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A proposed simple method for determining the outflow of ciliates from the reticulo-rumen

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ABSTRACT

Two adult male sheep were used to determine the outflow of protozoa from the reticulo-rumen. The outflow of ciliates was calculated on the basis of their concentration in reticular digesta and volume of effluent leaving the reticulo-rumen. The concentration of ciliates from the genus *Entodinium* and *Diplodinium* in reticulum was by 33-63 % and 32-73% lower than in the rumen. The concentration of *Holotricha* varied between 55.7 and 126.8% of the ruminal density. No differences were found between the concentration of protozoa in reticular digesta taken at the contraction and resting phases of the reticulum. The number of ciliates leaving the reticulo-rumen per day equaled 66-177 % of their number in the rumen, and the apparent residence time of protozoa varied between 13.8 and 36.6 h. It was longest in the case of ciliates from the genus *Dipolodinium* and the shortest in the case of *Isotricha*. A technique for the collection of reticular digesta at the contraction and resting phases of the reticulum is described.

KEY WORDS: rumen ciliates, outflow, residence time

INTRODUCTION

The forestomachs of ruminants are inhabited by numerous bacteria, fungi and protozoa. The protozoa are selectively retained in the rumen (Michałowski et al., 1986) but part of their population passes down from the reticulo-rumen to the omasum and then to the abomasum and duodenum. The outflow of protozoa from the

rumen has been measured indirectly, i.e as flow of protozoal protein at the duodenum (Harrison et al., 1979; Steinhour et al., 1982; Whitelaw et al., 1984; Cockburn and Williams, 1984; John and Ulyatt, 1984; Meyer et al., 1986) and by a more direct method, i.e. by counting ciliates in omasal liquid taken from omasum (Weller and Pilgrim, 1974; Punia and Leibholz, 1994) or in omasal effluent (Michałowski et al., 1986). No satisfactory method has, however, been developed yet.

On the other hand it has been shown that the concentration of ciliates in omasal influent and in reticular digesta are almost the same (Harmeyer and Michałowski, 1991). It was also found in the same study that only reticular contents passed down to the omasum and that inflow to omasum was observed only during the second phase of contraction of the reticulum. If this is the case the concentration of ciliates in the reticulum could be used for calculation the number of protozoa leaving the reticulo-rumen over the day. The data cited suggest also that the samples taken at the contraction of reticulum would be the best for this purpose.

The aim of the present study was to determine the daily outflow of protozoa from the reticulo rumen on the basis of their concentration in reticular digesta and compare the obtained results with the data of other authors who measured the outflow of ciliates to lower gut of ruminants.

MATERIAL AND METHODS

Animals and feeds

Two adult male sheep (L and R) weighing 77 and 85 kg and fitted with 100 mm permanent rumen cannulas were used. The animals were kept in separate pens and given 300 g pelleted concentrate (15.5 % protein) and 700 g hay at 8 a.m. and 4 p.m. Water was available all the time.

Experimental procedure

A 2 weeks adaptation period of animals to the food and feeding frequency was allowed before any collection of the samples of rumen and reticular digesta was started.

The samples of reticular digesta were taken *via* rumen cannula with the use of plastic tube of 10 mm ID and 500 mm in length. The tube was fitted with a tight rubber piston (Figure 1). A small balloon of an apparatus for recording contraction of the reticulum was fixed to the tube near its end and introduced into the reticulum during the sampling. The samples of reticular digesta were taken when the second phase of contraction of the reticulum wall had begun or during the resting phase. The samples were taken by suction made by pulling the piston to the opposite end of

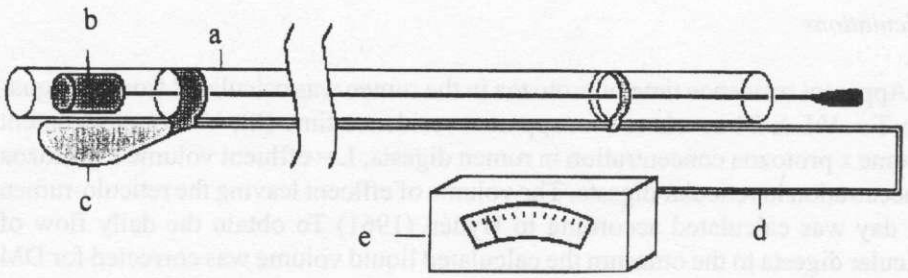

Figure 1. Device construction for collection of the reticular digesta *via* rumen cannula. a – collecting tube; b – rubber piston; c – ballon; d – connecting tube; e– recording apparatus

the collecting tube. The tube was then taken out and the sampled digesta was poured to a beaker. It was subsampled for protozoa counts (5 g) and for dry matter (DM) determination (the remaining part of the sampled material).

Rumen content (about 500 g) was taken from different places in the rumen. The sampled material was thoroughly mixed, subsampled for protozoa counts (2 x 5 g) and returned immediately to the rumen. The collection of rumen content was made immediately after sampling of reticular digesta. Both types of the material was collected at 2, 4, 6 and 8 h after morning feeding. The sampling was repeated four times on four different days.

The pool size of digesta was measured by removing and weighing all the rumen contents. The removed digesta was thoroughly mixed, subsampled for DM determination (20-30 g) and returned immediately to the rumen. The procedure was repeated four times, but no more than 2 times a week.

The volume of liquid leaving the reticulo-rumen was measured with the use of CrEDTA. A solution of marker (60 ml) containing 750 mg Cr was introduced into the rumen at 8 a.m. and samples of rumen fluid (20 ml) were taken at, 1, 2, 3, 4, 6, 8, 10, 14, 20 and 24 h thereafter. The collected samples were centrifuged at 20.000 g for 15 min and supernatant was used for Cr determination.

The experiment was repeated three times.

Analyses

Samples for protozoa counting, fixed in 4% formaldehyde solution (1:1 w/v) were analyzed under light microscope. *Entodinia* were counted in a Fuchs-Rosenthal chamber while other ciliates by the method described earlier (Michałowski, 1975). The protozoa were identified according to Dogiel (1927) and Grain (1966). Dry matter was determined by drying the samples at 95°C for 48 h. Cr was estimated by atomic absorption spectrometry as described by Michałowski et al. (1986).

Calculations

Apparent residence time of protozoa in the rumen was calculated from the equation T = V:L x 24 h; where T = apparent residence time (h), V = rumen content volume x protozoa concentration in rumen digesta, L = effluent volume x protozoa concentration in reticular digesta. The volume of effluent leaving the reticulo-rumen per day was calculated according to Hydén (1961) To obtain the daily flow of reticular digesta to the omasum the calculated liquid volume was corrected for DM content of reticular digesta.

All of the statistical calculations were made according to Ruszczyc (1970).

RESULTS

The weight of the digesta in the rumen of sheep varied between 12.1 and 17.1 kg and the volume of the effluent leaving the reticulo-rumen was 20.2-33.4 l/d. Mean values are given in Table 1. The concentration of ciliates in the rumen and reticulum of sheep "L" and "R" is presented in Tables 2 and 3, respectively. The „*Entodinium* group" composed of many species of which the most numerous were *Entodinium simplex, Entodinium caudatum* and *Entodinium longinucleatum. Anoplodinium denticulatum, Diploplastron affine, Polyplastron multivesiculatum* and *Ostracodinium obtusum* formed the *Diplodinium* group, while *Isotricha prostoma, Isotricha intestinalis* and *Dasytricha ruminantium* the „*Holotricha* group". Very large variations in the number of *Diplodinium* and *Holotricha* were observed during the sampling period. No *Diplodinia* other than *Polyplastron multivesiculatum* were found in the rumen of sheep "L" on the first sampling day and no more than 160 cells/g during the second collection. The concentration of ciliates from the genus *Diplodinium* in the rumen of sheep "R" increased during the collection period from 1400 to over 28000 per gram. On the other hand, the number of *Holotricha* not exceeded 500 cells/g during the second collection day while the highest concentra-

TABLE 1

Some reticulo-rumen parameters of sheep

Item	Sheep	
	L	R
Rumen content volume, kg	15.1±1.18	13.0±0.75
Rumen content DM, %	8.5±0.62	9.8±0.68
Effluent volume kg/d	28.6±3.36	22.5±1.88
Effluent DM, %	3.7±0.45	3.5±0.67
Rumen content, pH	6.2±0.21	5.8±0.33
Reticular content, pH	6.5±0.14	6.4±0.16

TABLE 2

The concentration of ciliates in the rumen (x 10^3/g) and reticular digesta (% ruminal concentration) of sheep"L" during the contraction and resting phases of reticulum and at different time after feeding (h)

Time	Rumen		Reticulum		
after feedig	Number	SD	A	B	SD
			Entodinium		
2	522.4	154.54	63.4	64.8	11.49
4	547.8	161.71	63.4	62.8	10.37
6	539.9	135.74	61.4	63.2	7.89
8	619.6	133.50	67.0	62.7	7.41
			Diplodinium		
2	2.7	2.63	45.7	35.0	7.06
4	2.8	1.75	60.4	51.7	18.49
6	4.2	3.23	29.2	27.1	6.44
8	3.4	2.15	68.4	58.5	23.31
			Holotricha		
2	12.5	8.24	80.0	58.3	25.51
4	5.2	3.36	93.7	105.8	48.86
6	3.5	2.47	126.8	122.0	36.98
8	5.0	3.76	78.4	59.9	14.16

A – contraction phase
B– resting phase

TABLE 3

The concentration of ciliates in the rumen (x 10^3/g) and reticular digesta (% ruminal concentration) of sheep "R' during the contraction and resting phases of reticulum and at different time after feeding (h)

Time	Rumen		Reticulum		
after feedig	Number	SD	A	B	SD
			Entodinium		
2	474.5	98.39	46.7	52.0	7.00
4	451.9	63.14	54.4	59.7	10.38
6	418.5	74.24	64.5	69.4	12.35
8	463.5	110.71	63.8	66.3	7.81
			Diplodinium		
2	11.7	7.37	62.3	54.6	10.91
4	11.3	8.27	36.6	49.5	15.80
6	12.4	9.55	59.1	53.9	10.23
8	13.7	8.82	50.5	46.5	13.02
			Holotricha		
2	13.0	5.94	55.7	63.1	6.04
4	6.0	2.68	62.9	71.6	20.73
5	5.2	1.25	83.3	85.8	19.96
8	10.3	4.64	96.1	99.9	28.57

A – contraction phase
B– resting phase

tion was over 22000/g. Due to the mentioned variations the standard deviations were commensurately high. Regardless of the day to day variations, the concentrations of *Entodinium* and *Diplodinium* in the reticulum were by about 33-63% and 32-73% lower, respectively as compared with their densities in the rumen. The higher concentration of *Holotricha* in the rumen was noted only 2 and 4 (sheep „L") or 2 and 8 (sheep „R") h after morning feeding. No significant differences were found between the concentration of ciliates in the reticulum contents taken at the contraction and resting phase. The densities of particular populations tended, however, to be, in majority of cases, higher in the samples taken at contraction. Of the *Diplodinium* and *Holotricha* the ciliates *Polyplastron multivesiculatum* and *Dasytricha ruminantium* were counted separately. The concentration of *Polyplastron multivesiculatum* in the rumen of sheep "L" varied from 360 to 3400 and in the reticulum from 160 to 2080/g. The respective concentration in sheep "R" was 800-4480 and 240-2160/g. The population density of *Dasytricha ruminantium* in the rumen and reticulum of sheep "L" and "R" were 1700-20500 and 1800-10800, and 1700-17100 and 1400-12200/g, respectively.

Total number of ciliates in the rumen of sheep "L" as well as outflow rate and apparent residence time of the different groups of protozoa are presented in Table 4. The number of ciliates leaving the reticulo-rumen during a day was equal to 66-174.2 % of their number in the rumen. According to this the apparent residence time varied from 14.7 to 36.6 h. The residence time of *Entodinium* was similar regardless of the calculation basis. The residence time of other populations tended to be longer when it was calculated on the basis of concentration of protozoa in the samples taken at the resting phase of the reticulum.

A significant difference, however, was found in the case of "other *Diplodinia*" and "*Dasytricha ruminantium*" only.

The number of ciliates passing down from the reticulum of sheep "R" varied from 77 to over 177% of their number in the rumen. The smallest outflow was noted

TABLE 4

Total number (x 10^7/rumen), outflow rate (% mean ruminal number/d) and apparent residence time (h) of ciliates in reticulo-rumen of sheep „L". Outflow rate and residence time were calculated on the basis of ciliate number in the raticular digesta during contraction (A) and resting (B) phases of reticulum

Protozoa group	Ciliates		Outflow rate			Residence time		
	Number	SD	A	B	SD	A	B	SD
Entodinium sp.	838.3	212.58	118.6	119.3	13.80	20.5	20.4	3.20
Polypl. multivesiculatum	3.5	0.58	102.8	96.6	21.92	23.8	27.2	6.56
„other *Diplodinia*"	4.0	1.58	86.1	66.0	13.42	28.4	36.6	5.31
Isotricha sp.	1.2	0.11	143.6	116.7	47.59	18.8	23.5	7.80
Dasytricha ruminantium	11.7	4.27	174.2	142.0	68.61	14.7	21.6	6.16

TABLE 5

Total number (x 10^7/rumen), outflow rate (% mean ruminal number/d) and apparent residence time (h) of ciliates in reticulo-rumen of sheep „R". Outflow rate and residence time were calculated on the basis of ciliate number in the reticular digesta during contraction (A) and resting (B) phases of reticulum

Protozoa group	Ciliates		Outflow rate			Residence time		
	Number	SD	A	B	SD	A	B	SD
Entodinium sp.	587.8	93.34	95.7	95.5	11.02	25.2	25.7	2.83
Polypl. multivesiculatum	3.1	1.78	75.6	77.3	18.31	32.7	34.6	9.75
„other *Diplodinia*"	12.4	10.72	95.6	91.6	30.61	27.4	30.6	10.55
Isotricha sp.	2.0	0.41	177.2	171.6	38.32	13.8	15.0	3.28
Dasytricha ruminantium	9.2	2.33	123.8	114.7	23.20	20.2	21.6	3.95

in the case of *Polyplastron multivesiculatum* while the largest in the case of *Isotricha sp.* All the results are presented in Table 5.

DISCUSSION

The results obtained provide direct evidence for reduced concentration of ciliates from the genus *Entodinium* and *Diplodinium* in reticular digesta compared with rumen contents of sheep. The observed reduction can result from selective retention of ciliates in the rumen due to attachment of these organisms to particulate fractions of rumen contents and their sequestration between large particles of food forming a sort of filter on the way from the rumen to reticulum. A similar phenomenon was already observed in earlier investigations (Michałowski, 1990). Conversely no sequestration seems occur in reticulum as the reticular digesta is composed of the liquid phase and very small food particles (unpublished). Due to this no differences were observed between the concentration of ciliates in reticular content and omasal influent (Harmeyer and Michałowski, 1991).

In contrast to *Entodinium* and *Diplodinium*, the concentration of *Isotricha* sp. and *Dasytricha ruminantium* in the rumen and the reticular digesta were sametimes similar. Holotrichs, however, seem to belong to organisms of the liquid fraction of rumen contents. Moreover the cuticula of these ciliates is not very rigid as of entodiniomorphs and, especially, of large *Diplodinium*. Due to this the cells of *Holotricha* are very flexible and this property could make any passage of these ciliates through the filter mentioned above much easier. Noteworthy is also fact that these protozoa temporarily attach to the reticulum wall (Abe et al., 1981). Thus their detachment could raise the concentration in reticular digesta.

The apparent residence time of protozoa in the rumen varied from about 13 to over 36 h. The residence time of *Polyplastron multivesiculatum* and "other *Diplod-*

inia" in the both sheep and *Entodinium* in sheep „R", were similar while that of *Entodinium* in sheep „L" was something shorter as compared with the results obtained by Michałowski et al. (1986). The residence time, however, was calculated there on the basis of concentration of ciliates in the omasal effluent thus any sequestration and/or lysis some of protozoa within the omasum can not be precluded and such a possibility has been suggested by the authors. The residence time of the both „*Holotricha* sp" and *Dasytricha ruminantium* were by two time shorter than calculated by cited authors. These protozoa, however, are very mobile and due to this they could escape of the passage to the omasum.The lysis of holotrichs within the omasum can not be, also precluded. Thus this phenomenon needs experimental explanation.

The aim of this investigation was to verify a suggestion that the concentration of ciliates in reticular digesta could be suitable to calculate the outflow of protozoa down the omasum. The basis for this assumption was similar concentration of ciliates in reticular digesta and in omasal influent (Harmeyer and Michałowski, 1991). Unfortunately there did not exist any possibility for simultaneous collection of the digesta from the reticulum and omasum. Thus it is only possible to compare the obtained results with the data of other authors. The mean concentration of ciliates from the genus *Diplodinium* in reticulum equaled 43-52% of their density in the rumen. The respective values for *Holotricha* were 75-95%. Similar differences were found between the concentration of holotrichs and large and medium entodiniomorphs in the ruminal and omasal digesta (Punia et al. 1992; Punia and Leibholz, 1994). Thus the cited findings suggest that outflow of *Diplodinium* and *Holotricha* could be calculated on the basis of their concentration in reticulum. The concentration of *Entodinium* in reticulum of sheep was by about 31-53% lower than in the rumen. The concentration of small ciliates (presumably, mainly *Entodinium*) in the omasal digesta account only for 26-45% of the density in the rumen (Punia et al., 1992; Punia and Leibholz, 1994). This suggest a possibility of some overestimation when the concentration of *Entodinium* in the reticulum would be the basis for calculation of the outflow of these ciliates down the omasum. On the other hand, however, no differences were found between the concentration of ciliates in the reticular digesta and omasal influent (Harmeyer and Michałowski, 1991). In fact, these last data concern only to the total number of protozoa but *Entodinium* accounted there for over 90% of the total count. Thus further investigation are necessary to explain this discrepancy.

The calculated daily outflow of protozoa equaled 66-180% of their number in whole rumen contents per day. These values are much higher in comparison with the findings of Weller and Pilgrim (1974) who calculated the outflow of ciliates on the basis of their concentration in the liquid taken from the omasal canal. It should be emphasized, however, that the outflow of protozoa described by the mentioned authors seems to be exceptionally low. It was not confirmed by other investigators

either by indirect or by more direct methods (Harrison et al., 1979; Steinhour et al., 1981; Michałowski et al., 1986; Punia et al. 1992; Punia and Leibholz, 1994). The outflow rate of ciliates in this experiment varied in high ranges in relation to the group of protozoa. The outflow of *Polyplastron multivesiculatum* and "other *Diplodinia*" was within the range calculated on the basis of concentration of ciliates in omasal effluent (Michałowski et al., 1986). The outflow rate, however, of *Entodinium* and especially *Holotricha* was faster. It is possible that these ciliates, having more delicate cuticlula, are more susceptible to disintegration in the omasum. Thus their inflow to and outflow from the omasum may not be equal. Such a possibility is suggested by low proportion of protozoal N in the microbial N leaving the omasum of the sheep (Michałowski, 1990). Protozoal N was calculated in that study on the basis of N content in single cells of ciliates and their number leaving the omasum. The obtained results are distinctly lower than those calculated from concentration of different markers or the amino acid profile (Harrison et al., 1979; Steinhour et al., 1982; Cockburn and Williams, 1984; Meyer et al., 1986) as well as on the basis of protozoa concentration in the omasal digesta (Punia et al., 1992).

The presented results and the data of other authors, cited above, seem to suggest that perhaps the concentration of ciliates in the reticulm could be the basis for calculation of the outflow of ciliates from the reticulo-rumen down the lower digestive tract. However, further investigations are necessary to confirm this hypothesis, and especially the experiments enabling simultaneous sampling of the reticular and omasal digesta from the same animals are necessary to confirm this hypothesis. A collection of numerous samples during each day of experiment seem to be also necessary to minimize any possibility of overestimation.

The concentration of ciliates in digesta taken from the reticulum at the contraction and resting phases were similar. This suggests that recording the motility of reticulum can be omitted in the proposed sampling technique. On the other hand, however, recording by the method described in this paper precludes any uptake of a sample of digesta from another compartment in the reticulo-rumen.

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STRESZCZENIE

Prosta metoda do oznaczania wypływu pierwotniaków z czepcożwacza

Dwie dorosłe owce, z trwałymi przetokami żwacza, użyto do badań nad intensywnością odpływu pierwotniaków z żwaczo-czepca do dalszych odcinków przewodu pokarmowego. Tempo odpływu pierwotniaków obliczano na podstawie ich gęstości w treści czepca i objętości płynu wypływającego z żwaczo-czepca. Stwierdzono, że gęstość pierwotniaków z rodzaju *Entodinium* i *Diplodinium* w czepcu była odpowiednio o 33-63 i 32-73 % mniejsza niż w żwaczu. Liczebność *Holotricha*, w czepcu wahała się od 55,7 do 126,8 % ich liczebności w żwaczu. Nie stwierdzono istotnej różnicy między gęstością populacji pierwotniaków w treści reticilum pobieranej w fazie spoczynku i podczas skurczu. Liczba orzęsków odpływających w ciągu doby do dalszych odcinków przewodu pokarmowego wahała się od 66 do 177 % ich średniej liczby w żwaczu, a przeciętny czas ich przebywania w żwaczu wahał się od 13,8, do 36,6 godzin, najdłuższej przebywały w żwaczu *Diplodinia*, najkrócej – *Isotricha*. W pracy opisano metodę pobierania prób treści czepca w fazie jego skurczu oraz w spoczynku.