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Composition and volume of the rumen microbiota of sheep fed on grass silage with different sucrose, starch and cellulose supplements

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Abstract. A comparative study was made of the effect of different sucrose, starch and cellulose supplements and the effect of different silage preservatives on the quality and quantity of the rumen microbiota of sheep fed on grass silage. The levels of the carbohydrate supplements were 15 % and 30 % of the dry matter of the daily rations, representing $2\,^1/_2$ and 5 g/kg animal live weight per day. The silages were prepared with three different preservatives: 1) AIV I solution (25 % formic acid and 20 % hydrocloric acid), 2) formic acid and 3) Viher solution (26 % formic acid and 70 % formalin).

The total number of ciliates was highest in the animals receiving sucrose with the silage, lower in those given starch and lowest in those given cellulose. On the pure silage diet, it was between those for the starch and cellulose diets. The total number of bacteria decreased in the opposite direction on the different diets.

The sucrose supplements increased the numbers of small ciliates especially and the cellulose supplements those of the bigger ciliates. The total volume of the ciliate fauna was thus highest in the animals on cellulose diets, lower in those on starch diets and lowest in those on sucrose diets.

The total microbe mass constituted the following percentages of the rumen content on the different diets: only silage 4.1, 15 % sucrose 3.5, 30 % sucrose 3.5, 15 % starch 4.0, 30 % starch 4.5, 15 % cellulose 4.6 and 30 % cellulose 5.2. Bacteria constituted 77—86 % of the total microbe mass on the different diets, the precentage being highest on the sucrose diets and lowest on the cellulose diets.

Only small differences were found between the different silage preservatives in the effect on the rumen microbiota.

In the fermentation processes occurring during ensiling of grass, the sugars and other soluble carbohydrates disappear from the grass more or less completely, depending on the preservation methods used, producing organic acids and gases. At the same time the solubility of the nitrogenous components increases. As readily fermentable carbohydrates are important as an energy source for rumen microbes, their scarcity or absence might be expected to affect the quality and quantity of the rumen microbiota, and also the utilization of the feed, in animals on grass silage-based diets. However, little information is available on these questions. Another subject that needs study is the effect

of the organic acids formed during silage fermentation and the pH of silage on the rumen microbes. Although the feeding value of lactic acid is almost as great as that of glucose (BARNETT 1954) and the value of volatile fatty acids is considerable, since they are absorbed both directly from the rumen and through the intestinal wall, these fermentation products are not an energy source for so large an amount of the rumen microbes as the soluble carbohydrates.

The use of different carbohydrate supplements with hay and similar forage has been investigated in many experiments (MITCHELL and HAMILTON 1940, HAMILTON 1942, BARNETT and REID 1961, KOMKRIS et al. 1965, SUTTON 1968, KELLOGG 1969, SYRJÄLÄ 1971, SALO et al. 1973 b), with widely varying results. Less attention has been paid to the effect of adding sugars or other readily available carbohydrates as an energy source for rumen microorganisms in silage diets. The digestibility coefficients of silage were found to decrease with increasing amounts of sucrose or starch supplements, whereas with cellulose supplements they tended to increase in some cases. Sucrose supplements seemed to promote the utilization of the nitrogenous components of silage better than starch or cellulose supplements at the same levels, animals on the sucrose diets showing a somewhat higher N balance and lower rumen NH_3-N contents than those on the other diets (SYRJÄLÄ 1972). SALO et al. (1973 a) found that varying the amounts of sucrose and starch supplements had little or no effect on the digestibility of silage by sheep. The capacity of sucrose and starch supplements to increase the live-weight gain, silage intake and feed utilization of growing lambs seemed to depend on the fermentation level of the silage (SYRJÄLÄ 1975).

The purpose of this experiment is to examine the quantity and quality of the rumen microbiota of sheep on pure silage diets and the effect on the microbiota of different sucrose, starch and cellulose supplements. Attention will also be paid to the effect of different silage preservatives. This study is closely connected with the earlier investigation on carbohydrate supplements (SYRJÄLÄ 1972), because it provided the rumen samples for the present experiment.

Experimental procedures

Experimental design and rations

The experiment was performed with nine rumen-fistulated adult Finnsheep rams according to a Latin-square design. The design of the experiment, the composition of the rations and the feeding of the animals are explained in detail in the earlier study (SYRJÄLÄ 1972).

The silages were prepared with three different preservatives: AIV I (25 % formic acid and 20 % hydrochloric acid), formic acid (86 %) and Viher solution (26 % formic acid and 70 % formalin). The carbohydrate supplements given with the silages were pure sucrose, potato starch and sulphite cellulose from the wood industry. Each carbohydrate supplement was given at two levels, constituting 15 % and 30 % of the dry matter of the daily rations, which averaged 928 g. The supplements thus represented about $2\,^1/_2$ g and 5 g per kg animal live weight. Pure silage diets were also given.

Sampling

The samples of rumen liquid were collected as described by SYRJÄLÄ (1972). Only the samples taken in the morning before feeding were used in this microbial work. Five millilitres of rumen liquid was transferred to a glass bottle containing 44 ml of 4 % formol (10 % formalin). Before the counting, 1 ml of methyl green (1 g methyl green and 2 ml glacial acetic acid and 100 ml aqua destillata) was added to each sample. In this way the original rumen sample was diluted to 1: 10, and the nuclei of the ciliates were stained deep bluish green. The staining was completed within 30 minutes.

Counting the ciliates

The ciliate cells were counted and identified by a modification of the method of WESTERLING (1970 a). The modified Fuchs-Rosenthal chamber B. S. 748 Chawksley & Sons Ltd, London), which is divided into 12 rows of 12 square fields, was used. The side of a square is 0.25 mm and the depth of the chamber is 0.2 mm. The total capacity of the chamber is thus 1.8 mm^3. Ten counts were performed on each rumen sample. In this way, the ciliates of 18 mm^3 of diluted rumen contents were counted.

Identification of the ciliates

The species were identified on the basis of the descriptions and figures of DOGIEL (1927), and KOFOID and MACLENNAN (1930, 1932, 1933) and according to the taxonomy of DOGIEL, account also being taken of the principles of NOIROT-TIMOTHEE (1960).

Counting the bacteria

The bacterial cells were counted as described by SYRJÄLÄ et al. (1973). Three preparations were made of each rumen sample for the counts.

Determination of the volume of the fauna and flora

The mean cell volume of each ciliate species was calculated according to the geometrical method introduced by SCHUMACHER (1962).

The mean dimensions used and the cell volumes for each ciliate species are shown in Table 1. The cell volumes are rounded up to two digits on account of computer processing.

For the bacterial cells the mean volume of 1 μ^3 was used (WARNER 1962).

Results and discussion

Effect of sucrose, starch and cellulose supplements

The results are combined for all the silages and are the averages of 54 samples for the 0 % carbohydrate diet and of 18 samples for each of the diets containing carbohydrate supplements. Altogether 162 samples were analysed.

Table 1. Lengths (L), widths (W) and thicknesses (T) of ciliate cells (μ) and the cell volumes (VOL) ($\mu^3 \times 10^4$).

	L	W	T	VOL	Reference
Charon	25	12	10	0.15	1
Entodinium vorax	95	68	60	20	2
E. longinucleatum	43	33	23	1.7	3
E. triacum	35	25	18	0.8	2
E. caudatum	43	33	23	1.7	2
E. loboso-spinosum	39	26	18	1.0	2
E. nanellum	28	15	11	0.25	3
E. dubardi	43	25	18	1.0	2
Diplodinium dentatum	71	56	44	9	3
Eudiplodinium maggii	140	91	83	55	3
E. affine	80	45	40	8	4
E. neglectum	79	47	40	8	3
Polyplastron multivesiculatum	161	95	91	73	2
Enoploplastron triloricatum	90	50	35	8	5

1. Our own measurements 2. DOGIEL (1927). 3. KOFOID and MACLENNAN (1930, 1932, 1933). 4. NOIROT-TIMOTHEE (1960). 5. WESTERLING (1970 b).

Number and kinds of ciliate and bacteria cells

Giliate species. The ciliates found in the rumen fluid of the experimental animals represented 14 different species (Table 2). Not all the species were found in every sample. For instance, *Entodinium vorax* and *Eudiplodinium neglectum* were found in only one third of the samples and *Entodinium triacum*, *E. loboso-spinosum*, *Diplodinium dentatum* and *Eudiplodinium maggii* in two thirds of the samples. Only *Entodinium nanellum* and *E. dubardi* were found in all the samples. *Charon* was the only representative of the holotrichs. It was found in large number and occurred in all the animals except one in one period.

On average, 50 % of the total ciliates were holotrichs *(Charon)* and 50 % entodiniomorphs, about 45 % belonging to the genus *Entodinium* and 5 % to the genus *Diplodinium*.

Descriptions of the ruminal fauna in the literature reveal large variations in the quality and quantity, depending on nutritional and many other factors (HUNGATE 1966). In domestic ruminants on normal feeding, the fauna sometimes comprises more than 30 different species and sometimes less than 10. On some special diets, such as high urea diets, all the ciliates may be lacking (VIRTANEN 1967).

Charon has been found to occur only occasionally in the rumen (HUNGATE 1966), whereas the holotrichs *Isotricha prostoma*, *I. intestinalis* and *Dasytricha ruminantium* occur frequently. However, these *Isotricha* and *Dasytricha* holotrichs were completely lacking in the animals in this experiment. Holotrichs require soluble sugars as a source of energy (HUNGATE 1966). For this reason they are present in especially large number in grazing ruminants and those on a diet containing a considerable fraction of good quality hay. Their absence from the animals on the pure silage diet is thus understandable, as its sugar

Table 2. The mean number of ciliate ($n \times 10^3$) and bacteria ($n \times 10^9$) cells per ml rumen content in sheep on different carbohydrate diets.

	Carbohydrate	Sucrose		Starch		Cellulose	
	0 %	15 %	30 %	15 %	30 %	15 %	30 %
Total ciliates	510.83	544.39	546.12	526.35	526.50	471.46	505.93
Charon	268.78	276.02	241.09	300.49	257.49	195.68	218.11
Entodinium vorax	0.64^{ac}	0.59^{ac}	0.43^{ac}	1.67^{ab}	2.97^{b}	0.12^{c}	0.06^{c}
E. longinucleatum	3.49^{a}	2.90^{a}	4.54^{ab}	5.81^{b}	5.90^{b}	3.58^{ab}	3.83^{ab}
E. triacum	10.43	1.88	0.90	2.60	4.91	1.61	1.58
E. caudatum	2.25^{a}	2.63^{a}	3.00^{a}	15.38^{b}	16.53^{b}	5.44^{a}	7.57^{a}
E. loboso-spinosum	3.76^{a}	4.76^{ab}	5.34^{ab}	1.82^{a}	1.61^{a}	10.19^{bc}	15.57^{c}
E. nanellum	29.37^{ac}	42.78^{bc}	52.57^{b}	22.39^{a}	27.21^{a}	35.74^{ac}	28.66^{ac}
E. dubardi	166.28^{ac}	190.37^{ab}	222.99^{b}	139.62^{c}	178.26^{abc}	185.43^{abc}	191.08^{ab}
Diplodinium dentatum	2.70	4.76	1.45	2.69	1.54	1.54	1.76
Eudiplodinium maggii	1.19	1.39	1.70	1.02	1.02	1.61	3.06
E. affine	4.91^{ac}	3.06^{a}	3.12^{a}	9.55^{bc}	12.29^{b}	5.50^{ac}	9.45^{bc}
E. neglectum	3.63^{ab}	2.07^{a}	1.67^{a}	7.41^{b}	0.37^{a}	0.31^{a}	0.65^{a}
Polyplastron multivesiculatum	1.97^{ab}	1.14^{a}	0.59^{a}	2.93^{ab}	3.98^{b}	8.03^{c}	7.75^{c}
Enoploplastron triloricatum	11.43^{ab}	10.04^{ab}	6.73^{a}	12.97^{ab}	12.42^{ab}	16.68^{b}	16.80^{b}
Bacteria	35.24^{ab}	29.09^{c}	30.18^{bc}	32.47^{bc}	35.76^{ab}	36.71^{ab}	39.52^{a}

Statistical analysis: The Tukey test (STEELE and TORRIE 1960) was applied to the differences between the averages. Different index letters in a horizontal row show that there are significant differences between the averages at the 95 % level of confidence.

content was rather low (SYRJÄLÄ 1972). But it is more difficult to explain why they did not occur when the sucrose supplements were given. One reason may be that before the experiment began the animals were kept for a long time on silage alone, during which period these ciliates probably disappeared. Moreover, during the experiment they were separated from other ruminants and there was no possibility of contamination.

Total numbers of cells. Some differences were found between the total numbers of ciliates in the animals on different carbohydrate diets, but these were not significant ($P > 0.05$). The sucrose and starch supplements increased the total numbers of rumen ciliates and cellulose supplements decreased them, as compared with the pure silage diet (Table 2, Fig. 1). If the number of ciliates on the pure silage diet is taken as 100, the relative values are 107 for the 15 % and 30 % sucrose diets, 103 for both the starch diets, and 92 and 99 for the cellulosa diets.

The numbers of bacteria were lower on the sucrose ($P < 0.05$) and starch diets than on the cellulose diets. When the number of bacteria on the pure silage diet is taken as 100, the relative values are 83 and 86 on the 15 % and 30 % sucrose diets, respectively, 92 and 101 on the starch diets and 104 and 112 on the cellulose diets.

The finding that the sucrose and starch supplements tended to increase the quantity of ciliate cells and decrease that of the bacteria cells, and that cellulose had the opposite effect can be at least partly explained by the different solubility of these carbohydrates. The rumen ciliates ingest sugars and starch very rapidly and thus remove them from bacterial attack (HUNGATE 1966). Cellulose is ingested more slowly, so that the bacteria are probably better able to compete for it. Besides being superior in the competition for food, protozoa can also use bacteria as their food, which explains the decreased bacterial population in faunated sheep as compared to defaunated (EADIE and HOBSON 1962).

The pH of the rumen fluid on the carbohydrate diets was somewhat lower than on the pure silage diets. Even when fermentation was strongest, however, it kept within the limits of 6.1 and 6.9 (SYRJÄLÄ 1972). This range is optimal for ciliates, which are rather sensitive to acidity (PURSER and MOIR 1959). *Entodinium* species are somewhat more resistant to acid than are the holotrichs (ABOU AKKADA et al. 1959), but all the ciliates die rapidly at very high rumen acidities (HUNGATE et al. 1952). The pH range outside which they do not survive has been found to be 5.5—8.0 (KROGH 1959, QUINN et al. 1962). According to HUNGATE (1955), ciliates die at pH 4.5.

Some variations in the numbers of the different ciliate species were found between the diets having different carbohydrate sources. The cellulose supplements decreased the numbers of *Charon*, compared with the pure silage diet, whereas the sucrose and starch supplements had little effect on them ($P > 0.05$). The total numbers of entodinia were highest on the sucrose diets and lowest on the starch diets, especially the 15 % starch diets. However, not all the entodinia species were affected in this way by the starch supplements; on the contrary, only *Entodinium loboso-spinosum*, *E. nanellum* and *E. dubardi* were decreased and the numbers of the other entodinia, especially *E. vorax*, *E. longinucleatum* and *E. caudatum*, were higher on the starch than on the other diets, sometimes significantly so ($P < 0.05$).

The total numbers of diplodinia decreased with the sucrose supplements, especially the 30 % supplement, but increased with the starch and cellulose supplements. On the 30 % cellulose diet the number was 2.6 times as high as on the 30 % sucrose diet.

In grass silage-based diets, the effect of the different carbohydrate supplements on the ruminal fauna can be explained by the nutritional requirements of the different genera (HUNGATE 1966). All the entodiniomorphs, except some small species of *Entodinium*, utilize starch, whereas holotrichs assimilate soluble sugars. Entodiniomorphs are thus usually abundant in the rumen of animals given a high-concentrate ration and holotrichs appear in greatest quantity in animals given hay or other forages rich in soluble sugars (WARNER 1965, ABE et al. 1973). The cultural, enzymatic, and microscopic evidence suggests that cellulose is also digested by many entodiniomorphs (HUNGATE 1966). OXFORD (1955) showed that rumen ciliates are capable of digesting both starch and cellulose and may utilize a number of soluble carbohydrates.

The differences in the numbers of the various ciliate species between the different levels of the same carbohydrate source were not significant ($P > 0.05$), except in the case of *Eudiplodinium neglectum*, which was more numerous ($P < 0.01$) on the 15 % starch than on the 30 % starch diet. This shows that neither the level of the carbohydrate nor the level of the protein in the diet had any clear effect on the rumen microbiota in this experiment. As the silage was the only source of protein in the diet, the protein content decreased when the carbohydrate supplement increased. The crude protein content averaged 18.7 % of dry matter in the pure silage diet, 15.9 % in the 15 % carbohydrate diets and 13.1 % in the 30 % carbohydrate diets (SYRJÄLÄ 1972). However, even at the lowest levels in this experiment, the protein content was too high to inhibit the growth of the rumen microbes. In experiments where a high positive correlation has been found between the level of protein in the diet and the total amount of microorganisms in the rumen, the protein content of the diet has been lower, the maximum being 12 % of dry matter (MOIR and WILLIAMS 1950).

Percentage composition of the fauna. The carbohydrate supplements caused some changes in the composition of the ciliate fauna as compared with that of the pure silage diet (Fig. 3). The representation of the genera and subgenera in the total fauna generally varied less than that of the single species.

Charon constituted more than half of the total ciliate numbers on the pure silage diet and on the 15 % sucrose and starch diets. The lowest percentages for *Charon* were obtained on the cellulose diets. Its representation on the cellulose diets was about 10 % units lower than on the pure silage diet. The proportion of the genus *Entodinium* was lowest on the 15 % starch diet, being sligtly more than one third of the total number of the fauna, and highest on the 30 % sucrose diet and both cellulose diets, where it constituted about half of the fauna. The genus *Diplodinium* composed 3—8 % of the fauna on the different diets, its contribution being lowest on the sucrose and highest on the cellulose diets.

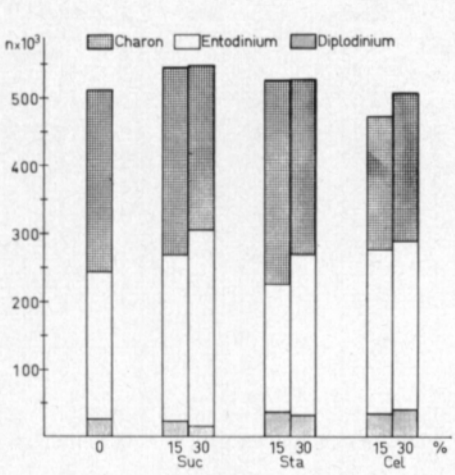

Fig. 1. The mean number of ciliate cells ($n \times 10^3$) per ml rumen content in sheep on different carbohydrate diets (O = only silage, Suc = sucrose, Sta = starch, Cel = cellulose).

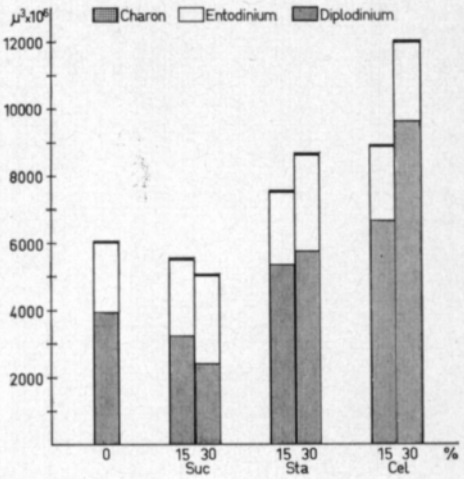

Fig. 2. The mean volume of ciliates ($\mu^3 \times 10^6$) per ml rumen content in sheep on different carbohydrate diets (O = only silage, Suc = sucross, Sta = starch, Cel = cellulose).

Volume of the microbe mass

Significant differences ($P < 0.05$) were found in the total volume of the ciliates between the different diets. The total volumes were highest on the cellulose diets, somewhat lower on the starch diets and lowest on the sucrose and pure silage diets (Table 3, Fig. 2). The changes in total volume thus went in the opposite direction to those in the total numbers of ciliate cells, which decreased from the sucrose diets to the starch diets to the cellulose diets. This is due to the size of the ciliates cells affected by the carbohydrate supplements. Cellulose increased the numbers of the bigger entodiniomorphs especially, whereas sucrose raised the numbers of the small holotrichs. The large ciliate volumes on the cellulose and starch diets, as compared with the sucrose diets, are attributable in particular to the massive *Polyplastron* (Fig. 4).

Table 3. The mean volume of ciliates ($\mu^3 \times 10^6$) per ml rumen content in sheep on different carbohydrate diets and the distribution by genera or subgenera.

	Carbohydrate	Sucrose		Starch		Cellulose	
	0 %	15 %	30 %	15 %	30 %	15 %	30 %
Total ciliates	6049^{c}	5547^{c}	5080^{c}	7541^{bc}	8676^{b}	8914^{ab}	12042^{a}
Charon	21^{ac}	22^{ac}	6^{b}	17^{ab}	20^{abc}	20^{abc}	33^{c}
Entodinium	2085^{a}	2285^{abc}	2637^{bc}	2185^{ab}	2880^{c}	2235^{abc}	2357^{ab}
Diplodinium	3943^{ad}	3240^{ad}	2437^{a}	5339^{abd}	5776^{bd}	6659^{b}	9652^{c}
Diplodinium	243	428	131	242	139	139	158
Eudiplodinium .	1340	1175	1317	1917	1752	1348	2490
Polyplastron	1436^{a}	834^{a}	428^{a}	2142^{ab}	2909^{ab}	3838^{bc}	5660^{c}
Enoplastron .	924^{abc}	803^{ac}	561^{a}	1038^{abc}	976^{abc}	1334^{bc}	1344^{b}

Meaning of index letters same as in Table 2.

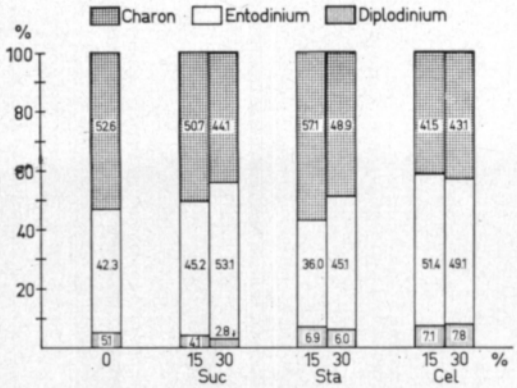

Fig. 3. The percentage composition of ciliate cells on the different carbohydrate diets.

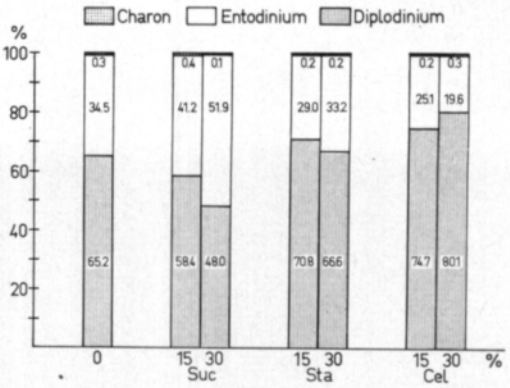

Fig. 4. The percentage composition of the ciliate volume on the different carbohydrate diets.

The total volume of the microbe mass varied from 3.5 % of the rumen content on the sucrose diets to 5.2 % on the 30 % cellulose diet (Fig. 5). The values were significantly higher ($P < 0.05$) on the two cellulose diets than on all the other diets, and significantly lower ($P < 0.05$) on the sucrose diets than on all the others except the 15 % starch diet.

The ciliates constituted a rather small proportion of the rumen content, between 0.5 and 1.2 %. One reason is that some large ciliate species, for instance *Isotricha* and *Dasytricha*, were completely lacking. Ciliate volumes of about this magnitude have found in cows (SCHUMACHER 1962), but the values in domestic ruminants have generally been higher (MOWRY and BECKER 1930, WARNER 1962, HARMEYER 1963).

The contribution of the bacteria to the total microbe volume was fairly high, the average for all the diets being 4.5 times as high as that of the ciliates. The ratio of bacteria to ciliate volumes was highest, 6.0, on the 30 % sucrose diet and lowest, 3,3, on the 30 % cellulose diet.

In earlier studies, the ciliate mass has been found to be roughly equal to that of the bacteria in domestic ruminants (ABOU AKKADA 1965) and 4.6 times as high in semidomestic reindeer (SYRJÄLÄ et al. 1973). In other experiments (OXFORD 1964, WARNER 1965), the bacteria have been found to compose the largest part of the microbial mass, as in the present study.

Effect of silage preservatives

The values for the diet consisting of silage alone are the averages of 18 rumen samples and those for the diets containing carbohydrate supplements are the averages of 54 samples.

Only slight and non-significant differences ($P > 0.05$) were found in the total numbers of ciliates and bacteria between the silages prepared with different preservatives (Table 4, Fig. 6). The differences in the numbers of the various ciliate species were significant ($P < 0.05$ or $P < 0.01$) in only a few cases.

The total numbers of both ciliates and bacteria tended to be higher on the Viher solution silage diets than on the other diets. The numbers of bacteria

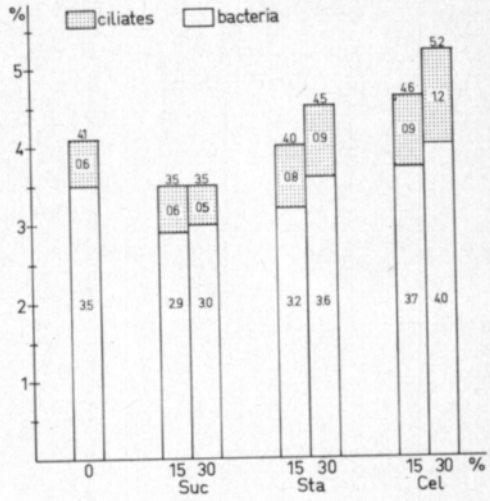

Fig. 5. The total microbe mass as a percentage of the rumen content in sheep on different carbohydrate diets (O = only silage, Suc = sucrose, Sta = starch, Cel = cellulose).

were higher on the formic acid silage diets than on the AIV I silage diets. The number of ciliates was higher on the AIV I silage diet than on the formic acid diet when only silage was given, but the situation was reversed with the diets containing the carbohydrate supplements.

Table 4. The mean number of ciliate ($n \times 10^3$) and bacteria ($n \times 10^9$) cells per ml rumen content in sheep on AIV I, formic acid and Viher solution silage diets.

	Diets consisting only of silage			Diets also containing carbohydrate supplements		
	AIV I	Formic acid	Viher solution	AIV	Formic acid	Viher solution
Total ciliates	516.74	483.13	532.62	488.84	526.96	535.29
Charon	288.10	251.53	266.72	248.15	250.29	266.63
Entodinium vorax	0.34[a]	1.30[b]	0.28[a]	0.78	0.90	0.91
E. longinucleatum	3.00	4.08	3.40	3.29	4.56	4.50
E. triacum	2.84[a]	4.11[a]	24.34[b]	1.46[a]	2.08[a]	11.38[b]
E. caudatum	2.50	1.64	2.63	7.44	6.21	5.46
E. loboso-spinosum	4.42	2.50	4.36	5.36	6.68	4.82
E. nanellum	27.71	31.20	29.19	30.30	36.28	32.57
E. dubardi	160.10	161.82	176.90	166.84	190.06	178.62
Diplodinium dentatum	2.72	2.38	3.00	2.48	2.77	2.03
Eudiplodinium maggii	0.80	1.26	1.51	1.87	1.19	1.39
E. affine	5.99	3.46	5.28	4.79[a]	4.35[a]	10.10[b]
E. neglectum	3.67	5.47	1.76	1.88[a]	4.77[b]	1.14[a]
Polyplastron multivesiculatum	2.38	1.88	1.64	3.36	3.68	3.08
Enopoplastron triloricatum	12.17	10.50	11.61	10.84	13.14	12.66
Bacteria	35.02	35.14	35.57	33.68	34.45	35.02

Statistical analysis performed separately for diets consisting only of silage and for diets also containing carbohydrate supplements. Meaning of index letters same as in Table 2.

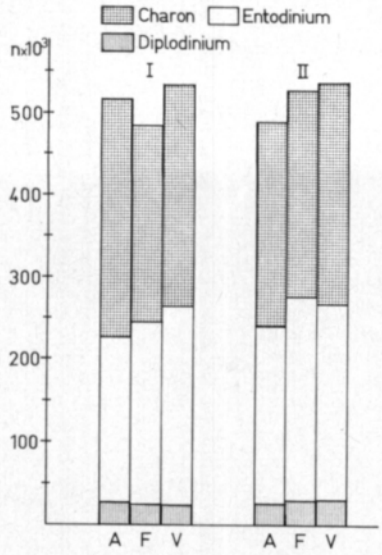

Fig. 6. The mean number of ciliate cells ($n \times 10^3$) per ml rumen content in sheep fed on AIV I (A), formic acid (F) and Viher solution (V) silage, alone (I) or with carbohydrate supplements (II).

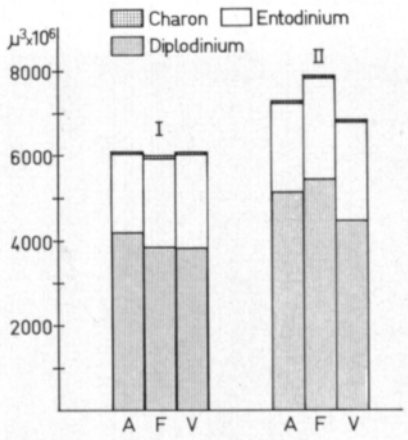

Fig. 7. The mean volume of ciliates ($\mu^3 \times 10^6$) per ml rumen content in sheep fed on AIV I (A), formic acid (F) and Viher solution (V) silage, alone (I) or with carbohydrate supplements (II).

The volumes of the different ciliates were about the same on the different silage diets, especially on the pure silage diet ($P > 0.05$, Fig. 7). The total volumes of ciliates on the pure AIV I, formic acid and Viher solution silages were 6094, 5975 and 6078 $\mu^3 \times 10^6$ per ml rumen content, respectively. The corresponding values on the diets containing carbohydrate supplements were 7284, 7881 and 6818. The genus *Entodinium* constituted the highest proportion of the ciliate volume on the Viher solution silage diets and a higher proportion on the formic acid silage diets than on the AIV I silage diets. The contribution of the genus *Diplodium* decreased in the opposition direction: AIV I > formic acid > Viher solution. The contribution of *Charon* was about the same on all the silage diets. The differences in the total microbe mass between the different silage diets were also non-significant ($P > 0.05$, Fig. 8).

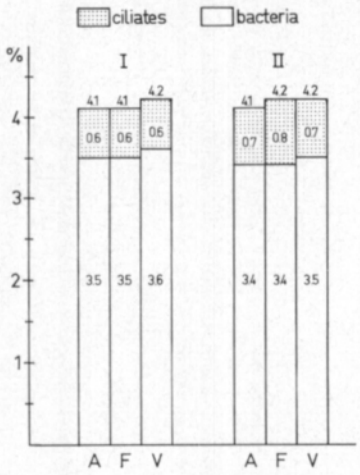

Fig. 8. The total microbe mass as a percentage of the rumen content in sheep fed on AIV I (A), formic acid (F) and Viher solution (V) silage, alone (I) or with carbohydrate supplements (II).

General considerations

The investigation reported in this paper concern the effect of sucrose, starch and cellulose supplements, at levels of $2\,^1/_2$ g and 5 g/kg live weight per day, on the composition and volume of the rumen microbiota of sheep fed on grass silage prepared with different preservatives.

The carbohydrate source affected the rumen microbiota more than the level at which it was used. The total number of ciliates was highest in the animals receiving sucrose with the silage, lower in those on starch diets and lowest in those given cellulose. On the pure silage diet, the ciliate number was between those for the starch and cellulose diets. The total number of bacteria was highest on the cellulose diets, lower on the pure silage and starch diets and lowest on the sucrose diets. These variations in the numbers of ciliate and bacteria cells can mainly be attributed to differences in the solubility of the carbohydrates.

The sucrose supplements increased the numbers of small ciliates especially and the cellulose supplements raised those of the bigger ciliates. The total volume of the ciliate fauna was thus highest in the animals on cellulose diets, lower in those on starch diets and lowest in those on sucrose diets. The contribution of the total microbe mass to the rumen content decreased in the same direction: cellulose > starch > sucrose.

In the earlier study (SYRJÄLÄ 1972) in which the same rumen samples were used as in this experiment, the average ammonia content of the rumen fluid was lower on the sucrose diets than on the starch and cellulose diets with the same levels of carbohydrate supplements. When this finding is considered together with present results, it appears that the decrease of the rumen ammonia content depends more on the numbers of the ciliate cells than on the volume of the ciliate fauna. Perhaps the bigger ciliates contain relatively more polysaccharides than the smaller ciliates, so that the protein content is relatively higher in a ciliate mass formed from smaller cells than in one formed from larger cells.

The average amounts of total volatile fatty acids behaved in the same way in relation to the different carbohydrate diets as the volume of the ciliate fauna, decreasing from cellulose to starch to sucrose. However, these results cannot be considered to reflect the fermentation activity of the ciliates alone or its dependence on, for instance, the volume of the ciliates, because the amount of bacteria was not the same on the different diets. The bacteria counts were highest on the cellulose diets, lower on the starch diets and lowest on the sucrose diets. The importance of the bacteria flora in this experiment is evident from its high contribution to the total rumen microbe mass compared with that of the ciliate fauna. It constituted 77—86 % of the mass on the different diets, the percentage being highest on the sucrose diet and lowest on the cellulose one.

The ciliates and bacteria were counted on samples taken only once a day, in the morning before feeding. In contrast, the pH, ammonia and VFA content were determined on samples taken three times a day (SYRJÄLÄ 1972). This investigation of the microbiota thus describes only the situation at the begin-

ning of feeding and does not give any information on qualitative and quantitative changes occurring during the day (PURSER and MOIR 1959, WARNER 1962). Consequently, the results cannot be used as a measure of the protein utilization of the host. They do, however, permit a comparison of the effects of the different diets on the rumen microbiota.

The other indices of the utilization of the nitrogenous compounds of silage, such as the nitrogen balance and the biological value of protein, were also somewhat better on the sucrose diets than on the corresponding starch and cellulose diets. In contrast, the apparent digestibility coefficients were generally higher on the cellulose diets than on the starch or especially on the sucrose diets. It must, however, be remembered that the metabolic nitrogen substances of the faeces are not separated when apparent digestibility is determined and that soluble carbohydrates have been found to increase its proportion in the faeces (HAMILTON 1942, FONTENOT et al. 1955, PALOHEIMO et al. 1968, SYRJÄLÄ 1971).

The different silage preservatives were found to have little effect on the composition and volume of the ruminal microbiota. Similarly, little variation was found in the digestibility values of the silages, except that of crude protein, which was lower for the Viher solution silage than the other silages (SYRJÄLÄ 1972). Some significant differences were found in the rumen fermentation products between the different silage diets. The ammonia concentration in the rumen fluid was, on average, lower on the Viher solution silage diets than on the other silage diets, which was attributed to the formaldehyde contained by the Viher solution (SYRJÄLÄ 1972). But according to the results of the present experiment, the formaldehyde did not have a notable effect on the rumen microbiota.

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SELOSTUS

Sokeri-, tärkkelys- ja selluloosalisäysten vaikutus erilaisilla säilörehuilla ruokittujen lampaiden pötsimikrobiston määrään ja koostumukseen

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Tutkimuksen tarkoituksena oli selvittää säilörehuannokseen lisättyjen eri suurien sokeri-, tärkkelys- ja selluloosamäärien sekä erilaisten säilöntäaineiden vaikutusta märehtijäin pötsin mikrobiston määrään ja laatuun.

Koe suoritettiin latinalaisten neliöiden mukaan yhdeksällä täysikasvuisella pässillä, joille oli tehty pötsifisteli. Alkueläinten ja bakteerien mikroskooppinen laskeminen tehtiin pötsinäytteistä, jotka oli otettu jokaisen koejakson lopulla kahtena päivänä aamulla ennen ruokintaa.

Hiilihydraattilisäyksillä korvattiin 15 % ja 30 % päivittäisen rehuannoksen kuiva-aineesta, joka oli keskimäärin 928 g. Hiilihydraattilisäykset olivat tällöin 2 $^1/_2$ ja 5 g elopainokiloa kohti päivässä. Kokeessa käytetyt säilörehut oli valmistettu kolmella eri säilöntäaineella: AIV I:llä, muurahaishapolla ja Viherliuoksella.

Tutkimuksessa havaittiin, että hiilihydraatin laadulla oli suurempi vaikutus pötsimikrobistoon kuin sen määrällä. Alkueläinten lukumäärä oli suurin silloin, kun eläimet saivat säilörehuruokinnan yhteydessä sokeria, seuraavaksi suurin tärkkelysdieeteillä ja alhaisin selluloosadieeteillä. Pelkällä säilörehudieetillä jäi alkueläinten lukumäärä tärkkelys- ja selluloosadieeteillä saatujen määrien väliin. Bakteerien lukumäärä oli suurin selluloosadieeteillä, seuraaviksi suurin pelkällä säilörehulla ja tärkkelysdieeteillä sekä alhaisin sokeridieeteillä.

Sokeridieeteillä alkueläimistö koostui muihin dieetteihin verrattuna suhteellisesti suuremmassa määrin kooltaan pienistä alkueläimistä, kun taas selluloosadieeteillä tavattiin suhteellisesti enemmän suurikokoisia alkueläimiä. Tästä oli seurauksena, että alkueläimistön kokonaistilavuus muodostui selluloosadieeteillä suuremmaksi kuin sokeridieeteillä tärkkelysdieetin jäädessä näiden väliin.

Koko mikrobimassan prosenttinen osuus pötsin sisällön tilavuudesta oli eri dieeteillä seuraava: pelkkää säilörehua 4.1, 15 % sokeria 3.5, 30 % sokeria 3.5, 15 % tärkkelystä 4.0, 30 % tärkkelystä 4.5, 15 % selluloosaa 4.6 ja 30 % selluloosaa 5.2. Bakteerien osuus koko mikrobimassan tilavuudesta oli suhteellisen korkea, 77—86 % eri dieeteillä, suurimman osuuden ollessa sokeridieeteillä ja pienimmän selluloosadieeteillä.

Eri säilöntäaineilla valmistetuilla rehuilla ruokittaessa ei havaittu mainittavia eroja pötsin mikrobiston määrässä ja laadussa.