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NOTES

Diurnal Changes and Effect of Ration on Concentrations of the Rumen Ciliate *Charon ventriculi*†

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Charon ventriculi comprised over 30% of the total protozoa observed in rumen contents of a Flamenga cow fed Rhodes grass hay (*Chloris gayana*). Both percentage of composition and concentration decreased markedly when concentrate was added to the ration or the animal was fed on pasture. Although *C. ventriculi* is classified as a holotrich, concentrations of this species in the rumen appear to follow a diurnal cycle more closely related to the entodiniomorph protozoa.

The rumen ciliate *Charon ventriculi* was first described by Jameson in 1925 (10). He examined rumen contents from 70 animals in England and observed low numbers of *C. ventriculi* in seven cattle and one sheep. The genus *Charon* was subsequently placed in the family *Blepharocordiae*, subclass *Holotricha*, by Hsiung (6). In later studies, Hsiung (7, 8) reported the occurrence of this species in both Chinese cattle and sheep; however, its occurrence was rare, and numbers were always low. The senior author has also observed low numbers of *C. ventriculi* in several cattle and sheep in Wooster, Ohio. Somewhat in contrast to the above observations, a recent study from Finland reported that the genus *Charon* comprised over 50% of the total rumen ciliate population in sheep fed grass silage (14). Neither of the more common genera of holotrichs, *Isotricha* and *Dasytricha*, was present in their animals. During the course of studies on rumen protozoa in Brazilian cattle, one animal was encountered which contained a very high percentage of *C. ventriculi*. Because the occurrence of this species is limited and numbers are generally quite low, it was desirable to investigate such factors as effect of ration and diurnal changes in concentrations. The results of this study constitute the present report.

The animal used in this work was a rumen-fistulated Flamenga cow, approximately 5 years old. Except for the period when the animal was on pasture, she was housed alone in a pen with concrete floor and walls, and fed once daily at 10:00 a.m. Composite samples of contents from various locations within the rumen were collected through the fistula by hand. Procedures for preservation of samples, total and differential counts, morphological studies, and cell measurements have been described (5). All protozoan counts are the mean of two or more replicate subsamples. Photomicrographs were taken with a Polaroid camera, model ED-10.

Measurements of 50 specimens of *C. ventriculi* are presented in Table 1. In his original description of the species, Jameson (10) reported a range in cell size from 24 to 36 μm for length and 12 to 15 μm for width. Thus, the organisms encountered in the present work would appear to be slightly larger than those he observed.

Figure 1a to f are photomicrographs showing the various morphological structures of this organism. Specimens in Fig. 1a to c were stained with Lugol iodine, and the camera has been focused to show the two tufts of cilia located near the posterior end of the body. The esophagus can readily be seen in Fig. 1a. The specimens in Fig. 1d to f were stained with methylene blue and illustrate the variation observed in location of the macronucleus.

The effect of ration on total protozoan concentrations and generic distribution are shown in Table 2. Samples were collected at 10:00 a.m. which was just before feeding except when the animal was on pasture. Some difficulty was encountered in feeding green chop, because the

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daily portion was never completely consumed. This is reflected in lower total protozoan numbers. In general, total protozoan concentrations and the percentage of *Entodinium* were low when the animal was fed grass hay or green chop. Concentration of all protozoa and the proportion of *Entodinium* both increased when the animal consumed pasture alone, grass hay plus concentrate, or pasture plus concentrate. In contrast, both the percentage and concentration of *C. ventriculi* were highest when grass hay was fed alone. *Diplodinium* percentages were higher on green chop, whereas *Dasytricha* percentages, except for the fourth sample on grass hay, were fairly consistent across all rations. These effects with respect to ration are similar to those observed by other workers for *Diplodinium* and *Dasytricha* (1, 9, 11). The percentage of *Entodinium* with Rhodes grass hay was much lower than values reported from the U.S. for cows fed alfalfa or orchard grass hays (H. C. Puch, M.S. thesis, Ohio State University, Columbus, 1977); however, Clarke (2) observed very low percentages of *Entodinium* in New Zealand cows fed fresh red clover or grass hay (8.0 to 35.4%).

TABLE 1. *Dimensions of C. ventriculi from rumen contents of a Flamenga cow fed Rhodes grass hay (C. gayana)*[a]

Dimension	Mean	σ	Observed limits of variation
Length	35.4 μm	±2.8	27.5–40.7 μm
Width	16.5 μm	±1.3	14.3–18.7 μm
Length/width ratio	2.16	±0.13	1.92–2.42

[a] Measurements of 50 specimens.

After 16 and 20 days on the grass hay ration, samples were taken at 23, 0, 0.5, 1.5, 3, 6, 12, 22, 23, 0.5, 1.5 and 3 h after feeding. The diurnal concentrations of *Entodinium, Diplodinium, Charon, Dasytricha,* and *Isotricha* are presented in Fig. 2 and 3. Previous studies have indicated that diurnal concentration changes differ between the entodiniomorphs and holotrichs

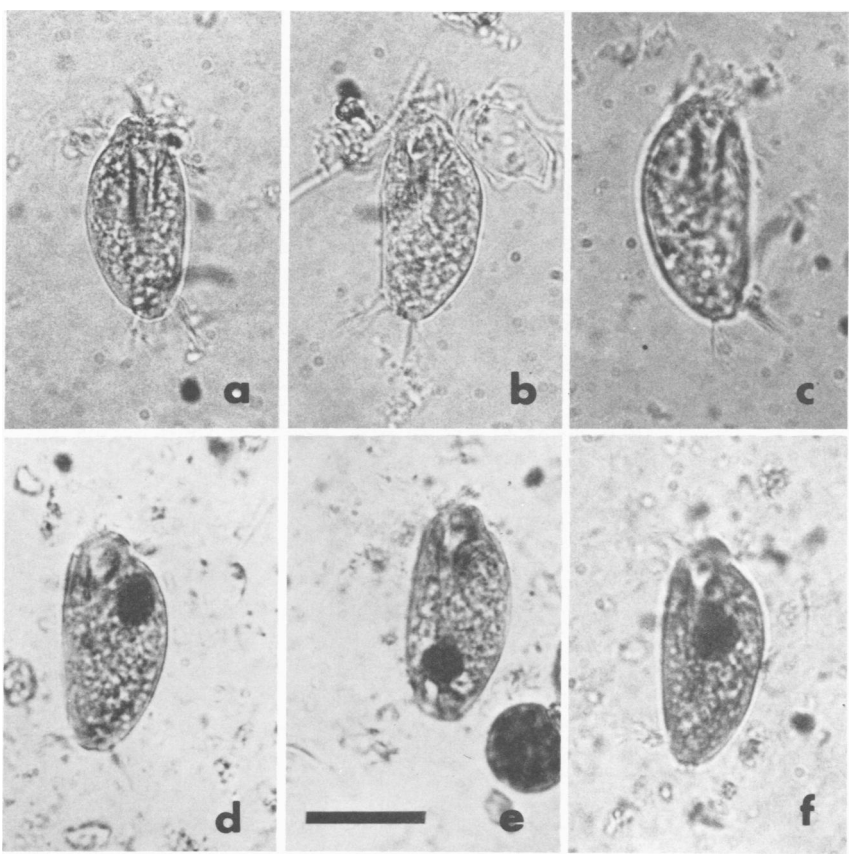

FIG. 1. *Photomicrographs of C. ventriculi. (a to c) Cells stained with Lugol iodine and focused on posterior tufts of cilia; (d to f) cells stained with methylene blue, showing variation in location of the macronucleus. Bar = 20 μm.*

TABLE 2. *Effect of ration on concentration of total protozoa and generic distribution*

Ration	Successive days on each ration	Total protozoa ($\times 10^{-3}$/ml)	Generic distribution (% of total protozoa)				
			Entodinium	*Diplodinium*	*Isotricha*	*Dasytricha*	*Charon*
Pasture + conc[a]	21	1,236	92.7	4.0	0.5	1.5	1.3 (16.0)[b]
Grass hay[c]	15	96	46.7	6.7	0	7.5	39.1 (37.5)
	16	118	44.3	15.9	0	3.4	36.5 (43.2)
	17	122	55.3	10.7	0.6	4.3	29.0 (35.5)
	20	195	44.4	8.2	1.2	15.7	30.5 (59.4)
	21	141	49.8	13.9	1.6	2.8	32.0 (45.2)
Pasture[d]	17	230	75.7	13.5	0.4	4.5	5.9 (13.6)
Green chop[e]	7	70	55.1	30.7	0	5.1	9.1 (6.4)
	11	60	50.8	19.9	2.0	6.0	21.3 (12.8)
Grass hay + conc[f]	9	344	86.6	7.6	0	5.5	0.3 (1.0)

[a] Native grass pasture plus 3 kg of concentrate (conc) per day.
[b] Numbers of *Charon* ($\times 10^{-3}$) per milliliter of rumen contents.
[c] Rhodes grass (*C. gayana*), 7 kg/day.
[d] Napier grass (*Pennisetum purpurem*).
[e] Napier grass (cut fresh daily and fed in the barn), 60 kg/day.
[f] 5.5 kg of Rhodes grass hay plus 1.5 kg of conc per day.

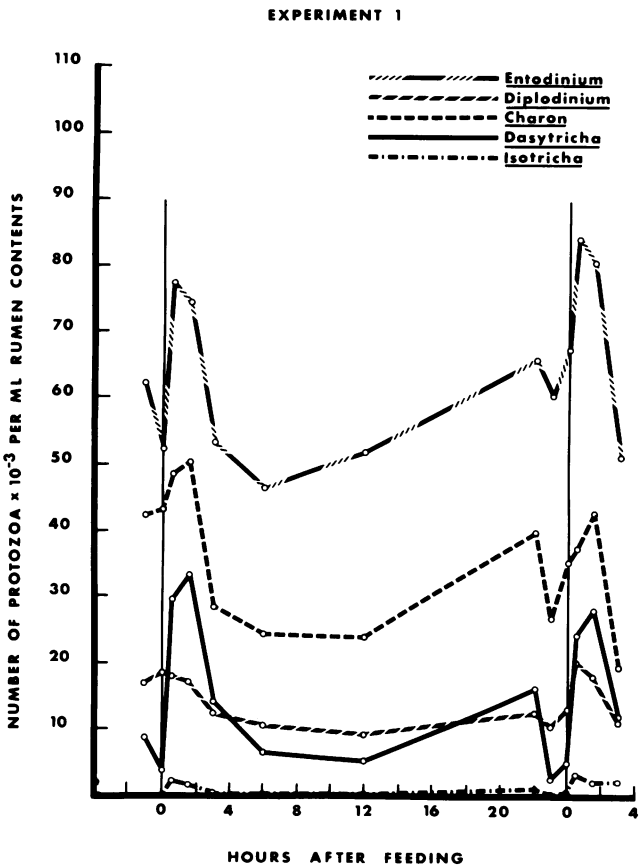

FIG. 2. *Diurnal variations in concentration of the different genera of rumen protozoa in a Flamenga cow fed Rhodes grass hay (C. gayana), experiment 1.*

(11–13, 15). In general, entodiniomorph concentrations are highest just before feeding, decrease immediately after feeding, presumably due to dilution effects, and then after 12 h begin to increase again up to prefeeding levels. On the other hand, the holotrichs begin to increase in

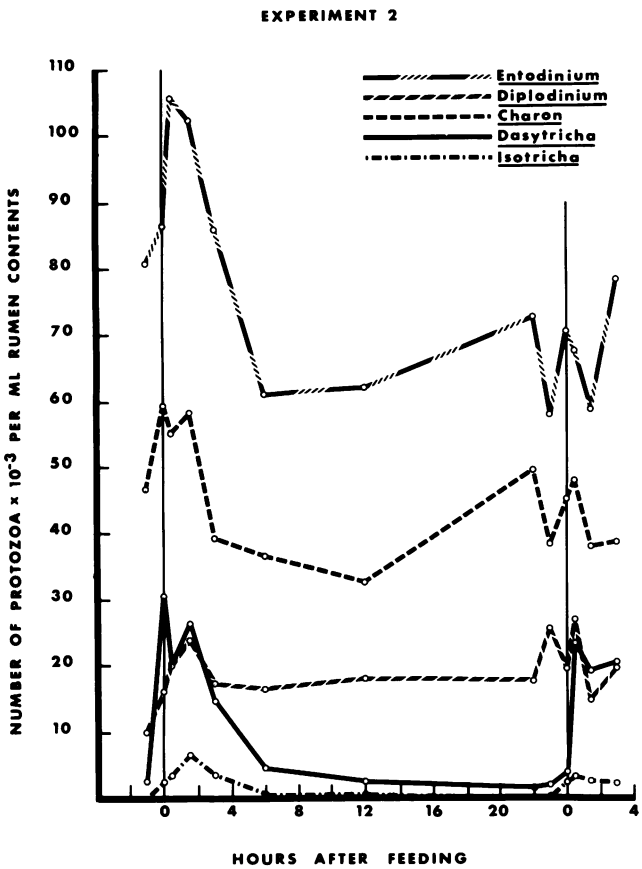

FIG. 3. *Diurnal variations in concentration of the different genera of rumen protozoa in a Flamenga cow fed Rhodes grass hay (C. gayana), experiment 2.*

concentration just before or at feeding, decrease shortly after feeding, and remain at this low level until 20 to 22 h postfeeding. In experiment 1 (Fig. 2) curves for all genera are somewhat similar in shape. However, in experiment 2 (Fig. 3) a definite difference can be noted between the holotrichs and entodiniomorphs, particularly *Entodinium,* during the 12- to 24-h postfeeding period. In this latter case, diurnal concentration changes of *Charon* are similar to those of *Entodinium.*

Another possible way of comparing diurnal variations in the different genera would be to calculate their percentage increase after feeding. The concentrations of each genus at feeding on both days 1 and 2 of experiment 1 were set at 100, and the concentration of organisms at 0.5, 1.5, and 3 h are expressed as a percentage of this value (Table 3). Both genera of holotrichs, *Isotricha* and *Dasytricha,* increased in concentration from four to seven times in the first 30 min after commencement of feeding, whereas none of the other genera even doubled their concentration. Similar results were obtained by Clarke (3), where two- to fourfold increases in the total numbers of rumen holotrichs occurred in the first 3 h after feeding. Values from experiment 2 showed an even higher rate of increase for the holotrich protozoa. It seems unlikely that the rapid increase in holotrich numbers immediately

TABLE 3. *Changes in concentration of protozoa after feeding, expressed as a percentage of their concentration at time of feeding*[a]

Day	Time after feeding (h)	% Concn at feeding				
		Entodinium	*Diplodinium*	*Isotricha*	*Dasytricha*	*Charon*
1	0.5	148	96	600	740	113
	1.5	141	92	475	828	117
	3	102	66	125	352	66
2	0.5	124	155	462	458	106
	1.5	119	138	300	528	121
	3	76	85	312	228	56

[a] Data from experiment 1, Fig. 2.

after feeding could be the result of multiplication. Generation times calculated for the 30-min postfeeding period from the present data were less than 15 min, which would be extremely fast even for a bacterium. Also, dividing forms were not that numerous during the period of increasing numbers. No experimental evidence has been obtained to support the theory of sequestration, either between various sites in the rumen, among feed particles, or among the papillae of the rumen wall (13, 15). Therefore, no explanation can be offered at present for this very rapid increase in numbers of both *Isotricha* and *Dasytricha*.

Based on the results of the two experiments on diurnal changes in concentration, *C. ventriculi* appears to follow a cycle similar to the entodiniomorphs. Further support for this conclusion was provided by their relatively low percentage increase in numbers after feeding, as compared with the holotrichs.

Of particular interest in this study was the observation that in almost all instances, concentrations of the individual genera increased with the commencement of feeding. This would contrast with previous studies (11–13, 15), which all report a decrease after feeding. The only exception would be an increase in either holotrich concentrations or total numbers observed by several investigators (3, 4). A possible explanation for this difference might be that the first sampling time in the previous studies was 2 h after commencement of feeding, as compared with 0.5 and 1.5 h in the present study. Because a sharp decrease in concentration occurred in most cases between 1.5 and 3 h in our data, similar results might have been obtained if the first sample after feeding had been taken at 2 h. The earlier sampling times were incorporated to follow possible increases in holotrich concentrations in response to feeding (4). A second possibility should be considered, that the quality of the grass hay was so poor that lack of energy was severely limiting protozoan growth. When energy became available, rapid growth occurred before the effects of dilution masked the increase in numbers. The extremely low concentrations of total protozoa observed while feeding the grass hay would suggest that its nutritive value was very low.

In both experiments on diurnal concentration changes, concentrations of *Entodinium* and *Charon* increased from 12 to 22 h, as expected for entodiniomorphs; however, concentrations of *Dasytricha* also increased in experiment 1 at 22 h. Concentrations of *Entodinium* and *Charon* decreased between 22 and 23 h in both experiments, but recovered considerably between 23 and 24 h. This might suggest that the animal drank after the 22-h sample was taken. For *Dasytricha* in experiment 1, a similar decrease was observed, but in contrast to the other two genera the concentration fell below that observed at 12 h and little recovery was noted between 23 and 24 h. The possibility that this difference could result from an unequal rate of passage out of the rumen seems unlikely, based on the data of Weller and Pilgrim (16). Also, the results of Wright and Grainger (17) indicate that fluid flow out of the rumen is minimal at this time.

The overall changes in percentage distribution of the different genera with changes in feed are consistent with previous results, i.e., increased concentrations of *Entodinium* when concentrate or starch is added, increases in *Diplodinium* with hay or pasture alone, and a relatively stable concentration of the holotrichs with both ration types (1, 9, 11). However, the sharp decrease in both percentage and concentration of *C. ventriculi* when concentrate was added to the daily ration differs from the results of Syrjälä, Saloniemi, and Laalahti (14). These authors, studying sheep in Finland with a ciliate population containing about 50% *Charon*, added supplements of sucrose or starch to grass silage at levels of 15% and 30% of the daily dry matter intake. No significant changes were observed in either total numbers of protozoa or numbers of *Charon*, although a slight decrease in the percentage of *Charon* was observed at the 30% supplement level for both sucrose and starch. It is difficult to reach any conclusions about energy sources available to *Charon* from their data. Although it would appear from the present data that *C. ventriculi* does not utilize concentrates to any extent for growth, the possibility of this species being more sensitive to lower rumen pH must also be considered.

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