The bacterial population adherent to plant particles in the rumen of reindeer fed lichen, timothy hay or silage

Monica Alterskjær Olsen[1] **& Svein Disch Mathiesen**[1, 2]

[1] Department of Arctic Biology and Institute of Medical Biology, Universiry of Tromsø, N-9037 Tromsø, Norway (monicao@fagmed.uit.no).
[2] Department of Arctic Veterinary Medicine, Norwegian College of Veterinary Medicine, N-9005 Tromsø, Norway.

Abstract: Male reindeer (*Rangifer tarandus tarandus*) calves taken from a narural winter pasture were given *ad.lib.* access to lichen (n=3), timothy silage (n=3) and hay (n=3) for 7 weeks. Median numbers of viable anaerobic bacteria adherent to the plant particles (cells/g wet weight of rumen solids), growing on a habitat simulating medium (M8V), were significantly higher (P = 0.05) in the rumen of reindeer fed lichen (26.5×10^9- 53.0×10^9) and hay (4.0×10^9- 40.5×10^9), compared to reindeer fed silage (1.15×10^9 - 3.25×10^9). Anaerobic bacterial strains (n=551) from the plant particles obtained from the rumen of the nine reindeer examined, were isolated using an acid swollen cellulose medium (M8SC) and tested for their ability to hydrolyse carboxymethyl cellulose (CMC). The proportion of CMC hydrolysing adherent bacteria isolated from M8SC was significantly higher in reindeer fed hay (21.5%) compared ro animals fed lichen (5.3%) and silage (2.7%) (P = 0.05). The CMC hydrolysing bacterial srrains (n=42) isolated from reindeer fed hay where characterised as non-cellulolytic *Butyrivibrio fibrisolvens* (9.5%), cellulolytic *B. fibrisolvens* (50.0%), *Clostridium* sp. (2.4%) and unknowns (38.1%), while CMC hydrolysing strains (n=11) isolated from animals fed lichen and strains (n=4) isolated from animals fed silage where all characterised as *B. fibrisolvens.* None of the bacterial strains isolated from the rumen solids of reindeer fed lichen or silage were found to be cellulolytic. This study suggests that both lichen and timothy silage have a negative influence, compared to hay, on the numbers of cellulolytic bacteria adherent to the plant particles in the rumen of reindeer.

Key words: *Rangifer tarandus tarandus,* rumen solids, cellulolytic bacteria.

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Introduction

Semi-domesticated reindeer (*Rangifer tarandus tarandus*) in northern Norway are intermediate, mixed feeders (Hofmann, 1985), exposed to large seasonal changes in vegetation types, climate and day length. High quality vascular plants are selected in summer, while lichens, primarily *Cladonia* sp., may constitute a large proportion of the winter diet. Lichen is mainly composed of hemicellulose, but also contain lichenin (lichen starch), which is in β-1,4 and β-1,3 linkages (Hale, 1961; Culberson, 1969). Compared to grass, lichens like *Cladonia stellaris* contains very little cellulose, constituting only 1.7% of its dry matter (DM) (Person *et al.,* 1980). Dehority (1975) isolated bacterial strains (n=21) from the rumen fluid of an Alaskan reindeer fed dried lichen, but these isolates were unable to digest cellulose. Likewise, Aagnes *et al.* (1995) reported that bacterial strains (n=90) isolated from the rumen fluid from two lichen-fed reindeer were

unable to hydrolyse carboxymethyl cellulose (CMC). Hence, prolonged feeding on a pure lichen diet seems to have a negative influence on the numbers of cellulolytic bacteria in the reindeer rumen. Rumen fluid of a lichen-fed reindeer may therefore also express less ability to digest cellulose compared to rumen fluid from a reindeer fed a grass diet. In winter, crusts of snow and ice often cover the pasture, exposing the reindeer for periods of starvation, during which supplementary feeding can prevent loss of animals. Different qualities of grass silage have been evaluated as food for reindeer (Aagnes & Mathiesen, 1995; Olsen *et al.*, 1995; Aagnes *et al.*, 1996; Moen *et al.*, 1998), revealing pronounced morphophysiological limitations towards digestion of fibrous timothy (*Phleum pratense*) silage (Aagnes *et al.*, 1996). Reindeer fed leaf rich silage made of *Phleum pratense, Agrostis tenuis* and *Poa* spp., demonstrated low *in vivo* apparent digestibility coefficients (62-66%) and *in vitro* dry matter digestibility (IVDMD) of the silage (only 67-71% after 72 h incubation in rumen fluid), with correspondingly low ruminal VFA concentrations (38-53 mM) and high pH (6.95-7.17), indicating a limitation in the ability of the reindeer and its microbiota to utilise this grass silage (Aagnes & Mathiesen, 1995). Depressed ability of the rumen microbiota to digest cellulose when fed grass silage (Olsen *et al.*, 1995) may have been caused by natural periods of starvation prior to the feeding experiment. Total numbers of rumen bacteria decrease dramatically during starvation and the composition of the bacterial population is changed (Mathiesen *et al.*, 1984; Aagnes *et al.*, 1995). Such changes in the ruminal ecosystem may reduce numbers of one or several of the synergistic bacteria acting together on fibre digestion, causing problems in adjusting to a new diet when re-fed. The unusual structure and chemistry of the lichen may also influence the composition of the rumen microbiota and its ability to digest timothy silage. Both the physical and chemical properties of the grass are changed during silage-making, when soluble carbohydrates are fermented to organic acids and nitrogenous compounds. Grass silage may therefore also in itself effect the bacterial population in the reindeer rumen in a different way than hay, even when harvested from the same crop. Studies of the bacterial population in the rumen fluid of reindeer fed two different qualities of timothy silage, however, revealed no correlation between the composition of the bacterial strains isolated and the ability of the rumen fluid to ferment cellulose *in vitro* (Olsen *et al.*, 1997). Cellulolytic bacteria were isolated from the rumen fluid of only one of the twelve silage-fed reindeer investigated (Olsen *et al.*, 1997). Cellulolytic bacterial species are, however, found in close association with the plant material in the rumen (Cheng *et al.*, 1977; Akin & Barton, 1983). Consequently, the present study was undertaken to investigate the bacterial population closely associated or adherent to the plant particles in the reindeer rumen, and how this population is influenced by a pure lichen diet, and by timothy hay and silage harvested from the same crop.

Materials and methods

Animals

Nine male reindeer calves (age 6 months, body mass (BM) 43.5 - 54.5 kg) were rounded up in November on a natural early winter pasture in northern Norway (68°N, 17°E) and brought to the Department of Arctic Biology at Tromsø. Three of the calves were given *ad lib.* access to lichen (47 days), and compared with three calves fed regrowth timothy with 79% leaves preserved as hay (50 days) and three calves fed regrowth timothy from the same crop, but preserved as silage (50 days) (Øksendal, 1994; Moen *et al.*, 1998). The chemical composition of the diets are presented in Table 1. Routine treatment against parasites were administered to all animals (Aagnes *et al.*, 1996). To allow expression of their normal seasonal appetite (Ryg & Jacobsen, 1982; Larsen *et al.*, 1985), all the reindeer calves were exposed to natural photo period in temperature (4 °C) regulated rooms. The animals were slaughtered at their regular feeding-time. The gastrointestinal tract was removed allowing incisions through the rumen wall for sampling of contents within 15 minutes of death.

Viable-cell counts

Numbers of anaerobic bacteria adhering to and living close to the rumen plant particles of reindeer fed lichen, timothy hay and silage were estimated according to Aagnes *et al.* (1995). Rumen solids were obtained by removing all rumen fluid through two layets of muslin. The dry rumen solids (2.5 g) were suspended for 30 min at room temperature in a M8 buffer solution (47.5 ml) (Olsen *et al.*, 1994) containing 0.1% (w/v) methyl cellulose (catalogue no. M 0262; Sigma) to detach the adherent bacteria from the food particles (Kudo *et al.*, 1987). The solution with the rumen solids were homogenised

Table 1. Chemical composition (%) of dry matter (DM) of lichen (*Cladonia stellaris*), timothy (*Phleum pratense*) hay and silage.

	Lichen[a]	Hay[b]	Silage[b]
DM, %	34.7	85.0	18.5
Crude protein[c]	2.7	15.7	14.0
True protein	-[d]	12.5	7.4
Cellulose	4.3	28.6	27.7
Hemicellulose	74.9	27.3	22.3
Lignin	3.2	1.9	2.1
Water soluble carbohydrates	1.2	8.5	8.2

[a] Data from Øksendal (1994).
[b] Data from Moen *et al.* (1998).
[c] Crude protein = total nitrogen x 6.25.
[d] - not determined.

in cortex tubes using a Polytron PT 10 OD homogenizer (Kinematica, GMB, Luzern, Schweiz) for 30-60 sec at a speed setting of 2. The homogenised rumen solids were diluted serially in ten fold steps in M8 buffer (Olsen *et al.*, 1994) in Hungate anaerobic culture tubes (catalogue no. 2047/16-125; Bellco, Vineland; N.J.), by techniques of Hungate (1950). Cultures of bacteria present at dilution's of 10^{-6} to 10^{-9} were made viable by inoculating 1 ml from each dilution in five parallels in petri-dishes in an anaerobic glovebox (COY Laboratory products, Inc., Ann Arbor, Michigan, U.S.A.) maintained with an atmosphere in the box of N_2, CO_2, and H_2, and a palladium catalyst present to remove O_2. Melted anaerobic rumen culture medium (M8V) or M8SC medium were added to the petri-dishes and mixed thoroughly with the inoculum. Viable count determinations were made in an anaerobic rumen culture medium (M8V) (Orpin *et al.*, 1985) designated to support the growth of a wide range of bacterial species based on a M8 anaerobic buffer (Olsen *et al.*, 1994). The M8V medium in addition to M8 buffer contained the carbohydrates: glucose, sucrose, maltose, cellobiose, starch, pectin and xylan, each at a concentration of 0.2% (w/v). It was solidified using 2.0% (w/v) agar. Vitamins (10.0 ml per l medium) (Rochè *et al.*, 1973) were sterile-filtered through a Millex R-GS single use filter unit (0.22 µm) (Millipore S.A., Molsheim, France) and added to the medium before use. M8SC medium were used to select for and isolate the cellulolytic bacteria. The preparation of the M8SC medium was similar to that of the M8V medium in all respects, except that the carbohydrates were replaced by acid swollen cellulose (0.2% v/v) (Schellhorn & Forsberg, 1984). Petri-dishes containing inoculated M8V or M8SC were incubated at 39 °C in anaerobic jars, gassed with CO_2 inside the anaerobic chamber for 48 h and 5-6 days, respectively.

Isolation of bacteria

A sector comprising 1/8 or 1/16 (depending on the density of viable bacteria) of the area of the petri-dish with the inoculated M8SC was marked, and all the bacterial colonies present within this sector were selected using sterile glass Pasteur pipettes. All the bacterial colonies within the sector was isolated on 4-5 of the parallels of M8SC containing the 10^{-7} or 10^{-8} dilution's. The bacteria were isolated and purified by streaking and plating repeatedly onto M8SC medium in new petri-dishes at 48 h intervals in the anaerobic chamber. Most of the bacterial strains were pure when first isolated, but some isolates required several transfers before pure. The bacterial strains were subsequently plated on M8 buffer containing 0.2% (w/v) cellobiose and 0.1% (w/v) low-viscosity carboxy methyl cellulose (CMC), incubated for 24 h at 39 °C, before staining the M8-CMC plates with Congo red (Teather & Wood, 1982). CMC-hydrolysing isolates showed a zone of clearing, unstained by Congo red, around the colony, and were transferred to individual Hungate tubes containing a slope of M8SC, incubated for 24-48 h and stored at -80 °C until analysis.

Identification of bacteria

Strains positive for the Congo red stain test, were identified by standard microbiological techniques (Holt *et al.*, 1994; Krieg & Holt, 1984; Ogimoto & Imai, 1981; Sneath *et al.*, 1986), by examination of morphology and motility in liquid M8C (M8 buffer added 0.2% (w/v) cellobiose) or M8G (M8 buffer added 0.2% (w/v) glucose), by Gram staining and identification of acidic fermentation products after growth in the same liquid media, and by spore formation. The concentrations of volatile fatty acids, lactate and succinate were determined by gas liquid chromatography (Sørmo *et al.*, 1994). Bacterial isolates were grown (24 h) in Hungate-tubes containing 9 ml liquid M8-medium added 0.2% (w/v) cellobiose and for strains not willing to grow on cellobiose, 0.2% glucose (w/v) was used instead. One ml of this culture was inoculated into another 9 ml liquid M8-media with the same carbohydrate added as in the previous growth tubes for an additional 24 h incubation period under standardised conditions

at 39 °C. Fermentation products were determined after acidification of the liquid phase (Sørmo *et al*. 1994). Aerobic growth was tested on Nutrient agat (Difco Laboratories, Detroit, Michigan, U.S.A.) containing 0.5% (w/v) glucose at 39 °C.

Statistical methods

Colonies of viable bacteria growing on M8V and M8SC media were counted and results presented as median (range) number of bacteria per gram wet weight of rumen solids. Median numbers of viable bacterial cells in the different groups of reindeer fed different diets were compared by the Wilcoxen rank-sum test for comparison of two treatments (Johnson & Bhattacharyya, 1992). The null hypothesis was rejected at $P \geq 0.05$.

Results

Median bacterial concentrations (cells / g wet weight) adherent to the rumen solids in reindeer fed lichen ($n=3$) (26.5×10^9 - 70.0×10^9) and hay ($n=3$) (4.0×10^9 - 40.5×10^9) was significantly ($P = 0.05$) higher than that of reindeer fed silage ($n=3$) (1.15×10^9 - 3.25×10^9) using a habitat-simulating medium (M8V) (Table 2). Median numbers of viable anaerobic bacteria adherent to rumen solids growing on M8SC medium was not significantly different compared to bacterial numbers growing on M8V in lichen-fed reindeer ($n=3$) and hay-fed reindeer ($n=3$). In reindeer ($n=3$) fed timothy silage, however, median numbers of viable anaerobic bacteria on rumen particles growing on M8SC was significantly higher ($P = 0.05$) compared to numbers growing on M8V (Table 2). Anaerobic bacterial strains adherent to the plant particles obtained from the rumen of the nine reindeer examined, were isolated on M8SC. A total number of 214 colonies were picked from the M8SC medium containing anaerobic bacteria from the rumen solids of lichen-fed reindeer calves ($n=3$), 97.7% of these grew up. Likewise, from reindeer ($n=3$) fed hay a total of 201 colonies were picked from which 97.0% were viable on isolation, and from reindeer ($n=3$) fed silage 191 colonies were picked from which 77.0% were viable. All the isolated anaerobic bacterial strains ($n=551$) were tested for their ability to hydrolyse carboxy methyl cellulose (CMC). The proportion of CMC hydrolysing adherent bacteria isolated from M8SC was significantly higher in reindeer calves fed timothy hay (21.5%) compared to both animals fed lichen (5.3%) and animals fed timothy silage (2.7%) ($P = 0.05$) (Table 3). In fact, CMC-hydrolysing bacteria were isolated from the M8SC-population of only one out of three silage-fed reindeer calves (Table 3). Cellulolytic strains of *B. fibrisolvens* were isolated from the rumen particles of all the individual reindeer fed timothy hay, but bacterial strains isolated from the rumen solids of reindeer fed lichen or timothy silage were not found to be

Table 2. Numbers of viable adherent anaerobic bacteria growing on M8V and M8SC medium and the proportion hydrolysing CMC and cellulose from the rumen of reindeer fed lichen (*Cladonia stellaris*), timothy (*Phleum pratense*) hay or silage.

		Viable bacterial cells ($\times 10^9$/ g wet weight of rumen solids)[a]		Isolated strains from M8SC		
	Animal	M8V	M8SC	Numbers tested	CMC-hydrolysing (%)	Cellulolytic (%)
Lichen	1	26.5 (24.0-30.5)	27.0 (23.5-37.0)	56	12.5	-
	2	30.0 (28.0-38.0)	28.5 (27.5-34.5)	76	3.95	-
	3	70.0 (50.0-85.0)	105.0 (75.0-130.0)	77	1.30	-
Hay	4	40.5 (35.5-67.0)	145 (100-150)	47	36.2	6.4
	5	33.5 (30.5-45.0)	175 (100-205)	71	19.7	16.9
	6	4.00 (3.35-5.00)	4.05 (3.10-4.35)	77	14.3	10.4
Silage	7	1.15 (0.70-1.40)	155 (110-175)	53	-[b]	-
	8	3.25 (2.75-3.70)	4.50 (4.25-5.30)	54	-	-
	9	2.0 (2.2-3.3)	68.0 (57.5-73.5)	40	10.0	-

[a] Data are medians, with ranges in parentheses; [b] -, not detected

Table 3. Morphological and biochemical characteristics of the individual CMC-hydrolysing stains from rumen solids from reindeer fed lichen, timothy hay or silage isolated using the M8SC medium.

	Result for indicated organism (no. of isolates)													
Substrate	Lichen			Hay										Silage
Animal	1	2	3	4				5				6		9
Parameter	*B. fibrisolvens* (7)	*B. fibrisolvens* (3)	*B. fibrisolvens* (1)	*B. fibrisolvens* (1)	*B. fibrisolvens* (3)	Unknown (2)	Unknown (11)	*B. fibrisolvens* (3)	*B. fibrisolvens* (11)	Unknown (1)	*Clostidium* sp. (1)	*B. fibrisolvens* (7)	Unknown (2)	*B. fibrisolvens* (4)
Characteristics[a]														
Cell morphology	Curved rod	Curved rod	Curved rod	Curved rod	Curved rod	Coccoid rod	Rod	Curved rod	Curved rod	Coccoid rod	Rod	Curved rod	Coccoid rod	Curved rod
Gram stain	–	–	–	–	ND	+	+	–	–	+	+	–	+	–
Spores	–	–	–	–	–	–	detected in two of the stains	–	–	–	ND	–	–	–
Motility	+	+	ND	+	+	–	+	+	+	–	+	–	–	+
Facultatively anarobic	–	–	–	–	–	+	+	–	–	+	–	–	+	–
Strictly anarobic	+	+	+	+	+	–	–	+	+	–	+	+	–	+
Cellulolytic	–	–	–	–	+	–	–	–	+	–	+	+	–	–
Fermentation product(s):	**B**, L	**B**, L	ND	**B**, L	**B**, L	L, A, v, i-c	**L**	**B**, P, 1/ **B**, L	**B**, L	**L**	**A**, l	**B**, L	**L**, **B**, a	**B**, L

[a] +, 100% of the strains positive; –, 100% of the strains negative; ND, not determined.

[b] VFAs produced when bacteria were grown in liquid M8C medium for 24 h are listed in order of concentraion from higherst to lowest. Boldface letters represent concentrations greater to or equal to 10 mM, lightface capital letters represent concentrations between 2 and 9 mM, lowercase letters represent 0.1–1.0 mM. Slaches indicate differences in production of individual VFAs between isolates of the same strain. Abbreviations: A, acetate; B, butyrate; I-C, iso-caproate; L, lactate; V, valerate; P, propionate; ND, none produced.

cellulolytic (Table 3). CMC hydrolysing strains isolated from animals fed timothy hay (n=42) where characterised as *B. fibrisolvens* (59.5%), *Clostridium* sp. (2.4%) and unknowns (38.1%), while CMC hydrolysing strains isolated from reindeer fed lichen (n=11) and timothy silage (n=4) where all characterised as non-cellulolytic *B. fibrisolvens*.

Discussion

Fibre-digesting bacteria interact and coexist with non-fibrolytic bacteria in the rumen ecosystem, and this synergistic interaction among the bacterial strains contributes to increased fibre digestion compared to the individual strains when grown in monocultures (Scheifinger & Wolin, 1973; Latham & Wolin, 1977; Fondevila & Dehority, 1994). The composition of the symbiotic bacterial population of the bovine rumen is influenced by the diet eaten by the host (Hungate, 1966). Starvation is also known to both change the composition and to reduce the numbers of bacteria in the reindeer rumen (Mathiesen *et al*, 1984; Aagnes *et al.*, 1995). A natural lichen diet combined with periods of starvation during winter may effect the ruminal microbial ecosystem of the reindeer in such a way that when fed silage or hay, the rumen microbiota is less capable of cellulose digestion. Lichen is very low in protein (Table 1), and even though reindeer have a very efficient recycling of urea (Hove & Jacobsen, 1975) reindeer fed lichen alone will be in a negative nitrogen (N) balance (Jacobsen & Skjenneberg, 1975). Microbial synthesis may be reduced at very low levels of N. Aagnes *et al.* (1995) characterised the rumen fluid bacterial population from two adult female reindeer with *ad lib.* access to lichen, finding strains of *Streptococcus* and *Clostridium* to be the most dominant. The low cellulose content of the lichen (Person *et al*, 1980; Øksendal, 1994: Table 4) may influence the composition of the rumen microbiota. In fact, Dehority (1975) and Aagnes *et al.* (1995) report that no cellulolytic bacterial strains were isolated from the rumen fluid of reindeer fed lichen. In the current study the bacterial population adherent to plant particles in reindeer calves (n=3) fed lichen were investigated. Total numbers of viable adherent bacteria (g wet weight of rumen solids) growing on M8V medium was higher in reindeer calves examined in this paper (Table 2), than in adult reindeer (n=2) (21.0×10^8 - 38.0×10^8) fed lichen (Aagnes *et al*, 1995). Only 11 of the bacterial strains (n=209) isolated from the rumen solids from lichen-fed reindeer using an acid swollen cellulose medium were CMC active, but none of these were found to be cellulolytic (Table 2-3). Similarly, cellulolytic bacteria appeared to be absent or depressed in Orkney sheep eating a diet of seaweed low in cellulose (Orpin *et al*, 1995b). The CMC-active strains isolated from lichen-fed reindeer were gram negative, motile, curved rods producing butyric acid as their main fermentation product (Table 3), and they were characterised as *B. fibrisolvens* (Bryant & Small, 1956). Dehority (1975) also isolated Butyrivibrio-like strains from the rumen fluid of reindeer fed lichen, but Aagnes *et al.* (1995) report no such findings. Strains of *B. fibrisolvens* have been found to solubilize hemicellulose more extensively than cellulose (Dehority & Scott, 1967;

Table 4. Dry matter (DM) food intake, reticulo-rumen wet weight relative to body mass (BM), ruminal pH, rotal concentration of volatile fatty acids (VFA), NH_4-N content and the *in vitro* DM digestibility (% IVDMD) of pure cellulose (Whatman filterpaper no. 1) after 48 h incubation in rumen fluid from reindeer calves fed lichen (*Cladonia stellaris*), timothy (*Phleum pratense*) hay or silage. Values are given as ranges, n=3 in each group.

		Characteristics of the rumen				
	Intake (g/kg BM/day)	Wet weight (% of BM)	pH	VFA (mM)	NH_4-N (mg/l)	% IVDMD of cellulose
Lichen	13.3-14.3[a]	8.4-12.1[a]	6.73-6.85[a]	57.3- 71.9[b]	300-400[a]	40.8-62.3[a]
Hay[c]	19.2-21.1	20.0-24.7	6.40-6.78	99.7-113.6	300-300	30.2-57.8
Silage[c]	9.2-14.4	23.7-35.9	6.97-7.30	57.7- 85.9	100-200	14.7-33.9

[a] Data from Øksendal (1994).
[b] Unpubl. data from H. Øksendal.
[c] Data from Moen *et al.* (1998).

Morris & van Gylswyk, 1980). The hemicellulose fraction of *Cladonia stellaris* has not been characterised. Hemicellulose are complex plant polysaccharides with β-1,4-linked xylose residues in the main core polymer (xylan) as a common factor, but with branches containing a variety of other glucosidic linkages (Van Soest, 1994). Degradation of hemicellulose therefore requires different enzymes capable of cleaving a variety of linkages. The similarities of β-1,4-xylosidic linkages of the hemicellulose and the β-1,4-glucosidic linkages of the cellulose may perhaps explain why many of the cellulolytic strains isolated from the rumen all seem able to degrade hemicellulose as well as cellulose (Dehorithy, 1965; Coen & Dehority, 1970; Kock & Kistner, 1969; Morris & van Gylswyk, 1980). A low intake of cellulose in lichen-fed reindeer may have suppressed the ability of the fibre-digesting bacteria to ferment cellulose, while the high intake of hemicellulose might have stimulated the production of enzymes cleaving hemicellulose. Hence, the lichen seems to have a negative influence on the numbers of cellulolytic bacteria present both in dilutions of the rumen fluid (Dehority, 1975; Aagnes *et al.*, 1995) and on the rumen solids (Table 2-3) in reindeer. Rumen fluid from the lichen-fed calves (animal 1-3) were allowed to ferment cellulose *in vitro*, and did not demonstrate a depressed cellulolytic activity (Øksendal, 1994: Table 4). This may be explained by the fact that the rumen fluid used in the digestibility trial was not diluted, hence cellulolytic microorganisms present in low concentrations may have been stimulated by the presence of the substrate resulting in a digestion of cellulose during the 48 h incubation period.

Grazers like cattle are known to have a higher DM intake of hay compared to silage prepared from the same crop (Campling, 1966; Ùden, 1984). Recently this has also been demonstrated for reindeer (Moen *et al.*, 1998; Table 4). Total concentrations of VFA in the rumen fluid was significantly lower and ruminal pH significantly higher in reindeer fed silage compared to hay, indicating a higher fermentation activity in the rumen of reindeer fed hay (Moen *et al.*, 1998; Table 4). Although the DM intake of hay is considerably higher than that of silage, this does not seem to have effected the rumen size of the animals fed the two different diets (Moen *et al.*, 1998; Table 4). Ruminal clearance rate is limited by plant particle density and size, which is influenced by mastication and microbial digestion (Lechner-Doll *et al.*, 1991). Rough timothy silage with a high fibre contents and stem/leaf ratio seems to accumulate in the reindeer rumen, and the capacity of the reticulo-rumen increases to compensate for this delay in digestion (Aagnes *et al.*, 1996). Even when fed silage prepared from leafy timothy an individual reindeer developed a large rumen (Olsen *et al.*, 1995). Rumen fluid from this animal demonstrated a delayed cellulolysis *in vitro* compared to animals that did not develop a large rumen (Olsen *et al.*, 1995).

The composition of the cellulolytic bacterial population adherent to the rumen particles in reindeer fed grass has not been examined earlier. The cellulolytic bacterial population in rumen contents from semi-domesticated reindeer in the Scottish highland ranged from 0.05-2.0% of the total viable population (Hobson *et al.*, 1976), resembling that of domestic ruminants like sheep and cow (van Gylswyk & Schwartz, 1984). Orpin *et al.* (1985) found the cellulolytic bacterial population in the rumen fluid of the high-Arctic Svalbard reindeer (*Rangifer t. platyrhynchus*) to constitute as much as 14.6 and 35.0% of the total viable population in summer and winter, respectively. The four major cellulolytic bacterial species *Ruminococcus albus, R. flavefaciens, Fibrobacter succinogenes* and *B. fibrisolvens* were all isolated from Svalbard reindeer on natural pastures (Orpin *et al.*, 1985). The same isolation technique was used in the present study, but bacterial isolations were made using an acid swollen cellulose medium and only CMC-hydrolysing bacteria were characterised. Numbers of bacteria adherent to the rumen solids obtained from the individual animals were generally higher on the M8SC medium which was incubated for 6-7 days, compared to the numbers on M8V medium incubated for 48 h (Table 2). The high total concentration of carbohydrates in the M8V medium allowed a very rapid growth of some of the bacterial strains, resulting in very large colonies after a 48 h incubation period, and the slow-growing strains may therefore have escaped counting on this medium. None of the CMC hydrolysing bacteria isolated from the silage-fed reindeer were found to be cellulolytic, contrasting the fact that cellulolytic *B. fibrisolvens* were obtained from all the hay-fed calves (Table 3). *B. fibrisolvens* is generally not considered to be a rapid cellulose digester compared to the other cellulolytic bacterial strains (Hungate, 1966; van Gylswyk & Labuschagne, 1971), but is found to be the most numerous strain in the rumen of the Svalbard reindeer (Orpin *et al.*, 1985). A cellulolytic *Clostridium*

sp. was also isolated from one of the hay-fed animals (Table 3). Cellulolytic members of the genus *Clostridium* have occasionally been isolated from the bovine rumen, but they appear to be of less importance (Hungate, 1957; Shane, 1969). *F. succinogenes* does not grow well in cellulose agar medium (Stewart *et al.*, 1981), and since isolations of CMC-hydrolysing bacteria from reindeer rumen feed particles were made from acid swollen cellulose agar medium, this might have excluded possible strains of this species present.

Growth of pure cultures of cellulolytic strains are inhibited at low pH values, the most resistant strain, *B. fibrisolvens,* is inhibited at pH 6.15 (Russel & Dombrowski, 1980). Re-feeding starved reindeer may induce ruminal acidosis (Bøe *et al.*, 1982; Sletten & Hove, 1990). Water soluble carbohydrates (WSC) may be present in high concentrations in timothy silage, and silage may therefore cause an initial drop in ruminal pH when given to reindeer due to rapid fermentation of the WSC. Such a drop in pH might cause changes in the ruminal cellulolytic bacterial population. The pH recorded in rumen fluid obtained from two adult female reindeer instrumented with rumen fistulas during the initial 47 hours of *ad lib.* access to leafy timothy silage with (on DM basis) 30% WSC, following a 7 weeks period of *ad lib.* access to lichen and 4 days of starvation, revealed no dramatic drop in pH, the lowest recorded pH being 6.90 and 6.45 in the two animals, respectively (M. A. Olsen & T. H. Aagnes, unpubl. data). Hence, reindeer seem to increase the intake of timothy silage, in such a way that rumen acidosis is prevented duting the initial feeding period. Compared to pelleted rations, structural grass will probably stimulate rumination and hence salivation, buffering the microbial VFA produced in the rumen. The differences in the composition of the adherent bacterial population in reindeer fed silage and hay is therefore unlikely to be explained by ruminal acidosis, more likely the chemical composition of the two different substrates. Addition of energy in the form of molasses, increasing the contents of WSC to 16% DM in the timothy silage, increased DM food intake significantly compared to when fed just a pure timothy silage diet (Moen *et al.*, 1998). Easily fermentable carbohydrates support microbial growth in the rumen and may therefore also increase cell wall digestion. A low content of WSC in the timothy silage (Table 1) may therefore have led to energy deficiency for the rumen microorganisms in these animals. The hay was rich in true protein compared to the timothy silage (Table 1). NH_4-N available for microbial synthesis was higher in hay-fed than in silage-fed reindeer (Moen *et al.*, 1998: Table 4). It is therefore concluded that low ruminal levels of N in reindeer calves fed timothy silage, compared to animals fed hay, seems to suppress ruminal microbial synthesis of cellulolytic bacteria, in particular when intake of easily available energy is low.

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