Effect of *Yucca schidigera* on Ruminal Fermentation and Nutrient Digestion in Heifers[1]

Alexander N. Hristov*, Timothy A. McAllister*,[2], Fred H. Van Herk*, K.-J. Cheng†, C. Jamie Newbold‡, and Peter R. Cheeke§

*Agriculture and Agri-Food Canada, Research Centre, P.O. Box 3000, Lethbridge, AB Canada T1J 4B1; †Department of Animal Science, University of British Columbia, Vancouver, BC Canada V6T 1Z4; ‡Rowett Research Institute, Aberdeen, U.K. AB21 9SB; and §Desert King International, Chula Vista, CA 91911

ABSTRACT: In a replicated 3 × 3 Latin square experiment, six heifers (443 ± 6.1 kg) fed a 61% barley grain:39% alfalfa silage diet (DM basis) were given intraruminal doses of powdered *Yucca schidigera* (YS). Doses of 0 (control), 20, or 60 g/d were given at 0800 daily. Ruminal content was sampled 0, 2, 4, and 6 h after dosing. Acidity, concentrations of reducing sugars, free amino acids, and peptides in the rumen were not affected ($P > .05$) by YS. Relative to control, ruminal ammonia concentration was reduced ($P < .05$) 2 h after YS dosing. Ruminal propionate concentration was increased ($P < .05$) by YS. Protozoal numbers in the rumen were lower ($P < .05$) with YS than without. Yucca did not affect ($P > .05$) rate or extent of in situ DM degradability. Fibrolytic, amylolytic, and proteolytic activities in ruminal contents were similar among treatments ($P > .05$). Dry matter intake, apparent digestibilities of DM, NDF, and CP, nitrogen balance, and microbial protein synthesis in the rumen were not affected ($P > .05$) by treatment. The effect of YS on ruminal ammonia concentration likely resulted from a decreased concentration of protozoa and, presumably, from ammonia binding by YS. The effect on ruminal propionate was probably a result of a selective inhibitory effect of YS on rumen microbial species.

Key Words: *Yucca schidigera*, Rumen Fermentation, Digestibility, Cattle

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Introduction

Steroidal saponins are present in a wide variety of plants, including the desert plant, *Yucca schidigera* (**YS**). Some of the physical and chemical properties of these compounds (e.g., surface active properties and ammonia binding capacity) have sparked research into their use in livestock production applications. Average daily gain by finishing steers fed high-grain diets was improved when they were supplemented with yucca saponins (Goodall et al., 1981, 1982). Grobner et al. (1982) observed reduced ammonia and increased propionate concentrations in continuous flow fermentors treated with yucca saponins. In subsequent studies, an extract of yucca decreased ruminal ammonia concentration (Hussain and Cheeke, 1995), increased propionate concentration (Kil et al., 1994) and in vivo OM digestibility (Valdez et al., 1986), and improved (Kil et al., 1994) or did not affect (Goetsch and Owens, 1985; Wu et al., 1994) animal performance.

Yucca saponins also have strong antiprotozoal activity and may serve as an effective defaunating agent for ruminants (Wallace et al., 1994). If digestibility of dietary fiber is not adversely affected, reducing protozoal populations in cattle could improve nitrogen utilization in the rumen and increase microbial protein flow to the intestine (Williams and Coleman, 1991), thereby enhancing overall growth performance. The purpose of the present experiment was to determine the effect of *Y. schidigera* on fermentation, protozoal populations, and enzyme activities in the rumen, as well as on total tract digestibility of a barley grain-based diet for cattle.

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[2]To whom correspondence should be addressed (phone: 403/317-2240; fax: 403/382-3156; E-mail: mcallister@em.agr.ca).

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Table 1. Ingredients and chemical composition of diet fed

Item	Percentage
Ingredients, %, DM basis	
Rolled barley grain	61.0
Alfalfa silage[a]	38.5
Mineralized salt[b]	.5
Chemical composition of the diet, mean ± SE	
DM, %	56.9 ± .47
NDF, % of DM	38.6 ± .36
CP, % of DM	15.2 ± .49

[a]Dry matter content of alfalfa silage was 37.8%.
[b]Containing: 92.6% NaCl, 1.1% $ZnSO_4$, .94% $MnSO_4$, .32% $CuSO_4$, .04% canola oil (as carrier of $CoSO_4$), .005% $CoSO_4$, 5.0% Dynamate (Pitman-Moore Inc., Oakville, ON), .0044% Na_2SeO_3, and .0013% ethylenediamine dihydroiodide (as an 80% preparation). Dynamate contains 22% S; 18% K; 11% Mg; .1% Fe; .0005% Pb (maximum).

Materials and Methods

Animals and Feeding

Animals involved in this experiment were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993). Six ruminally cannulated Angus heifers (443 ± 6.1 kg) were fed an alfalfa silage:barley grain-based diet (Table 1), offered as a total mixed ration (**TMR**), to 5% orts. Feed was provided in two meals, at 0800 and 1500. Heifers were randomly allotted to treatment sequences in two 3 × 3 Latin squares. They were weighed at the beginning of the experiment and at the end of each 28-d experimental period (14 d of adaptation to treatment and 14 d for sample collection). Treatments consisted of daily intraruminal doses of 0 (control), 20, or 60 g of a powdered (dried and pulverized whole plant) preparation of *Y. schidigera* (Desert King International, Chula Vista, CA). The yucca doses were given at 0800 daily, and treatments were designated **YS20** (20 g/d) and **YS60** (60 g/d). The YS contained 4.4% (wt/wt) saponins (Wang et al., 1998).

Sampling and Analyses

Feed Samples. Feed samples were collected weekly and combined for each period. Dry matter was determined by oven-drying at 65°C to constant weight. Samples were ground through a 1-mm screen and analyzed for NDF (Procedure A without sodium sulfite, Van Soest et al., 1991) and crude protein (N × 6.25). Nitrogen in all samples was analyzed on a Nitrogen analyzer 1500 (Carlo Erba Instruments, Milan, Italy). Prior to N analysis, samples were pulverized in a Pulverisette 7 Planetary micro-mill (Fritsch GmbH Laborgeratebau, Idar-Oberstein, Germany).

Ruminal Content Samples. On d 15, 16, and 17 of each period, samples of whole ruminal contents were collected 0, 2, 4, and 6 h after YS dosing. Approximately 200 g from the bottom of the ventral sac and 200 g from the reticulum were combined and squeezed through two layers of cheesecloth. After recording pH of the filtrate, subsamples of filtrate were prepared for VFA analysis as described by McAllister et al. (1992). For analysis of ammonia, total free amino acids (**TFAA**), peptides, and reducing sugars (**RS**), 30-mL subsamples of filtrate were added to 2.25 mL of 65% (wt/vol) trichloroacetic acid (**TCA**). The mixtures were incubated on ice for 30 min and then centrifuged (28,000 × *g*, 15 min, 4°C), and supernatants were stored at –40°C until analyzed. After collection of the 0- and 4-h samples only, additional subsamples of filtrate were frozen immediately for analysis of enzyme activities. Filtrate was also preserved for enumeration of protozoa by combining 2.5 mL of filtrate with 5 mL of methyl green:formalin: saline (**MFS**) solution (Ogimoto and Imai, 1981). Protozoa were counted using a Fuchs-Rosenthal counting chamber (Hausser Scientific Partnership, Horsham, PA). Triplicate subsamples of each filtrate:MFS preparation were counted (five randomly selected large squares per subsample).

The TCA-precipitated supernatants were analyzed for ammonia and TFAA as described by Broderick and Kang (1980). For peptide analysis, 2-mL subsamples were mixed with 10 mL of 7.2 N HCl in 30-mL Pyrex screw-capped test tubes. The tubes were flushed with N_2, sealed with Teflon tape, and incubated for 24 h at 110°C. Hydrolyzed samples were filtered through Whatman No. 1 filter paper, and 1.5 mL were evaporated to dryness in a rotary evaporator (SpeedVac SC100, Savant Instruments Inc., Farmingdale, NY). Residues were redissolved in 1.5 mL of deionized distilled water and analyzed for TFAA and ammonia. The amount of peptide-bound amino acids (expressed as mmol/L of L-leucine) was calculated as the difference in TFAA concentration before and after HCl-hydrolysis, after correction for the ammonia present in the HCl-hydrolysate.

Reducing sugars in the TCA-precipitated supernatants were determined semiautomatically with the ferricyanide method (Industrial method No. 389-76P, Technicon Instruments Corp., Tarrytown, NY) against a glucose standard. The ferricyanide reagent contained .6 g/L of $KFe(CN)_6$ and 38 g/L of Na_2CO_3. Washing solution was 5% (vol/vol) acetic acid with 1% Brij 35 (Sigma Chemical Co., St. Louis, MO), and sample and washing times were set at 25 and 50 s, respectively.

To determine ruminal enzyme activities, filtered ruminal content was thawed and sonicated, and centrifuged (28,000 × *g*) as described by Hristov et al. (1998a). The cell-free ruminal fluid supernatant was analyzed for deaminative, proteolytic, and polysaccharide-degrading enzyme activities.

Deaminative (amino acid oxidase) activity was measured by adding 2 mL of cell-free ruminal fluid to

4 mL of a .5% (wt/vol) solution of Amicase (an acid digest of casein, A-2427, Sigma Chemical) in .2 *M* sodium phosphate buffer (pH 6.0) and incubating for 2 h at 39°C. Proteins were precipitated by adding .75 mL of 65% (wt/vol) TCA and storing for 30 min on ice. After centrifuging at 10,080 × *g* for 15 min, supernatant was analyzed for ammonia (Broderick and Kang, 1980). Deaminative activity was expressed as nanomoles of ammonia released from Amicase per minute per milliliter of cell-free ruminal fluid, corrected against background ammonia release determined in samples treated with TCA prior to the 2-h incubation.

Proteolytic activity against casein (technical grade, C-7078, Sigma Chemical) was assessed by incubating 2 mL of cell-free ruminal fluid with 3.8 mL of .2 *M* sodium phosphate buffer (pH 6.0) and .2 mL of casein solution (10% [wt/vol] in 1 *N* NaOH) for 1 h at 39°C. The pH of the incubation mixture was 7.5. After incubation, .75 mL of 65% TCA (wt/vol) were added, and the tubes were held on ice for 30 min. The mixtures were centrifuged (10,080 × *g*, 15 min, 4°C) and analyzed for TCA-soluble N in the supernatant fraction (Nitrogen analyzer 1500, Carlo Erba Instruments). Proteolytic activity was expressed as micrograms of TCA-soluble N released from casein per minute per milliliter of cell-free ruminal fluid, corrected for TCA-soluble N in samples treated with TCA prior to incubation.

Carboxymethylcellulase (**CMCase**), xylanase, and amylase activities in cell-free ruminal fluid were determined as described by Hristov et al. (1998a). Activities are expressed as nanomoles RS released per minute per milliliter of cell-free ruminal fluid.

Ruminal Outflow Rates. Fractional outflow rates of ruminal fluid and solids were determined using Co and Yb markers, respectively. On d 21 and 24 of each period, each heifer was dosed intraruminally, at the time of the morning feeding, with 2.5 g of Co (as Co/Li-EDTA) dissolved in 500 mL of tap water (Udén et al., 1980). For measuring passage of solids, 18 g of Yb (as $YbCl_3 \cdot 6H_2O$) were dissolved in 1 L of water and sprayed onto 5 kg of TMR. The labeled feed was mixed, divided into six 1-kg portions, and introduced directly into the rumen of each heifer through the cannula. Samples of ruminal contents were collected for Co and Yb analyses at 0, 1.5, 3, 6, 9, and 12 h after dosing. Samples were filtered through polyester fabric with 105-μm pore size, and the liquid and solid fractions were frozen separately. Liquid samples were thawed and centrifuged (11,220 × *g*), and Co was determined in the supernatant. Solid samples were freeze-dried, ground through a 1-mm screen, and analyzed for Yb. Elemental analyses were done by atomic absorption (Perkin Elmer Corporation, 1976). Ruminal liquid and solid fractional outflow rates were computed by plotting ln [Co] and ln [Yb], respectively, against time after dosing.

All ruminal fluid analyses were conducted in duplicate, and values were averaged across the three days for each animal and sampling time.

In situ Dry Matter Digestibility. Ruminal disappearance of DM from alfalfa hay was determined using the nylon bag technique (Ørskov and McDonald, 1979). Alfalfa hay ground through a 4-mm screen was sieved, and particles retained between the 1,180- and 600-μm screens were used for in sacco incubation. On d 18, 5-g samples of alfalfa hay were incubated in polyester bags in the rumen of each heifer for 0, 3, 18, 24, and 48 h (in triplicates for each animal and time). Bag design and washing procedure were as described by Stanford et al. (1996). Parameters of ruminal disappearance were computed according to the nonlinear model:

$$P = a + b \times (1 - \exp^{-ct}) \quad (1)$$

where *P* (%) denotes proportion of the material degraded (lost from the bag) at time *t* (h); *a* (%) is the soluble, readily degradable fraction; *b* (%) is the insoluble, potentially degradable fraction; *c* (/h) is the degradation constant for fraction *b* (Ørskov and McDonald, 1979). Effective degradability of DM (*ED*) was calculated using the equation:

$$ED = a + [bc/(c + k)] \quad (2)$$

where *a*, *b*, and *c* are constants from the nonlinear model described above, and *k* is the measured particle passage rate for the treatment group: .070, .061, and .081/h, for control, YS20 and YS60, respectively (Table 2).

Plasma Glucose and Urea N. On d 18 and 19 of each period, blood samples were collected from the jugular vein 4 h after the morning feeding into heparinized tubes. Plasma was collected by centrifugation (1,500 × *g*, 40 min) and stored at –40°C until analyzed. Glucose (as RS) was determined as described above. Plasma urea N was determined after enzymatic conversion of urea to ammonia using Type III urease from jack beans (U-1500, Sigma Chemical). Plasma samples (.25 mL) were incubated with 1.5 mL of urease solution (4.03 U/mL in distilled water) for 10 min at 21°C. Reactions were stopped by adding .15 mL of 65% (wt/vol) TCA and incubating on ice for 30 min. Incubation mixtures were centrifuged (21,000 × *g*, 10 min), and ammonia in the supernatant was determined (Broderick and Kang, 1980).

Total Tract Nutrient Digestibility. Fecal and urine collections were conducted during the last 7 d of each experimental period. Once weighed, feces were subsampled (5%), dried at 65°C, ground through a 1-mm screen, and analyzed as described for feed samples. Additional subsamples of fresh fecal material were transferred to storage at –40°C, composited daily. After thawing, samples were thoroughly mixed, and 10 g of composited fecal sample were added to 20 mL of .5 *M* H_2SO_4, mixed by rotation (120 rpm) for 30 min, then centrifuged (17,500 × *g*, 15 min). Protein in the supernatant was precipitated by adding .125 mL/

mL 65% (wt/vol) TCA and incubating on ice for 30 min. After centrifuging again, ammonia concentration was determined in the supernatant (Broderick and Kang, 1980).

Urine was collected through Foley catheters (Bard Canada Inc., Mississauga, ON) into 500 mL of 1 *M* H_2SO_4. After volume was recorded, subsamples were diluted 1:5 with distilled water for analyses of total nitrogen and uric acid. Additional subsamples were diluted 1:50 for allantoin analysis. All urine samples were stored at –40°C until analyzed. Uric acid was analyzed enzymatically using a commercially availa-

Table 2. Effect of powdered *Yucca schidigera* on ruminal fermentation parameters, protozoal counts, and rate of passage of ruminal solids and liquid in heifers

	Treatment[a]				Pr > F[b]	
Item	Control	YS20	YS60	PSE[c]	Treatment	Time
pH	6.28	6.18	6.19	.02	NS	***
0 h	6.37	6.28	6.28	.03		
2 h	6.27	6.17	6.21	.03		
4 h	6.26	6.15	6.14	.05		
6 h	6.23	6.12	6.13	.04		
Reducing sugars, m*M*	.248	.243	.220	.01	NS	***
0 h	.215	.208	.183	.01		
2 h	.331	.342	.278	.02		
4 h	.218	.209	.220	.02		
6 h	.230	.215	.199	.02		
Ammonia, m*M*	6.29	5.59	4.95	.42	NS	***
0 h	6.78	6.72	6.02	.45		
2 h[d]	10.71[y]	9.75[yz]	7.12[z]	.56		
4 h	4.72	3.40	3.77	.42		
6 h	2.94	2.47	2.88	.37		
TFAA, mmol/L[e]	.832	.795	.840	.04	NS	**
0 h	.984	.889	.958	.04		
2 h	.814	.832	.735	.05		
4 h	.682	.656	.814	.04		
6 h	.850	.803	.851	.05		
Peptides, m*M*	2.88	3.07	2.95	.09	NS	**
0 h	2.85	3.07	2.87	.19		
2 h	2.30	3.02	2.82	.18		
4 h	3.21	3.13	3.04	.19		
6 h	3.18	3.07	3.07	.17		
Ruminal VFA, m*M*						
Total	80.7	83.1	83.8	1.63	NS	***
0 h	77.8	82.1	81.3	2.97		
2 h	86.2	86.3	86.9	3.31		
4 h	82.5	84.2	84.7	3.41		
6 h	76.2	79.6	82.2	3.33		
Acetate	49.7	50.0	50.1	.96	NS	***
Propionate	16.5[y]	19.3[z]	19.5[z]	.54	*	NS
iso-Butyrate[f]	1.00[y]	.95[z]	.95[z]	.01	*	***
Butyrate	11.0[y]	10.2[z]	10.3[z]	.19	*	***
iso-Valerate	1.24	1.22	1.13	.02	NS	***
Valerate	1.38	1.42	1.34	.03	NS	***
Acetate:Propionate[f]	3.13[y]	2.85[z]	2.77[z]	.05	*	NS
Protozoa, millions/cm^3	.69[y]	.40[z]	.55[yz]	.030	*	*
0 h	.74	.43	.57	.043		
4 h	.64	.37	.53	.041		
Fractional rate of passage, /h						
Liquid	.18	.16	.17	.001	NS	—
Solid	.070	.061	.081	.006	NS	—

[a]Treatments were intraruminal doses of dried and pulverized whole *Yucca schidigera* plant (YS), given once daily. Control, YS20, and YS60 provided 0, 20, and 60 g/d YS, respectively.
[b]*,**, ***: ($P < .05$), ($P < .01$), and ($P < .001$), respectively; NS: nonsignificant ($P > .05$).
[c]Pooled standard error of the mean.
[d]Linear response to YS at 2 h after treatment ($P < .05$).
[e]Total free amino acids.
[f]Linear response to YS treatment ($P < .05$).
[y,z]Within a row, means lacking a common superscript differ ($P < .05$).

ble diagnostic kit (685-20, Sigma Chemical). Allantoin concentration was determined using a semiautomated method (Lindberg and Jansson, 1989) modified as follows: Tween 20 (1 mL/L) was omitted from all reagents except the sampler washing solution, cooling bath was set at 4°C, and chromophore was developed at 25°C. Excretion of microbial purine derivatives (MPD) in the urine was estimated using the equation of Verbic et al. (1990):

$$MPD = [PD - (.385 \times BW^{.75})]/.85 \quad (3)$$

where PD (mmol/d) is excretion of the purine derivatives, allantoin and uric acid; BW (kg) is body weight; .85 is the recovery of MPD; and .385 (mmol/d) is the contribution of endogenous PD. Rumen microbial protein synthesis (MPSR, g N/d) was calculated according to Chen et al. (1992):

$$MPSR = (MPD \times 70)/(.83 \times .116 \times 1000) \quad (4)$$

where 70 is the N content of purines, in mg/mmol; .83 is the digestibility of microbial purines; and .116 is the ratio of purine N to total N in rumen bacteria.

Statistical Analyses

Data were analyzed with repeated measures analysis. Sphericity test was used to test the assumption for the correlation matrices. Because the sphericity test was not significant, data were analyzed as a split-plot in time using GLM procedure. Treatment × time interactions were not significant. When the effect of diet was significant, orthogonal contrasts were used to test for linearity of the response to YS dose, and the least significance difference test was used to separate treatment means (SAS, 1989).

Results

Acidity of ruminal fluid increased after feeding ($P < .001$) in all treatments (Table 2). From 0 to 6 h after feeding, ruminal pH was numerically lower ($P > .05$) in heifers receiving yucca than in the control animals. Reducing sugars in ruminal fluid increased ($P < .001$) 2 h after feeding and declined thereafter, but YS did not affect ($P > .05$) average RS concentrations.

Ruminal ammonia concentrations fluctuated with time after feeding ($P < .001$), but average concentrations were not affected by YS ($P = .397$). The increase in ruminal ammonia concentration in the first 2 h after feeding was larger ($P < .05$, linear response) in heifers receiving no YS (increased by 3.9 m*M*) than in those receiving 20 g/d (3.0 m*M* increase in NH_3) or 60 g/d (1.1 m*M* increase). Heifers receiving 60 g/d of YS had lower ruminal ammonia concentrations 2 h after feeding than did those receiving no YS ($P < .05$). After 4 h, ammonia concentrations were still numerically lower in YS20 and YS60 heifers (by 1.3 and .9 m*M*, respectively) than in the control animals ($P > .05$).

Yucca did not affect ($P > .05$) TFAA or peptide concentrations in ruminal fluid. Total concentrations of VFA increased ($P < .001$) after feeding but were not affected by treatment ($P > .05$). Ruminal dosing with YS significantly altered the patterns of ruminal fermentation. Concentration of acetate was not changed by YS ($P > .05$), but average ruminal propionate concentrations were 2.8 and 3.0 m*M* higher ($P < .05$) in heifers receiving 20 and 60 g/d of YS, respectively, than in the controls. Concentrations of *iso*-butyrate and butyrate were decreased ($P < .05$, linear response for *iso*-butyrate) with YS, compared to control diet. The ratio of acetate to propionate was reduced ($P < .05$, linear response) by YS.

The concentration of protozoa in the rumen was reduced ($P < .05$) by YS treatment. On average, protozoa were 42% less numerous in ruminal fluid from heifers receiving 20 g/d of YS, than in fluid from control heifers. The higher dose of YS did not further decrease the protozoal population in the rumen. Outflow rates of solid and fluid phases of ruminal contents were not affected by YS treatment ($P > .05$).

Ruminal deaminative and proteolytic activities were not affected ($P > .05$) by YS (Table 3). Deaminative activity was numerically lower ($P = .435$), however, with both YS treatments than with control (by 19 and 12%, respectively, with YS20 and YS60). Ruminal CMCase, xylanase, and amylase activities were lower ($P < .01$, $P < .001$, and $P < .001$, respectively) 4 h after feeding than immediately prior to feeding. Yucca did not affect ($P > .05$) these polysaccharide-degrading activities in cell-free ruminal fluid, which contains about 35% of the activities present in whole ruminal content (Hristov et al., 1998b). Average amylase activities in the YS20 and YS60 groups were numerically higher ($P > .05$) than in the control group, by 29 and 20%, respectively.

The extent of disappearance of alfalfa hay DM from the rumen (Figure 1) was not affected by treatment ($P > .05$). In heifers given 0, 20, and 60 g/d of YS, respectively, average parameters of ruminal disappearance of alfalfa hay DM (followed by pooled standard error and effect of YS) were 15.4, 12.9, and 12.8 (.90, NS) for (a); 55.1, 55.7, and 55.6 (.95, NS) for (b); .074, .089, and .094 (.004, $P < .05$, linear effect) for (c); and 43.6, 45.2, and 42.4 (.82, NS) for *ED*. The soluble DM fraction (a) was numerically lower, and the rate of degradation (c) of insoluble, potentially degradable fraction b was higher ($P < .05$) with YS than without. Effective degradability of alfalfa hay DM was unaffected by treatment.

Yucca did not affect DM intake by the heifers ($P > .05$; Table 4). Apparent digestibilities of DM, NDF, and CP also were similar among treatments. Ammonia concentrations in fecal DM were unaffected by YS ($P > .05$). Approximately 50% of consumed N was excreted in the urine. No significant differences in N

Table 3. Enzyme activity in cell-free ruminal fluid from heifers receiving *Yucca schidigera*

	Treatment[a]				Pr > F[b]	
Activity	Control	YS20	YS60	PSE[c]	Treatment	Time
Carboxymethylcellulase[d]	47.0	49.9	48.8	2.09	NS	**
0 h	50.3	53.2	50.1	1.97		
4 h	43.6	46.7	47.5	1.68		
Xylanase[d]	167.5	168.3	166.7	6.87	NS	***
0 h	181.6	187.2	176.4	24.7		
4 h	153.4	149.4	157.1	20.9		
Amylase[d]	56.3	72.7	67.9	3.32	NS	***
0 h	62.1	84.3	75.0	3.52		
4 h	50.1	61.1	60.7	2.78		
Deaminative activity[e]	9.33	7.54	8.26	.58	NS	NS
0 h	8.80	8.25	8.50	.53		
4 h	9.86	6.84	8.01	.72		
Proteolytic activity[f]	4.93	4.76	5.46	.40	NS	NS
0 h	5.03	4.79	4.29	.26		
4 h	4.79	4.81	6.64	.69		

[a]Treatments were intraruminal doses of dried and pulverized whole *Yucca schidigera* plant extract (YS), given once daily. Control, YS20, and YS60 provided 0, 20, and 60 g/d YS, respectively.
[b]**, ***: ($P < .01$) and ($P < .001$), respectively; NS: nonsignificant ($P > .05$).
[c]Pooled standard error of the mean.
[d]Expressed as release of reducing sugars, nmol/(mL·min).
[e]Expressed as release of ammonia, nmol/(mL·min).
[f]Expressed as release of TCA-soluble N from casein, μg/(mL·min).

retention among treatments were observed ($P > .05$), although the amount of N excreted in the urine was numerically higher ($P = .219$) with 60 g/d of YS than without YS, compared to control, and N retention was 13% lower ($P = .206$). The extent of excretion of allantoin and uric acid in urine, and the computed MPSR, were similar among treatments ($P > .05$), as were blood glucose or urea levels ($P > .05$).

Discussion

Decreased ruminal pH associated with YS supplementation has been reported previously (Grobner et al., 1982; Goetsch and Owens, 1985). Wu et al. (1994) observed a quadratic decline in ruminal pH when 0 to 8 g/d of YS were administered to dairy cows and observed the largest effect at 6 g of YS/d. In the present experiment, ruminal pH was slightly decreased in heifers receiving YS, as compared to control animals, but the difference was not significant ($P > .05$).

Reductions in ruminal ammonia concentrations associated with YS have been observed in vitro (Wallace et al., 1994) and in vivo (Hussain and Cheeke, 1995). The glycofractions in YS are known to bind ammonia (Headon et al., 1991). Grobner et al. (1982) found a 15% reduction in ammonia concentration in vitro when saponins were included at 60 ppm in the incubation medium. In vivo, responses have been varied, apparently related to dosing levels and time of sampling. In studies with dairy cows, treatments of up to 77 ppm (dietary DM basis) of saponins (Goetsch and Owens, 1985; Valdez et al., 1986) or 8 g/d YS (Wu et al., 1994) did not significantly affect ruminal ammonia concentrations. Lu and Jorgensen (1987) treated sheep with high levels of alfalfa saponins (2 and 4% of dietary DM; i.e., 20,000 and 40,000 ppm), which were primarily of the tripterpenoid type, and recorded highly significant negative effects on rumen ammonia concentrations. Hussain and Cheeke (1995) included YS at 78 ppm (DM basis) in diets for steers, and observed a numerical depression in ruminal ammonia concentration, with a significant effect of time of sampling. Depending on time after feeding, concentration of ammonia in the rumen can vary greatly (Hristov and Broderick, 1996). In the present experiment, the range for ruminal ammonia concentration was large (minimum = .12 and maximum = 18.2 mmol/L). Nonetheless, at the relatively higher levels of YS administered in this study (YS20 and YS60 yielded 1,961 and 5,825 ppm dietary DM, respectively), ruminal ammonia concentrations were reduced ($P < .05$), compared with control, 2 h after YS dosing.

As pointed out by Wallace et al. (1994), the ammonia-binding potential of YS is negligible in solutions containing ammonia at concentrations typical of the rumen; thus, it cannot fully explain the decrease in rumen ammonia concentration observed in the present experiment. Reduced ammonia concentrations in the rumen are typical when protozoa are inhibited (Williams and Coleman, 1991), presumably as a result of depressed bacterial lysis. Inhibition of

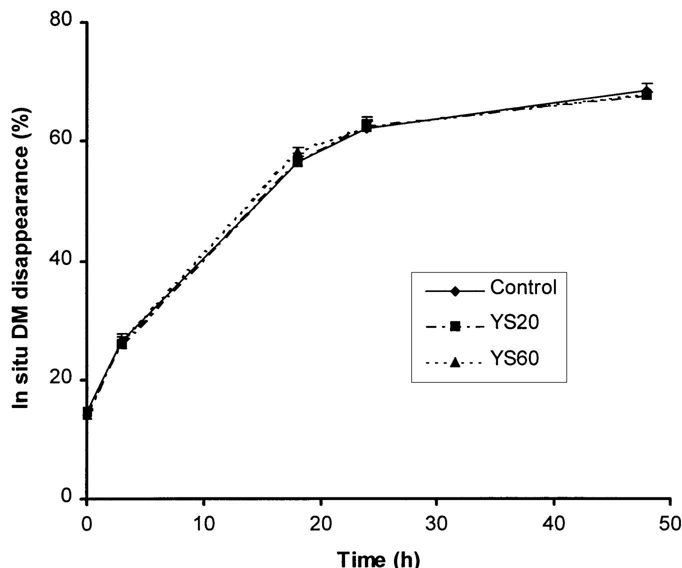

Figure 1. Effect of a powdered preparation of *Yucca schidigera* plant (YS) on in situ disappearance of dry matter from alfalfa hay incubated in heifers fed a barley grain:alfalfa silage diet. Treatments were once daily intraruminal doses of YS. Control, YS20, and YS60 provided 0, 20, and 60 g/d YS, respectively. Data are means ± SE. Where not visible, error bars fall within symbols. Ruminal DM disappearance parameters (*a*), (*b*), (*c*), and *ED* (from Ørskov and McDonald, 1979) are given in the text.

bacterial lysis is probably only partially responsible for the decreased ruminal ammonia concentrations observed in defaunated animals, however, given that ruminal degradation of feed protein is also lower (Ushida et al., 1986). The effect of YS on ruminal ammonia in this study likely results from decreased bacterial lysis (as a consequence of inhibited protozoal growth) and, to a lesser extent, from inhibited deaminative activity and direct binding of ammonia in the rumen (supported by the significantly lower concentration of ammonia 2 h after YS treatment).

Alfalfa silage accounted for 38.5% of the heifers' dietary DM, which equated to intake of approximately 3.95 kg of alfalfa DM per day. Alfalfa is a rich source of triterpenoid saponins; its saponin content has been reported as .12% (USDA, 1957), .24% (Kalac et al., 1996), or higher (Bickoff et al., 1972). Had it been fed as fresh forage, alfalfa would have significantly increased the background level of saponins in the diet. However, depending on forage variety and ensiling technique, 92 to 100% of the major saponins in alfalfa are destroyed during ensiling (Kalac et al., 1996).

Other major effects of YS on ruminal fermentation were the increased concentration of propionate and decreased acetate-to-propionate ratio. Reported effects of saponins on ruminal propionate have varied with diets and application levels. Grobner et al. (1982) reported a significant increase (by 3.8 mmol/d, $P < .05$) in propionate production in vitro with 60 ppm of saponins. Valdez et al. (1986) found no significant change in propionate production in vivo with 77 ppm of sarsaponin. Kil et al. (1994) found a significant increase ($P < .05$) in propionate concentration in vitro when supplying 125 ppm of YS with a mixed forage-concentrate diet, but reported a marginal effect with a concentrate diet. In an in vivo study, Hussain and Cheeke (1995) found numerically decreased ruminal propionate concentrations on both high-forage and high-concentrate diets when YS (as Deodorase, Alltech Biotechnology, Nicholasville, KY) was included at 75 ppm (as-fed basis). Wu et al. (1994) reported a nonsignificant increase in ruminal propionate with up to 8 g/d (396 ppm) of YS. In the present experiment, the effect of YS on propionate concentration in the rumen was persistent over the course of sampling and was evident even before the daily dose of YS was introduced into the rumen; 0-h propionate concentrations were 15.5, 19.1, and 18.8 m*M* for control, YS20, and YS60, respectively.

Increased molar proportions of propionic acid in the rumen are often found in studies with defaunated sheep (Williams and Coleman, 1991). As the single most important rumen bacterium involved in decarboxylation of succinate, *Selenomonas ruminantium* is apparently responsible for most of the propionate production in the rumen arising from the randomizing pathway (Wolin and Miller, 1988). Wallace et al. (1994) determined that growth of *S. ruminantium* was not affected by yucca saponins, whereas growth of some other rumen bacterial species (*Streptococcus bovis* and *Butyrivibrio fibrisolvens*) was strongly inhibited. It is possible that, by inhibiting bacteria and protozoa not involved in propionate production in the rumen, the high levels of YS used in the present experiment promoted species such as *S. ruminantium* to fill the niche, thereby increasing the accumulation of propionic acid in the rumen.

By supplementing an incubation mixture with 1% YS (10,000 ppm), Wallace et al. (1994) almost completely inhibited protozoal activity in vitro. In the same experiment, .1% YS (1,000 ppm) was far less detrimental to ruminal protozoa. *Yucca schidigera* was administered to the heifers at 1,961 and 5,825 ppm in the present experiment, and average protozoal numbers in cheesecloth-strained ruminal fluid were significantly reduced ($P < .05$). This effect was apparent prior to administering the daily dose of YS (i.e., in the 0-h samples), which indicates persistence of the YS-mediated decrease in ruminal protozoal concentration.

In the present experiment, YS was introduced into the rumen on a daily basis for 14 d prior to commencement of sampling. Newbold et al. (1997) found strong antiprotozoal properties associated with the saponin component of an African multipurpose tree (*Sesbania sesban*) fed to sheep. After 10 d,

however, the protozoal population in the rumen recovered completely. Because the sensitivity of protozoa to *S. sesban* was determined not to have changed, the authors concluded that rumen bacteria may have adapted to degrade saponins and eliminate their antiprotozoal activity. If adaptation of the ruminal microflora to saponins had occurred in the present experiment, it would be expected to have begun during the 14 d prior to sampling and would be expected to persist, given the continued exposure to YS through daily dosing. Reduced protozoal populations with YS20 and YS60 indicate, however, that such adaptation was either not occurring in this study or was occurring to a lesser extent.

A decreased concentration of rumen protozoa could increase the flow of microbial protein to the intestine, benefiting the ruminant by increasing the amount of amino acids available for absorption (Williams and Coleman, 1991). With the technique used in the present experiment (urinary excretion of purine derivatives), however, we found no significant effect of YS on microbial protein flow to the intestine. As demonstrated by Lu and Jorgensen (1987), alfalfa saponins (at dietary concentrations of 20,000 to 40,000 ppm) could be detrimental to ruminal microorganisms, significantly decreasing microbial protein synthesis in the rumen.

The comparatively high fractional rates of passage for the solid phase of the ruminal contents (average of .071/h for the three diets) probably reflect an inadequacy in the Yb-labeling procedure used in this experiment. Migration of marker can be significantly reduced if feed-soluble DM is extracted before labeling and the labeled material is soaked in acidic solution to remove loosely bound Yb (Hristov and Broderick, 1996).

The extent of ruminal degradation of dietary DM was not affected by treatment, although the rate of degradation of insoluble DM was increased ($P < .05$) with YS. Goetsch and Owens (1985) measured an 8% increase ($P < .05$) in apparent digestibility of OM in cattle fed 44 ppm of yucca saponins, but they also observed trends toward depressed ruminal degradability of ADF (by 13%, $P > .05$) and efficiency of microbial synthesis in the rumen (by 36%, $P > .05$). In a subsequent experiment, Valdez et al. (1986) recorded increased apparent digestibility of OM ($P < .05$), together with a tendency toward increased digestibility of ADF ($P > .05$) with increasing application levels of YS (up to 77 ppm). Similarly, Lu and Jorgensen (1987) recorded significant increases in hemicellulose and cellulose digestion associated with inclusion of alfalfa saponins in a concentrate-based diet for sheep. In the latter experiment, degradability of cellulose in the rumen was depressed by added saponins, but degradability of fiber fractions in the hindgut was greatly increased. Fiber degradability in the rumen is usually observed to decrease in defaunated animals (Williams and Coleman, 1991). Because YS is reasonably soluble, it is possible that a portion of the ruminally administered saponins could pass out of the rumen with the liquid phase of ruminal

Table 4. Effect of *Yucca schidigera* (YS) on feed intake and digestibility in heifers

Item	Treatment[a]			PSE[b]	Treatment Pr > F[c]
	Control	YS20	YS60		
Dry matter intake, kg/d	10.3	10.2	10.3	.15	NS
Apparent digestibility, %					
DM	74.2	74.0	73.3	.38	NS
NDF	60.0	59.0	58.3	.85	NS
Crude protein	70.7	70.2	70.5	.72	NS
Fecal ammonia, mg NH_3/g fecal DM	.80	.87	.96	.07	NS
Nitrogen balance					
Intake, g/d	251.7	249.8	250.1	6.2	NS
Excreted in feces, g/d	73.3	73.4	71.6	1.7	NS
Excreted in urine, g/d	125.0	122.0	131.8	5.8	NS
Retained, g/d	53.5	54.5	46.7	6.5	NS
Urinary excretion					
Allantoin, g/d	40.1	39.9	38.0	1.6	NS
Uric acid, g/d	2.8	2.6	2.9	.16	NS
Computed MPSR, g N/d[d]	198.9	200.2	187.8	9.2	NS
Blood glucose, m*M*	3.54	3.55	3.56	.03	NS
Blood urea, m*M*	5.04	5.08	5.05	.10	NS

[a]Treatments were intraruminal doses of dried and pulverized whole *Yucca schidigera* plant (YS), given once daily. Control, YS20, and YS60 provided 0, 20, and 60 g/d YS, respectively.

[b]Pooled standard error of the mean.

[c]NS: Nonsignificant ($P > .05$).

[d]Microbial protein synthesis in the rumen (see Materials and Methods).

contents and, upon reaching the intestine, could affect the postruminal digestibility of nutrients.

In this experiment, YS did not affect nitrogen utilization. Relative to the control (no YS given), YS at 60 g/d increased urinary excretion of nitrogen by 5% ($P = .219$); as a result, N retention was numerically lower (13%) with this treatment ($P = .206$). Lu and Jorgensen (1987) found high levels of alfalfa saponins to be strongly inhibitory of N digestion in the forestomach ($P < .05$) when a forage-based diet was fed. Those researchers also reported a trend toward reduced N retention with increasing saponin levels. At a much lower application rate (77 ppm), Valdez et al. (1986) also observed depressed apparent digestibility of protein (49.8%, compared to 67.4% for the control) but due to large variation, the difference was not statistically significant. A similar negative effect on nitrogen digestibility (17% lower, $P < .05$) has been reported for equine weanlings (Glade, 1992).

Implications

Feeding 20 to 60 g/d of *Yucca schidigera* to cattle may decrease the postprandial concentration of ammonia in the rumen and increase the ruminal propionate concentration. These effects apparently result from partial elimination of ruminal protozoa. This study suggests that including the yucca plant in high-protein diets may improve ammonia utilization in the rumen and, by reducing the number of protozoa in the rumen, may enhance microbial protein flow to the intestine.

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