Effect of Sampling Site on Protozoa and Fermentation End Products in the Rumen of Dairy Cows[1]

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ABSTRACT

Three ruminally cannulated Holstein cows fed a diet containing 55% corn silage and 45% concentrate (DM basis) were used to compare rumen contents obtained from three sampling sites in the rumen. Rumen contents were collected from the dorsal, ventral, and anterior sacs of the rumen prior to (0 h) and at 2, 4, and 8 h after the a.m. feeding. Strained and unstrained rumen contents were preserved for protozoa count. Volatile fatty acids, NH_3 N, and pH analyses were performed on strained rumen contents. Total protozoa numbers (entodiniomorphs and holotrichs), VFA, and NH_3 N concentrations were higher and rumen pH lower in rumen contents obtained from the dorsal sac than in rumen contents from ventral and anterior sacs; however, holotrich numbers were not affected by rumen sampling site. Numbers of protozoa in strained preparation of rumen contents were greater than those in unstrained rumen contents. Data from this study suggest that differences in concentration of protozoa among ruminal sampling sites are related to end products of microbial fermentation.

INTRODUCTION

Concentrations of NH_3 N (23, 24), VFA (23), and bacteria (7) are higher in rumen samples taken from the dorsal sac than from the ventral sac in dairy cattle. However, information is not available on location of protozoa in the rumen and their relationship to fermentation end products. Because protozoa in the rumen can be associated with both the liquid and particulate fractions of rumen digesta, the manner in which rumen digesta is prepared (strained versus unstrained) may affect protozoa concentration. The objective of this experiment was to determine effects of location of sampling site in the rumen and method of preparation of rumen contents on protozoa numbers in lactating dairy cows.

MATERIALS AND METHODS

Three multiparous lactating Holstein cows, fitted with ruminal cannula, were used in a randomized complete block design. Cows were housed in individual tie stalls and were fed at 0730 and 1830 h and milked at 1000 and 1900 h daily. The diet fed was 55% corn silage and 45% concentrate on a DM basis and formulated to meet or exceed NRC nutrient recommendations (19). Ingredients and chemical composition of the total mixed diet are presented in Table 1. Weighbacks were removed and weighed daily just prior to the 0730 h feeding. Feed offered was adjusted to obtain at least 10% weighback. Water was available at all times.

Rumen contents were collected from the dorsal, ventral, and anterior sacs of the rumen via the rumen cannula prior to 0730 h feeding (0 h) and at 2, 4, and 8 h thereafter. Collection of rumen contents was replicated three times for each cow. Capped plastic containers (120 ml) were used to obtain rumen contents from the ventral and anterior sacs to minimize contamination with rumen contents from other locations. A portion of the rumen contents was strained through four layers of cheesecloth. Measurement of pH on strained and unstrained rumen contents was made by glass electrode,

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TABLE 1. Diet ingredients and chemical composition of the total mixed ration.

Components	% DM
Ingredients	
Corn silage	55.0
Shelled corn, cracked	26.1
Liquid molasses	1.9
Soybean meal, 48%	14.5
Dicalcium phosphate	.48
Limestone	.53
Sodium bicarbonate	.64
Salt	.39
Trace mineral premix[1]	.13
Selenium premix[2]	.04
Calcium sulfate	.19
Magox[3]	.10
Chemical composition	
DM	61.6
	(% of DM)
Crude protein	15.3
Acid detergent fiber	14.4
Neutral detergent fiber	26.8
Ash	5.6

[1]Contained 26% Ca, 1.4% Mg, .6% Cu, 5.4% Zn, and 2.7% Fe.

[2]Contained .02% Se.

[3]Contained 54.5% Mg.

immediately after sample collection. One gram of strained or unstrained rumen contents was preserved in 3 or 5 ml of formalin-saline solution, one part formaldehyde (37.8%) and two parts saline (.9%). For ciliated protozoa count, preserved samples were initially vortexed and then suspended in a 30% glycerol solution to obtain adequate dilution. Numbers of protozoa were determined under a light microscope at 100× magnification using a Levy-Hausser counting chamber (Thomas Scientific, Philadelphia, PA). Protozoa count was grouped into entodiniomorphs and holotrichs.

A second portion of the strained rumen contents was acidified with concentrated HCl to pH 4.0 and frozen until analyzed for NH_3 N by a modified procedure (10) of Chaney and Marbach (8). For analysis of VFA, 5 ml of strained rumen contents were added to 1 ml of 25% metaphosphoric acid solution and 1 ml of .6% 2-methyl-butyric acid internal standard solution, shaken, and then frozen until analyzed. The VFA were analyzed using GLC with a column 2-m long × 2 mm i.d. packed with Carbowax 20 M on 80/100 Carbopack B-PA.

To maintain the 55:45 ratio of silage to concentrate DM in the diet, DM determinations for corn silage were conducted weekly. After determining DM, corn silage and concentrate samples were ground through a 1-mm screen in a Wiley mill (Wiley, Philadelphia, PA) for subsequent analyses. Dietary CP was measured by the Kjeldahl procedure (4). Acid detergent fiber was determined by the method of Goering and Van Soest (11), and NDF was analyzed according to the modified procedure of Mertens (15). Ash content was determined as described by AOAC (4).

Data were analyzed as a randomized complete block design with three replications by the analysis of variance as described by Steel and Torrie (22) using the SAS General Linear Model program (12). Changes in ruminal measures over sampling time within sampling site were analyzed as a split-plot design (22). Means were compared using *t* test analysis (22).

RESULTS AND DISCUSSION

Animals

The cows were in good health throughout the experiment with no mastitis or digestive problems. Average DM intake and milk production were 20.8 and 20.9 kg/d, respectively.

Effect of Sampling Site

Total protozoa (entodiniomorphs and holotrichs) and entodiniomorph numbers were higher ($P<.01$) across sampling times in the dorsal sac than in the ventral or anterior sacs (Table 2). However, holotrich numbers were similar among different locations (Table 2).

Rumen contents taken from the dorsal sac had the lowest ($P<.05$) pH followed by the ventral and anterior sacs in the rumen (Table 2). Concentration of total VFA was higher ($P<.01$) in the dorsal sac than in the ventral or anterior sac but were not different between the ventral and the anterior sacs (Table 2). Individual VFA concentrations (Table 2) followed a trend similar to that of total VFA concentrations with the exception of isovalerate, which

TABLE 2. Effect of rumen sampling site on ruminal protozoa numbers, pH, and VFA and NH_3 N concentrations in lactating dairy cows.

Measurement	Sampling site			SE
	Dorsal	Ventral	Anterior	
Protozoa[1]				
Entodiniomorphs, $\times 10^4$/g	83.3[a]	42.8[b]	36.1[b]	3.62
Holotrichs, $\times 10^4$/g	.56	.46	.46	.082
Total protozoa, $\times 10^4$/g	84.4[a]	43.3[b]	36.5[b]	3.63
pH	5.76[a]	6.19[b]	6.30[c]	.033
VFA, μmol/ml				
Total	141.2[a]	121.0[b]	125.9[b]	3.45
Acetate	88.4[a]	74.9[b]	78.6[b]	2.14
Propionate	31.2[a]	26.5[b]	27.5[b]	.81
Isobutyrate	1.37[a]	1.16[b]	1.17[b]	.026
Butyrate	17.0[d]	14.7[e]	14.9[e]	.48
Isovalerate	1.48	1.35	1.43	.094
Valerate	2.72[a]	2.28[b]	2.25[b]	.076
Acetate:propionate	2.87	2.85	2.89	.020
NH_3 N, mg/dl	14.4[a]	10.9[b]	11.7[b]	.35

[a,b,c]Means with different superscripts differ ($P<.01$).

[d,e]Means with different superscripts differ ($P<.05$).

[1]In strained rumen contents.

did not differ among sampling sites. In contrast, with the exception of valerate, molar percentages of all VFA were not different among sampling sites (data not shown). Mean NH_3 N concentrations (Table 2) were higher ($P<.01$) in rumen fluid from the dorsal sac than from the ventral or anterior sac and were similar between the ventral and anterior sacs.

Wiedmeier et al. (23) and Wohlt et al. (24) also reported higher VFA and NH_3 N concentrations in the dorsal sac than in the ventral sac in dairy cows, which was similar to the results of the present study. Higher concentration of fermentation end products in the dorsal sac than in the ventral or anterior sac was associated with the predominance of protozoa in the dorsal rumen in the present study (Table 2). A greater bacteria concentration has also been observed in the dorsal sac than in the ventral sac in Holstein heifers (7). These observations indicate that microbial fermentation activity is highest in the dorsal region of the rumen.

The lower pH in the dorsal sac in this study appeared to be associated with the higher VFA concentration in the dorsal region than in the other regions of the rumen (Table 2). The highest rumen pH occurred in digesta from the anterior sac. The contribution of saliva may influence pH values in the anterior sac because of the proximity of the anterior sac to the esophageal orifice. In addition to higher microbial population density, the lower pH in the dorsal sac may contribute to the higher NH_3 N concentration. In a lower rumen pH, more NH_3 in the dorsal sac would be in the ionized form, which would result in less NH_3 diffusing across the rumen wall (13, 24).

Entodiniomorph protozoa numbers were affected by sampling site within the rumen. It is difficult to interpret this observation, particularly since limited information is available regarding the location of protozoa in the rumen. However, many factors may be involved. Attachment or sequestration (5) of entodiniomorphs to particulate matter, which is abundant in the dorsal sac, may offer an explanation. It is also probable that more substrates, both feed and bacteria, in the dorsal region may serve as chemotactic factors that attract entodiniomorph protozoa (2, 5, 21).

Holotrich protozoa numbers were distributed equally among ruminal locations (Table 2). Holotrichs have been reported to be more susceptible to extremely low pH (below 6.0) than are entodinia (6). A more acidic dorsal environment observed in the present study may have

inhibited the growth or survival of holotrichs even though there is abundant substrate in the dorsal rumen. In addition, maintenance of a holotrich population is independent of bacterial ingestion as a source of food (3). The structural nature of protozoa cells may also explain the phenomena of holotrich distribution in the rumen. A more flexible cell structure and greater motility of the holotrichs may facilitate their migration in stratified rumen contents (16). In contrast, with a more rigid cell structure and lower motility, most entodiniomorph protozoa may be retained within the fibrous dorsal pad during ruminal contractions.

Effect of Sampling Time

Holotrich numbers varied ($P<.05$) with time after the morning feeding when data across sampling sites were pooled. However, total protozoa numbers (entodiniomorphs and holotrichs) were not affected by time of sampling ($P>.10$).

Total ruminal VFA concentration (Table 3) tended to increase 2 h after feeding and remained elevated for 8 h after the morning feeding. Variations in individual VFA were found in which acetate and propionate concentrations followed a similar trend as total VFA after the morning feeding. Butyrate and valerate concentrations increased linearly ($P<.05$), and isovalerate varied in quadratic ($P<.05$) and cubic ($P<.05$) fashions over sampling time. When expressed as molar percentage (data not shown), the results for isovalerate and valerate were unchanged. Other VFA showed no difference in molar percentage or concentration over time. The ratio of acetate to propionate tended to decrease after the 0730-h feeding. Concentrations of NH_3 N in rumen fluid fluctuated in a cubic ($P<.05$) pattern with time after feeding (Table 3).

Dennis et al. (9) also observed a similar postprandial trend for changes of rumen holotrichs in heifers fed a restricted supply of semipurified diets. Holotrichs sequester on the wall of reticulum and migrate into the rumen for only a few hours after feeding in cattle, resulting in a postprandial increase in ruminal holotrich numbers (1). Despite the variation in holotrich protozoa over sampling time, total protozoa (entodiniomorphs plus holotrichs) numbers observed in the present study did not change after feeding since holotrichs contributed less than 1.5% of total protozoa numbers. Some studies (17, 18) have found that total protozoa numbers are maximal during the prefeeding period and decrease to a minimum 4 to 12 h after feeding in sheep fed at restricted intakes. Michalowski and Muszynski (18) suggested that decreased postprandial protozoa numbers in rumen fluid may be due to an increase in dilution rate of rumen fluid and aggregation and attachment of protozoa to feed particles after feeding. A postfeeding reduction in total protozoa numbers was not observed in this experiment; however, animals, intake, and method of feeding in the present experiment differed from the previous studies mentioned. Few studies are available that examine postprandial changes of rumen protozoa in ruminants consuming the amount of DM as in the present experiment.

Peak rumen NH_3 N concentration in this experiment occurred 2 h after the morning feeding. The higher NH_3 N immediately before feeding than at 8 h after feeding may be due to endogenous metabolism of nongrowing microbes releasing NH_3, and energy sources such as soluble carbohydrates may be minimal for microbial growth and protein synthesis prefeeding (14). In addition, cytolytic microorganisms that digest other rumen microorganisms with the release of NH_3 may be a significant source of NH_3 before feeding (20). However, saliva secretion may also be a contributing factor to the prefeeding increase in the concentration of NH_3 N in the rumen, as indicated by Wohlt et al. (24).

There was an interaction effect ($P<.1$) of sampling site × sampling time on NH_3 N (Table 4) as rumen contents from the dorsal sac contained a higher NH_3 N concentrations than rumen contents in the ventral and anterior sacs immediately before and at 2 and 4 h after the morning feeding. However, at 8 h after feeding, NH_3 N was similar among rumen sampling sites. It appears that a representative rumen contents sample can be obtained at 8 h postfeeding, regardless of rumen sampling site, for NH_3 N determination. A source of variation for NH_3 N values is added by rumen sampling site if rumen digesta samples are taken through rumen cannula within 4 h after feeding. Our

TABLE 3. Effect of sampling time on ruminal protozoa numbers, pH, and VFA and NH_3 N concentrations in lactating cows.

Measurement	Sampling time[1]				SE
	0 h	2 h	4 h	8 h	
Protozoa[2]					
Entodiniomorphs, $\times10^4$/g	52.6	54.6	54.4	55.4	4.22
Holotrichs, $\times10^4$/g	.52	.76	.23	.6	$.96^{L,Q,C}$
Total protozoa, $\times10^4$/g	53.1	55.3	54.6	55.9	4.24
pH	6.40	6.04	6.00	5.87	$.038^{L}$
VFA, μmol/ml					
Total	118.2	133.5	135.9	131.1	3.91
Acetate	75.4	83.5	83.7	80.0	2.44
Propionate	25.3	28.9	30.0	29.4	.91
Isobutyrate	1.21	1.28	1.24	1.20	.030
Butyrate	12.8	15.9	16.7	16.7	$.54^{L}$
Isovalerate	1.42	1.28	1.65	1.31	$.106^{Q,C}$
Valerate	1.98	2.58	2.65	2.46	$.086^{L}$
Acetate:propionate	3.03	2.90	2.82	2.74	.023
NH_3 N, mg/dl	11.0	18.2	12.2	8.0	$.42^{L,Q,C}$

[L,Q,C]Linear, quadratic, and cubic effect, respectively ($P<.05$).

[1]Hours postfeeding.

[2]In strained rumen contents.

data indicate that major fluctuations in NH_3 N concentrations occur in the dorsal sac in relation to feeding time. The largest differences in rumen NH_3 N concentration between the dorsal sac and other regions occurred at 2 h after the morning feeding. There was no significant sampling site by sampling time interaction effect on ruminal protozoa numbers or VFA concentrations.

Method of Rumen Contents Preparation

The effect of sample preparation on ruminal protozoal numbers and pH is presented in Table 5. Strained preparation of rumen contents contained higher ($P<.01$) total protozoa numbers, particularly when sampled from the dorsal and ventral sacs. Numbers of entodiniomorphs in the anterior sac, and numbers of holotrichs in the ventral sac were not different when strained and unstrained rumen contents were compared.

In this experiment, protozoa in the strained rumen contents were those present in the liquid phase and associated with small particulate matter in the rumen. However, protozoa counts obtained in the unstrained preparation of rumen contents would presumably represent protozoa associated with large particulate matter in the

TABLE 4. Mean concentration (mg/dl) of NH_3 N in rumen fluid for sampling site versus sampling time.

Sampling site	Sampling time[1]				Mean
	0 h	2 h	4 h	8 h	
Dorsal sac	12.8^{a}	21.7^{a}	14.6^{a}	8.8	14.4^{a}
Ventral sac	9.7^{b}	16.1^{b}	10.7^{b}	7.1	10.9^{b}
Anterior sac	10.6^{b}	16.7^{b}	11.3^{b}	8.2	11.7^{b}

[a,b]Means in the same column with different superscripts differ ($P<.05$).

[1]Hours postfeeding; SE = .78.

TABLE 5. Effect of sample preparation on protozoa numbers and pH in rumen contents.

Measurement	Sample preparation		SE
	Strained	Unstrained	
Entodiniomorphs, $\times 10^4$/g			
Dorsal	83.8[a]	29.9[b]	2.46
Ventral	43.2[a]	32.5[b]	2.36
Anterior	36.1	31.5	2.46
Mean	54.4[a]	31.3[b]	1.40
Holotrichs, $\times 10^4$/g			
Dorsal	.56[a]	.13[b]	.081
Ventral	.43	.46	.078
Anterior	.46[a]	.18[b]	.081
Mean	.48[a]	.25[b]	.046
Total protozoa, $\times 10^4$/g			
Dorsal	84.4[a]	30.1[b]	2.48
Ventral	43.6[a]	33.0[b]	2.38
Anterior	36.5	31.7	2.48
Mean	54.4[a]	31.6[b]	1.41
pH	6.09	6.11	.02

[a,b]Means with different superscripts differ ($P<.01$).

rumen, especially when samples were from the dorsal sac. High numbers of protozoa in the unstrained rumen contents indicated their strong association with particulate matter in the rumen. Ruminal pH was similar between strained and unstrained rumen contents.

CONCLUSIONS

The results of this experiment demonstrate that in lactating dairy cows receiving a corn silage-concentrate diet, rumen sampling site and sampling time are essential variables that should be considered when obtaining or comparing ruminal measures of protozoa concentration and fermentation end products. The presence of high numbers of protozoa observed in the dorsal rumen was associated with high concentrations of microbial fermentation end products. The manner in which rumen contents are prepared can affect the estimation of protozoa numbers and is dependent on rumen sampling site.

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