selection of these additives may allow the manipulation of protein degradation in the rumen.

Key Words: Ruminal Fermentation, Plant Extract, Protein Degradation

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Introduction

Ruminal microbial activity is essential for the use of structural carbohydrates and the synthesis of high-quality protein in ruminants. However, microbial fermentation may result in considerable energy and protein losses as methane and ammonia N (NRC, 2001). Many feed additives have been developed to improve

the efficiency of nutrient use by decreasing the total amount of methane or ammonia N produced, among which ionophore antibiotics have been very successful (Hutjens, 1992). However, the risk of the presence of antibiotic residues in milk and meat and its effects on human health have led to its prohibition for use in animal feeds in the European Union in 2006 (Official Journal of the European Union, 2003). Industry is seeking alternative additives that would improve the efficiency of nutrient use in the rumen. Many plants produce secondary metabolites such as phenolic compounds, essential oils, and sarsaponins (Chesson et al., 1982; Wallace et al., 1994; Kamel, 2001) that affect microbial activity. Sarsaponins are secondary compounds of yucca (*Yucca schidigera*) that have been reported to decrease ammonia N concentration and to

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alter the acetate and propionate proportions (Grobner et al., 1982; Ryan et al., 1997) in ruminal fluid. However, other authors found no effect of yucca extract on ammonia N concentration (Wang et al., 1997; Hristov et al., 1999). Thymol, a secondary compound present in oregano (*Origanum vulgare*), decreased the acetate and propionate concentrations and increased the acetate:propionate ratio in in vitro mixed ruminal fluid incubations (Evans and Martin, 2000). Although many other plant extracts have been shown to affect microbial activity (Cowan, 1999), few have been tested for their effects on ruminal microbial fermentation.

The objective of this study was to evaluate the effects of several natural plant extracts on VFA concentrations and protein degradation in a dual-flow continuous culture system.

Materials and Methods

Apparatus and Experimental Design

Eight 1,320-mL dual flow continuous culture fermenters (Hoover et al., 1976) were used in four consecutive 10-d periods. On the first day of each period, all fermenters were inoculated with ruminal fluid obtained from two ruminal fistulated dairy cows fed a total mixed diet (35.7% alfalfa hay, 7.6% ryegrass hay, 15.5% ground corn, 11.6% barley grain, 11.9% corn gluten feed, 8.1% cottonseed, 4.6% molasses, 1.6% soybean meal, 1.3% calcium soaps of fatty acids, and 2.1% mineral and vitamin mixture, DM basis). During the 10 d of each period, all fermenters were fed 95 g of DM/d of the diet (18.9% CP; 36.6% NDF; 17.6% ADF; DM basis) in three feedings per day (0800, 1600, and 2400). The diet was ground to pass a 1.5-mm screen (Hammer mill, P. Prat SA, Sabadell, Spain), and consisted (DM basis) of alfalfa hay (27%), dehydrated whole corn plant (20%), barley straw (5%), soybean meal (16%), ground corn grain (15%), ground barley grain (15%), and a vitamin and mineral mixture (2%; 1 kg DM of vitamin and mineral mixture contained 1,000,000 IU of vitamin A; 200,000 IU of vitamin D; 1,333 mg of vitamin E; 300 g of magnesium oxide; 67 g of sodium chloride; 33 g of sulfur; 2.7 mg of manganese sulfate; 7 mg of cobalt sulfate; 167 mg of copper sulfate; 2.7 g of zinc methionate; 2 g of zinc sulfate; 33 mg of iodine; 27 mg of selenium; and 267 g of urea). The diet was designed to meet or exceed nutrient recommendations for a Holstein cow (600 kg) producing 25 kg of milk (NRC, 2001). Temperature (38.5°C), pH (6.4 ± 0.05), and liquid (10%/h) and solid (5%/h) dilution rates were kept constant in the fermenters. Fermentation parameters were monitored and controlled by a computer and a programmable linear controller (National Instruments, Austin, TX), and fermentation conditions were programmed with LabView Software (National Instruments). Anaerobic conditions were maintained by infusion of N₂ at a rate of 40 mL/min. Artificial saliva (Weller and Pilgrim, 1974) containing 0.4 g/L of urea to simulate recycled N

was continuously infused into flasks at rate of 2.2 mL/min. Treatments were assigned to fermenters at random within periods, and were 1) no extract (**CTR**), 2) a mixture of equal proportions (vol/vol) of all extracts (**MIX**), and 3) extracts of garlic (*Allium sativa*, **GAR**; 0.7% of allicin), cinnamon (*Cinnamomum cassia*, **CIN**; 59% of cinnamaldehyde), yucca (*Yucca schidigera*, **YUC**; 8% of sarsaponin), anise (*Pimpinella anisum*, **ANI**; 86% of anethole), oregano (*Origanum vulgare*, **ORE**; 64% of carvacrol and 16% of thymol), and pepper (*Capsicum annuum*, **PEP**; 12% of capsaicin). Raw materials were provided by AXISS France SAS (AXISS France SAS, Archamps, France). The levels of inclusion were 15 mg/kg DM for the MIX and 7.5 mg/kg DM for each of the individual extracts. Under the experimental conditions defined in this work, 7.5 mg/kg was equivalent to 0.71 mg of extract/d or 0.22 mg/L of ruminal fluid. The lipid-soluble extracts (all except YUC) were dissolved in sunflower oil at a 1:250 dilution. Yucca was dissolved in water at the same proportion. All extracts were stored at 5°C in 200-mL smoked glass bottles. One-third of the daily dose of extracts was dosed into the fermenters 1 min before each feeding. The CTR and YUC treatments were also dosed with the equivalent amount of sunflower oil.

Each experimental period consisted of 10 d (8 d for adaptation and 2 d for sample collection). On the adaptation days, 8 mL of fermenter fluid was taken 2 h after the morning feeding to determine the effects of natural plant extracts on ruminal ammonia N and VFA concentrations. During the last 2 d of the experiment, 4 mL of fermenter fluid were taken 2 h after the morning feeding to determine VFA concentrations. Samples (36 mL in each sampling time) were also taken at 0, 2, 4, 6, and 8 h after the morning feeding to determine tungstic acid soluble N (**TA N**), trichloroacetic acid soluble N (**TCA N**), and ammonia N. Results were used to calculate peptide, AA, and ammonia N concentrations in fermenters.

Chemical Analyses

Samples for VFA were prepared as described by Jouany (1982). One milliliter of a solution comprising a 0.2% (wt/wt) solution of mercuric chloride, 0.2% (wt/wt) of 4-methylvaleric acid as an internal standard, and 2% (vol/vol) orthophosphoric acid was added to 4 mL of ruminal fluid and frozen. Samples were centrifuged at 3,000 × g for 30 min, and the supernatant fluid was analyzed by gas chromatography (model 6890, Hewlett Packard, Palo Alto, CA) using a polyethylene glycol nitroterephthalic acid-treated capillary column (BP21, SGE Europe Ltd., Kiln Farm Milton Keynes, U.K.) at 275°C in the injector and a 29.9 mL/min gas flow rate.

For ammonia N determination, a 4-mL sample of fermenter fluid was acidified with 4 mL of 0.2 N HCl and frozen. Samples were centrifuged at 25,000 × g for 20 min, and the supernatant was analyzed by spectro-

photometry (UV-120-01, Shimadzu, Kyoto, Japan) for ammonia N (Chaney and Marbach, 1962).

Peptide and AA N were determined as described by Winter et al. (1964). A 16-mL sample of fermenter fluid was added to 4 mL of 10% (wt/vol) sodium tungstate and 4 mL of 1.07 *N* sulfuric acid. After allowing the tubes to stand at 5°C for 4 h, they were centrifuged at $9,000 \times g$ for 15 min. The supernatant was frozen until analyzed for TA N by the Kjeldahl procedure (AOAC, 1990). To determine TCA N, 4 mL of 50% (wt/vol) TCA was added to 16 mL of fermenter fluid. After 4 h at 5°C, tubes were centrifuged at $9,000 \times g$ for 15 min. The supernatant was frozen until analyzed for TCA N. Results were used to calculate in mg/100 mL: 1) Peptide N = (TCA N) – (TA N); and 2) AA N = (TA N) – (ammonia N).

Statistical Analyses

All statistical analyses were conducted using SAS (SAS Inst., Inc., Cary, NC, version 8.1). Results were analyzed using PROC MIXED for repeated measures (Littell et al., 1998). The model accounted for the effects of treatments and days (for VFA and ammonia N concentration in d 1 to 10), or treatment and hours of sampling (for the protein fractions in d 9 and 10), and the interaction of treatment with day or treatment with hours. The period was considered a random effect. The statistical analyses of results of VFA and ammonia N concentrations day by day, and protein fractions hour by hour, was performed using the compound symmetric covariance structure that yielded the largest Schwarz's Bayesian criterion. Orthogonal contrasts were used to compare 1) treatment means vs. CTR, 2) each day vs. previous day for VFA and ammonia N concentration during the adaptation days, and 3) hour after feeding vs. 0 h (for the protein fractions in d 9 and 10). Differences were declared at $P < 0.05$.

Results

The treatment \times day (during d 1 to 8) or treatment \times hour (during d 9 and 10) interactions were not significant for any of the measurements. Therefore, main effects are discussed.

Effect during the Adaptation Period

Total VFA concentrations decreased ($P < 0.05$) in all treatments from d 1 to 2 (Table 1), and in all treatments except GAR and ORE in d 3, remaining constant thereafter (Table 1). There were no treatment effects on total VFA concentrations. Changes in total VFA concentration with time in continuous culture system in experiments conducted in our laboratory (unpublished observations) indicated that this reduction is normal, probably due to the adaptation of ruminal microorganisms to *in vitro* fermentation conditions. Results suggest that 3 d was sufficient for the adaptation of the ruminal

flora to the fermentation conditions *in vitro*. The molar proportion (mol/100 mol) of acetate (Table 1) was higher ($P < 0.05$) in CIN, GAR, ANI, and ORE on d 2 to 6 compared with CTR. These differences disappeared after 6 d of fermentation. The molar proportion (mol/100 mol) of propionate (Table 1) was lower ($P < 0.05$) in CIN and GAR from d 2 to 4, and in ANI and ORE from d 2 to 5 compared with CTR; however, all of these differences disappeared after 6 d of fermentation. The molar proportion (mol/100 mol) of butyrate (data not shown) was lower ($P < 0.05$) in CIN, GAR, ANI, and ORE (average of 11.4 ± 0.93 , 10.6 ± 0.86 , 11.3 ± 0.75 , and 10.5 ± 0.88 , respectively) from d 2 to 4 compared with CTR (average of 14.8 ± 0.75), but these differences also disappeared after 4 d of fermentation. The acetate:propionate ratio (data not shown) was higher ($P < 0.05$) in CIN (average of 2.4 ± 0.13), GAR (average of 2.2 ± 0.17), ANI (average of 2.4 ± 0.12), and ORE (average of 2.1 ± 0.11) from d 2 to 4 compared with CTR (average of 1.6 ± 0.17). After d 5, the acetate:propionate ratio was similar among treatments. Treatments had no effect on the proportions of isobutyrate, isovalerate, and valerate during the 8 d of adaptation.

Total ammonia N concentrations (mg/100 mL) for all treatments decreased ($P < 0.05$) in the first 2 d of fermentation from an average of 14.5 ± 3.14 in d 1 to 7.5 ± 2.09 in d 2, and remained constant thereafter (average of 7.4 ± 2.11 ; data not shown). The decrease in ammonia N concentration in the first days of fermentation is a normal observation in the dual-flow continuous culture system (unpublished observations) and is attributed to the adaptation of ruminal flora to the fermentation conditions *in vitro*. During the adaptation period, treatments had no effect on ammonia N concentration compared with CTR (data not shown). However, the ammonia N concentration (mg/100 mL) tended to be lower ($P = 0.06$) in YUC (average of 5.5 ± 0.86) from d 4 to 8, and tended to be higher ($P = 0.07$) in ANI (average of 10.4 ± 0.93) from d 3 to 7 compared with CTR (average of 7.3 ± 0.66).

Effects after the Adaptation Period

Total VFA concentration was similar in all treatments (average of 109.4 ± 4.8 mM; Table 2). The proportion of each individual VFA was not affected by treatments except for the valerate proportion, which was lower ($P < 0.05$) in PEP compared with CTR.

The peptide N concentration of CTR decreased ($P < 0.05$) in the first 2 and 4 h after feeding and returned to prefeeding levels thereafter (Table 3). Peptide N concentration in MIX and GAR followed a similar pattern. At time of feeding (0 h), the peptide N concentrations in ORE and PEP were lower ($P < 0.05$) compared with CTR. The peptide N concentrations 2 h after feeding in MIX, CIN, GAR, YUC, and ANI were higher ($P < 0.05$) compared with CTR. These differences disappeared 4 h after feeding. The average peptide N concentration throughout the 8-h feeding interval was higher ($P <$

Table 1. Effect of natural plant extracts on total volatile fatty acids concentrations and acetate and propionate proportions 2 h after feeding from d 1 to 8 of fermentation^a

Item	Treatments ^b								SEM ^c
	CTR	MIX	CIN	GAR	YUC	ANI	ORE	PEP	
Total VFA, mM									
d 1	155.4	155.4	155.4	155.4	155.4	155.4	155.4	155.4	0.02
d 2	134.9 ^y	139.6 ^y	130.6 ^y	132.1 ^y	139.6 ^y	138.2 ^y	138.2 ^y	145.2 ^y	8.67
d 3	119.4 ^y	117.5 ^y	122.8 ^y	129.6	126.7 ^y	117.8 ^y	127.1	121.9 ^y	4.81
d 4	121.3	121.6	118.9	125.2	119.6	122.4	121.6	115.0	3.36
d 5	124.1	117.5	124.4	119.7	112.9	126.2	119.3	121.1	5.62
d 6	118.7	114.7	113.6	110.0	111.1	115.5	110.4	115.1	4.55
d 7	119.9	112.8	109.0	117.4	114.8	115.5	114.3	111.3	5.56
d 8	114.0	116.9	106.2	113.0	112.5	110.7	113.4	107.0	8.70
SEM	6.34	7.01	4.62	7.18	5.85	5.07	5.83	5.82	
Acetate, mol/100 mol									
d 1	63.9	63.9	63.9	63.9	63.9	63.9	63.9	63.9	0.01
d 2	53.1 ^y	52.0 ^y	60.1 ^{yz}	59.3 ^{yz}	53.6 ^y	58.9 ^{yz}	58.6 ^{yz}	55.7 ^y	2.92
d 3	50.7	51.1	59.7 ^z	58.7 ^z	55.6	60.9 ^z	59.5 ^z	55.1	2.91
d 4	51.1	49.1	57.6 ^z	57.9 ^z	53.4	59.3 ^z	56.6 ^z	54.8	2.37
d 5	52.2	50.8	58.6 ^z	58.2 ^z	53.5	59.9 ^z	57.6 ^z	54.8	3.07
d 6	52.3	50.8	57.4 ^z	57.3 ^z	57.0	59.5 ^z	57.3 ^z	55.8	2.81
d 7	54.1	54.4	55.5	54.1	56.9	58.1	58.4	55.0	1.90
d 8	55.5	52.6	56.2	56.1	57.3	56.2	58.7	55.8	2.13
SEM	2.33	2.77	1.62	1.91	1.94	1.33	1.96	1.76	
Propionate, mol/100 mol									
d 1	18.7	18.7	18.7	18.7	18.7	18.7	18.7	18.7	0.01
d 2	31.4 ^y	33.0 ^y	24.1 ^{yz}	25.8 ^{yz}	31.8 ^y	26.5 ^{yz}	27.0 ^{yz}	30.4 ^y	2.53
d 3	33.3	33.9	25.7 ^z	27.0 ^z	30.2	24.4 ^z	26.5 ^z	31.2	2.52
d 4	31.5	34.3	26.7 ^z	26.1 ^z	25.9 ^y	26.3 ^z	26.8 ^z	29.6	2.25
d 5	29.1	32.1	25.3	27.2	23.2	24.3 ^z	24.2 ^z	29.0	3.29
d 6	27.4	30.6	24.8	26.5	23.1	22.9	22.2	26.3	3.20
d 7	24.5	26.2	23.4	26.8	22.5	22.9	21.6	24.5	2.13
d 8	23.5	27.4	22.9	22.9	21.9	22.9	21.2	24.8	2.77
SEM	2.34	3.89	1.94	1.96	2.03	1.57	1.76	1.86	

^aThere were no treatment × day interactions for total VFA concentration ($P < 0.98$), acetate ($P < 0.63$), and propionate ($P < 0.85$) proportions.

^bCTR = Control, MIX = mixture of equal proportions of all extracts, CIN = cinnamon, GAR = garlic, YUC = yucca, ANI = anise, ORE = oregano, and PEP = pepper.

^cStandard error of the mean ($n = 32$).

^yOrthogonal contrast: Within a column, means with the superscript differ from the previous day, $P < 0.05$.

^zOrthogonal contrast: Within a row, means with the superscript differ from Control, $P < 0.05$.

Table 2. Effect of natural plant extracts on volatile fatty acids concentrations 2 h after the morning feeding after 8 d of adaptation

Item	Treatments ^a								SEM ^b
	CTR	MIX	CIN	GAR	YUC	ANI	ORE	PEP	
Total VFA, mM	105.2	110.7	102.3	114.5	109.9	111.7	108.7	112.7	4.77
Individual, mol/100 mol									
Acetate	56.9	55.7	58.1	58.3	59.6	57.2	58.7	55.9	2.16
Propionate	23.8	25.5	22.3	21.6	21.3	23.5	20.3	25.7	2.19
Butyrate	12.1	12.7	12.7	12.4	12.3	12.0	13.8	12.5	1.03
Isobutyrate	0.8	0.7	0.7	0.7	0.7	0.8	0.8	0.7	0.06
Isovalerate	3.0	3.0	2.4	3.9	2.9	3.0	3.4	2.5	0.59
Valerate	3.5	3.1	3.9	3.1	3.1	3.5	3.1	2.8 ^y	0.27
BCVFA ^c	4.0	3.7	3.2	5.2	3.9	4.2	4.5	3.6	2.63
C2:C3 ^d	2.5	2.5	2.8	2.9	2.9	2.5	3.0	2.2	0.32

^aCTR = Control, MIX = mixture of equal proportions of all extracts, CIN = cinnamon, GAR = garlic, YUC = yucca, ANI = anise, ORE = oregano, and PEP = pepper.

^bStandard error of the mean, $n = 64$.

^cBranched-chain VFA; includes isobutyrate and isovalerate.

^dAcetate:propionate ratio.

^yOrthogonal contrast: Means within a row with the superscript differ from Control, $P < 0.05$.

Table 3. Effect of natural plant extracts on hour-by-hour and average nitrogen fractions in a dual flow continuous culture measured after the morning feeding^a

Hours	Treatments ^b								SEM ^c
	CTR	MIX	CIN	GAR	YUC	ANI	ORE	PEP	
Peptide N, mg/100 mL									
0	9.8	10.1	9.2	10.8	9.2	8.0	5.9 ^z	6.1 ^z	1.53
2	3.9 ^y	8.6 ^z	7.9 ^z	7.9 ^{yz}	8.9 ^z	6.9 ^z	6.0	6.7	1.41
4	6.1 ^y	3.4 ^y	7.6	4.9 ^y	7.6	8.5	5.8	5.6	1.29
6	7.8	8.3	7.6	8.5 ^y	7.6	8.1	8.5	7.2	1.73
8	7.2	8.2	8.9	7.9 ^y	7.1	7.6	6.8	6.0	1.14
SEM	1.31	1.40	1.24	1.12	1.22	1.46	1.53	1.37	
Average	6.5	8.5 ^z	8.2 ^z	8.0 ^x	8.2 ^z	7.8	6.6	6.3	1.07
Amino acid N, mg/100 mL									
0	5.7	4.3	4.5	6.7	5.8	6.3	7.1	7.1	2.03
2	10.4 ^y	9.6 ^y	9.8 ^y	10.3 ^y	9.9 ^y	11.5 ^y	10.1 ^y	8.7	1.46
4	7.9	8.3	6.5	10.5 ^{yz}	6.5	8.9	7.8	8.2	1.19
6	6.6	5.8	6.1	7.4	5.2	7.1	5.2	6.3	1.07
8	6.3	5.5	4.9	7.2	5.5	6.3	6.2	7.5	1.45
SEM	1.68	1.23	1.14	1.14	1.07	1.29	1.40	1.36	
Average	7.2	6.7	6.3 ^x	8.4 ^z	6.4	8.3 ^z	7.3	7.6	0.77
Ammonia N, mg/100 mL									
0	7.1	7.9	6.4	5.4 ^z	6.6	9.1 ^z	8.1	6.8	1.05
2	6.7	5.6	5.5	5.2	4.9 ^{yz}	8.4 ^z	7.2	6.3	0.84
4	3.6 ^y	3.3 ^y	4.6	2.3 ^y	4.0 ^y	5.4 ^{yz}	5.5 ^{yz}	3.8 ^y	0.76
6	3.7	3.5 ^y	3.8	3.0	3.8 ^y	6.2 ^{yz}	5.0 ^y	3.5 ^y	0.88
8	6.2	5.7 ^y	5.8	4.8	6.1	7.1 ^y	6.3	5.5 ^y	1.00
SEM	0.63	0.68	0.69	0.85	0.65	0.80	1.12	0.66	
Average	5.5	5.2	5.2	4.1 ^z	5.1	7.2 ^z	6.4	5.2	0.51

^aThere were no treatment × hour interactions for peptide ($P < 0.21$), AA ($P < 0.97$), or ammonia ($P < 0.94$) nitrogen.

^bCTR = Control, MIX = mixture of equal proportions of all extracts, CIN = cinnamon, GAR = garlic, YUC = yucca, ANI = anise, ORE = oregano, and PEP = pepper.

^cStandard error of the mean (n = 96 for treatment; n = 60 for hours; n = 480 for average).

^xOrthogonal contrast: Within a row, means with the superscript tended to differ from Control, $P < 0.10$.

^yOrthogonal contrast: Within a column, means with the superscript differ from 0 h, $P < 0.05$.

^zOrthogonal contrast: Within a row, means with the superscript differ from Control, $P < 0.05$.

0.05) in MIX, CIN, and YUC, and tended to be higher ($P = 0.07$) in GAR compared with CTR (Table 3).

The AA N concentration in CTR increased ($P < 0.05$) from 0 to 2 h after feeding, and returned to prefeeding levels thereafter (Table 3). Similar pattern was observed for all other treatments except for PEP. At 4 h after feeding, the AA N concentration was higher ($P < 0.05$) only in GAR compared with CTR. The average AA N concentration between feedings was higher ($P < 0.05$) in ANI and GAR, and tended to be lower ($P = 0.06$) in CIN compared with CTR (Table 3).

The ammonia N concentration in CTR was lower ($P < 0.05$) at 4 and 6 h after feeding, and returned to prefeeding levels thereafter (Table 3). Similar pattern was observed in all treatments. At time of feeding (0 h), the ammonia N concentration in ANI was higher ($P < 0.05$), and in GAR was lower ($P < 0.05$) compared with CTR. The ammonia N concentration was higher ($P < 0.05$) at 2, 4 and 6 h after feeding in ANI, and at 4 h after feeding in ORE, and lower ($P < 0.05$) at 2 h after feeding in YUC, compared with CTR. The average ammonia N concentration throughout the 8-h feeding interval was higher ($P < 0.05$) in ANI, and lower ($P < 0.05$) in GAR compared with CTR (Table 3).

Discussion

Natural plant extracts have antimicrobial properties that may provide an alternative to ruminal modifiers for their ability to improve energy or protein use in the rumen (Kamel, 2001). However, there is very limited information on the effects of plant extracts on ruminal microbial fermentation. Total VFA concentration was not affected by treatments compared with CTR, suggesting that these additives at the doses used did not modify diet fermentability. It is likely that the use of high doses of plant extracts with antimicrobial activity would decrease microbial activity and diet fermentability. In fact, Evans and Martin (2000) observed that the use of 400 mg/L of thymol (a main component of oregano) decreased total VFA concentration in vitro, but the dose was 1,800 times higher than the dose used in the present experiment (0.22 mg/L). The lack of effect of plant extracts on total VFA indicates that the doses used were not toxic to ruminal microbes. The addition of YUC did not affect total VFA concentration, which agrees with other in vivo (Wu et al., 1994; Hussain and Cheeke, 1995; Hristov et al., 1999) and in vitro studies (Wang et al., 1997). There is no information available

on the effect of the other natural plant extracts on total VFA production in the rumen.

The proportions of individual VFA were not affected by treatments compared with CTR, except for valerate proportion in PEP. The effects of YUC on individual VFA proportions are contradictory. Although the lack of effect observed in the present trial agrees with some *in vivo* (Wu et al., 1994) and *in vitro* (Wang et al., 1997) reports, others (Grobner et al., 1982 and Ryan et al., 1997, *in vitro*; Hristov et al., 1999, *in vivo*) found that YUC modified the acetate and/or propionate proportions. However, it is noteworthy that during the adaptation period, CIN, GAR, ANI, and ORE affected the molar proportions of acetate, propionate, and butyrate between d 2 to 6 of fermentation, although all these difference disappeared after d 6. These results suggest that, although these additives had a short-term effect on ruminal microbial fermentation, ruminal microbes were adapted after 6 d. Evans and Martin (2000) reported that thymol (400 mg/L), a secondary compound of ORE, modified the acetate and propionate molar proportions in mixed ruminal microorganisms in *in vitro* 24-h incubations. In the present trial, the addition of ORE also affected the molar proportions of acetate and propionate after 24 h of fermentation, but differences disappeared after 6 and 5 d of fermentation, respectively. These results indicate that, at the doses used, ruminal microbes built up a tolerance to these additives, and suggest that results from short-term fermentation studies should be interpreted with caution. Although similar changes were observed for CIN, GAR, and ANI, there are no other reports available on the effect of these additives on individual proportion of VFA in the rumen. In the dual-flow continuous culture, an adaptation period of 6 d seemed to be sufficient to test the long-term effects of this type of products on ruminal microbial fermentation.

The concentrations of the different N fractions in the CTR after feeding provide evidence of the dynamics of N use by ruminal microbes. The decrease in peptide N concentration after feeding agrees with *in vivo* results (Hristov et al., 1999), but other *in vivo* studies (Chen et al., 1987; Broderick and Wallace, 1988) indicated that peptide N concentration increases after feeding as a result of protein degradation. The inconsistencies among reports may be attributed to differences in the rate of protein degradation, the rate of peptide use by ruminal microbes, which is dependent on energy availability, and the rate of peptide N passage to the lower tract. The increase in the AA N concentrations during the 2 h after feeding may be attributed to a high rate of peptide degradation or a low rate of AA N use by ruminal microbes. The ammonia N concentration in CTR in the current study decreased in the 4 h after feeding, and returned to prefeeding levels after 8 h. *In vivo*, ruminal ammonia N concentration after feeding may increase (Hristov et al., 1999) or decrease (Devant et al., 2000) depending on the amount of degradable

protein and on the amount and type of dietary carbohydrates available for microbial use (Russell et al., 1983).

There is limited information on the effect of natural plant extracts on peptide metabolism in the rumen. The addition of 7.5 mg/kg DM of YUC extract (containing 8% of sarsaponin) in the present trial increased the average peptide N concentration by 26.2% throughout the 8-h feeding interval. This result suggests that proteolysis was stimulated or peptidolysis was inhibited. In contrast, Hristov et al. (1999) supplemented heifers with 1,961 and 5,825 mg/kg of YUC (containing 4.4% of sarsaponin) and found no effect on peptide N concentration during the 6 h after feeding. The lack of agreement may be attributed to the approach used (*in vivo* vs. *in vitro*), the doses used, or the level of forage in the diet. In the present trial, YUC did not affect the AA N concentration and only decreased the ammonia N concentration 2 h after feeding without affecting the overall average between feeding. Previous studies have found inconsistent effects of YUC on ammonia N concentrations over a wide range of levels of inclusion in the diet. Ryan et al. (1997) reported that the addition of 6,250 mg/kg of YUC decreased the ammonia N concentration from 17.0 to 15.6 mmol/L after 48 h in *in vitro* incubation. Grobner et al. (1982) found a 15% decrease ($P < 0.08$) in ammonia N after 7 d of continuous culture fermentation trial when 60 mg/kg of pure sarsaponin (secondary compound of YUC) were added to the fermenters. Wallace et al. (1994) also found a 6% reduction ($P < 0.05$) in ammonia N in an *in vitro* incubation with strained ruminal fluid containing 10 mg/L of YUC. In contrast, other *in vivo* (Wu et al., 1994, level of inclusion 400 mg/kg of YUC; Hristov et al., 1999, level of inclusion 5,825 mg/kg of YUC) and *in vitro* (Wang et al., 1997; level of inclusion 44,000 mg/kg of YUC) studies found no effects of YUC extract on ammonia N concentration. Our results suggest that YUC extract may affect protein or peptide degradation more than deamination. However, it is relevant that the level of inclusion of YUC used in the present trial (7.5 mg/kg DM of YUC containing 8% of sarsaponin) was lower than the levels of inclusion tested in previous reports, which may explain the lack of effect of YUC on ammonia N concentration in ruminal fluid. The addition of 7.5 mg/kg DM of CIN (containing 59% of cinnamaldehyde) increased peptide N concentration by 102.5% 2 h after feeding, increased the average peptide N concentration by 26.2%, and numerically decreased the average AA N concentration by 12.5% throughout the 8-h feeding interval. These results suggest that CIN extract stimulated proteolysis or inhibited peptidolysis. The addition of 7.5 mg/kg DM of GAR (containing 0.7% of allicin) increased the peptide N concentration by 102.5% 2 h after feeding, and increased numerically the average peptide N concentration by 23%, suggesting that GAR extract stimulated proteolysis or inhibited peptidolysis. In addition, the 33% increase in the AA N concentration 4 h after feeding, the 17% increase in the average AA N, and the 25.5% decrease in the average ammonia

N concentration throughout the 8-h feeding interval suggest that GAR extract inhibited deamination. The addition of 7.5 mg/kg DM of ANI (containing 86% of anethole) increased the peptide N concentration by 79% 2 h after feeding, but did not affect the average peptide N concentration throughout the 8 h feeding interval. The higher average concentration of AA N, the higher ammonia N concentration at 0, 2, 4, and 6 h after feeding, and the higher average ammonia N concentration throughout the 8-h feeding intervals, suggests that ANI extract stimulated peptidolysis and deamination. To our knowledge, this is the first report on the effects of CIN, GAR, and ANI extracts on peptide, AA, or ammonia N degradation in ruminal microbial fermentation. The accumulation of peptides and AA in ruminal fluid may stimulate microbial protein synthesis or improve the flow of AA to the small intestine (Griswold et al., 1996). The effects of ORE, PEP, or MIX on N metabolism were small and inconsistent.

Implications

The cinnamon, garlic, oregano, and anise extracts modified the proportion of acetate, propionate, and butyrate between d 2 to 6 of fermentation in a dual-flow continuous-culture fermenter system, but all these effects disappeared after d 6, probably because of the adaptation of the bacteria to these additives. Results suggest that data from short-term in vitro studies may lead to erroneous conclusions. The accumulation of peptide nitrogen in yucca suggests that proteolysis was stimulated or peptidolysis was inhibited. The accumulation of amino acid and ammonia nitrogen in anise suggests that peptidolysis and deamination were stimulated. The accumulation of amino acid nitrogen and the reduction of ammonia nitrogen in garlic suggest that deamination was inhibited. The accumulation of peptide nitrogen and the numerical decrease in amino acid nitrogen in cinnamon suggest that peptidolysis was inhibited. Careful selection and combination of these additives may allow for the manipulation of protein degradation in the rumen.

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