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Ruminal Cellulolytic Bacteria and Protozoa from Bison, Cattle-Bison Hybrids, and Cattle Fed Three Alfalfa-Corn Diets

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Ruminal cellulolytic bacteria and protozoa and in vitro digestibility of alfalfa fiber fractions were compared among bison, bison hybrids, and crossbed cattle (five each) when they were fed alfalfa and corn in a ratio of 100:0, 75:25, and 50:50, respectively. The total number of viable bacteria (2.16×10^9 to 5.44×10^9/ml of ruminal fluid) and the number of cellulolytic bacteria (3.74×10^7 to 10.9×10^7/ml) were not different among groups of animals fed each diet. The genera of protozoa in all of the animal groups were similar; however, when either the 100:0 or 50:50 diet was used the percentage of *Entodinium* sp. was lower and the percentage of Diplodiniinae was higher ($P < 0.05$) in bison than in bison hybrids or cattle. *Bacteroides succinogenes* made up the largest number of cellulolytic isolates from bison (58 and 36%, respectively, on the 100:0 and 75:25 diets), which were more numerous ($P < 0.05$) than those from bison hybrids (36 and 12%) and cattle (33 and 18%). This was offset by a lower number of cellulolytic *Butyrivibrio* isolates. The numbers of *Ruminococcus albus* and *R. flavefaciens* isolates, in general, were similar among the bovid species, although *R. flavefaciens* generally made up less than 10% of the cellulolytic isolates. In vitro digestibility coefficients were greater ($P < 0.05$) for the bison when the 75:25 diet was used and similar for the other two diets. The concentration of ruminal volatile fatty acids was larger ($P < 0.05$) in bison than in bison hybrids and cattle when the 50:50 diet was used. Results from this study indicate that the percentages of protozoan genera and cellulolytic bacterial species in bison are different from those of bison hybrids and cattle, suggesting that metabolic differences exist among these animal groups.

Various studies suggest that the North American buffalo or bison (*Bison bison*) has a superior ability, when compared with domestic cattle (*Bos taurus*), to digest low-quality forages. Hawley et al. (14) found that the digestion coefficients of all nutrients, including dry matter, crude protein, fat, and neutral and acid detergent fibers from sedge hay were significantly greater ($P < 0.05$) in bison than in Hereford steers. They suggested that digestibility comparisons between bison and cattle favor the bison when poor-quality, low-protein diets are used. Richmond et al. (31) observed greater digestibilities for sedge and grass hays which contained 7 to 8% crude protein but similar digestibilities for alfalfa hay containing 19% crude protein. However, when bison and Hereford steers were fed a grain diet supplemented with grass and alfalfa, Peters (29) found that bison were least efficient in digesting total nutrients. Explanations offered for the superior digestion coefficients when poor-quality diets were used include greater recycling of nitrogen to the rumen and a reduced rate of passage (14). It was speculated that zebu cattle (*Bos indicus*) are able to utilize poor quality forage more efficiently than are European cattle (*B. taurus*). Hungate et al. (17) concluded that this may be due to a greater ruminal fermentation rate per unit of solids in zebu than in European cattle.

The above-described studies did not examine the ruminal cellulolytic populations in the various animal species. A study with Indian buffalo (*Bos bubalis*) (34) did not report any cellulolytic microorganisms that have not been previously described. Interestingly, *Clostridium lochheadii* and *C. longisporum* were observed sporadically in that study. Hungate (15, 16) has previously described these organisms in cattle and found them to be more active in digesting cellulose than are previously described species. He was not able to conclude whether they play a major role in cellulose digestion in the rumen. Recently, Kelly et al. (21) described an isolate closely related to these two species, *C. chartatabidum*. It was isolated from chloroform-treated ruminal contents; however, no indication was given about its relative efficiency in degrading cellulose.

The objectives of our study were to compare in vitro digestion, cellulolytic populations, and ruminal parameters, including pH, ammonia, and volatile fatty acid concentrations among bison, bison hybrids, and crossbred cattle. These analyses were made on each of five animals from each group fed three levels of alfalfa: 100% alfalfa, 75% alfalfa-25% corn, and 50% alfalfa-50% corn. Protozoa were enumerated and identified when the 100% alfalfa and 50:50 diets were used.

MATERIALS AND METHODS

Animals, diets, and sampling. Five steers, 225 to 250 kg, from each of three groups, bison (*Bison bison*), bison hybrids (1/2 bison × 1/2 cattle, breed Charolais), and crossbred cattle (*Bos taurus*), were fed three levels of alfalfa hay with a trace mineral salt supplement; 100% ground alfalfa, 75% alfalfa–25% corn, and 50% alfalfa–50% corn (Table 1). Animals were penned by species and fed individually ad libitum with electronic headgates. Each diet was used for 12 weeks. Samples of ruminal fluid (750 ml) were obtained by stomach tube and vacuum pump from all of the animals at approximately 0900 h or just before daily feeding between weeks 5 and 7 of each diet used. Three ruminal fluid samples, one

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TABLE 1. Analytical composition of diets on a dry-matter basis

Diet[a]	Gross energy (kcal/kg)	% Crude protein	% Cell walls	% Cellulose	% Hemicellulose	% Lignin
100	4,339	13.4	59.7	38.1	11.3	11.4
75:25	4,352	18.2	41.4	22.5	12.6	7.7
50:50	4,437	15.0	34.2	19.5	11.1	5.3

[a] Diets: 100, 100% alfalfa; 75:25, 75% alfalfa–25% corn; 50:50, 50% alfalfa–50% corn.

from each animal species, were processed in 1 day. The datum set is thus representative of 5 ruminal samples per diet per animal group or a total of 45 samples for the three diets. Ruminal fluid pH was measured immediately after fluid was obtained, and subsamples were acidified with phosphoric acid to pH 2.0 for volatile fatty acid and ammonia analyses. Ruminal fluid samples were transported to the laboratory in 1-liter rubber-stoppered flasks, and culture analyses were performed.

Culture analyses and media. In the laboratory, a subsample of ruminal fluid was preserved for protozoal enumeration and identification by adding an equal part of an 18.5% formaldehyde solution to ruminal fluid. Protozoa were enumerated, and percent generic distribution was determined by the procedures of Purser and Moir (30) and Dehority (8). The remaining ruminal fluid was blended for 1 min with a Waring blender with vigorous gassing with CO_2. The fluid was filtered through two layers of cheesecloth, and serial dilutions were made in anaerobic buffer (1). A 0.2-ml sample from the 10^{-8} dilution was used to inoculate four roll tubes to determine total viable bacteria, and 0.2 ml from each of the 10^{-5} and 10^{-6} dilutions was used to inoculate three cellulose roll tubes. A 6-ml volume of undiluted ruminal fluid was used to inoculate in vitro alfalfa cell wall medium to determine the digestibility of cell walls, cellulose, and hemicellulose.

The medium used to determine total viable bacteria contained the following (per 100 ml): clarified cattle ruminal fluid, 30.0 ml; glucose, cellobiose, maltose, starch, xylose, and glycerol, 0.03 g each; Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 0.2 g; resazurin, 0.0001 g; mineral S2, 5 ml (32); purified agar (BBL), 1.75 g. Sodium carbonate (0.4%) and cysteine hydrochloride (0.05%) were added as sterile anaerobic solutions after the medium was autoclaved (3). This and other media were prepared under a CO_2 gas phase by the Hungate anaerobic culturing method as described by Bryant (3). Roll tubes were incubated at 37°C, and colonies were counted after 7 days. The composition of the cellulose agar roll tube medium was as follows (per 100 ml): clarified, preincubated cattle ruminal fluid, 15 ml; Trypticase, 0.2 g; yeast extract, 0.05 g; mineral S2, 5 ml; cellulose (Whatman no. 1 filter paper ball milled with flint pebbles for 18 h), 0.2 g; resazurin, 0.0001 g; Na_2CO_3, 0.4 g; cysteine hydrochloride, 0.05 g; purified agar, 0.7 g. These tubes were incubated for 2 weeks before zones of clearing were counted. However, isolates were picked between 48 and 120 h to avoid contamination from spirochetes, which was a frequent problem with long-term incubation. Approximately 10 to 12 isolates per animal were picked and presumptively identified by cell morphology, Gram stain, digestion of filter paper, and fermentation products (15). Thus, 50 to 60 cellulolytic isolates were presumptively identified per group of animals on each diet. Ruminal fluid from cattle was used in all medium preparations because preliminary studies indicated that the source of ruminal fluid had no effect on total viable bacteria.

The composition of the in vitro digestibility medium was (per 30 ml) 24 ml of anaerobic buffer (1), 0.5 g of alfalfa cell walls, and 6 ml of ruminal fluid as the inoculum. The cell walls were prepared by grinding alfalfa to 1-mm-diameter particles in a Wiley mill and boiling it for 1 h with neutral detergent, followed by extensive washing of the insoluble residue to remove the detergent (39). For replicates of the medium in glass tubes (25 by 142 mm) with rubber stoppers were used per ruminal sample; these were incubated in a tube press (Bellco, Glass, Inc., Vineland, N.J.) for 48 h. The samples were vigorously shaken at 0800 and 1600 h daily. After 48 h of fermentation, samples were centrifuged at 2,500 $\times$ g for 20 min. The residue pellet was frozen and later analyzed for fiber content by the sequential-detergent system (39). Sulfuric acid (72%) was used to solubilize cellulose and isolate crude lignin plus ash. The total cell wall was defined as neutral detergent fiber. Hemicellulose and cellulose were calculated by weight difference as follows: neutral detergent fiber − acid detergent fiber = hemicellulose, and acid detergent fiber − acid detergent lignin = cellulose. Digestibility was calculated as the disappearance of the component during fermentation relative to the initial concentration. Correction was made for addition of the components in the inoculum by using controls which did not contain the cell wall substrate.

The colorimetric procedure of Chaney and Marbach (5) was used to determine ruminal ammonia once these samples were neutralized to pH 7.0 with NaOH. Volatile fatty acids were determined on ruminal fluid samples that were acidified to pH 2.0 with H_3PO_4 and centrifuged at 45,000 $\times$ g for 20 min. The gas chromatograph used was a Hewlett-Packard 5840A with a coiled glass column (182.9 by 0.2 cm [inside diameter]) packed with 15% SP 1220–1% H_3PO_4 on 100/200-mesh Chromosorb W, acid washed (13). The injection port, column, and detector temperatures were 200, 125, and 250°C, respectively. A nitrogen carrier gas was used at a flow rate of 40 ml/min.

Statistics. Data were analyzed by one-way analysis of variance within each diet by using the Statistical Analysis System (SAS Institute, Inc.) with the animal as the experimental unit and the animal species as the error term (33). Means were separated by the least-significant-difference technique. Statistical significance was considered to be at $P < 0.05$.

RESULTS

Viable and cellulolytic bacteria. The total numbers of viable or cellulolytic bacteria were not different among animal groups fed diets containing three proportions of alfalfa (Table 2). Total viable bacteria ranged from 2.16×10^9 to 5.44×10^9/ml of ruminal fluid, and the cellulolytic bacteria ranged from 3.74×10^7 to 10.9×10^7/ml. The cellulolytic population represented 1.2 to 3.6% of the total viable bacteria.

When the 100% alfalfa diet was used, a greater percentage ($P < 0.05$) of *Bacteroides succinogenes* isolates were obtained from the bison (58%) than from the bison hybrids (36%) or cattle (33%) (Table 3). On this diet, *Butyrivibrio* isolates represented only 7% of the cellulolytic isolates from the bison, which was significantly lower ($P < 0.05$) than the 29% for bison hybrids but not the 13% for cattle. *Ruminococcus albus* represented 43% of the cellulolytic isolates from cattle; however, this was not statistically different from

TABLE 2. Comparison of total viable and cellulolytic bacteria from the rumens of bison, bison hybrids, and cattle fed diets containing three proportions of alfalfa

Species	No. of bacteria from indicated diet[a]					
	Total (10^9/ml)[b]			Cellulolytic (10^7/ml)[c]		
	100	75:25	50:50	100	75:25	50:50
Bison	3.02	2.92	5.44	10.9	5.06	6.64
Bison hybrids	4.22	2.16	3.54	7.5	3.74	6.60
Cattle	4.62	2.62	2.80	7.0	4.54	6.74

[a] Diets: 100, 100% alfalfa; 75:25, 75% alfalfa–25% corn; 50:50, 50% alfalfa–50% corn.
[b] The standard errors obtained with the 100, 75:25, and 50:50 diets were 0.45, 0.97, and 0.51, respectively.
[c] The standard errors obtained with the 100, 75:25, and 50:50 diets were 1.4, 0.53, and 1.08, respectively.

the 29 and 32% found in bison and bison hybrids, respectively. *R. flavefaciens* represented less than 3% of the cellulolytic isolates from all of the animals fed the 100% alfalfa diet.

With the addition of 25% corn to the diet, the number of *B. succinogenes* decreased in all groups of animals, although bison still had a larger ($P < 0.05$) percentage than bison hybrids or cattle. The proportion of *Butyrivibrio* isolates increased in all animals. The largest increase was in cattle, in which 48% of the cellulolytic organisms were *butyrivibrios*; this was concomitant with a decrease (43 to 10%) in the number of *R. albus* isolates. The percentage of *R. albus* isolates from bison and bison hybrids changed little from that obtained with the 100% alfalfa diet. *R. flavefaciens* increased in all animal groups yet still represented 9% or less of the total number of cellulolytic bacteria. When a diet containing 50% corn was used, the percentage of *B. succinogenes* isolates continued to decrease (36 to 16%) in bison, rose in bison hybrids (12 to 26%), and stayed the same in cattle (18%). *Butyrivibrio* species continued to increase in all of the animals, representing 50% or more of all cellulolytic isolates in bison hybrids and cattle. The numbers of *R. albus* decreased in all of the animals, yet a higher ($P < 0.05$) number (17%) was observed in bison than in cattle (6%). *R. flavefaciens* declined in bison hybrids and cattle but increased from 7 to 17% in bison, which was greater ($P < 0.05$) than the percentage observed in cattle (17 versus 3%). Some cellulolytic isolates were not identified; however, they made up only a small percentage of the total in all cases (Table 3).

Protozoa. The total numbers of protozoa were not different ($P > 0.05$) among the animal groups fed 100% alfalfa or 50% alfalfa–50% corn diets (Table 4). The genera of protozoa in all of the animal groups were similar, and no unusual ones were observed. However, as observed with the bacterial species, the percentages of distribution of the genera were different. When the 100% alfalfa diet was used, the percentage of *Entodinium* sp. was different ($P < 0.05$); cattle > bison hybrids > bison. Diplodiniinae and *Dasytricha* sp. were greater ($P < 0.05$) in bison than in bison hybrids or cattle. The number of *Epidinium* sp. organisms was significantly less in cattle than in bison and bison hybrids. When corn was added to the diet, the percentage of *Entodinium* sp. in bison was less than 1% and was significantly different from those of bison hybrids (68.2%) and cattle (51.1%). In contrast, the percentage of Diplodiniinae from bison (80.7%) was significantly greater than those of Diplodiniinae from bison hybrids (12.7%) and cattle (19.6%).

Ruminal fluid parameters. No differences in ruminal pH among the animal groups were observed when 100% alfalfa was used (pH 6.94 to 7.05; data not shown). Ruminal pH was lower ($P < 0.05$) for bison (7.00 and 6.68, respectively, with the 75:25 and 50:50 diets) than for bison hybrids (7.21 and 7.05) and cattle (7.15 and 7.17). It is unknown why some of the pH values were higher than what one might expect in a typical ruminal fermentation (pH 6.5 to 6.8), although sampling by stomach tube may include some saliva in the ruminal fluid sample and sampling just before feeding could contribute to higher-than-normal pH values. Ruminal ammonia-nitrogen concentrations were similar among the animal groups on each diet, with the exception of the 50:50 diet, with which the concentration for bison (26.9 mM) was higher ($P < 0.05$) than those of bison hybrids (18.0 mM) and cattle (17.9 mM).

The major ruminal volatile fatty acids were not different among the animal groups when the 100% alfalfa or 75:25 diet was used (Table 5). When 50% corn was used, the concentrations of all volatile fatty acids, with the exception of isovalerate, were greater ($P < 0.05$) in bison than in bison hybrids and cattle.

Table 6 gives 48-h in vitro digestibility coefficients. No differences between the animal groups were observed when the 100% alfalfa or 50:50 alfalfa-corn diets was used. Microorganisms from bison fed the 75:25 alfalfa-corn diet degraded more ($P < 0.05$) of the alfalfa cell walls, hemicellulose, and cellulose than did those from bison hybrids but not more than those from cattle ($P > 0.05$). However, with this diet there was a trend for higher digestion coefficients with bison than with cattle.

TABLE 3. Percentages of ruminal cellulolytic bacteria from bison, bison hybrids, and cattle fed diets containing three proportions of alfalfa

Species	% of ruminal cellulolytic bacteria that were[a]:														
	Butyrivibrios			*R. albus*			*R. flavefaciens*			*B. succinogenes*			Unknown[b]		
	100	75:25	50:50	100	75:25	50:50	100	75:25	50:50	100	75:25	50:50	100	75:25	50:50
Bison	7^c	15^c	39	29	29	17^c	1.8	7	17^c	58^c	36^c	16	4	12	11
Bison hybrids	29^d	$30^{c,d}$	50	32	36	$13^{c,d}$	1.0	9	$5^{c,d}$	36^d	12^d	26	2.4	12	5
Cattle	$13^{c,d}$	48^d	57	43	10	6^d	2.6	7	3^d	33^d	18^d	18	8	17	16

[a] Diets: 100, 100% alfalfa; 75:25, 75% alfalfa–25% corn; 50:50, 50% alfalfa–50% corn. The standard errors obtained with the three respective diets were as follows: butyrivibrios, 3.8, 6.3, and 8.8%; *R. albus*, 4.0, 6.2, and 2.3%; *R. flavefaciens*, 1.4, 3.5, and 2.9%; *B. succinogenes*, 4.2, 4.7, and 4.3%; unknown, 2.1, 3.9, and 3.0%.
[b] Not identified or culture was lost before it could be characterized.
[c,d] Means in each column not having the same superscript differ significantly ($P < 0.05$).

TABLE 4. Comparison of protozoan numbers and distribution among bison, bison hybrids, and cattle fed diets containing two proportions of alfalfa

Diet and animals (no.)	Total protozoa (10^4)/ml[a]	% of total protozoa belonging to[b]:					
		Entodinium	Diplodiniinae	*Epidinium*	*Isotricha*	*Dasytricha*	*Beutschlia*
100% Alfalfa							
Bison (4)	6.1	5.4[c]	35.2[c]	39.8[c]	5.4	14.1[c]	
Hybrids (3)	13.7	55.4[d]	3.0[d]	39.2[c]	13.6	1.4[d]	0.4
Cattle (3)	16.0	79.6[e]	9.9[d]	5.8[d]	2.6	2.1[d]	
50% Corn–50% alfalfa							
Bison (4)	48.6	0.5[c]	80.7[c]	16.4	0.4	2.2	
Hybrids (3)	41.4	68.2[d]	12.7[d]	3.2	3.5	0.3	0.1
Cattle (2)	19.1	51.1[d]	19.6[d]	27.6	0	0.9	

[a] The standard errors of the values associated with the 100% alfalfa and 50% corn–50% alfalfa diets were 2.8×10^4 and 13.8×10^4 protozoa per ml, respectively.
[b] The standard errors at the values associated with the 100% alfalfa and 50% corn–50% alfalfa diets, respectively, were as follows: *Entodinium*, 10.2 and 9.6%; Diplodiniinae, 2.9 and 5.0%; *Epidinium*, 7.7 and 7.4%; *Isotrichia*, 3.8 and 1.3%; *Dasytricha*, 1.8 and 0.6%.
[c,d,e] Values in each column not having the same superscript differ significantly ($P < 0.05$).

DISCUSSION

Numbers of total viable ruminal bacteria ranged from a low 2.16×10^9/ml in bison hybrids fed 75% alfalfa–25% corn to 5.44×10^9/ml in bison fed 50% alfalfa–50% corn. Microscopic counts of total ruminal bacteria ranging from 5.88×10^9/ml to 8.52×10^9/ml have been reported from seven bison killed during the regular hunting season in southern Utah (28). In general, total microscopic counts are generally 5- to 10-fold greater than viable counts (4, 40); however, the quality and availability of feed may have been limited for the wild bison.

The percentage of cellulolytic isolates from the three animal groups varied with the composition of the diets and may reflect the ecological role of these species in ruminal fermentation. *B. succinogenes* and *R. albus* isolates generally declined with increasing corn in the diet (Table 3), possibly reflecting their limited ability to utilize substrates other than cellulose, cellobiose, and glucose, while *Butyrivibrio* isolates increased, which may reflect their ability to utilize starch and other carbohydrates. *R. flavefaciens* isolates represented no more than 9% of the cellulolytic isolates when any of the diets were used, with the exception of the 50:50 alfalfa-corn diet in bison. While this cellulolytic organism is known to hydrolyze cellulose and cellobiose, it does not utilize glucose, which may limit its ability to compete with *B. succinogenes* and *R. albus*.

Dehority and Scott (10) found that *B. succinogenes* digested appreciably larger amounts of cellulose from forage than did ruminococci or strains of *B. fibrisolvens*. Other studies (26, 27, 35) support this. Halliwell and Bryant (12) reported similar findings on the digestibility of purified celluloses with *B. succinogenes*. However, Kolankaya et al. (22) found that *R. albus* may be the most rapidly acting organism against straw, and Chesson et al. (6) found this to be true when ryegrass was the substrate. The high percentage of *B. succinogenes* isolates observed in the bison group (58%; Table 3) when a diet of 100% alfalfa was used suggests that a higher digestion coefficient might be expected if one assumes that *B. succinogenes* is a more efficient degrader of crystalline cellulose (36). We did not observe this from our in vitro digestibility data (Table 6) when the animals were fed the 100% alfalfa diet. An earlier study also indicated that in vitro digestibility was unrelated to fibrolytic bacterial numbers or proportions (20). Except in one bison, cellulolytic clostridia were not found as predominant organisms. Thus, the superior ability that that bison may have to degrade low-quality forages is not likely to be attributed to these organisms.

Somewhat surprisingly, the total and cellulolytic bacterial numbers changed little when corn was added to the diet (Table 2). One might expect fewer cellulolytic organisms with 50% corn added to the diet, because this diet contained 19.5% cellulose, compared with 38.1% for the 100% alfalfa diet (Table 1). Other studies support numbers of cellulolytic bacteria similar to those found in this study, with little change in number when all-forage or up to 60% grain diets were used (4, 23–25, 37). Similarly, the numbers of total viable bacteria have been shown to be higher when grain diets are used (4, 11, 34). The percentage of cellulolytic microorganisms among the total viable microorganisms found in our study (1.2 to 3.6%) is comparable to those of other studies of cows (16, 24), sheep (38), and buffalo from India (34). Bryant and Burkey (4) reported higher percentages of cellulolytic bacteria in cattle, ranging from 5 to 28% of the total culturable count.

For bison, total protozoan numbers varied in individual animals from 2.08×10^4/ml when they were fed 100% alfalfa to 76.16×10^4/ml with 50% alfalfa–50% corn. This compares

TABLE 5. Comparison of ruminal volatile fatty acid concentrations among bison, bison hybrids, and cattle fed diets containing three proportions of alfalfa

Diet and animals	Volatile fatty acid concn (mM)[a]					
	Acet	Prop	i-Buty	Buty	i-Val	Val
100% Alfalfa						
Bison	71	12	1.0	7.0	0.9	0.9
Bison hybrids	75	14	1.4	6.7	1.4	1.0
Cattle	72	12	1.3	6.5	1.5	0.9
75% Alfalfa–25% corn						
Bison	51	9	0.9	5.4	0.7	0.6[b]
Bison hybrids	54	10	1.0	4.7	0.9	0.9[c]
Cattle	47	9	1.0	4.0	0.8	0.7[b,c]
50% Alfalfa–50% corn						
Bison	70[b]	15[b]	1.5[b]	13.7[b]	1.8[b]	1.4[b]
Bison hybrids	45[c]	10[c]	1.0[c]	6.9[c]	1.2[c]	0.9[c]
Cattle	46[c]	10[c]	0.8[c]	6.8[c]	1.0[c]	0.9[c]

[a] The standard errors associated with the 100% alfalfa, 75% alfalfa–25% corn, and 50% alfalfa–50% corn diets, respectively, were as follows: acetate (Acet), 3.0, 2.7, and 3.4 mM; propionate (Prop), 0.5, 0.5, and 1.0 mM; isobutyrate (i-Buty), 0.06, 0.05, and 0.06 mM; butyrate (Buty), 0.3, 0.4, and 0.6 mM; isovalerate (i-Val), 0.08, 0.05, and 0.1 mM; valerate (Val), 0.05, 0.06, and 0.05 mM.
[b,c] Values in a column not having the same superscript differ significantly ($P < 0.05$).

TABLE 6. Comparison of 48-h in vitro digestibilities of alfalfa meal fractions with ruminal inocula from bison, bison hybrids, and cattle fed diets containing three proportions of alfalfa

Species	Mean digestibility of fiber component (%)[a]								
	Cell walls			Hemicellulose			Cellulose		
	100	75:25	50:50	100	75:25	50:50	100	75:25	50:50
Bison	25.4	29.2[b]	30.8	37.4	39.4[b]	39.6	25.9	31.9[b]	36.5
Bison hybrids	27.7	23.8[c]	32.0	38.6	34.4[c]	42.7	30.5	24.8[c]	36.7
Cattle	29.0	26.7[b,c]	30.0	40.1	36.0[b,c]	40.4	31.4	28.2[b,c]	33.7

[a] Diets: 100, 100% alfalfa; 75:25, 75% alfalfa–25% corn; 50:50, 50% alfalfa–50% corn. The standard errors associated with the respective diets were as follows: cell walls, 0.5, 0.8, and 0.6%; hemicellulose, 1.0, 0.7, and 0.9%; cellulose, 2.5, 0.5, and 0.6%.

[b,c] Means in each column not having the same superscript differ significantly ($P < 0.05$).

well with the report by Pearson (28) on wild bison, in which the numbers varied in seven animals from 30×10^4/ml to 86×10^4/ml, and the species were similar to those that occur in domestic ruminants. No unusual species of protozoa were observed in the bison in this study; however, generic distribution varied in comparison with bison hybrids and cattle.

In general, the percentage of *Entodinium* sp. was low and those of Diplodiniinae and *Epidinium* sp. were high in bison fed an alfalfa diet. The changes observed when bison were switched to a 50% concentrate diet were opposite to what one might expect (15, 30). It might be expected that with a lower ruminal pH (6.68 with the 50:50 diet compared with 6.94 with the 100% alfalfa diet) the more acid-tolerant *Entotinium* sp. would increase and the Diplodiniinae would decrease. However, the decrease in pH may not have been large enough to affect the growth of these protozoa.

Large differences in percentage distribution for a number of the individual animals in this study were observed which represented extremes that exceed those previously reported in a wide range of ruminants (2, 7, 9, 15, 18, 19). Two of the bison and one bison hybrid fed the 50:50 diet did not contain *Entodinium* sp. We are unaware of any previous reports of a protozoan population devoid of the genus *Entodinium*. Imai (18) recently reported an *Entodinium* percentage of 1.8% in zebu cattle from Kenya. Previous low *Entodinium* values were observed in New Zealand (8%), Senegal (14.9%), Ceylon (27.3%), and India (30.5%) (2, 15). A corresponding increase in the percentage of Diplodiniinae in the two bison (77.4 and 99.4%) was also much higher than in previous reports, in which a greater percentage of the population occurred as *Isotricha*, *Dasytricha*, and *Epidinium* species.

The low ruminal pH observed in bison (6.68) when they were fed the 50:50 alfalfa-corn diet reflects the higher concentrations of volatile fatty acids (Table 5) than in bison hybrids and cattle. The concentrations of ammonia were also higher in bison on this diet, which suggests a different microbiological or animal nutritional response by bison than bison hybrids or cattle when the concentrate diet is used.

Based on previous studies (14, 31) which suggest that bison are superior to cattle in the digestion of nutrients from poor-quality, low-protein feeds, our 100% alfalfa diet, which contained 13.4% protein (Table 1), may have been too high in protein for the microflora from the bison to demonstrate superiority in the in vitro fermentation analyses. However, in a preliminary study 2 years earlier (R. M. Koch and H. G. Jung, unpublished data), in which bison were fed bromegrass with 8% crude protein, many of the animals were not able to maintain body weight; thus, the higher-protein diet was used in this study.

In summary, our study suggests that there are differences in the percentages of cellulolytic bacteria and protozoa inhabiting the bison rumen compared with those of cattle, yet consistent differences in in vitro digestibility coefficients were not observed as a result of this. When a diet mixture of 50:50 alfalfa-corn was used, ruminal pH, volatile fatty acids, and ammonia nitrogen were different between bison and cattle, supporting potential different microbiological or animal nutritional responses to this diet. Growth efficiency studies with these animals which could assist in the interpretation of our data have not been completed (Koch et al. unpublished results data). Fiber degradation is dependent on retention time; thus, it should be a parameter measured with bison. The bison used in our study were in close contact with cattle, possibly causing some microbial cross-contamination. On the other hand, if bison are nutritionally or physiologically different, one would assume that their respective ecologically developed microflora would be maintained. It would be interesting to study the bison ruminal microflora when these animals graze native range grass.

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