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Variation in Numbers and Mass of Ciliate Protozoa in the Rumens of Sheep Fed Chaffed Alfalfa (*Medicago sativa*)

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Masses and numbers of rumen ciliate protozoa were markedly different in individual sheep fed chaffed alfalfa hay under different feeding regimens. Studies on the ciliate contribution to specific aspects of rumen fermentation should take into account the size of members of each genus in individual animals as well as the numbers present.

Ciliate protozoa have long been known to be an integral part of the rumen microbiota of ruminant animals on most diets, but there are still difficulties in defining their role and importance in the breakdown and digestion of ingested feedstuffs and their contribution to microbial protein passing from the rumen. Assessment of the ciliate population of the rumen by counting has long provided data for estimating the contribution of the protozoa to digestion and overall fermentation and to microbial protein synthesis. Because each ciliate genus has a characteristic metabolism, it has been possible, by considering the numbers present, to estimate the contribution of various genera to specific aspects of digestion (8, 11). However, progress in defining the role of the ciliates has been slow, partly because workers often fail to define the ciliate populations they are studying. As with bacteria, ciliate populations may be qualitatively and quantitatively different in individual animals in an otherwise apparently homogeneous group (3, 6, 13) and thus may contribute in different fashions to the many metabolic activities of the microbiota. Also, there have been relatively few determinations of actual masses of ciliate genera on a total rumen basis (7, 8, 10, 11, 15).

The study described here shows remarkable variability in the numbers, sizes, and masses of ciliate protozoa in the rumens of individual sheep in groups fed chaffed alfalfa hay under different regimens.

Protozoa were counted and measured in formolized samples of rumen contents from 32 sheep individually housed indoors in metabolism crates and fed chaffed alfalfa hay (*Medicago sativa* L.) at two levels of dry matter (DM) intake, 1,000 or 700 g. Within each intake level there were two feeding frequencies, hourly and once daily. The sheep fed once a day were trained to consume their daily ration in 3 to 4 h, commencing at 0900 h. Any residual food was removed at 1200 h (700-g intake group) or 1300 h (1,000-g intake group), dried, and weighed so that DM intake could be calculated. Water was available continuously.

After at least 4 weeks of controlled feeding the sheep were slaughtered, and the contents of the rumens and reticulums were removed separately and weighed. The sheep fed hourly were slaughtered at 0900 h, whereas those fed once daily were slaughtered at one of three times: 0830 h, 1330 h, or 2200 h. Each combination of feeding regimen and time of slaughter was repeated consecutively with four animals. The samples of mixed rumen contents taken for counting and DM determination were assumed to be representative of the overall contents of the rumens and reticulums.

The weighed samples in Formalin were diluted to 4:1 with tap water and thoroughly mixed by shaking by hand. The numbers of ciliate protozoa present were determined three times in a counting chamber constructed of microscope slides (2). About 200 ciliates were identified and counted. The numbers of each genus of ciliate in the original sample of rumen contents were calculated. Entodinia were counted as two groups, large and small, with cells less than 29 μm in length being designated as small. Errors were calculated as ±5% (2). Few (<4%) ciliates remained attached to the plant fragments after being shaken with water in the dilution flask (4). Numbers in the rumens and reticulums were obtained by multiplying the weight of the contents by the ciliate concentration. Formulas for the calculation of the cell volume (V) of individual ciliate genera were derived for each genus from a detailed consideration of the basic shape and the length (L), width (W), and depth of the cells. These formulas were: *Dasytricha*, $V = 0.48\ LW^2$; *Entodinium*, $V = 0.45\ LW^2$; *Epidin-*

TABLE 1. Concentrations, volumes, and masses of ciliates in rumens of sheep fed chaffed alfalfa hay under various regimens

Sheep no. and regimen[a]	No. and vol for each ciliate genus[b]								Rumen contents (g)	Rumen DM (g)	Ciliates in rumen (g [dry wt])[c]
	Entodinium		*Epidinium*		*Eudiplodinium*		*Dasytricha*				
	10^3 g^{-1}	Vol (ml)	10^3 g^{-1}	Vol (ml)	10^3 g^{-1}	Vol (ml)	10^3 g^{-1}	Vol (ml)			
1-H24	606	32	165	114	35	86	43	30	3,829	496	29
2-H24	366	22	125	114	33	96	22	26	4,968	643	28
3-H24	401	31	0	0	28	123	24	58	7,135	860	23
4-H24	367	24	0	0	45	136	20	22	4,819	603	20
5-Hla	278	14	83	66	21	77	57	60	4,696	442	24
6-Hla	321	19	0	0	15	45	46	52	4,882	497	13
7-Hla	179	9	69	70	14	32	32	28	4,318	496	15
8-Hla	668	33	169	218	50	145	126	183	5,539	557	64
9-Hlb	138	15	18	25	7	33	14	57	9,265	1231	14
10-Hlb	138	18	31	63	7	69	32	147	9,834	1256	33
11-Hlb	152	20	36	80	12	60	33	142	9,505	1286	33
12-Hlb	347	37	148	262	49	195	52	142	7,595	1058	70
13-Hlc	155	13	37	47	33	77	8	15	6,188	824	17
14-Hlc	226	18	47	60	33	112	16	26	5,811	777	24
15-Hlc	240	27	28	54	6	26	27	65	7,105	863	19
16-Hlc	134	14	49	82	10	33	0	0	7,435	1030	14
17-L24	482	23	55	37	35	102	35	26	3,958	501	21
18-L24	191	6	79	37	30	37	21	7	2,581	281	10
19-L24	336	18	0	0	22	47	17	16	4,422	513	9
20-L24	133	9	35	49	9	37	28	50	6,127	525	16
21-Lla	124	5	20	15	8	21	50	42	4,206	305	9
22-Lla	521	20	54	32	23	65	41	28	3,772	317	16
23-Lla	242	16	0	0	10	26	85	101	5,008	435	16
24-Lla	678	33	249	247	45	100	168	145	4,259	305	58
25-Llb	119	9	33	55	14	51	14	31	6,887	870	16
26-Llb	126	8	24	21	14	32	37	46	5,096	726	12
27-Llb	160	16	0	0	10	41	17	41	7,159	952	11
28-Llb	122	9	32	54	9	34	21	52	7,196	868	16
29-Llc	91	7	24	41	7	25	34	57	5,926	611	14
30-Llc	189	12	43	55	2	53	47	57	5,057	564	19
31-Llc	255	23	19	42	13	41	13	30	6,910	776	15
32-Llc	434	27	95	115	118	321	46	59	5,202	538	57

[a] Sheep were given 1,000 g (H) or 700 g (L) of chaff daily at two feeding frequencies: hourly (24) or once a day (1). Samples were obtained at slaughter from the latter group at 0830 h (a), 1330 h (b), or 2200 h (c). The samples of mixed rumen contents taken for counting and DM determination were assumed to be representative of the overall contents of the rumen and reticulum.

[b] Volume = total number of cells × cell volume.

[c] Mass = (volume [milliliters] × 1.1)/10.

ium, $V = 0.61\ LW^2$; and *Eudiplodinium*, $V = 0.52\ LW^2$. Depth was a nearly constant proportion of width in all cases, and its measurement was not needed for volume calculations. Separate formulas were developed for each ciliate genus, because a preliminary study of the formulas used by others (5, 9, 10, 12, 14) for entodiniomorphs as a group gave differing results, particularly with different individual genera.

Protozoal masses were calculated from cell volumes by assuming a specific gravity of 1.1 and a DM content of 10% (7), although the content of reserve polysaccharide could cause these values to vary (6). For the calculation of mass, a single volume (47×10^3 μm^3) was used for all dasytrichs (L, 40 to 52 μm; W, 28 to 34 μm; mean of 30 cells). Two size groups of entodinia were used because of the relatively large number of species (about eight) and the wide range of sizes represented in each animal, but a single formula was applied to both groups. The basic shape of the species in each group was the same, and *Entodinium bursa* was not present. Within each group the size range and distribution varied only slightly, and a single cell volume was calculated for each group. This volume was 20×10^3 μm^3 for the large group (L, 29 to 42 μm; W, 18 to 29 μm; mean of 30 cells) and 7×10^3 μm^3 for the small group (L, 20 to 28 μm; W, 13 to 17 μm; mean of 30 cells). The mean sizes of *Epidinium* and *Eudiplodinium* varied considerably among the sheep, necessitating the calculation of separate cell volumes (from measurements of 10 cells) for each individual animal. The volumes of single epidinia, calculated on that basis, ranged from 148×10^3 to 316×10^3 μm^3, with L ranging from 108 to 139 μm and W ranging from 46 to 61 μm. Eudiplodinial volumes ranged from 376×10^3 to 776×10^3 μm^3, with L ranging from 121 to 167 μm and W ranging from 77 to 100 μm. This variation could reflect differences between recently divided and older cells. The total numbers of each genus in each treatment and slaughter group, the volume occupied by each genus, the calculated masses of the total ciliates, and the total ciliate mass as a percentage of the total rumen DM are shown in Table 1.

There were marked differences in the nature and magnitude of the ciliate populations in individual animals, even within treatment groups. Four genera of ciliates were represented in the 32 sheep: *Entodinium* sp. (Stein), *Epidinium* sp. (Crawley), *Eudiplodinium* sp. (Dogiel), and *Dasytricha* sp. (Schuberg). Not all of these genera were present in each sheep. All of the animals contained entodinia and eudiplodinia, but dasytrichs did not occur in one animal and epidinia did not occur in six. The total concentration of ciliates found (up to 1.1×10^6 g^{-1}) was within the range normally found in the rumens of sheep (6), with entodinia being the most numerous.

The calculated ciliate DM in the rumens of the individual sheep ranged from 9 to 70 g. The absolute ciliate mass in each animal could have been 10% lower. Westerling (15) determined that suspension of ciliates in formolized water can increase their volume by up to 10%. Four sheep had ciliate masses (57 to 70 g) considerably higher than all of the other animals, but in all of the animals but one (Table 1, sheep no. 24) the proportions of ciliate DM to the DM of total rumen contents were within the few reported values for sheep (8, 11), goats, guanacos (5), and reindeer (14).

The differences in ciliate masses in individual sheep found in this experiment suggest considerable differences in the contribution of individual ciliate genera to fermentation in individual sheep and reflect differences in both numbers and volume. The size of individual ciliates is undoubtedly important in terms of enzyme activity, quantities of cell constituents, and capacity for ingesting bacteria and plant fragments. The variation in the mass of any one genus in different animals in these experiments certainly does not allow any accurate prediction on the extent of any specific protozoal activity. It must be remembered also that even relatively minor changes in ciliate populations may substantially affect the bacterial population and its fermentation (3).

The results emphasize that animals must be considered individually if a detailed analysis is to be made of the interrelations within overall rumen fermentation. This means that when certain quantitative aspects of the protozoal contribution to rumen fermentation are being considered in relation to other microbial activities, as will be required for example in modeling exercises (1), ciliate numbers and the size of the individual cells in the populations in individual animals should be taken into account.

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