Key note address:

Microbiology of digestion in the Svalbard reindeer *(Rangifer tarandus platyrhynchus)*

Colin G. Orpin [1,2] and **Svein Disch Mathiesen** [2]

[1]CSIRO Division of Tropical Crops and Pastures, 306 Carmody Road, St Lucia QLD 4067, Australia and
[2]Department of Arctic Biology and Institute of Medical Biology, University of Tromsø, 9001 Tromsø, Norway

Introduction

The Svalbard reindeer *(Rangifer tarandus platyrhynchus)* lives on the high arctic archipelago of Svalbard (77 to 81° N). It is the only indigenous herbivorous mammal found there, and with the exception of possible contact with introduced Musk-ox *(Ovibos moschatus)* in Spitsbergen, has been isolated from other ruminants for possibly 40,000 y (Hakala, Staaland, Pulliainen & Røed, 1985).

Both climatic and nutritional conditions for the Svalbard reindeer are extreme. During winter for a period of about three months there is continuous darkness, and snow covers much of the ground from October to late May. Although snow cover rarely exceeds 30 cm (Tyler, 1987), maritime climatic influences cause the ambient temperature in winter to occasionally rise above zero for short periods. This results in cycles of thawing and refreeezing of the snow which turns into a hard ice layer over much of the vegetation on the tundra. Before the vegetation is locked in the ice, the animals can dig in the snow to find forage, but after the ice forms they are forced to abandon these areas and seek food on the more windswept areas of the valley sides and mountains which are devoid of ice (Tyler, 1987). In these regions the standing plants may provide only a few grams of forage per square metre (Tyler, 1987). In some years when the animals are forced into these nutritionally inadequate areas, over 25% mortality due to starvation occurs before the onset of plant growth in the spring (Tyler, 1987).

Unlike many reindeer or caribou populations, the Svalbard reindeer does not have the option of migrating to better feeding areas in winter because of its geographically isolated habitat. In Svalbard plant growth is restricted to about eight weeks in the summer when mean ambient temperatures are in the range of 1-6°C. From mid-June to September, when the snow returns, food is abundant and of high nutrient value (White & Staaland, 1983,; Staaland *et al.* 1983). During this period the animals have to restore depleted body reserves of fat and protein used up during the winter period,

in addition to providing energy for maintenance and for lactation in the females. The deposition of up to 30% of their body weight as fat in the autumn, however, is not sufficient to guarantee their survival over winter (Mathiesen *et al*, 1987, Tyler 1987).

In the summer the animals have access to a wide range of plant species. By September, many of these plants, particularly grasses and sedges, have formed seed heads which comprise a major part of the diet which is consequently rich in non-structural carbohydrates and protein (Staaland *et al*, 1983). In winter, mostly mosses and other fibrous plants such as the woody *Dryas octopetala* are available as food; thus, the annual food cycle ranges from young forage in spring, to a concentrated diet including seed heads in September, to a highly fibrous diet including mosses in winter (Person *et al*, 1983; Punsvik *et al*, 1980). In order to survive under these nutritional conditions the animals must make maximum use of the high quality summer forage, in addition to being able to digest the poor quality forage which is often the only food available during the eight month winter period.

Since the Svalbard reindeer occupies such an unusual ecological position, it was of considerable interest that the microbiology of digestion be examined. It was considered that the possession of a rumen and/or caecal microflora particularly adapted to a high-fibre diet could be critical to the survival of these animals (Orpin *et al*, 1985), since the caecum in Svalbard reindeer is relatively large, comprising 11% of the alimentary tract in summer and 7% in winter (Staaland *et al*, 1979) and in domestic ruminants, caecal fermentation may provide up to 30% of the volatile fatty acids entering the blood stream (Williams, 1965; Faicheny, 1986).

This paper summarises research into the digestive microbiology of the Svalbard reindeer, done in this area, and compares the results with what is known of the microbiology of digestion in other reindeer and caribou, and in domestic ruminants. The work was undertaken in April (late in the high-arctic winter) and September (late in the high-arctic summer). Full details are

Table 1. Population densities of rumen large bacteria from the Svalbard reindeer, estimated by direct microscopy of diluted rumen fluid, in September (high-arctic summer) and April (high-arctic winter) (Orpin & Mathiesen, 1985).

Counting method, bacteria	Mean population density + SD ($\times 10^8$) in:		April populations as % of September populations
	September	April	
Total large bacteria	5.97 ± 1.53	0.54 ± 0.13	9.0
Oxcillospira guilliermondii	0.07 ± 0.03	0.008 ± 0.032	11.4
Magnoovum eadii	0.25 ± 0.08	0.064 ± 0.018	25.6
Quin's Oval	0.94 ± 0.21	0.082 ± 0.021	8.7
Large *Selenomonas* sp.	3.75 ± 0.72	0.280 ± 0.047	7.4
Other large bacteria	0.96 ± 0.14	0.109 ± 0.033	11.4

given by Orpin *et al* (1985) and Mathiesen *et al* (1987).

Ruminal and caecal pH and volatile fatty acid levels

The low pH values recorded in the rumen of the free-ranging Svalbard reindeer (Table 1) and the relatively high total concentration of volatile fatty acids when compared with grazing domestic ruminants suggested that active fermentation of dietary materials occurred in both seasons. The rumen pH in cattle fed a high concentrate diet is usually in the range of 5.5-6.7 (Hungate, 1966), thus, the pH of the reindeer rumen in summer, grazing a diet rich in seedheads, is within this range. The rumen pH in winter was high and may be caused by reduced volatile fatty acid production in winter. The winter volatile fatty acid concentration of 98.7 mM was surprisingly high considering the high pH and paucity of food available; it was comparable with that found in both sheep and cattle fed good pasture, and better than found in domestic ruminants fed high roughage diets where the volatile fatty acid concentrations may fall to as low as 55 mM (Hungate, 1966). The acetate to propionate ratio, higher in winter than in summer, might reflect the increased fibre content of the diet in winter compared with that in summer.

In the caecum the pH was above 7.0 in winter, suggesting that little acid production occurred in this organ in that season. A drop in caecal pH to 6.81 in summer, when food availability was high indicated that more fermentable material was reaching the caecum compared to that in the winter. The caecal pH in the Svalbard reindeer was thus similar to that of domestic ruminants, which is close to neutrality, ranging from 6.6 to 7.8 in sheep fed lucerne, and from 5.7 to 7.2 in sheep fed grain diets (Hungate, 1966).

Rumen digesta and epithelial populations

Electron microscopic examination of digesta fragments (K.J. Cheng *et al*, unpublished results) undergoing digestion in the rumen revealed that the major cellulolytic bacteria adherent to plant fragments were *Fibrobacter succinogenes* and *Ruminococus albus,* identified by their characteristic glycocalyces. Some adherent *Butyrivibrio fibrisolvens* cells were observed, and these may be representative of cellulolytic strains. These cells were relatively few, particularly in relation to the high proportion of cellulolytic butyrivibrios cultured from the rumen liquor.

Direct examination by SEM of sites on the rumen walls of the summer animals showed that only about 30% of their epithelial surfaces were covered by adherent bacteria, compared to 75% in well-fed cattle (K-J Cheng, personal communications). Many epithelial cells were partially sloughed. The adherent bacterial populations consisted largely of curved rod-shaped cells, and cocci which resembled *Ruminococcus* spp. by their possession of a condensed glycocalyx on the cell surface. Other small cocci (<0.3 μm) were observed amongst the larger bacteria (<8 μm) in the adherent populations. In contrast, less than 10% of the epithelial surfaces were covered by adherent bacteria in samples of rumen epithelium taken from animals shot in April. This value is low and corresponds to that found in starved cattle (K-J Cheng, personal communications). No individual epithelial cells were extensively colonized, and fewer cells were in the process of sloughing than observed on the rumen epithelium from summer- shot animals. Colonized regions were principally occupied by cocci possessing less glycocalyx than observed in summer- harvested epithelium, and the majority of cells were smaller in size (<0.3 μm). Bacteria of this type were not, however, identified amongst strains isolated during the viable culturing techniques employed to characterize the bacterial population of the rumen liquor. No comparative data is available for other reindeer subspecies.

While the removal of rumen contents, the washing of the rumen wall and complete starvation of domestic cattle, reduced their rumen wall-associated bacterial populations to <3% of their original values (K-J Cheng, personal communication), the natural seasonal changes in food quality and food intake of Svalbard reindeer reduced both the number and cell size of the rumen wall-associated bacterial population from summer to winter and eliminated the glycogen-rich bacterial population associated with celulose fibres undergoing bacterial digestion in winter. Starvation-induced reductions have been documented in rodents and in marine verebrates and invertebrates.

However, the Svalbard reindeer retain residual bacterial populations on their rumen surfaces, aided perhaps by reductions in the rates of epithelial cell sloughing. Cellulosic material in the rumen contents is effectively colonized and digested by adherent bacterial consortia of which cellulolytic *B. fibrisolvens* do not appear to be major members, despite their occurrence at high populations densities in the rumen liquor.

Protozoal and fungal populations of the rumen

Microscopic examination of the rumen contents of the Svalbard reindeer revealed that as in domestic ruminants, bacteria, protozoa and anaerobic fungi were present. Direct microscopic counts of protozoa in diluted rumen fluid stained with iodine (Coleman, 1987) showed that protozoal population varied from 10^5 in summer to 10^4 in winter, and that it consisted of only entodiniomorphid ciliates. No holotrich ciliates were observed. The major species of entodiniomorphs identified were *Entodinium simplex*, *E. triacum triacum*, *E. longinucleatum*, *Polyplastron multivesiculatum*, *Eremoplastron bovis* and *Eudiplodinium maggii*. The population density of the ciliates in summer was similar to that found in domestic ruminants fed a good quality diet (Hungate, 1966) falling to about 10% of the summer value in winter, The species of protozoa detected are also found in domestic ruminants althoug *E. triacum triacum* is rare and has not been recorded previously from reindeer. There is little evidence to suggest that the *Entodinium* spp. are important in fibre digestion in ruminants utilizing principally starches and bacteria as carbon source (Williams & Coleman, 1988), but *E. maggii, Polyplastron multivesiculatum* and *Eremoplastron bovis* are all known to ingest plant particles, and contain cellulase (Coleman, 1985). Among the other subspecies of the reindeer/caribou complex, *E, maggii* has only been found in reindeer in Russia and *P. multivesiculatum* in Russia and Alaska (Dehority, 1986).

Holotrich ciliates, whilst common in domestic sheep and cattle, have been found in reindeer in Russia, Finland and Alaska, and in Alaskan caribou but not in Canadian or wild Alaskan reindeer (Dehority, 1986, Westerling 1970). Thus, their absence from the Svalbard reindeer is not exceptional and, since they metabolize only soluble carbohydrates and starch (Williams & Coleman, 1988), play little direct role in fibre digestion. There absence may reflect the repeated starvation cycles to which the animals are exposed.

Anaerobic fungi are now known to be common inhabitants of the rumen and alimentary tracts of large herbivores (Orpin & Joblin, 1988). Several different species and unnamed morphological types are known to exist, but only one type was found in the rumen of the Svalbard reindeer. This was a monocentric species with polyflagellated zoospores, endogenous development and a branching rhizoidal system characteristic of *Neocallimastix* spp. The population density of rumen fungi as determined by enumeration of thallus-forming units (Joblin, 1981) was in the range of 10^3-10^4 in summer and 10^2-10^3 in winter. Using the same methods the population density of fungal thallus forming units in forage- fed cattle and sheep is 10^3-10^5 (Orpin & Joblin, 1988) depending on the diet; thus, the winter population density is low compared with cattle and sheep.

Table 2. Culturable bacteria from the rumen liquor of Svalbard reindeer (Orpin *et al* 1985) and from the caecum (Mathiesen *et al* 1986). Results are the means from six animals in each season.

Rumen	Summer	Winter
Total viable bacteria	$2.1 \pm 1.3 \times 10^{10}$	$3.6 \pm 2.8 \times 10^{9}$
Lactate utilizers	$2.6 \pm 3.0 \times 10^{9}$	$3.9 \pm 1.9 \times 10^{7}$
Spirochetes	$1.9 \pm 2.6 \times 10^{8}$	$2.5 \pm 3.0 \times 10^{7}$
Cellulolytic species	$3.0 \pm 5.4 \times 10^{9}$	$1.3 \pm 8.7 \times 10^{9}$
Methanogenic species	10^{4}	10^{7}

Caecum	Summer	Winter
Total viable bacteria	$8.9 \pm 5.3 \times 10^{8}$	$1.5 \pm 0.7 \times 10^{8}$
Lactate utilizers	ND	$1.1 \pm 0.3 \times 10^{6}$
Cellulolytic species	$8.7 \pm 3.2 \times 10^{-7}$	$9.9 \pm 3.8 \times 10^{7}$

ND = not determined

Ciliated protozoa were not observed in the caecum of these reindeer, and attempts to isolate anaerobic fungi from caecal contents were not made. Flagellated protozoa, however, occurred at 10^{2}-10^{3} cells ml^{-1} in the caecum and 10^{3}-10^{4} cells ml^{-1} in the rumen. This is similar to levels found in domestic ruminants (Hungate, 1966).

Bacterial populations of the rumen and caecum

All of the recognised species of rumen large bacteria, i.e. *Oscillospira guilliermondii, Magnoovum eadii* (Orpin, 1976), Quin's Oval, and large strains of *Selenomonas* sp. were present in the rumen during both seasons (Table 2). The population density in winter showed a greater decrease when compared with the summer value than did the small bacteria., with Quin's Oval and large selenomonads showing a greater decrease in population density than the other species. An unidentified large bacterium (classified as 'other large bacteria' in Table 1) similar in size to Quin's Oval, but differing in its motility pattern was observed in both seasons. The rumen large bacteria are known to ferment only soluble carbohydrates (Orpin, 1972, 1973) and their lower population density in the winter is most likely due to a reduction in dietary soluble carbohydrates and starch in the diet in winter compared with summer.

Conventional normal bacterial populations

The population density of the conventional bacteria of the rumen decreased in winter to about 17% of the summer population density (Table 3) probably reflecting the reduced availability and quality of diet in winter. A similar decrease in the population density of caecal bacteria was recorded in winter compared with the summer values.

Table 4 presents the species distribution of bacteria other than large bacteria, present in rumen and caecum fluid of the Svalbard reindeer in both summer and winter; their main fermentation niches are presented in Table 5. Population densities in general showed a large decrease in winter compared with the summer values, with most species declining to 10-50% of the summer values. Exceptionally, increases in population density of individual species occurred in winter, for example, *Fibrobacter succinogenes* in the rumen showed a 107% increase and *Lactobacillus* sp. in the caecum showed a 143% increase compared with summer values. It is difficult to compare the species distribution

Table 3. Culturable ruminal and ceacal bacterial populations expressed as a percentage of total culturable bacteria in winter and summer.

	Rumen			Caecum		
	summer	winter	winter as % of summer	summer	winter	winter as % of summer
B. fibrisolvens	22*	30	24	23	18	14
B. fibrisolvens[a]	10	18	33	7	1	3
Selenomonas ruminantium	12	14	21	10	8	14
Selenomonas ruminantium subsp. *lactilytica*	4	2	7	ND	ND	—
Lacbnospira multiparus	2	ND	—	1	ND	—
Lactobacillus sp.	8	3	7	1	7	143
Megasphera elsdenii	1	1	29	2	3	22
Bacteroides ruminicola	4	10	49	10	26	45
Bacteroides amylophilus	10	3	6	7	5	13
Fibrobacter succinogenes	1	8	107	ND	ND	—
Ruminococcus albus	3	7	39	3	5	28
Ruminococcus flavefaciens	1	2	57	ND	ND	—
Ruminococcus bromii	ND	ND	—	3	1	7
Succinivibrio dextrinosolvens	3	1	5	2	ND	—
Streptococcus bovis	*17*	*4*	*5*	*17*	*5*	*5*
Streptococcus faecium	ND	ND	—	5	7	22
Eubacterium ruminantium	2	1	7	ND	ND	—

a = cellulolytic strains ND = not detected

Table 4. Ecological niches of the major bacterial species isolated from the rumen and caecum of Svalbard reindeer.

	Major substrates
Butyrivibrio fibrisolvens	Pectin, hemicelluloses, some strains cellulose and starch
Selenomonas ruminantium	Soluble carbohydrates, most strains utilize starch
S. ruminantium lactilytica	Soluble carbohydrates, lactate, glycerol, and many strains starch
Lacbnospira multiparus	Soluble carbohydrates, pectin
Lactobacillus sp.	Soluble carbohydrates, some strains starch
Megasphera elsdenii	Starch, lactate, soluble carbohydrates
Bacteroides ruminicola	Hemicelluloses, xylan, pectin, some strains starch
Bactroides amylophilus	Starch, soluble carbohydrates
Fibrobacter succinogenes	Cellulose
Ruminoccus albus	Cellulose, xylan
R. flavefaciens	Cellulose, xylan
R.bromii	Starch, amylopectin
Streptococcus bovis	Starch, amylopectin, soluble carbohydrates
Succinivibrio dextrinosolvens	Pectin, soluble carbohydrates
Eubacterium ruminantium	Soluble carbohydrates, xylan

Table 5. Population densities of some cellulolytic bacteria in the rumen of Svalbard reindeer in September (summer) and April (winter).

Bacteria	Population densities[a]	
	in September	April
Ruminococcus albus	3.0 ± 1.1	6.7 ± 3.3
Ruminococcus flavefaciens	0.7 ± 0.5	2.2 ± 1.0
Fibrobacter succinogenes	1.3 ± 1.5	8.0 ± 5.2
Butyrivibrio fibrisolvens	9.5 ± 7.1	18.2 ± 7.7
Total viable cellulolytic species as % of total visible population	14.6 ± 5.4	35.0 ± 8.7
Cellulolytic *B. fibriosolvens* as % of total *B. fibrisolvens*	44.6	60.1

[a] values are expressed as % viable bacterial populations

found with that occurring in other animals since we know of no comparable detailed work giving full quantitative data.

The viable bacterial population of the rumen in summer was dominated by *Butyrivibrio fibrisolvens* and *Streptococcus bovis*, together representing 39% of the total isolates. In winter, ruminal *S. bovis* declined considerably, but *B. fibrisolvens* increased from 22% to 30% of the population. Amongst the other species, an increase in the differential population of the celluloytic species was recorded in winter, as was the case with *Bacteriodes ruminicola*, a hemicellulose digester. Similar seasonal fluctuations were also found amongst the caecal bacterial population, which reflected the rumen population in species composition with the exception that more *Streptococcus* spp., including *S.faecium* and *S.faecalis* were present, and *Ruminococcus bromii*, a starch digester, which was not identified amongst the ruminal isolates (Table 4).

Methanogenic bacteria in rumen contents were estimated to be present at 10^4 cells ml^{-1} in the summer and 10^7 cells ml^{-1}in the winter; in the caecum their population densities during the same seasons were 10^7 and 10^5 cells ml^{-1}respectively. These levels are similar to those found in domestic ruminants with the exception of the summer value which is very low and may have been caused by cultivation problems.

When the bacteria isolated from the viable count medium were individually assessed for their ability to ferment the major plant components in the diet, a large proportion were found to be fibrolytic, able to use cellulose, xylan or both as carbon sources. In the rumen, 31% and 74% of isolates were fibre fermenters in summer and winter respectively, and in the caecum the corresponding values were 36% and 48% respectively. Indeed, in the rumen, cellulolytic bacteria alone accounted for about 15% of the total population density in summer and 35% in winter, (Table 6). The dominant cellulolytic bacteria in the rumen were *B. fibrisolvens* with *Ruminococcus albus*, *R. flavefaciens* and *Fibrobacter succinogenes* present at lower population densites. The latter three species are commonly the most abundant cellulolytic bacteria in the rumens of domestic animals (Hungate, 1966), with cellulolytic *B. fibrisolvens* being rare, having been isolated routinely only from sheep in South Africa (van Gylswyk & Roche, 1970). Cellulolytic *B. fibrisolvens*, however, have been found abundantly in the rumen of African Antelopes (Margherita & Hungate, 1963) and was found by Dehority (1986) in lucerne-fed

Table 6. Distribution of cellulase (CMCase) activity in cultures of *Butyrivibrio* A46

Carbon source	Glucose		Cellobiose		Cellulose[a]	
Age of culture (h)	Activity against CMC[b]					
	cells	supt.	cells	supt.	cells	supt.
6	—	1.9	0.6	5.1	4.8	1.2
16	0.2	6.5	0.5	11.8	11.7	2.4
64	0.4	9.7	0.4	8.7	12.3	14.1
88	0.3	9.2	0.4	13.2	9.1	18.6

[a] acid-swollen Sigmacell

[b] units mg cell protein^{-1}or ml supt^{-1} (1 unit = 1 μ mole glucose equivalent h^{-1})

reindeer in Alaska. In the caecum, the only cellulolytic species identified other than *B. fibrisolvens* was *Ruminococcus albus*, representing 3 and 5% of the total population in summer and winter respectively compared with cellulolytic *B. fibrisolvens* which comprised 7 and 3% of the summer and winter populations respectively.

Degradation of plant cell walls is a function of both celluloytic and hemicellulolytic organisms. All of the celluloytic species except *F. succinogenes* also degraded xylan, a major hemicellulose of grasses, but in addition a number of xylan- utilizing strains of *Bacteriodes ruminicola* and *Eubacterium ruminantium* were found.

Starch digestion

There was little difference in the proportion of the bacterial population able to utilize starch in the two seasons, but the population density of starch digesting bacteria in winter was only 16.6% of the summer value. The dominant starch-digesting bacterium in summer was *Streptococus bovis*, whilst in winter it was *Butyrivibrio fibrisolvens*. In winter the *S. bovis* population decreased to only 5% of its summer value, with starch-utilizing *Bacteroides ruminicola* increasing from 4% in summer to 10% in winter. In winter only 85% of *B. ruminicola* strains utilized starch, compared with 100% in summer. Similar decreases in starch-digesting ability of *Selenomonas ruminantium*, *Sr lactilytica* and *B. fibrisolvens* was evident. Of the total viable population, 68% could utilize strach in the summer, compared to 64% in winter. Many of the *B. fibrisolvens* could also utilize xylan, cellulose or both as substrates (Orpin *et al*, 1985). In the ceacum, the proportion of the bacterial starch digesters was *Butyrivibrio fibrisolvens* in summer, and *Bacteroides ruminicola* in winter. Other starch utilizers present were *Streptococcus bovis*, *Selenomonas ruminatium*, *Bacteroides amylophilus* and *Ruminococcus bromii*, each showing a decrease in numbers in winter compared with the summer populations (Mathiesen *et al*, 1987).

Proteolytic and ureolytic bacteria

Isolates with proteolytic activity or ureolytic activity were common in both seasons. In summer, 51% of the population and in winter, 28% showed proteolytic activity. All of the *Streptococus bovis* isolates were proteolytic with azocasein as substrate, with over 80% of these able to use plant fraction 1 protein as sole nitrogen source. Other species found to be commonly proteolytic were *Bacteroides amylophilus* (100% in sumer, 67% in winter) all of which could also use fraction 1 protein as sole nitrogen source, and hydrolysed up to 1 mg/ml of protein in laboratory culture in 24 h. Of the *Selenomonas ruminantium* isolates, 80% of the summer and 43% of the winter isolates were proteolytic when tested with azocasein, but

none hydrolysed plant fraction 1 protein *in vitro*, which suggests they many not be important in proteolysis in the rumen.

The summer population density of proteolytic bacteria was higher than previously recorded for any ruminant. Fulghum & Moore (1963) recorded up to 46% of proteolytic bacteria in cattle, but the proteolytic population of domestic ruminants is normally smaller (Wallace & Cotta, 1988). The lower proteolytic population in winter compared to summer coincides with a lower protein content in the winter diet.

Ureolytic bacteria were also common in the rumen of Svalbard reindeer, with about 40% of strains in winter and 54% in summer possessing weak urease activity. All the *Bacteroides ruminicola* and *Succinivibrio dextrinosolvens* isolated in both seasons were ureolytic together with large numbers of *Selenomonas ruminantium* and *Butyrivibrio fibrisolvens*. However, the urease activity of all these isolates was low compared with *Streptococcus faecium* (Cook, 1976) and other rumen epithelium-associated bacteria (Cheng *et al*, 1979).

Nitrogen recycling via urea could be of significance in these animals in maintaining their nitrogen balance in winter where food quality and availability is low. The large ureolytic bacterial population supports this suggestion, and agrees with the finding of large weakly ureolytic bacteria populations in cattle fed a diet containing urea (Jones *et al*, 1964).

Ammonia can be used by many rumen bacteria as a source of nitrogen. Some species require peptides or amino acids for growth (Wallace & Cotta, 1988). During periods of starvation, ammonia produced by hydrolysis of urea (by urease), which enters the rumen via the saliva and crosses the rumen epithelium from the blood, would satisfy the nitrogen requirements of the ammonia-utilizing species. A large proteolytic population of bacteria in the rumen may be necessary to supply peptides by hydrolysis of proteins, from the diet, dead rumen microbes, or sloughed eptihelial cells during the winter. Protein hydrolysis would also result in the generation of branched-chain amino acids which would aid in the maintenance of the bacterial population under conditions of semi-starvation (Bryant, 1974).

Studies on the cellulolytic Butyrivibrio

We have examined the cellulase activity of *Butyrivibrio fibrisolvens* A46, isolated from one of the Svalbard reindeer shot in September, and have cloned a fragment of DNA coding for endo-ß-1,4-ß-glucanase (carboxymethyl cellulase) activity in *Escherichia coli* (Hazlewood, Clarke, Davidson, Romeniec & Orpin, *et al*, 1990).

Table 7. Activity of *Butyrivibrio fibrisolvens* A46 cellulase on polysaccharides

Polysaccharide	Activity[a]
Carboxymethyl cellulose	0.54
Lichenan	0.00
Laminarin	0.31
Avicel[b]	0.00
ß-1,3-glucan	2.17
Arabinogalactan	0.96
Galactomannan	0.24

[a] Activity expressed as μ mols of reducing sugar mg protein^{-1} h^{-1}.

[b] Avicel is a highly crystalline cellulose

Cellulase activity was produced constitutively by A46 when grown on glucose or cellobiose, but at lower concentrations than when grown on cellulose. The activity was present in both the cells and the cell-free supernatant, with the proportion of cell-bound enzyme greatest when grown on cellulose (Table 7). Since cellulase was produced when A46 was grown on soluble carbohydrates, it is likely that it is produced continuosly in the rumen and not subject to major catabolite repression as occurs with other cellulolytic rumen bacteria (Chesson & Forsberg, 1988; Taylor *et al*, 1987). Cellulase (carboxymethyl cellulase; CMCase) was purified from *Butyrivibrio* A46 cells to a specific activity of 700 U mg protein^{-1} or from cell-free culture supernatant that had an apparent molecular mass of 57 kDa. Purified enzyme was most active against CMC and aryl-cellobiosides but also hydrolysed acid-swollen Sigmacell to a lesser extent. Activity was optimal at pH 5.6 and was thermolabile, being rapidly inactivated at 55°C. Hydrolysis of CMC resulted in the production of reducing sugar and a rapid fall in viscosity, characteristics of endo-ß-1,4-glucanase activity. A gene coding for CMCase activity was contained on a 2.4 kbp *EcoRI* fragment cloned from *Butyrivibrio* A46 in lambda phage gt11, and was subcloned in *Escherichia coli* using the plasmid vector pUC12. CMCase encoded by the cloned fragment of DNA was purified to a specific activity of 142 Umg protein^{-1}. In general, the properties of the cloned CMCase were similar to those described for the enzyme purified from cultures of *Butyrivibrio* A46.

The cloned enzyme showed activity against laminarin (a ß-1.6-glucan), ß-1,3-glucan, arabinogalactan and galactomannan (S.P. Mann, personal communications; Table 8) as well as carboxymethyl cellulose, but not avicel, a highly crystalline form of cellulose. Thus, the probability exists that the enzyme produced *in vivo* is a multi-functional enzyme with cellulase activity, produced with limited regulation; its activity at any particular time being controlled by substrate availability in its immediate environment. Such an enzyme, would be of great benefit in the rumen of an animal that needed to digest food rapidly and which fed on diets ranging greatly in quality, such as the Svalbard reindeer.

As yet there is not proof that the cellulolytic *Butyrivibrio fibrisolvens* adheres to the plant tissue like the other major rumen cellulolytic bacteria, *Ruminococcus albus, R. flavefaciens* and *F.succinogenes.* In these species adherence is a prerequisite to cellulolysis, and the production of cellulase tightly regulated. In the *B. fibrisolvens*, the continuous production of cellulase may stimulate cellulolysis by the other cellulolytic species by removal of oligosaccharides from the immediate vicinity of the digesta fragments being degraded. This would minimise the chance of the oligosaccharides being hydrolysed to glucose and cellobiose, catabolite repressors of cellulase, and thus help maintain cellulolysis by the adherent cellulolytic population. However, adherence by cellulolytic *B. fibrisolvens* has been demonstrated *in vitro* (Cheng, Mathiesen & Orpin, unpublished).

In vitro digestibility experiments

In vitro digestibility trials of food plants, including mosses, were conducted using plants, collected from the range in both seasons. The methods used were based on those of Tilley & Terry (1963) scaled to 100 mg of plant material, and using centrifugation to separate residual plant tissues from bacteria after incubation. Since mosses form a large part of the diet of these reindeer in winter, and large fragments of moss were observed in the caecal contents (Mathiesen *et al*, 1987), particular attention was paid to the digestion of mosses in the caecum. Comparable data for the digestibility of these species in domestic ruminants are not available, but in the Svalbard reindeer rumen moss digestion was in the range of 11-36%, and caecal moss digestion 11-27%. The *in vitro*

DMD of standard samples of Timothy *(Phleum pratense)* of high (74.7% *in vivo)* DMD and low (57.6% DMD *in vivo* DMD) in sheep, gave similar DMD values *in vitro* using reindeer rumen fluid in summer, but in winter the DMD of the poorer quality Timothy grass in caecum fluid was marginally greater than in sheep.

Thomas & Kroeger (1980) investigated the *in vitro* ruminal range plant in Peary caribou *(Rangifer tarandus pearyi)*. Whilst the apparent digestibility of vascular plants in summer was in the range of 50-80%, that of mosses was 11-35%, in close agreement with the results found for the Svalbard reindeer. However, the Svalbard reindeer rumen fluid digested mosses condsiderably more extensively in winter (16-31% over 48 h of incubation) than fluid from Peary caribou (3-11% over 60 h).

These results suggest that moss digestion in both the rumen and the ceacum may be of value in the nutrition of the animal, especially with *Callergon sarmentosum,* but its quantitative significance is still not known.

In vitro digestibility values for mosses collected in the summer and incubated with summer caecum fluid were 11-28%, suggesting that some moss digestion in the caecum would occur if mosses reach the caecum in that season. In winter, when the diet is relatively rich in moss, ceacal digestiblity was lower, in the range of approximatley 14% in the two species tested.

In winter the rumen and caecal contents smelt strongly of earth, and sand grains were found in the rumen. The occurrence in one animal of 10^8 cells ml^{-1} of *Serratia marcescens,* a soil bacterium, is indicative of soil ingestion, which could happen when the animal uproots the whole plant including some soil, when eating.

Conclusion

The rumen bacterial population of the Svalbard reindeer is very specialised in relation to fibre digestion and nitrogen recycling with large numbers of fibrolytic, proteolytic, and ureolytic strains present in both summer and winter. The high population density of species such as *Butyriribrio fibrisolvens* that are capable of fibre and starch digestion enables the animals to make best use of the very variable diet that the animals eat. The ability of these organisms to ferment starch in the summer as well as fibre may allow the maintenance of high population densities of cellulolytic bacteria in the presence of the concentrated diet. The high ruminal bacterial population density in the winter despite apparent starvation of the animals, may in part be supported by urea recycling ensuring a sufficient nitrogen supply to enable the bacterial population to maintain high activity despite the low protein diet.

The low population density of methanogenic bacteria in the summer compared with winter would ensure that additional energy is conserved from the diet in summer. In addition, the high concentrate diet would produce additional propionate in summer and lead to increased gluconeogenesis in the animal.

Winter survival of the Svalbard reindeer is aided by some moss digestion in the rumen and possibly the caecum.

Our results indicate that the caecal bacteria may contribute substantially to forage utilization in the Svalbard reindeer, but their precise role cannot be determined until particle flow rate and compositon measurements have been made.

References

Bryant, **M.P** 1974. Nutritional features and ecology of predominant anaerobic bacteria of the intestinal tract. - *American Journal of Clinical Nutrition,* 27:1313-1322.

Chesson, **A.** & Forsberg, **C.W.** 1988. Polysaccharide degradation by rumen microorganisms. In: P. Hobson (ed) - *The Rumen Microbial Ecosystem* pp.251-284.

Cheng, **K-J.**, McCowan, **R.P** & Costerton, **J.W.** 1979. Adherent epithelial bacteria in ruminants and their roles in digestive tract function. *American Journal of Clinical Nutrition,* 32:139-48.

Coleman, G.S. 1985. The cellulase content of 15 species of entodiniomorphid protozoa, mixed bacteria and plant debris isolated from the ovine rumen. - *Journal of Agricultural Science,* 104: 349-360.

Coleman G.S. 1987. Rumen entodiniomorphid protozoa. - In: *In vitro methods for parasite cultivation,* ed A.E.R. Taylor & J.R. baker. London: Academic Press, pp. 29-51.

Cook, A.R. 1976. Urease activity in the rumen of sheep and the isolation of ureolytic bacteria. *Journal of General Microbiology,* 92: 32-48.

Dehority, B.A. 1986. Microbes in the foregut of arctic ruminants. pp. 307-327 - In: L.P. Milligan, W.L. Grovum and A. Dobson, *Control of Digestion and Metabolism in Ruminants.* Prentice-Hall, Englewood Cliffs, New Jersy.

Faicheny, G.J. 1986. Volatile fatty acids in the caecum of sheep. - *Australian Journal of Biological Sciences,* 21: 177-180.

Fulghum, R.S. & Moore, W.E.C. 1963. Isolation, enumeration and characteristics of proteolytic ruminal bacteria. - *Journal of Bacteriology,* 85: 808-815.

Hakala, A.V.K., Staaland, H., Pulliainen, A. and **Røed, K.H.** 1985. Taxonomy and history of arctic island reindeer with special reference to Svalbard reindeer. - *Auilo* (zool.), 23:1-11.

Hazlewood, G.P., Clarke, N.G., Davidson, K., Romaniec, M.P.M. & Orpin, C.G. 1990. Cellulase activity of a rumen *Butyrivibrio* sp. and the cloning and expression of an endo-ß-1,4-glucanase gene in *Escherischia coli.* - *Journal of General Microbiology,* in press.

Hungate, R.E. 1966. The rumen and its microbes. New York: Academic Press.

Jones, G.A., McLeod, R.A. & Blackwood, A.C. 1964. Ureolytic rumen bacteria: characterisation of the microflora from a urea-fed sheep. - *Canadian Journal of Microbiology,* 39: 1144-1147.

Joblin, K.N. 1981. Isolation, enumeration and maintenance of rumen anaerobic fungi in roll tubes. - *Applied & Environmental Microbiology,* 42: 1119-1122.

Margherita, S.S. & Hungate, R.E. 1963. Serological analysis of *Butyrivibrio* from the bovine rumen. - *Journal of Bacteriology,* 86: 855-860.

Mathiesen, S.D. & Orpin, C.G. 1986. Mikrobiell fordøyelse i Svalbardrein. In: N.A. Øritsland (ed) Svalbardreinen og dens livsgrunnlag. Oslo: Universitetsforlaget AS, pp. 111-117.

Mathiesen, S.D., Orpin, C.G. & Blix, A.S. 1985. Evidence for an important digestive function of the ceacum in the Svalbard Reindeer. - *Acta Pbysiologica Scandinavia 124,* Suppl. 542.

Mathiesen, S.D., Orpin, C.G., Greenwood, Y. & Blix, A.S. 1987. Seasonal change in the ceacal microflora of the high-arctic Svalbard reindeer *(Rangifer tarandus platyrhynchus). -Applied and Environmental Microbiology,* 53: 114-118.

Orpin, C.G. 1972. The culture of the rumen organism Eadie's Oval *in vitro.* - *Journal of General Microbiology,* 70: 321-329.

Orpin, C.G. 1973. The culture *in vitro* of the rumen bacterium Quin's Oval. - *Journal of Gereral Microbiology,* 73: 523-530.

Orpin, C.G. 1976. The characterisation of the rumen bacterium *Magnoovum eadii. Archives of Microbiology,* 111: 153-159.

Orpin, C.G. & Joblin, K.N. 1988. Anaerobic fungi. In Hobson, P.N. (ed). - *The Rumen Microbial ecosystem,* pp. 129-150. London: Elsevier.

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).* - *Applied and Environmental Microbiology,* 50: 144-151.

Person, E., Holand, Ø., Leifseht, A.B. & Staaland, H. 1983. Reindeer grazing on Brøggerhalvøya, Svalbard. - *Acta Zoologica Fennica,* 175:

Punsvik, T., Syversten, A. & Staaland, H. 1980. Reindeer grazing in Advent dalen, Svalbard, p 115-124. *in* E. Reimers, E. Gaare and S. Skjenneber (eds.). - *Proceedings of the 2nd International Reindeer and Caribou Symposium,* Røros, Norway, 1979.

Staaland, H., Brattback, E., Ekern, K & Kildemo, K. 1983. Chemical composition of reindeer forage plants in Svalbard and Norway. - *Holarctic Ecology,* 6: 109-122.

Taylor, K.A., Crosby, B., Mc Gavin, Forsberg, C.W. & Thomas, D.Y. 1987. Characteristics of the endoglucanase encoded by a *eel* gene from *Bacteroides succinogenes* expressed in *Escherichia coli.* - *Applied and Environmental Microbiology,* 53: 41-46.

Thomas, D.C. & Kroeger, P. 1980. *In vitro* digestibilities of plant in rumen fluids of Peary caribou. - *Arctic,* 33: 757-767.

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

Tyler, N.J.C. 1987. Body compositon and energy balance of pregnant and non-pregnant Svalbard reindeer during winter. - *Symposia of the zoological Society of London* (1987) No. 57: 203-229.

Van Gylswyk, N.O. & Roch, C.E.G. 1970. Characteristics of *Ruminococcus* and cellulolytic *Butyrivibrio* species from the rumens of sheep fed differently substituted teff *(Eragrostis tef.)* hay diets. - *Journal of General Microbiology,* 64: 11-17.

Wallace, R.J. & Cotta, M.A. 1988. Metabolism of nitrogen-containing commpounds. - In: P.N. Hobson (ed) *The Rumen Microbial Ecosystems,* pp. 217-249. London: Elsevier.

Westerling, B. 1970. Rumen ciliate fauna of semidomestic reindeer *(Rangifer tarandus* L.) in Finland: composition, volume and some seasonal variation. - *Acta Zoologica Fennica,* 127: 1-76.

White, R.G & Staaland, H. 1983. Ruminal vilatile fatty acid production as an indicator of forage quality in Svalbard reindeer. - *Acta Zoologica Fennica,* 175: 35-37.

Williams, V.J. 1965. Microbial metabolism in the forestomachs and the large intestine of sheep. - *Australian Journal of Agricultural Research,* 16: 77-91.

Williams, A.G. & Coleman, G.S. 1988. The rumen protoza. - In: P.N. Hobson (ed) *The Rumen Microbial Ecosystem,* pp. 77-128. London: Elsevier.