A study of nutritional status of Finnish reindeer (Rangifer Tarandus L.) in differents months

I. Composition and volume of the rumen microbiota

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Abstract. The rumen microbiota were studied in free-ranging semi-domestic reindeer in Finnish Lapland under the nutritional conditions obtaining at two different sampling times. Qualitative and quantitative investigations were made of the rumen ciliate fauna and quantitative investigations of the rumen bacterial flora. The volume coefficients for rumen ciliates obtained by WESTERLING (1970) and that for rumen bacteria obtained by WARNER (1962) were used to obtain an indication of the volume of the rumen microbe mass in reindeer.

The rumen samples were collected in connection with the round-up and slaughter of reindeer, being taken from 30 animals in December and 29 animals in March. The reindeer slaughtered in December had normal access to food,but those slaughtered in March had grazed on better pastures and received a supplementary feed of hay.

The total number of ciliate cells was over six times as high in March as in December, the numbers being 1 182 900 and 188 300 per ml rumen contents, respectively. The corresponding total numbers of bacterial cells were 9.65×10^9 in March and 6.65×10^9 in December. The reason for the statistically significantly ($P < 0.01$) higher numbers in March than in December is probably the better nutritional conditions of the herd slaughtered in March, not the time of the year.

The ciliate fauna consisted of 19 different species, although not all the species were found in every sample. The percentage composition of the ciliate fauna did not vary considerably between the two sampling times.

The volume of the total microbe mass constituted 8.2% of the rumen contents in March and 1.9 % in December, the average being 5.1 %. The proportion of the ciliate volume in the total microbe mass was clearly higher than that of the bacteria at both sampling times: 7.2 times as high in March and 1.7 times in December, the average being 4.7 times.

Since the importance of the rumen microbiota for the utilization of food by ruminants was established, many investigations have been made of the numbers and kinds of rumen microorganisms and the factors affecting them (WARNER 1965, HUNGATE 1966). The majority of the studies have been devoted to the rumen microbiota of domestic animals, but some have also dealt with semi-domestic or wild animals (GIESECKE 1970). An extensive review of the

rumen ciliate fauna of reindeer is given by WESTERLING (1970), but the rumen bacteria of reindeer appear to have received less attention.

The purpose of this work was to study the quality and quantity of the rumen ciliate fauna and the quantity of the bacterial flora of free-ranging semi-domestic Finnish reindeer *(Rangifer Tarandus L.)* under the nutritional conditions prevailing in two different months.

Experimental procedures

Sampling

The rumen samples studied were collected on 3.XII 1969 and 11.III 1970 from free-ranging reindeer in two areas of Finnish Lapland. The samples taken in December were from animals in Lokka and those in May from animals in Savukoski. The distance between these two areas is about 50 km. The samples were obtained in connection with ordinary reindeer round-ups and slaughter.

The samples from the rumen were collected immediately after the animal had been killed and the forestomachs removed. The wall of the rumen was cut open and the contents were mixed thoroughly with a scoop, after which 25 ml of the rumen contents were measured with a glass measure and transferred to a glass bottle containing 25 ml of 8 % formalin. As a rule, the rumen contents were thickflowing, finely divided and fairly homogeneous, the colour being gray-brown. No stratification was found.

Ciliate cell counts

A Wild-20 microscope with phase-contrast lighting was used for counting the microbe cells.

The ciliate cells were counted and identified in a Fuchs-Rosenthal chamber, as described by WESTERLING (1970). This chamber is divided into 16 rows of 16 square fields. The side of a square is 0.25 mm and the depth of the chamber 0.2 mm. The total capacity of the chamber is thus 3.2 mm^2. Usually the cells in every second square row of the chamber were counted. Four counts were performed on each rumen sample and thus the ciliates of $4 \times (3.2 : 2) = 6.4$ mm^3 of diluted rumen contents were counted. The largest organisms were counted in the whole chamber and half of the result was recorded. The original rumen sample was kept in 8 % formalin solution. It was further diluted with water to make the counting easier to perform. No staining was used, since the ciliates were most easily identified in phase-contrast lighting without staining. The total number of ciliates was calculated by adding the results for the separate species.

Bacteria cell counts

The bacterial cells were counted in a microscope whose ocular was marked with a grid. The numbers of bacterial cells in a given amount of sample can

be calculated, if the area of the coverslip is known and also the area of the grid squares at a certain magnification. The procedure was as follows (SYRJÄLÄ 1967): 0.01 ml of rumen sample, diluted with water and well mixed, was pipetted on to the object slide and covered with a coverslip having an area of 24×24 mm^2 = 576 mm^2. The preparation was checked to see that the cells were evenly distributed under the coverslip, and discarded if the distribution appeared unequal. The ocular grid consisted of 25 squares. When an objective with a magnification of × 40 was used, the area of each square was 24.2×24.2 μ^2 = 586 μ^2. The area of the coverslip thus contained about 982 900 squares. The cells of 24 squares were counted on each preparation. The choice of squares was intended to be as representative as possible of the whole preparation. For instance, the first squares were always taken at the same distance from the margin of the coverslip.

The amount of cells in 0.01 ml of sample was obtained by multiplying the sum of the cells in 24 squares by 40 950 (982 900: 24). Three preparations were made from each rumen sample for the bacteria cell counts. Only the total number of cells was counted, no attempt being made to identify the bacteria.

Determination of the volume of the microbe mass

The volume of the ciliate fauna was calculated by using the cell volume coefficients for each cilliate species determined by WESTERLING (1970):

Dasytricha ruminantium	3.86 $\mu^3 \times 10^4$
Entodinium simplex	1.07 »
E. dilobum	1.51 »
E. damae	0.33 »
E. anteronucleatum	3.60 »
E. quadricuspis	0.78 »
E. bicornutum	0.60 »
E. exiguum	0.26 »
E. longinucleatum	1.21 »
Diplodinium dogieli	17.57 »
D. rangiferi	151.73 »
Eudiplodinium impalae	6.89 »
E. spectabile	21.99 »
Ostracodinum magnum	70.87 »
O. obtusum	21.22 »
O. confluens	26.06 »
Enoploplastron triloricatum	8.49 »
Epidinium ecaudatum	12.09 »
E. gigas	51.92 »

In determining these coefficients, Westerling used the geometrical method introduced by SCHUMACHER (1962):

$$\text{Cell volume} = \frac{\text{Length}}{2} \times \frac{\text{Width}}{2} \times \frac{\text{Thickness}}{2} \times \frac{3}{4}\pi$$

For the bacterial cells the mean volume of 1 μ^3 was used (WARNER 1962). The volume of the whole microbe mass in the rumen was taken as the sum of the volumes of ciliates and bacteria.

Results and discussions

Number and kinds of ciliate and bacteria cells

Ciliate species. The ciliates found in the rumen of the reindeer represented 19 different species (Table 1), which are the same as those found

Table 1. The mean number of ciliate ($n \times 10^3$) and bacteria ($n \times 10^9$) cells per ml rumen contents and the percentage composition of the ciliate fauna.

	Mean numbers		Percentage composition of fauna			
	December	March	December		March	
Number of samples	30	29				
Ciliates, total	188.3	1 182.9	100		100	
Dasytricha ruminantium	0.2	2.3	1.0	1.0	0.2	0.2
Entodinium simplex	10.1	104.4	5.4		8.8	
E. dilobum	5.2	63.5	2.8		5.4	
E. damae	8.1	92.6	4.3		7.8	
E. anteronucleatum	88.0	428.3	46.7		36.2	
E. quadricuspis	28.9	134.3	15.3		11.4	
E. bicornutum	2.9	104.1	1.5		8.8	
E. exiquum	6.1	50.8	3.2		4.3	
E. longinucleatum	3.1	16.6	1.6	80.8	1.4	84.1
Diplodinium dogieli	8.0	21.3	4.3		1.8	
D. rangiferi	0.2	9.8	0.1	4.4	0.8	2.6
Eudiplodinium impalae	6.7	38.3	3.6		3.2	
E. spectabile	2.8	21.3	1.5	5.1	1.8	5.0
Ostracodinium magnum	1.4	3.1	0.7		0.3	
O. obtusum	2.6	32.2	1.4		2.7	
O. confluens	1.8	2.5	1.0	3.1	0.2	3.2
Enoploplastron triloricatum	2.1	18.1	1.1	1.1	1.5	1.5
Epidinium ecaudatum	3.9	12.9	2.1		1.1	
E. gigas	6.2	26.5	3.3	5.4	2.2	3.3
Bacteria, total	6.65	9.65				

by WESTERLING (1970). According to the review of GIESECKE (1970) concerning the rumen protozoa of different ruminant animals, species of the genera *Dasytricha* and *Enoploplastrum* are not found at all in the rumen of reindeer. The 19 species found did not all occur in every animal. Especially in the samples taken in December, some species were completely lacking. The more or less casual absence of some ciliate species from the rumen of some animals in the herd seems to be very common and may depend on chance or some temporary fluctuation (QUINN et al. 1962, WESTERLING 1970).

It has been shown on many experiments (WARNER 1965) that even in animals given the same dietary and environmental treatment the rumen microbial populations may differ greatly both qualitatively and quantitatively. When the same animal is kept under constant conditions, the rumen microbial population may still show marked temporal variation. Besides changes in the diet, starvation has an important influence on the rumen microorganisms. During some days' starvation the microorganisms die out at different rates (MEISKE et al. 1968, WARNER 1965). This is important from the point of view of the present study, since the samples were taken from animals rounded up for slaughtering. During the round-up they are subject to extra exertion and often have to go some days without any food, their only »food» being snow.

Total numbers of cells. Considerable variation was found between the samples in the amounts of different micoroorganisms. The results thus give information about the conditions in the herds rather than in the individual reindeer.

The total numbers of ciliates and bacteria in the samples of March are significantly ($P<0.01$, tested by t-test) higher than in the samples of December (Table 1). This is probably due more to the nutritional conditions than to the time of year. Contrary to the usual situation the nutritional conditions were more favourable for the herd slaughtered in March than for that slaughtered in December. Before slaughtering the animals in the former herd had each received a supplementary feed of about one kilogram of dried hay per day. They were also kept throughout the winter on pasture that had been fenced off and protected from grazing for the previous years. The reindeer herd slaughtered in December had received no extra feed and its pasture had been in continuous use for many years. If the two herds had been kept under normal nutritional conditions, the total numbers of ciliate and bacteria cells could be expected to be higher earlier than later in the winter. It should be mentioned here, that in winter 1969 a thick permanent snow cover was established unusually early in Lapland, so that the reindeer had difficulty in obtaining food as early as in November. Normally the food of reindeer becomes less varied when winter arrives, which can be reflected in the rumen microorganisms. When the animals have normal access to food, it has been noticed (WESTERLING 1970) that the total number of ciliates decreases from summer to winter.

Percentage composition of the fauna. The percentage composition of the ciliate fauna did not show such large variations between the two months as the amounts of cells (Table 1). Especially the representation of different genera or subgenera in the total fauna seemed to vary less than that of the single species. The small entodinia formed the highest proportion of the fauna, on the average 82.5 %. This value is of about the same magnitude as that obtained by WESTERLING (1970). He also found that *Entodinium anteronucleatum* was the only one of the entodinia whose representation did not decrease from summer to winter, which indicates that a lichen diet suits it better than the other species of the genus. In the present study, the entodinia other than *E. anteronucleatum* were better represented in the samples of March than in those of December. *E. anteronucleatum* constituted 43 % of the numbers of *Entodium* in March and 58 % in December. This is understandable, because,

since the animals received hay in March, the proportion of the diet consisting of lichens was presumably smaller then than in December.

The proportions of *Diplodinium*, *Ostracodinium*, *Enoploplastron* and *Epidinium* in the ciliate fauna are of the same magnitude as in earlier experiments (WESTERLING 1970), but the proportion of *Eudiplodinium* is less than half that of the values obtained by Westerling.

Volume of the microbe mass

In the volumes of both ciliates and bacteria there were found statistically significant ($P<0.01$) differences between the two months. The total volume of ciliates per ml rumen contents (Table 2) was about 6 times as high in March

Table 2. The mean volume of ciliates ($\mu^3 \times 10^6$) per ml rumen contents and its percentage distribution by genera or subgenera.

	Volume		Percentage distribution	
	December	March	December	March
Ciliates, total	12 354	72 365	100.0	100.0
Dasytricha	8	89	0.1	0.1
Entodinium	3 678	19 805	29.8	27.4
Diplodinium	1 709	18 612	13.8	25.7
Eudiplodinum	1 077	7 323	8.7	10.1
Ostracodinium	2 013	9 681	16.3	13.5
Enoploplastron	178	1 537	1.4	2.1
Epidinium	3 691	15 318	29.9	21.2

as in December. The largest proportions of the ciliate volume in both months related to the genera *Entodinia* and *Epidinium*. In March the genus *Diplodinium* was also well represented, consitituting 25.7 % of the volume, whereas in December it amounted to only 13.8 %.

The proportion of the total volume of the microbe mass in the rumen contents (Table 3) was found to be 8.2 % for samples in March and 1.9 % in

Table 3. Microbe mass volume as % of rumen contents

	December	March	Average
Ciliates	1.2	7.2	4.2
Bacteria	0.7	1.0	0.9
Total	1.9	8.2	5.1

December, the average being 5.1 %. These values are of about the same magnitude as those obtained for other ruminants (WARNER 1965), but are lower

than the values for rumen ciliates alone reported for reindeer by WESTERLING (1970). He found the total volume of rumen ciliates in reindeer to be about 14 % of the rumen contents which is almost 100 % more than that demonstrated in other hosts and did not observe any variations attributable to diet or season when the animals had normal access to food. This indicates that the magnitude of the ciliate mass in the reindeer forestomachs does not diminish when the animal changes from a protein-rich summer diet to a winter diet poor in protein.

The microbe mass in the rumen is a good index of rumen microbial activity and the protein economy. According to several estimates more than half of the rumen nitrogen is in the form of microbial cells (GRAY et al. 1953, WELLER et al. 1958). The rumen microbe mass can be very important as a protein reserve especially in wild ruminant animals such as reindeer, whose nitrogen intake can be very unstable in the winter. This explains, why the volume occupied by microorganisms in the rumen of wild animals has been observed to be notably high.

The proportion of ciliates in the total volume of the microbe mass is clearly higher in both months than that of bacteria: 7.2 times as high in March and 1.7 times in December, the averege being 4.6 times. In domestic ruminants the ciliate mass has been found to be be roughly equal to that of the bacteria (ABOU AKKADA 1965). The present results indicate that the amount of ciliates decreases more than that of bacteria when the food supply diminishes or the food becomes less varied. This relatively faster decrease in the representation of ciliates can be explained by the fact that they are more sensitive than the bacteria to any changes in the environmental and nutritional conditions (HUNGATE 1966). However, apart from some exceptions, the percentage composition of the ciliate fauna (Tables 1—2) was about the same in the two months. The changes in question thus relate to the total numbers or volume of the ciliate fauna.

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SELOSTUS

Porojen ravitsemustilaa koskeva tutkimus
I. Pötsimikrobiston koostumus ja volyymi

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Helsingin yliopiston kotieläintieteen laitos

Tutkimuksessa selvitetään luonnonvaraisena elävien porojen pötsin alkueläinten määrää ja laatua, bakteerien määrää sekä mikrobimassan osuutta pötsin tilavuudesta kahtena eri ajankohtana. Näytteet pötsistä otettiin erotuksen yhteydessä teurastetuilta poroilta, joulukuussa 1969 30 eläimeltä ja maaliskuussa 1970 29 eläimeltä. Joulukuussa teurastetuilla poroilla oli ollut normaalit luonnonvaraisten porojen ruokinnalliset olosuhteet, kun taas maaliskuussa teurastetut porot olivat olleet paremmilla laitumilla sekä saaneet muutamia päiviä ennen teurastusta lisärehuna heinää.

Alkueläinten kokonaislukumäärä oli maaliskuussa yli 6 kertaa suurempi kuin joulukuussa, vastaavien lukuarvojen ollessa 1 182 900 ja 188 300 per ml pötsin sisältöä. Alkueläimistön prosenttinen jakautuminen eri lajeihin oli sitävastoin lähes samanlainen kumpanakin kautena. Alkueläinlajeja tavattiin yhteensä 19. Bakteerien kokonaislukumäärä oli maaliskuussa 9.65×10^9 ja joulukuussa 6.65×10^9. Syy merkitsevästi ($P < 0.01$) korkeampiin mikrobisolujen lukumääriin maaliskuussa verrattuna joulukuuhun johtuu todennäköisesti laumojen erilaisista ruokinnallisista olosuhteista eikä niinkään vuodenajasta. Koko mikrobimassan osuus pötsin sisällön tilavuudesta oli maaliskuun näytteissä 8.2 % ja joulukuun näytteissä 1.9 % eli keskimäärin 5.1 %. Alkueläinten osuus mikrobimassan tilavuudesta oli selvästi suurempi kuin bakteerien osuus: maaliskuussa 7.2 kertaa ja joulukuussa 1.7 kertaa suurempi, keskiarvon ollessa 4.7.