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Moderation of Ruminal Fermentation by Ciliated Protozoa in Cattle Fed a High-Grain Diet†

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The objective of this study was to assess the influence of ciliated protozoa on ruminal fermentation in cattle fed high-grain diets. Six ruminally cannulated steers fed a corn-based grain diet (85% concentrate plus 15% alfalfa hay) at 12-h intervals were assigned randomly to two groups, ciliate free and faunated, in a crossover design. Defaunation was by ruminal emptying, omasal flushing, and treatment with sodium sulfosuccinate. Two to 3 weeks after defaunation, the ruminal contents of all steers were sampled before the morning feeding (0 h) and at 1, 2, 4, 6, 8, and 12 h after feeding to measure pH, analyze fermentation products, and monitor counts of ciliated protozoa and lactic acid-producing and -fermenting bacterial groups. Total numbers of ciliated protozoa in the faunated steers averaged 4.3×10^5/g, and the protozoa consisted of nine genera. Ciliate-free steers had lower ($P < 0.01$) ruminal pHs (pH 5.97) than faunated cattle (pH 6.45); however, the treatment-time interaction was not significant. Ruminal lactate and ammonia concentrations were similar in both groups. The total volatile fatty acid concentration was higher ($P < 0.05$) in the ciliate-free steers than in the faunated steers and exhibited a treatment-time interaction ($P < 0.05$). The acetate-to-propionate ratio was higher ($P < 0.05$) in the faunated group than in the ciliate-free group and showed a treatment-time interaction ($P < 0.05$). Total anaerobic bacterial counts were about fourfold higher in the ciliate-free group than in the faunated group. Although counts of lactic acid producers were higher and counts of lactic acid fermenters were lower in ciliate-free steers than in faunated steers, the differences were not significant. Ciliated protozoa in cattle fed high-grain diets apparently moderate the ruminal fermentation rate as evidenced by higher ruminal pH values and lower volatile fatty acid concentrations in faunated cattle than in ciliate-free cattle. It appeared that the moderation of the ruminal fermentation rate by ciliated protozoa was attributable to reduced bacterial numbers and possibly reduced activity.

Ciliated protozoa constitute an important fraction of the total microbial population in the ruminal ecosystem. Because ciliated protozoa are preferentially retained in the rumen (16) and do not significantly contribute to the postruminal nutritive supply, their overall value to the host is debatable (13, 35). The impact of the presence or absence of ruminal ciliated protozoa on the host may depend on the diet and on the numbers and kinds of ciliates. In animals fed low-protein diets, ciliated protozoa apparently have a negative effect on growth and performance (4). However, in animals fed high-grain diets, ciliated protozoa may have a beneficial role, primarily because of their ability to influence ruminal starch and lactic acid metabolisms (12, 35). The presence of ruminal ciliated protozoa in animals fed high-grain diets is associated with decreased accumulation and increased fermentation of lactic acid (20, 22, 28, 40). Because of their influence on ruminal lactate accumulation, it is hypothesized that ciliated protozoa play an important role in the moderation of ruminal fermentation in ruminants fed high-grain diets. Our objective was to compare ruminal pH values, fermentation products, and bacterial numbers in ciliate-free and faunated steers fed a high-grain diet.

MATERIALS AND METHODS

Animals, diet, and defaunation technique. Six ruminally cannulated steers (body weight, 220 to 265 kg each) were assigned randomly to two groups, ciliate free and faunated, in a crossover design. Steers were fed a high-grain diet formulated to contain 85% concentrate and 15% alfalfa hay. The concentrate consisted of (as percentage of dry matter) cracked corn (87.8%), soybean meal (10.5%), salt (1.0%), dicalcium phosphate (0.3%), trace mineral mix (0.2%), and vitamins A, D, and E (0.1%). Steers were fed twice a day at 12-h intervals in equal portions to provide 1.75 net energy for maintenance per day. All steers were defaunated by withholding feed for 24 h, completely emptying the ruminal contents, flushing the omasum with tepid tap water, and spraying 1,000 ml of 0.4% dioctyl sodium sulfosuccinate solution (Aerosol OT, Fisher Scientific) on the reticuloruminal wall and into the reticulo-omasal orifice (31). After normal feed intake resumed, three steers were inoculated with ruminal fluid containing ciliated protozoa from a donor animal fed a high-grain diet. The remaining three steers remained ciliate free throughout the sampling period. Ciliate-free steers were housed separately in a metabolism room, and faunated steers were brought into the same room the evening before the sampling day. After the completion of the first sampling period, treatment groups were reversed and subjected to defaunation as described before.

Sampling and processing of ruminal contents. Two to 3 weeks after defaunation, the ruminal contents of all steers were sampled before the morning feeding (0 h) and at 1, 2, 4, 6, 8, and 12 after feeding to measure pH, analyze fermentation products, and monitor changes in lactic acid-producing and -fermenting bacterial groups. Samples were sealed in an insulated thermos and transported to the laboratory. The sample pH was recorded, and a portion of each mixed

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sample from the faunated steers was fixed with 10% formalin for enumeration of ciliated protozoa. Ruminal contents from the defaunated group were examined microscopically to confirm the absence of ciliated protozoa. The remaining sample was blended for 1 min and strained through four layers of cheesecloth under CO_2 gas. It was used for dry matter determination; analyses of fermentation products [volatile fatty acids (VFA), ammonia, and L-(+)- and D-(−)-lactic acid]; and enumerations of total viable anaerobic bacteria, lactic acid-producing bacteria (*Streptococcus bovis*, anaerobic lactobacilli, and amylolytic bacteria), and lactic acid-fermenting bacteria.

Analytical procedures for analyses of fermentation products and bacteriological procedures for enumerating total and functional groups of bacteria have been described previously (3). The procedure for the dilution, generic identification, and enumeration of ciliated protozoa was the method of Towne et al. (32).

Statistical analysis. One animal died after the first sampling period for reasons unrelated to the experiment (abomasal ulcer and abscess). Thus, the second sampling period had only five animals (two ciliate free and three faunated). Because of the missing animal, data were analyzed as a split-plot design by using the GLM procedure of SAS (29). The whole plot consisted of animal and treatment (faunated versus ciliate free), with animal-treatment interaction as the error term for treatment effects. The subplot consisted of sampling time and its interactions with animal and treatment. The whole-plot residual error served as the error term for the subplot. Least-square means were separated with a protected least-significant-difference test when significant treatment or treatment-time interactions were observed.

RESULTS

The total number of ciliated protozoa in the faunated steers averaged 4.3×10^5/g of ruminal contents and was not affected by sampling time ($P > 0.1$). Among the holotrichid ciliates (*Isotricha*, *Dasytricha*, and *Charonina* spp.), only *Isotricha* and *Dasytricha* spp. were affected by sampling time, with 1-h-postfeeding counts higher ($P < 0.01$) than those at all other sampling times. The entodiniomorphid ciliates included (as percentage of total number) *Entodinium* spp. (73.4%), *Diplodinium dentatum* (1.3%), *Epidinium caudatum* (6.7%), *Ophryoscolex purkeynei* (4.6%), and *Polyplastron multivesiculatum* (0.3%).

Ruminal pH and fermentation products. Ruminal pH values declined in both groups after feeding and were lower ($P < 0.05$) at 1, 2, 4, and 6 h postfeeding than at prefeeding (Fig. 1A). The average ruminal pH was lower ($P < 0.01$) for ciliate-free steers than for faunated steers (5.97 versus 6.45); however, the treatment-time interaction was not significant ($P > 0.1$). Ruminal lactate concentrations [L-(+) and D-(−)] were similar in both groups of steers, but a sampling time effect ($P < 0.1$) was observed. Samples collected at 1 and 2 h postfeeding had higher lactate concentrations than the 0-h sample ($P < 0.05$) in the ciliate-free group, but concentrations in the faunated group did not change over time (Table 1). Average ammonia concentrations were not different between the ciliate-free and faunated steers (10.2 versus 9.1 mM). The total VFA concentration was higher ($P < 0.05$) in the ciliate-free steers than in the faunated steers (92.3 versus 64.8 mM) and exhibited a treatment-time interaction ($P < 0.05$). Ruminal VFA concentrations were higher at 2, 4, and 6 h postfeeding in the ciliate-free group and higher at 2 and 4 h in the faunated group than at prefeeding (Fig. 1B). The

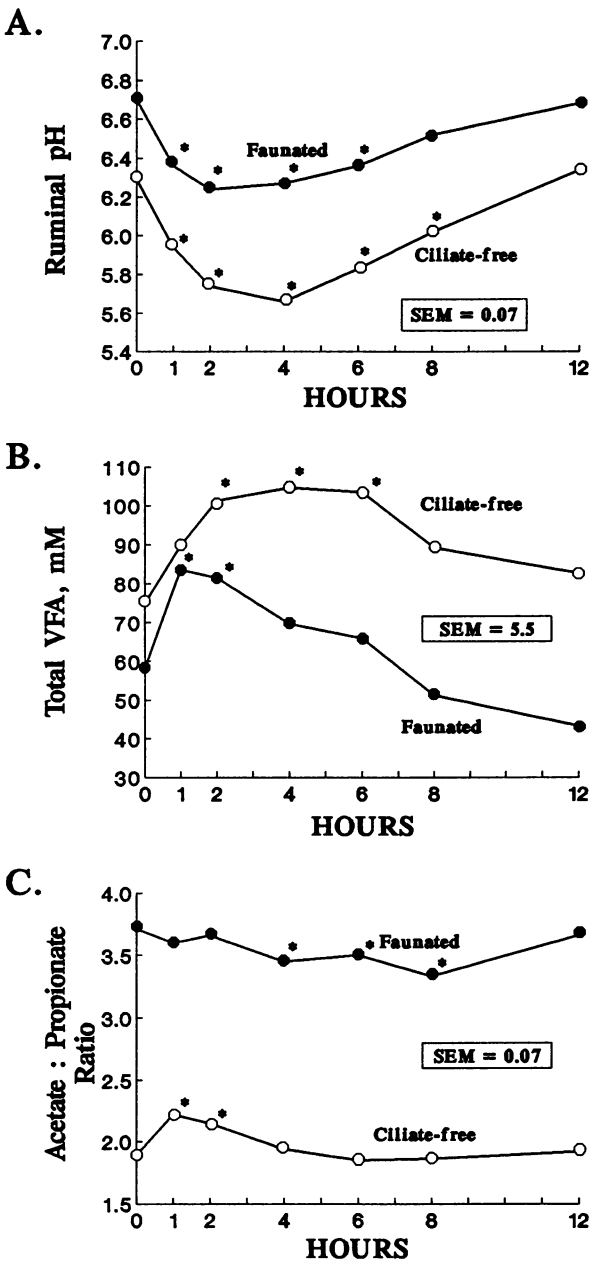

FIG. 1. Ruminal pH values (A; treatment effect $P < 0.01$), VFA concentrations (B; treatment effect $P < 0.01$; treatment-sampling time interaction $P < 0.05$), and acetate-to-propionate ratios (C; treatment effect $P < 0.05$; treatment-sampling time interaction $P < 0.05$) in faunated and ciliate-free steers fed a high-grain diet. *, different from time zero at $P < 0.05$); treatment-sampling time interaction $P < 0.05$.

acetate concentration increased after feeding and exhibited a treatment-time interaction ($P < 0.05$). The average propionate concentration was higher in the ciliate-free group than in the faunated group (27.2 versus 12.2 mm) and also showed a significant treatment-time interaction ($P < 0.05$). The acetate-to-propionate ratio was significantly higher in the faunated than in the ciliate-free group and showed a significant treatment-time interaction (Fig. 1C). Butyrate and isovalerate concentrations were similar in both groups. The butyrate concentration increased at 4 and 6 h postfeeding in the

TABLE 1. Ruminal concentrations of lactate, ammonia, and volatile fatty acids in ciliate-free or faunated steers fed a high-grain diet

Fermentation product	Steers	Ruminal concn (mm) at sampling time							SEM[a]
		0 h	1 h	2 h	4 h	6 h	8 h	12 h	
Lactate	Ciliate free	0.02	0.25[b]	0.30[b]	0.09	0.05	0.09	0.01	0.07
	Faunated	0.01	0.09	0.07	0.10	0.04	0.02	0.01	
Ammonia	Ciliate free	12.5	12.3	13.4	9.7	7.0	7.1	9.2	2.1
	Faunated	9.9	15.9	13.8	6.7	8.1	4.2	5.1	
Acetate[c]	Ciliate free	41.1	53.1[b]	59.5[b]	58.8[b]	56.7[b]	49.4	46.5	3.4
	Faunated	37.6	56.0[b]	54.1[b]	44.9	43.3	33.1	28.6	
Propionate[c,d]	Ciliate free	23.0	25.1	28.7[b]	30.8[b]	31.3[b]	27.4[b]	24.3	1.4
	Faunated	10.4	15.6[b]	15.2[b]	12.7	12.5	10.1	7.9	
Butyrate	Ciliate free	6.0	6.9	8.2	10.3[b]	10.7[b]	8.5	7.3	1.0
	Faunated	7.4	8.6	8.9	8.3	7.7	6.1	5.0	
Isobutyrate[c,e]	Ciliate free	1.4	1.2[b]	1.2[b]	1.1[b]	1.1[b]	1.0[b]	1.1[b]	0.05
	Faunated	0.9	0.9	0.9	0.8	0.7[b]	0.6[b]	0.5[b]	
Isovalerate[c]	Ciliate free	2.1	1.9	1.9	1.9	1.9	1.7[b]	2.1	0.1
	Faunated	1.4	1.5	1.4	1.2	1.1[b]	1.0[b]	0.9[b]	
Valerate[d]	Ciliate free	1.3	1.6	1.8	1.9	1.8	1.4	1.3	0.1
	Faunated	0.6	0.9	1.1	0.7	0.6	0.5	0.4	

[a] SEM, standard error of the mean.
[b] Within each row, the mean is different from time zero ($P < 0.05$).
[c] Treatment-sampling time interaction ($P < 0.05$).
[d] Treatment effect ($P < 0.01$).
[e] Treatment effect ($P < 0.05$).

ciliate-free group but did not change in the faunated group after feeding. Average valerate and isobutyrate concentrations were higher ($P < 0.05$) in the ciliate-free group than in the faunated group (Table 1).

Because no significant time effect was observed ($P > 0.1$), bacterial counts were pooled across sampling times (Table 2). Total anaerobic bacterial counts were about fourfold higher ($P = 0.11$) in the ciliate-free group than in the faunated group. Counts of lactic acid-producing bacteria (*S. bovis*, lactobacilli, and amylolytic bacteria) tended to be higher in the ciliate-free group than in the faunated group. Lactate-fermenting bacterial counts were unaffected by the absence of ciliated protozoa. However, lactate fermenters as a percentage of the total bacterial population were lower ($P < 0.05$) in the ciliate-free group than in the faunated group (Table 2).

DISCUSSION

The contribution of ciliated protozoa to the ruminal metabolism of feedlot cattle (fed a 75 to 95% grain diet) historically has been considered to be insignificant because grain diets presumably reduce or completely eliminate ciliated protozoal populations (10, 18, 19, 30, 34). This reduction or elimination usually has been attributed to low ruminal pH, hypertonicity, and faster passage rates of ruminal contents (1, 7, 10, 28). However, Towne et al. (33) reported that feedlot cattle were not characteristically defaunated and often possessed relatively high protozoal concentrations. Total numbers of ciliated protozoa in feedlot cattle averaged 1.59×10^5/g of ruminal contents. Only 13% of feedlot cattle were defaunated, whereas 15% had protozoal concentrations greater than 10^5/g (33). Defaunation in feedlot cattle apparently is transitory, and individual animals harbor a dynamic

TABLE 2. Counts of ruminal total anaerobic, lactic acid-producing, and lactic acid-fermenting bacteria in ciliate-free or faunated steers fed a high-grain diet

Bacterial group	Bacterial count (CFU/g of DM)		Pooled SEM[a]	P
	Ciliate free steers	Faunated steers		
Total bacteria	13.0×10^{10}	2.8×10^{10}	3.4×10^{10}	0.11
Streptococcus bovis	36.8×10^7	23.7×10^7	15.7×10^7	0.59
Lactobacillus spp.	27.7×10^8	6.4×10^8	11.7×10^8	0.28
Amylolytic bacteria	10.3×10^{10}	2.3×10^{10}	2.7×10^{10}	0.11
Lactate fermenters	33.6×10^9 (35.8)[b]	13.8×10^9 (55.4)[b]	10.2×10^9 (4.8)[b]	0.25

[a] SEM, standard error of the mean.
[b] The values in parentheses indicate percentages of total bacteria. The P value of these percentages is 0.05.

protozoal population that fluctuates in response to changing ruminal conditions (32). The paucity of information on the exact role and extent of contribution of ciliated protozoa to the ruminal ecosystem is probably due to the difficulty of cultivating ciliates in vitro. Therefore, the assessment of the ciliated protozoal contribution is made indirectly by comparing the ruminal metabolism of ciliate-free animals with that of faunated animals. Conventional procedures to eliminate ciliated protozoa from ruminants have involved dosing the rumen with an antiprotozoal compound (2, 5, 26). Despite their widespread use, however, chemical defaunating agents are not always successful, and the persistent reappearance of ciliates after a defaunation procedure is often acknowledged (4, 5, 36). Although this reappearance of ciliates is often blamed on exogenous contamination, Towne and Nagaraja (31) proposed that relatively high concentrations of ciliates residing in the omasum appear to be responsible for reinoculating defaunated rumens. Therefore, the defaunation technique in this study involved omasal flushing in conjunction with chemical defaunation. This procedure was effective in maintaining ciliate-free animals.

Any effects, direct or indirect, that ciliated protozoa have on host nutrition result from the changes they induce in ruminal metabolism. However, ruminal changes associated with defaunation cannot be attributed solely to the absence of ciliated protozoa. Because of the predatory role of ciliated protozoa, the presence of ciliated protozoa in the rumen is associated with reduced bacterial density (9, 15). In this study, a four- to fivefold increase in total bacterial numbers was observed in the ciliate-free group in comparison with those of the faunated group. Other researchers have suggested that ciliates selectively engulf amylolytic bacteria (15). However, the selectivity may not be from amylolytic bacteria engulfment per se but from ingestion of starch granules with adherent amylolytic bacteria. Although counts of amylolytic and other lactic acid-producing bacteria tended to be higher in the ciliate-free group than in the faunated group, the difference was not significant.

Because of the negative interaction between bacteria and ciliates, bacterial activity is lower in the presence of ciliates than in their absence. This is evidenced by lower ruminal pH values in ciliate-free steers than in faunated steers in our study. These results agree with other reports comparing faunated ruminants with ciliate-free ruminants on a wide variety of diets (8, 17, 36). Although no treatment-time interaction was observed, the postprandial decline in pH tended to be lower in the ciliate-free group than in the faunated group. Veira et al. (36) reported that ciliated protozoa prevented a sharp decline in postprandial pH in sheep that were meal-fed with starch-containing diets. The pH effect was attributed to the influence of ciliates on ruminal lactate metabolism (35) because faunated animals had faster clearances of exogenous lactate and lower levels of endogenously produced lactate than ciliate-free animals did (6, 23). Although the relative contribution of ciliated protozoa to ruminal lactate pool size is not known, an inverse relationship exists between ciliated protozoa and lactate accumulation or production in the rumen (22, 24). Differences in lactate concentrations have been attributed to ciliate uptake of readily fermentable sugars and starch, thereby sequestering them from immediate bacterial fermentation (27), and to enhanced lactate clearance by ciliated protozoa (24, 25). In this study, the ruminal lactate concentration increased postprandially in the ciliate-free group, but there was no significant difference between the concentrations of the ciliate-free and faunated groups, and the overall lactate concentration in both groups was extremely low.

The difference in ruminal pH between the faunated and ciliate-free groups was related to total VFA and not to lactate concentrations. The increase in total VFA concentration in ciliate-free steers reflects increased bacterial numbers and activity. Additionally, defaunation produced a significant increase in propionate, isobutyrate, and valerate concentrations but did not affect acetate, butyrate, and isovalerate concentrations. Defaunation is often associated with a decrease in butyrate concentration (11, 21, 39). However, shifts in VFA concentrations or proportions may not be entirely due to ciliates per se but to concurrent changes in bacterial populations. Ciliates have been reported to be the major methane-producing fraction in ruminal contents (14) because of their adherent methanogens (37, 38). The difference in propionate concentrations reflects the amount of hydrogen available in the absence or presence of ciliates.

Ciliated protozoa in cattle fed high-grain diets apparently moderate the ruminal fermentation rate. This is supported by higher ruminal pH values and lower VFA concentrations in faunated cattle than in ciliate-free cattle. Although protozoa possibly sequester some starch, the moderation is more likely due to reduced bacterial numbers and activity. Therefore, when high-grain diets are fed, protozoa may exert a buffering effect by slowing the rate of starch fermentation and, hence, may play a beneficial role in the ruminal ecosystem.

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