Failure of cellulolysis in the rumen of reindeer fed timothy silage

Monica A. Olsen, Tove H. Aagnes & Svein D. Mathiesen

Department of Arctic Biology and Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway

Abstract: Three male reindeer (*Rangifer tarandus tarandus*) calves were brought from mountain pastures in April and fed regrowth timothy (*Phleum pratense*) silage with 76 % leaves and 24.0 % dry matter (DM) *ad libitum*. The silage contained (on DM basis) 25.4 % cellulose, 12.0 % crude protein and 19.6 % water soluble carbohydrates. After an initial period of 11 days the daily silage intake rose to almost similar values for all animals, but independently of food intake, body mass (BM) increased by as much as 13.3 kg for animal R3 during the first 21 days, compared to 4.4 kg and 2.8 kg for R1 and R2, respectively. At slaughter the wet weight of the rumen contents of animal R3 constituted 30.2 % of the total BM, compared to 18.5 % and 19.1 % in animals R1 and R2, respectively. A reduced ability of the rumen microbiota to ferment pure cellulose *in vitro* was observed in R3. The ruminal pH was 7.07 and the concentration of volatile fatty acids was only 50.0 mM in R3, indicating a low rate of fermentation. The initial rates of *in vitro* dry matter digestibility of timothy silage and standard hay were also affected by the rumen fermentation failure in animal R3. Depressed rumen cellulolysis, which may be related to natural periods of starvation prior to the feeding experiment, could have caused the low rate of fermentation and the large rumen size observed in this animal.

Key words: *Rangifer*, digestion, grass silage.

Rangifer, 15 (2): 79–86

Introduction

Reindeer rely on symbiotic rumen micro-organisms to ferment polysaccharides like cellulose and hemicellulose in plant cell walls. The development of the rumen and its symbiotic microflora depend on the diet and on which genetic feeding type the ruminant belongs to. Only few of the predominant rumen bacterial species in reindeer exhibit cellulolytic activity (e.g. Hobson *et al.*, 1976; Orpin *et al.*, 1985). Semi-domesticated reindeer in northern Norway are exposed to seasonal variations in quality and availability of food, factors which greatly affect the ruminal ecosystem. Unstable weather conditions in the winter sometimes result in ice and crust, which covers the pasture, and the animals may therefore be exposed to periods of acute starvation. The density and composition of the bacterial population in the rumen of the reindeer is influenced by the substrate eaten (e.g. Dehority, 1975; Syrjälä *et al.*, 1973; Hobson *et al.*, 1976; Orpin *et al.*, 1985; Aagnes *et al.*, 1995), and the numbers of bacteria decrease dramatically during periods of starvation (Mathiesen *et al.*, 1984; Aagnes *et al.*, 1995). After a period of starvation changes in both the physiological state of the animal and its ruminal ecosystem may cause problems in adjusting to a new diet if refed.

Different qualities of grass silage have been evaluated as food for reindeer in winter. Silage of leaf-rich regrowth of *Phleum pratense, Agrostis tenuis* and *Poa* sp. seemed to be of limited nutritional value to adult female reindeer, due to poor utilisation with corresponding low ruminal volatile fatty acids (VFA) concentrations and low food intake (Aagnes & Mathiesen, 1995). Reindeer are intermediate mixed feeders (Hofmann, 1985), and have pronounced limitations in the ability to digest fibrous timothy silage with only 27 % leaves and 30.4 % dry matter (DM) cellulose (Aagnes *et al.*, 1994). However, Aagnes *et al.* (1994) found that regrowth timothy silage consisting mainly of leaves (90 %) and as much as 30 % DM water soluble carbohydrates (WSC) seemed to satisfy ruminal metabolism and the energy requirements of reindeer calves both summer and winter.

The purpose of this study was to investigate the relationships between ruminal fermentation, ruminal cellulose digestion, rumen content fill, food intake and body mass (BM) in reindeer calves taken from a natural winter pasture and fed regrowth timothy silage with 76 % leaves and 19.6 % DM WSC.

Materials and methods

Animals

Male reindeer calves (n=3) were rounded up on a natural winter pasture in northern Norway (68°N, 17°E) and taken to Department of Arctic Biology, University of Tromsø in mid April 1993 (age 11 months). The animals were initially offered pelleted timothy for 72 hours, but even when mixed with lichen (1.5-2.6 kg wet weight during the whole 72 hour period) they refused to eat it. After this period they were offered regrowth timothy silage *ad libitum* once a day. Food intake was recorded daily for each animal, except for the first 16 hour period (17:00–9:00). The animals were kept in metabolism cages (70 cm wide, 148 cm long, 97 cm high) in a temperature regulated room (0–5°C) and exposed to a natural photo period allowing expression of their natural seasonal appetite (Ryg & Jacobsen, 1982; Larsen *et al.*, 1985). The animals were treated for parasites using albendazol (Valbazen vet.® Smith Kline Beecham, pic, England) and fenbendazol (Panacur® Hoechst Veterinär, GmbH, München, Norske Hoechs a/s, Økern, Oslo) which were administered orally on day 7 and 12 of the experiment, respectively At the end of the silage feeding trial which lasted for 48 d the animals were slaughtered at the time when they normally would have been fed. Samples of rumen ingesta were taken for analyses and *in vitro* digestion studies.

Silage

Regrowth timothy (*Phleum pratense*), which consisted of 76 % leaves, was harvested on the 21 August 1992 at the Norwegian Crop Research Institute, Tromsø. Fertiliser had been applied to the sward at a rate of 50 kg/1000m² (10.8 kg N, 1.8 kg P and 9.0 kg K) in the spring and 30 kg/1000m² (4.5 kg N, 0.75 kg P and 3.75 kg K) after the first cut, which was harvested 23 June. The grass was cut using a mower conditioner (JF 190 Freudendahl A/S, Sønderborg, Denmark) and wilted for six to eight hours. It was subsequently picked up by a flail forage harvester (Serigstad 134, Serigstad A/S, Bryne, Norway) cutting the grass to a length between 3–10 cm and packed in bales (600-700 kg) by a fixed chamber press, Orkel GP 1200 (Gjønnes Mekansike Verksted, Norway). Natuferm® solution (homofermentative lactic acid bacteria, Apothekernes Laboratorium, Norway) was added to the grass during packing (5 litres/bale). The bales were mechanically wrapped with six layers of plastic with a Kvernland silowrap 7550 (Kvernland, Sandnes, Norway). After fermentation bales used in the feeding experiments were packed in plastic bags (30 kg) sealed and stored at -20°C prior to use. The silage was thawed before being fed to the reindeer.

Chemical analysis

The chemical composition of the silage was determined in a random sample from the bale. It was analysed for DM, ash, nitrogen, ether extract, WSC and fibres. DM was determined after preheating for 24 h at 80°C and then heating at 103–105°C for 4 h. The grass silage was ashed at 550°C for 12 h (Horwitz, 1980). Nitrogen was determined using the Kjeldahl method (Horwitz, 1980) and crude protein estimated after multiplying by 6.25. True protein was determined by boiling the material in water to remove the soluble protein and the remaining nitrogen was analysed by the Kjeldahl method. The ether extract was determined using the Soxhlet method and extracting with diethyl ether. NH_3-N was liberated from an aqueous extract with magnesium oxide. The distillate was collected in H_2SO_4 and excess acid was titrated with NaOH (Horwitz, 1980). To determine WSC the material was extracted with NaOH (Smith & Grotuleschen, 1966), deproteinsed with zinc sulphide/barium

Table 1. Chemical composition of baled regrowth timothy (*Phleum pratense*) silage with 76% leaves and of two different standards of hay (*Phleum pratense*) (A, high quality and B, poor quality).

	Silage	Hay A[1]	Hay B[1]
Dry matter (DM), %	24.0	–[2]	–
Chemical composition, % of DM:			
Ash	6.3	9.8	4.5
Crude protein	12.0	22.1	6.9
True protein	7.8	–	–
Ether extract	4.7	3.4	2.1
Cellulose	25.4	25.2	32.9
Hemicellulose	22.9	31.9	30.2
Lignin	2.5	2.3	4.4
Water soluble carbohydrates	19.6	4.9	15.5
Formic acid	0.88	–	–
Acetic acid	0.92	–	–
Propionic acid	<0.04	–	–
Butyric acid	<0.04	–	–
Lactic acid	9.25	–	–
NH_3-N, % of tot. N:	2.6	–	–
pH	3.8	–	–

[1] Data from Aagnes & Mathiesen (1995)
[2] –, not determined

hydroxide, and the WSC content assayed using the ferricyanide method (Furuholmen *et al.*, 1964). The fibre fraction (cellulose, hemicellulose and lignin) was calculated from values of NDF, ADF and ADL by methods of Van Soest (1963 a, b), Van Soest & Wine (1967) and Goering & Van Soest (1970). The fermentation products of the silage were detected by gas-liquid chromatography (Carlo Erba Strumentazione, Fractovap 4200/42, Milano, Italy; integrator, Perkin-Elmer, LCI-100). The material was chopped while frozen and extracted with water. Acetate, lactate, propionate and butyrate were determined by analysing the water extract with a Carbopack/Carbowax stationary phase and detected with a flame-ionisation detector (Fussel & McCalley, 1987; Supelco, 1985). Formic acid was determined in an ether extract of the aqueous extract which was analysed by GLC with SP1220/H_3PO_4 as stationary phase and detected with a thermal conductivity detector (TCD) (Hauser & Zabransky, 1975; Supelco, PA, 1975).

Digestibility

The *in vitro* dry matter digestibility (IVDMD) of cellulose (Whatman no. 1 filterpaper), timothy silage and of two standard qualities of timothy hay (A, high quality and B, poor quality) were determined with rumen fluid from the three reindeer, using a modification of the Tilley & Terry (1963) method as described by Aagnes & Mathiesen (1995). Silage, standards of hay and cellulose were dried at 60°C and milled using a mesh size of 0.75 mm. The DM content of the silage and standards of hay were determined after drying at 100°C. Samples of plant material (approx. 100 mg) were placed in previously dried, cooled and weighed Hungate anaerobic culture tubes fitted with a screw cap and a butyl rubber septum. Artificial saliva (pH 6.9) was prepared under CO_2 according to McDougall (1948) with the modifications of Aagnes & Mathiesen (1995), and added (9 ml) to each Hungate tube. Rumen fluid (1 ml), obtained by straining the rumen contents through two layers of muslin, was subsequently added by syringe to each tube and the tubes were incubated in a water bath at 39°C. After 6, 24, 48 and 72 h of microbial fermentation, 1.1 ml of 2M HCl, 1 ml pepsin solution (5 mg/ml distilled water) and 1.9 ml water were added by syringe. The tubes were incubated for another 48 hours to simulate the enzymatic digestion of the abomasum. All experiments were conducted in quadruplicate. The IVDMD was calculated as percent DM disappearance in each tube (Aagnes & Mathiesen, 1995). The IVDMD of cellulose (Whatman no. 1 filter paper) was calculated in a similar way, but addition of water, HCl and pepsin solution were omitted.

Ruminal pH, VFA and lactic acid concentration

Rumen fluid was sampled within 11 minutes after death and strained through two layers of muslin. The pH was recorded and 5 ml portions were acidified with 1.25 ml 0.5M HCl, mixed and stored at -20°C for subsequent analyses of VFA and lactic acid. Total VFA were determined according to Sørmo *et al.* (1994).

Body mass and composition

The animals were weighed once weekly between 10:00 and 11:00 to 0.5 kg. Carcass weight and wet weights of the reticulum-rumen tissue and digesta were measured to 0.01 kg. The DM of the reticulum-rumen contents was determined by drying at 106°C.

Statistics

The IVDMD of the silage was compared with the IVDMD of the standard hay A and B using the Wilcoxen rank-sum test for comparing two treatments (Johnson & Bhattacharyya, 1992). The null hypothesis was rejected at $p \leq 0.05$. Regression line analysis of the fermentation rates of cellulose and hay were calculated with 99 % confidence intervals.

Results

Silage

The chemical composition of the silage and standard hay A and B is presented in Table 1. The silage contained more WSC than both hay A and B. The cellulose content of the regrowth timothy silage resembled that of the high quality hay, while the crude protein and lignin contents of the silage fell between the two standards of hay (Table 1).

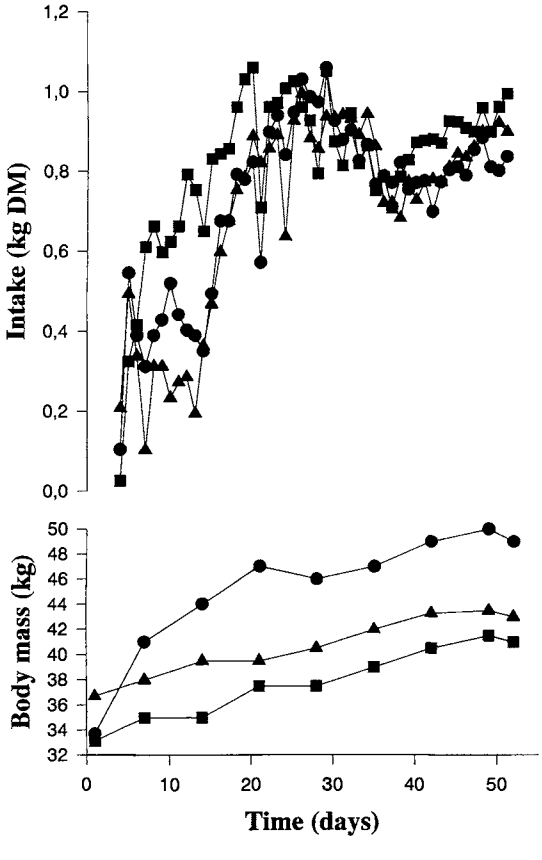

Fig. 1. Food intake and body mass in reindeer calves fed *ad libitum* regrowth timothy (*Phleum pratense*) silage. Animal R1 (■); R2 (▲) and R3 (■).

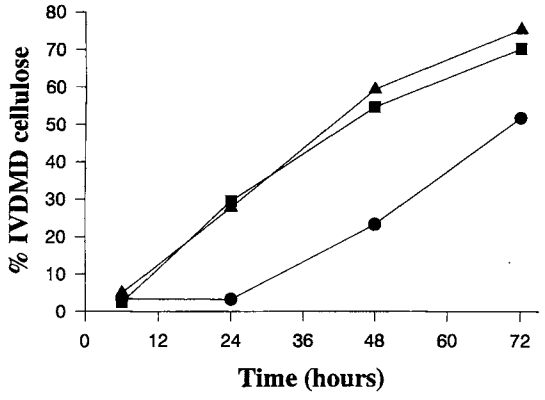

Fig. 2. Median *in vitro* dry matter digestibility (% IVDMD) of pure cellulose (Whatman no. 1 filter paper) in rumen fluid from reindeer calves fed *ad libitum* regrowth timothy (*Phleum pratense*) silage in winter. Animal R1 (■); R2 (▲) and R3 (■).

Food intake

The daily intake of silage rose to similar values for all three animals after an initial adaptation period of approximately 11 days (Fig. 1). The median (range) daily DM intake of timothy silage increased from 104 g (26-208 g) in the first 16 h, to 442 g (273-663 g) on day eight and to 941 g (892-941 g) on day 20 (Fig. 1).

Digestibility

The median IVDMD of pure cellulose in rumen fluid from reindeer calves fed regrowth timothy silage increased from 27.8 % (range 3.3–29.5 %) after 24 h microbial digestion, to 70.4 % (range 51.8–75.5 %) after 72 h digestion (Fig. 2). A marked delay in cellulolysis was found in animal R3, but during the 24–72 h incubation period the rate of cellulose fermentation was similar in all animals. Omitting the 6 h value for animal R3, regression line analysis for the three individual curves in Fig 2. showed similar slopes (median 1.02, range 1.01–1.09) for all curves (correlation coefficients 0.97–0.99).

The median IVDMD of timothy silage using rumen fluid from the reindeer calves increased from 52.5 % (range 51.0–52.5 %) after 6 h incubation, to 76.9 % (range 76.3–77.4 %) after 48 h. The IVDMD of standard hay A was significantly lower than the IVDMD of silage after 48 h (W_s=6, n_1 and n_2=3, $p \leq 0.05$), and the IVDMD of hay B was significantly lower than that of the silage at both 6, 24, 48 and 72 h (W_s=6, n_1 and n_2=3, $p \leq 0.05$), increasing from 28.4 % (range 26.8-28.4 %) after 6 h to

Table 2. Ruminal pH, total concentration of volatile fatty acids (VFA) and lactic acid, percent of acetate, propionate and butyrate and the acetate/propionate ratio in reindeer fed baled regrowth timothy (*Phleum pratense*) silage.

Animal	R1	R2	R3
pH	6.82	6.95	7.07
Total VFA (mM)	75.5	60.7	50.0
Lactic acid (mM)	0.23	0.67	0.25
% of total VFA			
Acetate	69.9	73.0	75.1
Propionate	20.7	19.4	18.4
Butyrate	9.4	7.6	6.5
Aceate/propionate	3.37	3.76	4.08

53.6 % (range 52.6–54.9 %) after 48 h. The initial rate of digestion of the silage and hay were lower in animal R3 than in the other animals (Fig. 3).

Ruminal pH, VFA and lactic acid concentration

The ruminal pH, VFA and lactic acid concentration are presented in Table 2. The ruminal ratio of acetate/propionate (4.08) was higher in animal R3, compared to 3.37 and 3.76 in animal R1 and R2, respectively.

Body mass and composition

At arrival at the Department of Arctic Biology the median (range) BM of the three calves were 33.7 kg (33.1-36.7 kg). At the end of the feeding trial it had increased to 43 kg (41-49 kg) (Fig. 1, Table 3). A dramatic increase in BM observed for animal R3 (Fig. 1) was related to the large amount of ingesta found in the reticulum-rumen after slaughtering the animal (Table 3). The carcass weight and femur length of animal R3 resembled that of the other two animals in the trial (Table 3).

Discussion

In the current experiment one animal, R3, failed to adapt to high quality timothy silage (Table 1) and a decreased ability of it's rumen fluid to ferment cellulose *in vitro* was demonstrated (Fig. 2). Depression of rumen cellulolysis in reindeer fed silage in winter may be caused by several factors, like the animals physiological state and the chemical composition of the food. Grass undergoes processes which alter its physical and chemical properties during silage-making. Soluble carbohydrates in the grass are fermented to organic acids, mainly lactate, but also significant amounts of acetate, and many nitrogenous compounds such as amines. The silage used in this experiment contained 19.6 % DM WSC. A high content of WSC may reduce fibre digestion due to preference by rumen micro-organisms for WSC rather than fibre components (e.g. Mould & Ørskov, 1984; Simpson, 1984). Furthermore, fermentation of WSC could reduce ruminal pH, and reduction of ruminal pH from 6.8 to approximarely

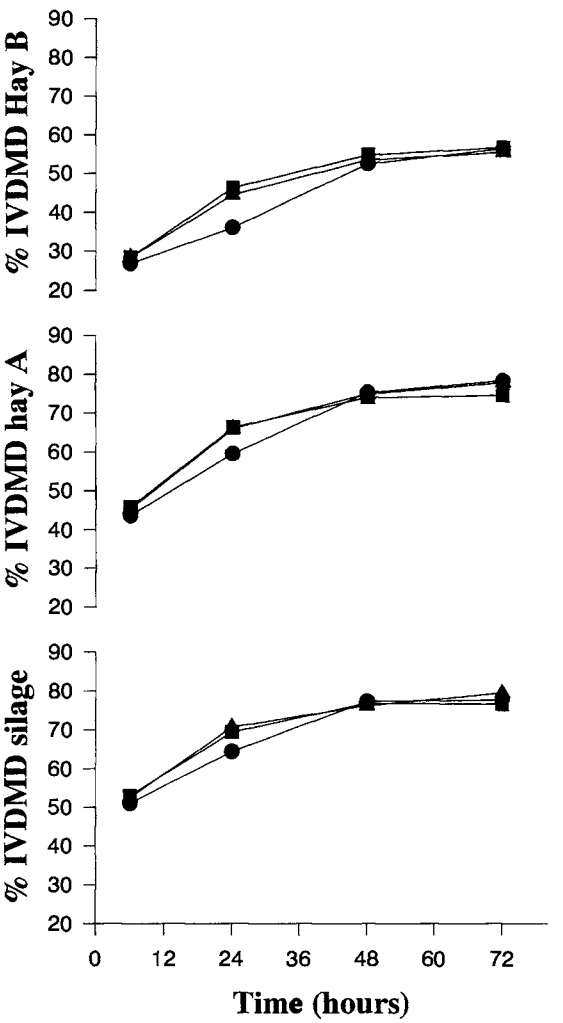

Fig. 3. Median *in vitro* dry matter digestibility (% IVDMD) of baled regrowth timothy (*Phleum pratense*) silage and of two different qualities of hay (*Phleum pratense*) (A, high quality and B, poor quality), in rumen fluid from reindeer calves fed *ad libitum* timothy silage in winter. Animal R1 (■); R2 (▲) and R3 (●).

Table 3. Body mass, carcass weight, femur length and rumen size of reindeer fed baled regrowth timothy (*Phleum pratense*) silage in winter.

Animal	R1	R2	R3
Body mass (kg)	41	43	49
Carcass weight (kg)	19.3	19.8	19.8
Femur length (cm)	23	23.4	23.7
Reticulum rumen:			
Contents (kg wet weight)	7.6	8.2	14.8
(kg dry weight)	0.67	0.77	1.29
Tissue (kg wet weight)	0.97	1.10	1.25

6.0 is known to give a moderate depression in fibre digestion, whereas a decrease in pH below 6.0 cause severe inhibition (Mould *et al.*, 1984). The ruminal pH recorded (Table 2) was, however, well above the pH threshold for cellulolysis (Mould *et al.*, 1984). The IVDMD of regrowth timothy silage with as much as 30 % DM WSC (T. H. Aagnes, unpubl. data) was even higher than that of our silage (Fig. 3) in winter, indicating that the rumen fermentation is stimulated by WSC.

Chemical components in grass silage may inhibit bacterial activity and, hence, breakdown of fibre. The timothy silage used in this experiment was, however, of high quality (Table 1), and since all animals were offered the same food, grass chemistry was probably not the major cause of reduced rumen cellulolysis and the large ruminal contents wet weight observed in animal R3 (Fig. 2).

The reindeer calves were taken directly from a winter pasture in northern Norway, where the availability of food may change dramatically during the winter. Animal R3 seemed to be in a poor condition, looking more skinny and resting more, compared to the other animals upon arrival at Department of Arctic Biology. This may have been due to previous starvation or periods of reduced food and water intake. A dramatic increase in BM during the first week of the experiment (Fig. 1) could be related to a large weight of the rumen contents in this animal observed at slaughter (Table 3), even though daily food intake of this animal resembled that of the other animals (Fig. 1) and the mean (±SE) water intake (kg/d, sampled day 36-45 of the silage feeding trial) was not particularly high in R3 (0.16±0.05) compared to R1 (0.45±0.07) and R2 (0.12±0.04) (Olsen, unpublished data). The ruminal VFA concentration of animal R3 was low compared to the other animals (Table 2) and resembled the mean (±SD) concentration of VFAs observed by Aagnes and Mathiesen (1995) in reindeer calves (n=5) fed silage of mixed grasses in winter (47.06±5.21 mM). Starvation prior to the experiment may have affected the physiological state of the animal and the rumen microbiota in such a way that rumen fermentation and cellulolysis was depressed. A short period of starvation is known to reduce the bacterial population densities and change the bacterial species composition in the rumen of reindeer (Mathiesen *et al.*, 1984; Aagnes *et al.*, 1995). In fact, the population densities of bacteria associated with rumen solids decreased by 52.5 % after only one day of starvation and as much as 95.9 % after four days (Aagnes *et al.*, 1995). Fibre fermenting bacteria interact and coexist with each other and other bacteria not able to ferment fibre (e.g. Dehority, 1973; Scheifinger & Wolin, 1973; Latham & Wolin, 1977; Fondevila & Dehority, 1994). In such a synergistic system different bacterial species have complementary activities resulting in greater formation of products than when growing alone. *In vitro* studies have demonstrated a synergistic increase in forage cellulose digestion by combining several different cellulolytic bacteria with the non-cellulolytic bacteria, *Bacteroides ruminocola* (Dehority & Scott, 1967). A change in the composition of the ruminal bacterial population, with reduced numbers of one or several of these synergistic bacteria may therefore influence on the cellulolysis when re-feeding the animal. Data from domestic ruminants like sheep and cattle shows that *in vitro* rumen cellulolysis is greatly affected by starvation (2–4 days) but is restored to normal when re-fed (Meiske *et al.*, 1958; Quin, 1951). The effect of starvation and subsequent feeding on *in vitro* rumen cellulolysis in reindeer still remains to be evaluated. It is, however, evident that cellulolytic bacteria are present in the rumen fluid obtained from animal R3 (Fig. 2) although at low numbers. The rumen micro-organisms seems to be unable to digest the plant particles at high enough rate to match the food intake, and this may explain the increase in rumen contents (Fig. 1). The rumen contents (Table 3) may have increased to the extent that efficient mixing of the contents and movement of fibre-digesting bacteria were prevented, causing accumulation of undigested material in the rumen. The DM percentage of the reticulum-rumen in R3 was, however, not higher than in the other two animals (Table 3). A longer retention time of ingested silage

in R3 may have led to a lower ammonia-N level in the rumen of R3 compared to R1 and R2, which may have contributed to the delayed cellulose fermentation.

Acknowledgements

We thank the Eira family from Gielas reindeer pasture district for providing reindeer, and the staff at the Norwegian Crop Research Institute, Tromsø, for producing the silage. The work was supported by the Reindeer Research Fund under the auspices of the Norwegian Ministry of Agriculture.

References

Aagnes, T. H., Olsen, M. A. & Mathiesen, S. D. 1994. Digestive limitations in reindeer fed timothy silage. *Abstract. – Proc. Soc. Nutr. Physiol.* 3: 209.

Aagnes, T. H., Sørmo, W. & Mathiesen, S. D. 1995. Ruminal microbial digestion in free-living, in captive lichen-fed, and in starved reindeer (*Rangifer tarandus tarandus*) in winter. – *Appl. Environ. Microbiol.* 61(2): 583–591.

Aagnes, T. H. & Mathiesen, S. D. 1995. Round baled grass silage as food for reindeer in winter. – *Rangifer* 15(1):27–35.

Dehority, B. A. & Scott, H. W. 1967. Extent of cellulose and hemicellulose digestion in various forages by pure cultures of rumen bacteria. – *J. Dairy Sci.* 50(7):1136–1141.

Dehority, B. A. 1973. Hemicellulose degradation by rumen bacteria. – *Fed. Proc.* 32(7): 1819–1825.

Dehority, B. A. 1975. Characterization studies on rumen bacreria isolated from Alaskan reindeer (*Rangifer tarandus L.*). – *In:* Luick, J. R., Lent, P. C., Klein, D. R. and White, R. G. (eds.). *Proceedings of the First International Reindeer and Caribou Symposium,* pp. 228–240. University of Alaska, Fairbanks, Alaska.

Fondevila, M. & Dehority, B. A. 1994. Degradarion and utilization of forage hemicellulose by rumen bacteria, singly in coculture or added sequenrially. – *J. Appl. Bacteriol.* 77: 541–548.

Furuholmen, A. M., Winefordener, J. D., Knapp, F. W. & Dennison, R.A. 1964. The qualitative analysis of glucose and fructose in potatoes. – *J. Agric. Food. Chem.* 12: 109–112.

Fussel, R. J. & McCalley, D. V. 1987. Determinarion of volatile fatty acids (C_2-C_5) and lactic acid in silage by gas chromatography. – *Analyst* 112: 1213–1216.

Goering, H. K. & Van Soest, P. J. 1970. *Forage fibre analyses (Apparatus, Reagents, Procedures, and some Applications).* Agriculture Handbook, no. 379, Agriculture Research Service, United States Department of Agriculture, Washington, D.C.

Hauser, K. J. & Zabransky, R. J. 1975. Modification of the gas-liquid chromatography procedure and evaluation of a new column packing material for the identification of anaerobic bacteria. – *J. Clin. Microbiol.* 2: 1–7.

Hofmann, R. R. 1985. Digestive physiology of the deer – their morphophysiological specialisation and adaptation. *In: Biology of Deer Production. – Roy. Soc. N.Z. Bull.* 22: 393–407.

Horwitz, W. 1980. *Official methods of analysis of the association of analytical chemists,* 13th ed. AOAC, Washington.

Hobson, P. N., Mann, S. O. & Summers, R. 1976. Rumen micro-organisms in red deer, hill sheep and reindeer in rhe Scottish highlands. – *Proc. Roy. Soc. Edinb.* 75(B)(11): 171–180.

Johnson, R. A. & Bhattacharyya, G. K. 1992. *Statistics, principles and methods.* 2nd ed. John Wiley & Sons, Inc. USA.

Larsen, T. S., Nilsson, N. Ö. & Blix, A. S. 1985. Seasonal changes in lipogenesis and lipolysis in isolared adipocytes from Svalbard and Norwegain reindeer. – *Acta Physiol. Scand.* 123: 97–104.

Latham, M. J. & Wolin, M. J. 1977. Fermentation of cellulose by *Ruminococcus flavefaciens* in the presence and absence of *Methanobacterium ruminantium.* – *Appl. Environ. Microbiol.* 34(3): 297–301.

Mathiesen, S. D., Rognmo, A. & Blix, S. D. 1984. A test of the usefulness of a commercially available mill "waste product" (AB-84) as feed for starving reindeer. – *Rangifer* 4 (1):28–34.

McDougall, E. I. 1948. Studies of ruminant saliva. 1. The composition and output of sheep's saliva. – *Bioch. J.* 43: 99–109.

Meiske, J. C., Salsbury, R. L., Hoefer, J. A. & Luecke, R. W. 1958. The effect of starvation and subsequent refeeding on some activities of rumen microorganisms *in vitro.* – *J. Anim. Sci.* 17: 774–781.

Mould, F. L. & Ørskov, E. R. 1984. Manipulation of rumen fluid pH and irs influence on cellulolysis *in sacco,* dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. – *Anim. Feed Sci. Technol.* 10:1–14.

Mould, F. L., Ørskov, E. R. & Mann, S. O. 1984. Associative effects of mixed feeds. I. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis *in vivo* and dry matter digestion of various roughages. – *Anim. Feed. Sci. Technol.* 10: 15–30.

Orpin, C. G., Mathiesen, S. D., Greenwood, Y. & Blix, A. S. 1985. Seasonal changes in the ruminal microflora of the high-arctic Svalbard reindeer (*Rangifer tarandus platyrhynchus*). – *Appl. Environ. Microbiol.* 50(1): 144–151.

Quin, J. I. 1951. Studies of the alimentary tract of the Merino sheep in South Africa. XVIII.- The effect of fasting on the activity of the ruminal flora of sheep and cattle. – *Onderspoort J. Vet. Res.* 25(1): 51–58.

Ryg, M. & Jackobsen, E. 1982. Effects of castration of growth and food intake cycles in young male reindeer (*Rangifer tarandus tarandus*). – *Can. J. Zool.* 60(5): 942–945.

Scheifinger, C. C. & Wolin, M. J. 1973. Propionate formation from cellulose and soluble sugars by combined cultures of *Bacteroides succinogenes* and *Selenomonas ruminantium*. – *Appl. Microbiol.* 26(5): 789–795.

Simpson, M. E. 1984. Protective effect of alternative carbon sources toward cellulose during its digesrion in bovine ruminal fluid. – *Dev. Ind. Microbiol.* 25: 641–649.

Smith, D. & Grotuleschen, R. D. 1966. Carbohydrates in grasses. I. Sugar and fructosan composition of the stem bases of several northern-adapted grasses at seed maturity. – *Crop Science,* 6: 263–266.

Supelco. 1975. Analysis of VFAs from anaerobic fermentation, *Bulletin 748F.*

Supelco. 1985. GC analyses of lactic acid in the presence of other volatile free acids. – *The Supelco Reporter, Chromatography and Chemical Standards News* 4: 9–10.

Syrjälä, L., Kossila, V. & Sipilä, H. 1973. A study of nutritional status of Finnish reindeer (*Rangifer tarandus L.*) in different months. I. Composition and volume of the rumen microbiota. – *J. Sci. Agric. Soc. Finl.* 45: 534–541.

Sørmo, W., Aagnes, T. H., Olsen, M. A. & Mathiesen, S. D. 1994. The bacteriology of the small intestinal mucosa of free-living reindeer. – *Rangifer* 14(2): 65–78.

Tilley, J. M. A. & Terry, R. A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. – *British Grassland Society* 18: 104–111.

Van Soest, P. J. 1963a. Use of detergents in the analyses of fibrous feeds. I. Preparation of fibre residues of low nitrogen content. – *J. Assoc. Off. Agric. Chemists.* 46(5): 825–829.

Van Soest, P. J. 1963b. Use of detergent on the analyses of fibrous feeds. II. A rapid method for the determination of fibre and lignin. – *J. Assoc. Off. Agric. Chemists.* 46(5): 829–835.

Van Soest, P. J. & Wine, R. H. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determinarion of plant cell-wall constituents. – *J. Assoc. Off. Agric. Chemists.* 50(1): 50–55.

Manuscript received 2 October 1995, accepted 31 January 1996