

## *In Vitro* Digestibilities of Plants Utilized by Barren-Ground Caribou

DONALD C. THOMAS<sup>1</sup>, PETER KROEGER<sup>1</sup>, and DAVID HERVIEUX<sup>1,2</sup>

**ABSTRACT.** Rumen fluids of barren-ground caribou (*Rangifer tarandus groenlandicus*) were used with standard *in vitro* procedures in March 1981 to investigate the relative digestibilities of forages collected on caribou winter ranges in the southern Northwest Territories. *In vitro* dry matter disappearance (IVDMD) of the three most abundant arboreal lichens, when fermented in test tubes for 60 h, averaged 67% compared with 43% for the seven most common terricolous lichens. The DMD of leaves of the most common shrubs, *Vaccinium vitis-idaea*, *Empetrum nigrum*, *Arctostaphylos* spp., and *Ledum* spp. averaged 46% (37-51%). Eight bryophyte species averaged 17% (7-28%) DMD. The DMD of species of three lichen genera with low protein contents, *Cladina*, *Cladonia*, and *Cetraria*, continued to increase with increasing fermentation periods up to 180 h. Nine species of lichens averaged 49% DMD when fermented for 60 h in test tubes, 64% when fermented in Erlenmeyer flasks, and 76% when 60 mg of urea was added to flasks. DMDs of 22 plant species were significantly higher in March 1981 than in similar tests conducted one year earlier. This annual variation in the digestive capacities of ruminal fluids was associated with the physical condition of the caribou and may have been related to their nutritional history.

**Key words:** Canada, caribou, digestibilities, *in vitro*, lichens, nutrition, *Rangifer*

**RÉSUMÉ.** Les fluides ruminiaux du caribou de la toundra arctique (*Rangifer tarandus groenlandicus*) furent utilisés suivant des méthodes *in vitro* standards en mars 1981 afin d'étudier la digestibilité relative des fourrages recueillis dans les pâturages hivernaux dans le sud des Territoires du Nord-Ouest. La disparition *in vitro* de matière sèche (DIVMS) des trois lichens arboricoles les plus abondants, lorsque fermentés dans des éprouvettes pendant 60 h, était en moyenne de 67% à comparer à une moyenne de 43% pour les sept lichens à croissance terrestre les plus communs. La DMS des feuilles des arbustes les plus communs, *Vaccinium vitis-idaea*, *Empetrum nigrum*, *Arctostaphylos* spp. et *Ledum* spp. était en moyenne de 46% (37-51%). Huit espèces de bryophytes avaient pour moyenne 17% (7-28%) en DMS. La DMS de trois genres de lichens à faible contenu en protéines, *Cladina*, *Cladonia* et *Cetraria* continua à augmenter suivant l'extension de la période de fermentation jusqu'à 180 h. Neuf espèces de lichens avaient une moyenne de 49% en DMS lorsqu'elles étaient fermentées pendant 60 h dans des éprouvettes, de 64% lorsque fermentées dans des erlenmeyers, et de 76% lorsque 60 mg d'urée étaient ajoutées aux erlenmeyers. Les DMS de 22 espèces de plantes étaient significativement plus élevées en mars 1981 que dans les essais effectués un an plus tôt. Cette variation annuelle des capacités digestives des fluides ruminiaux furent associées à la condition physique du caribou et peut avoir été reliée à son histoire alimentaire.

**Mots clés:** Canada, caribou, digestibilité, *in vitro*, lichens, nutrition, *Rangifer*

Traduit pour le journal par Maurice Guibord.

### INTRODUCTION

The primary objective of this study was to obtain data on the relative digestibilities and rates of digestion of plant species utilized by barren-ground caribou (*Rangifer tarandus groenlandicus*) in winter. Data were available on winter diets of that subspecies in north central Canada (Scotter, 1967; Kelsall, 1968; Miller, 1976, Thomas, unpubl.) and on the chemical composition of forages (Scotter, 1965, 1972; Kelsall, 1968; Parker, 1975; Miller, 1976; Person *et al.*, 1980a). There was no information on the relative digestibilities of forages by barren-ground caribou in Canada before our 1980 *in vitro* digestibility study (Thomas and Kroeger, 1981). Data from studies in Alaska on *in vitro* and *in vivo* digestibilities of forages in caribou and captive reindeer (Person *et al.*, 1980b; Trudell *et al.*, 1980) may be extrapolated to northern Canada only in a general way. In addition, variations in diets among caribou populations and seasonal variability in diets are likely to affect *in vitro* dry matter disappearance (IVDMD) results (Thomas and Kroeger, 1980; Trudell *et al.*, 1980). Our overall objective was to obtain a better understanding of the winter ecology of barren-ground caribou as a basis for management decisions.

In March 1980, we used the Tilley and Terry (1963) *in vitro*

technique, and rumen fluids from barren-ground caribou collected in north central Canada, to assess the relative digestibilities and rates of digestion of 32 species of plants (Thomas and Kroeger, 1981). The IVDMD of eight lichen species continued to increase over fermentation intervals of 30-120 h, in contrast to non-lichen species whose IVDMD values stabilized after 60 h of fermentation. The addition of 63 mg of urea per tube increased the IVDMD of two lichen species by 88 and 67%, and it had positive (one species) and negative (three species) effects on vascular plant species. Technical problems resulted in much higher variability between duplicates than found in similar trials with ruminal fluids from Peary caribou (*R. t. pearyi*) and plant species from the Canadian Arctic Islands (Thomas and Kroeger, 1980).

In this paper we present the results of further studies of *in vitro* plant digestibilities conducted in March 1981. Our objectives were to check the 1980 results by using better procedures, to include species not tested in 1980, to investigate extended fermentation periods for lichens, to explore the effect of adding variable amounts of urea, to compare test tubes and Erlenmeyer flasks as fermentation vessels, to explore changes in digestibility of plant material stored for one year, and to assess the effect of cooling ruminal fluids to 20°C before fermentation.

<sup>1</sup>Canadian Wildlife Service, #1000, 9942 - 108 Street, Edmonton, Alberta, Canada T5K 2J5

<sup>2</sup>Present address: Department of Biology, University of New Brunswick, Bag Service No. 44555, Fredericton, New Brunswick, Canada E3B 6C2

## METHODS

Plant material was obtained from three sources: (1) samples collected on the winter range of caribou in March 1980 that were milled and stored for one year; (2) plants stored since March 1980 and milled and dried just before use in March 1981; and (3) fresh samples obtained from caribou feeding sites in March 1981. The fresh samples of living plant tissues, except where noted, were dried at 55°C for 2-12 h, milled twice (no. 20 screen, 0.85-mm opening), and further dried at 55°C for at least 12 h. Duplicate 0.5-g samples were placed in 75-mL test tubes and inserted side by side in racks. Two blanks (no plant material) were placed centrally among sets of 18 test tubes.

Rumen fluids were pooled from five caribou collected on 19 March at 60°41'N, 107°53'W (fluid A) and from an equal number killed on 23 March at 61°04'N, 107°57'W (fluid B). The caribou were transported by aircraft to the laboratory within 3 h of death, the stomachs were excised and taken to a room heated to 30°C, and fluids were obtained by squeezing rumen samples through four layers of cheesecloth. The fluids were mixed with warm (39°C) buffer (ratio 1:4) and 50 mL of this mixture was added to each test tube and Erlenmeyer flask containing plant samples. Anaerobic conditions were maintained at all stages by use of air valves and periodic infusion of CO<sub>2</sub> gas. Procedures used in previous trials were repeated (Thomas and Kroeger, 1980, 1981) except that the final weights of undigested plant matter and filter papers were obtained after being dried at 90°C rather than 55°C. We also conducted duplicate tests on 10 species of lichens using 250-mL Erlenmeyer flasks as fermentation vessels. Urea in 15, 30, and 60 mg amounts was added to duplicate tubes and flasks containing three species of lichens. Five lichen species were fermented for 15, 30, 60, 90, 120, 150, and 180 h. The effect on DMD of allowing rumen fluid to cool for one hour to room temperature (20°C) and remain there for two hours before fermentation commenced was tested on three species of lichens.

Differences among sets of data were tested for significance with paired *t*-tests and analyses of variance, as appropriate. Variability about a mean is expressed as plus and minus one SE.

## RESULTS

*Comparative Digestive Capacities of Rumen Fluids A and B*

Tests on four plant species collected in 1981 gave the following IVDMDs (percent, after 60 h fermentation) for fluids A and B (parentheses): *Vaccinium vitis-idaea* var. *minus* leaves, 57 (51); *Empetrum nigrum*, leaves and stems, 54 (46); *Ledum* spp. leaves 35 (38); and mixed *Parmelia centrifuga* and *P. sulcata* 48 (56). There was no significant difference ( $P > 0.1$ ) between the rumen fluids in their *in vitro* digestive capacities.

*Effect of Storing Lichens for One Year*

There was no difference ( $P = 0.1$ ) among IVDMDs ob-

tained from fresh samples and samples stored for one year in plant form (Table 1). The values for fresh samples were higher than those of stored plant samples in four cases, and lower in three. Some variation is expected because all the samples were from different locations. The IVDMDs of two species of lichens stored in powder form were similar to the results for fresh material and stored plants (Table 1).

TABLE 1. Comparative dry matter disappearance (mean percent  $\pm$  SE) of lichens (60 h fermentation in tubes with caribou ruminal fluids) handled in three ways in 1981 and comparative values obtained in March 1980<sup>a</sup>

	1981			1980	
	Fresh plants	Stored <sup>b</sup> plants	Stored <sup>b</sup> powder	Fresh plants 60 h	63 h
<i>Usnea hirta</i>	65 $\pm$ 4	—	—	39 $\pm$ 8	52 $\pm$ 6
<i>Evernia mesomorpha</i>	76 $\pm$ 5	—	—	52 $\pm$ 11	43 $\pm$ 3
<i>Alectoria americana</i>	59 $\pm$ 2	—	—	42 $\pm$ 5	42 $\pm$ 5
<i>Hypogymnia physodes</i>	47 $\pm$ 3	—	—	32 $\pm$ 4	—
<i>Cladonia mitis</i>	48 $\pm$ 2	53 $\pm$ 3	56 $\pm$ 0	43 $\pm$ 3	44 $\pm$ 6
<i>Cladonia rangiferina</i>	35 $\pm$ 2	37 $\pm$ 13	—	—	45 $\pm$ 0
<i>Cladonia amaurocraea</i>	47 $\pm$ 1	—	—	30 $\pm$ 3	41 $\pm$ 5
<i>Cladonia uncialis</i>	55 <sup>c</sup>	41 $\pm$ 1	—	—	—
<i>Cladonia</i> spp.	—	39 $\pm$ 3	—	—	44 $\pm$ 3
<i>Cladonia cornuta</i>	44 $\pm$ 0	36 $\pm$ 2	—	—	—
<i>Cetraria nivalis</i>	55 $\pm$ 1	38 $\pm$ 0	—	20 $\pm$ 4	34 $\pm$ 4
<i>Cetraria cucullata</i>	64 <sup>c</sup>	—	—	—	—
<i>Cetraria ericetorum</i>	67 <sup>c</sup>	—	—	—	—
<i>Stereocaulon paschale</i>	45 $\pm$ 6	47 $\pm$ 4	48 $\pm$ 1	30 $\pm$ 6	24 $\pm$ 0
<i>Peltigera aphthosa</i>	44 $\pm$ 6	32 $\pm$ 1	—	33 $\pm$ 3	38 $\pm$ 5
<i>Lasallia pennsylvanica</i>	49 $\pm$ 3	—	—	—	—
<i>Parmelia centrifuga</i> and <i>P. sulcata</i> (mixed)	52 $\pm$ 3	—	—	—	—

<sup>a</sup>Thomas and Kroeger (1981).

<sup>b</sup>Stored for one year.

<sup>c</sup>Single tube only.

*Comparative Digestibilities in 1980 and 1981*

The IVDMD values for lichens were higher ( $P < 0.01$ ) in 1981 than in 1980. They averaged 44% (7-73%) higher in 1981 than in 1980 for 9 of 11 species of lichens, where average values or single values for 60-h and 63-h fermentations were used (Table 1). The largest differences between years (73 and 72%) were for *Stereocaulon paschale* and *Cetraria nivalis*. Between-year differences for non-lichen species ( $n = 11$ , Table 2) were not significant ( $P > 0.2$ ) but between-year differences among all species ( $n = 22$ ) were significant ( $P < 0.01$ ).

We reduced the average standard errors of the duplicates from 3.4 ( $n = 129$ ) in the 1980 trials to 2.2 ( $n = 128$ ) in 1981 by varying some techniques. In 1981 the rumen fluid-buffer mixture was stirred vigorously before adding it to each tube, blanks were placed centrally to 10-20 test tubes containing plant material, and duplicates were placed side by side in the racks. In the 1980 trials, the duplicates were scattered in the racks and two blanks were associated with 38 test tubes. The location of duplicates, and blanks in relation to them, is important because fragments of different specific gravities separated horizontally even in mixtures that were stirred vigorously.

TABLE 2. *In vitro* dry matter disappearance (mean percent  $\pm$  SE) of stems (S), leaves (L), and other parts of non-lichen species fermented for 60 h in ruminal fluids of barren-ground caribou in March 1981 and comparative values obtained in 1980

Plant species	<i>In vitro</i> dry matter disappearance (percent)		
	1981, 60 h	1980, 60 h	1980, 63 h
<b>Trees, high shrubs</b>			
<i>Picea mariana</i>	47 $\pm$ 2 L	44 $\pm$ 3 L	40 <sup>e</sup> L
<i>Pinus contorta</i>	14 $\pm$ 1 L	—	—
<i>Picea &amp; Pinus</i> spp., bark	16 $\pm$ 5	—	—
<i>Salix</i> sp.	36 $\pm$ 4 S	29 $\pm$ 1 S	33 $\pm$ 1 S
<i>Betula</i> sp.	35 $\pm$ 1 S, 11 $\pm$ 1 L	28 $\pm$ 2 S	32 $\pm$ 1 S
<i>Larix larix</i>	41 $\pm$ 3 S	—	—
<b>Low shrubs</b>			
<i>Chamaedaphne calyculata</i>	45 $\pm$ 1 L, 29 $\pm$ 3 SL	27 $\pm$ 5 S, 47 $\pm$ 4 L	32 $\pm$ 1 S, 49 $\pm$ 5 L
<i>Ledum</i> spp.	19 $\pm$ 6 S, 38 $\pm$ 1 L <sup>a</sup> , 35 $\pm$ 1 L	31 $\pm$ 8 L	31 $\pm$ 4 S, 37 $\pm$ 2 L
<i>Empetrum nigrum</i>	54 $\pm$ 3 LS, 46 $\pm$ 0 LS <sup>a</sup> , 49 $\pm$ 3 L	—	30 <sup>e</sup> L
<i>Arctostaphylos</i> spp.	40 $\pm$ 2 S, 46 $\pm$ 1 L <sup>a</sup>	—	—
<i>Vaccinium vitis-idaea</i>	33 $\pm$ 2 S, 51 $\pm$ 4 L <sup>a</sup>	25 $\pm$ 10 S, 36 $\pm$ 11 L	43 $\pm$ 1 S, 44 $\pm$ 4 L
<b>Sedges</b>			
<i>Carex rostrata</i> , cured	41 $\pm$ 1 L	43 $\pm$ 7 L	49 $\pm$ 0 L
<i>Carex rostrata</i> , green	57 $\pm$ 0 LS	67 $\pm$ 3 LS	63 $\pm$ 4 LS
<i>Carex</i> sp., cured <sup>b</sup>	28 $\pm$ 3 L, 29 $\pm$ 5 L	—	—
<b>Mosses and moss-like</b>			
<i>Sphagnum</i> spp.	22 $\pm$ 1, 16 $\pm$ 4	26 $\pm$ 4	19 $\pm$ 2
<i>Dicranum undulatum</i>	25 $\pm$ 1	—	—
<i>Dicranum polysetum</i>	7 <sup>c</sup>	—	—
<i>Pleurozium schreberi</i>	17 $\pm$ 0, 16 $\pm$ 3, 14 $\pm$ 2	—	—
<i>Polytrichum juniperinum</i>	18 <sup>c</sup>	—	—
<i>Polytrichum strictum</i>	15 $\pm$ 2	—	—
<i>Polytrichum piliferum</i>	12 $\pm$ 1	—	—
<i>Ptilidium ciliare</i> <sup>c</sup>	30 <sup>c</sup> , 29 $\pm$ 2, 24 $\pm$ 1	29 $\pm$ 3	24 $\pm$ 2
<b>Lycopodium spp.</b>			
(club moss) <sup>d</sup>	34 $\pm$ 1	—	—

<sup>a</sup>Digested with rumen fluid A; others with fluid B.

<sup>b</sup>Separate unidentified species.

<sup>c</sup>A liverwort.

<sup>d</sup>A vascular plant.

<sup>e</sup>Single tube only.

Proportionately more heavy fragments entered the tubes as the mixture was used from a beaker. We used only the top half of the mixture in any container in an attempt to reduce that source of variability. Thus, the greater the number of blanks, the greater the accuracy, because they compensate for the particulate matter in the rumen fluids.

#### Comparative IVDMD of Plant Species

As a group, the common arboreal lichens (excluding *Hypogymnia physodes*) were more digestible than the terricolous and rock lichens (Table 1). IVDMD (in tubes) of the 11 species of terricolous lichens were similar ( $\bar{x}$  = 48  $\pm$  3) with *Cladina rangiferina* (36%) and *Cetraria ericetorum* (67%) at the extremes.

Low DMDs characterized the leaves of *Betula* spp. (11%) and *Pinus banksiana* (14%), and the bark of *Picea mariana* and *Pinus Banksiana* (16%) (Table 2). The aromatic leaves of *Picea mariana* and *Ledum* spp. were moderately digestible *in vitro* (47 and 35%, respectively). They produced a pungent aroma during fermentation, as did the leaves of *Chamae-*

*daphne* and the stems of *Salix*. The leaves of *Vaccinium vitis-idaea*, *Empetrum nigrum*, *Arctostaphylos* spp., and *Ledum* spp., the most common species of shrubs on the tundra and forest floor in the range of the Beverly herd, were moderately digestible (37-51%) in the 1981 trials. Green parts of a sedge were about twice as digestible as the cured parts (Table 2).

The mosses were poorly digested *in vitro* ( $\bar{x}$  = 16%  $\pm$  2,  $n$  = 7), with the extremes occupied by *Dicranum polysetum* (7%) and *D. undulatum* (25%) (Table 2). Digestions of a liverwort (28%) and a club moss (34%) were slightly higher.

#### The Digestion Rate of Lichens In Vitro

In general, the IVDMDs of *Cladina mitis*, *Cladonia amaurocraea*, and *Cetraria nivalis* increased with time through seven fermentation periods of 15-180 h (Table 3). In contrast, the IVDMDs of *Peltigera* spp. and *Stereocaulon paschale* stabilized after 30 h and 60 h of fermentation, respectively. The trend of the data (Table 3) suggests that the values for 90 h were somewhat depressed for all five species.

TABLE 3. Effect of variable fermentation periods on the *in vitro* dry matter disappearance (mean percent  $\pm$  SE) of five lichen species in ruminal fluids of barren-ground caribou

Lichen species	Fermentation period (hours)							Correlation coefficient (30-180 h)
	15	30	60	90	120	150	180	
<i>Cladina mitis</i>	17 $\pm$ 2	44 $\pm$ 7	53 $\pm$ 3 (54 $\pm$ 4) <sup>a</sup>	50 $\pm$ 0	59 $\pm$ 2	63 $\pm$ 0	69 $\pm$ 3	0.96 (0.96) <sup>b</sup>
<i>Cladonia amaurocraea</i>	13 $\pm$ 8	30 $\pm$ 3	47 $\pm$ 1 (41 $\pm$ 1) <sup>a</sup>	40 $\pm$ 3	54 $\pm$ 1	57 $\pm$ 0	57 $\pm$ 2	0.88 (0.95) <sup>b</sup>
<i>Cetraria nivalis</i>	28 $\pm$ 0	38 $\pm$ 1	38 $\pm$ 0	38 $\pm$ 7	57 $\pm$ 2	65 $\pm$ 2	70 $\pm$ 2	0.93
<i>Stereocaulon paschale</i>	20 $\pm$ 2	28 $\pm$ 2	46 $\pm$ 4 (36 $\pm$ 3) <sup>a</sup>	34 $\pm$ 3	37 $\pm$ 2	42 $\pm$ 1	41 $\pm$ 1	0.45 (0.89) <sup>b</sup>
<i>Peltigera aphthosa</i>	19 $\pm$ 0	35 $\pm$ 1	32 $\pm$ 1	29 $\pm$ 3	36 $\pm$ 1	36 $\pm$ 1	25 <sup>c</sup>	-0.43

<sup>a</sup>Ruminal fluid cooled to 20°C for 2 h before fermentation commenced.

<sup>b</sup>Includes value in parentheses for 60-h fermentation.

<sup>c</sup>Single tube only.

### Type of Fermentation Vessel

Dry-matter disappearance (DMD) averaged 46% higher ( $P < 0.01$ ) in Erlenmeyer flasks than in test tubes for seven species of terricolous lichens (Table 4). Differences for the three species of arboreal lichens were small. Nevertheless, DMDs of all 10 species were higher ( $P < 0.05$ ) in flasks than in tubes.

TABLE 4. Comparative dry matter disappearance (means  $\pm$  SE) of lichens fermented for 60 h with caribou ruminal fluids in 125-mL test tubes, and in 250-mL Erlenmeyer flasks with and without 60 mg of exogenous urea

Lichen species	Dry matter disappearance (percent)		
	Tubes	Flasks	Flasks with urea
<i>Usnea hirta</i>	65 $\pm$ 4	72 $\pm$ 1	89 $\pm$ 2
<i>Evernia mesomorpha</i>	76 $\pm$ 5	67 $\pm$ 2	94 $\pm$ 0
<i>Alectoria americana</i>	59 $\pm$ 2	65 $\pm$ 0	—
<i>Cladina mitis</i> <sup>a</sup>	53 $\pm$ 3	66 $\pm$ 2	77 $\pm$ 2
<i>Cladina rangiferina</i> <sup>a</sup>	37 $\pm$ 13	52 $\pm$ 6	49 $\pm$ 4
<i>Cladonia amaurocraea</i>	47 $\pm$ 1	62 $\pm$ 1	74 $\pm$ 2
<i>Cladonia uncialis</i> <sup>a</sup>	41 $\pm$ 1	54 $\pm$ 1	76 $\pm$ 1
<i>Cladonia</i> spp. <sup>a</sup>	39 $\pm$ 3	48 $\pm$ 1	67 $\pm$ 1
<i>Stereocaulon paschale</i>	47 $\pm$ 4	67 $\pm$ 1	66 $\pm$ 3
<i>Cetraria nivalis</i> <sup>a</sup>	38 $\pm$ 0	87 $\pm$ 3	91 $\pm$ 0

<sup>a</sup>Stored plants used for test.

Most lichen species have a low specific gravity and some of the milled fragments appear to be anhydrous, especially those of the rock lichens *Lasallia* and *Parmelia*. Gas production (CO<sub>2</sub>) during fermentation caused the fragments of fruticose lichens to rise in test tubes and lose contact with the rumen fluid-buffer mixture, resulting in low IVDMD values relative to those obtained in Erlenmeyer flasks. Fragments of *Cetraria nivalis* rose above the fluid in test tubes most quickly, which resulted in a DMD of 38% compared with 87% in flasks.

### Effect of Adding 15, 30, and 60 mg of Urea

The addition of 60 mg of urea to Erlenmeyer flasks caused the IVDMDs of six of nine species of lichens to increase by

17-42% ( $\bar{x} = 30\%$ ) (Table 4). Changes in the IVDMDs of the other three species were less than 5%. The mean values for all nine species increased from 64 to 76% ( $P < 0.01$ ). Addition of 60 mg of urea to flasks (Table 4) and tubes (Table 5) caused inconsistent changes to the digestibilities of *Cladina mitis*, *C. rangiferina*, and *Stereocaulon paschale*.

Variable levels of urea (15, 30, and 60 mg) caused insignificant changes in the IVDMDs of *Cladina mitis*, *C. rangiferina*, and *Stereocaulon paschale* fermented for 30 h and 60 h in tubes (Table 5). However, the 60-h IVDMD values were higher ( $P < 0.05$ ) than the 30-h values for each level of added urea. There was no urea effect but there was a time effect.

TABLE 5. Effect of adding 15, 30, and 60 mg of urea per tube on the *in vitro* dry matter disappearance (mean percent  $\pm$  SE) of three lichen species after fermentation periods of 30 and 60 h in ruminal fluids of barren-ground caribou

Lichen species	Fermentation period (h)	Mg urea/tube			
		0	15	30	60
<i>Cladina mitis</i>	30	44 $\pm$ 7	32 $\pm$ 1	41 $\pm$ 1	41 $\pm$ 2
	60	53 $\pm$ 3 <sup>a</sup>	16 $\pm$ 2	50 $\pm$ 3	54 $\pm$ 5
<i>Cladina rangiferina</i>	30	—	27 $\pm$ 1	34 $\pm$ 3	30 $\pm$ 3
	60	37 $\pm$ 13 <sup>a</sup>	29 $\pm$ 2	40 $\pm$ 2	47 $\pm$ 1
<i>Stereocaulon paschale</i>	30	28 $\pm$ 2	38 $\pm$ 0	48 $\pm$ 2	45 $\pm$ 1
	60	47 $\pm$ 4 <sup>a</sup>	44 $\pm$ 1	56 $\pm$ 0	62 $\pm$ 2

<sup>a</sup>Stored plants used for test.

### Deletion of Stage 1 or 2

Omission of stage 2 (pepsin digestion) of the Tilley and Terry (1963) technique resulted in DMDs for four plant species ranging from 58 to 85% ( $\bar{x} = 76\%$ ) of the two-stage values (Table 6). One spurious value, caused by loss of plant material through the air valve, was 35% higher after stage 1 (fermentation) than after both stages. Omission of stage 1, i.e., treatment with acid pepsin only, led to DMD values of only 23-40% ( $\bar{x} = 30\%$ ) of the values obtained after both stages. In contrast, the second stage added only 2-4% to the DMDs of lichens incubated with reindeer inoculum in Norway (Trudell *et al.*, 1980). The variability in the effect on DMD of the second stage of the Tilley and Terry (1963) technique suggests that it should not be deleted.

TABLE 6. Observed *in vitro* dry matter disappearance (mean percent  $\pm$  SE) of five species of lichens after 60 h fermentation (stage 1), after only pepsin digestion (stage 2), and after both stages of the Tilley and Terry (1963) technique

Plant species	Stage 1 only	Stage 2 only	Both stages <sup>a</sup>
<i>Cladina mitis</i>	41 $\pm$ 2	12 $\pm$ 0	52 $\pm$ 2
<i>Cladina rangiferina</i>	21 $\pm$ 7	11 $\pm$ 1	36 $\pm$ 5
<i>Cladonia amaurocraea</i>	40 $\pm$ 3	13 $\pm$ 0	47 $\pm$ 1
<i>Stereocaulon paschale</i>	39 $\pm$ 4 <sup>b</sup>	19 $\pm$ 0	47 $\pm$ 2
<i>Cetraria nivalis</i>	62 $\pm$ 6 <sup>b</sup>	12 $\pm$ 1	46 $\pm$ 5

<sup>a</sup>Standard two-stage trials (see Table 1).

<sup>b</sup>Some loss of plant material from the tubes.

### Effect of Cooling Ruminal Fluids

The IVDMDs (percent), using warm (32-39°C) and cooled (20°C for 2 h) (parentheses) ruminal fluids to begin fermentation in test tubes at 38-39°C for 60 h, were as follows: *Cladina mitis* 53 (54), *Cladonia amaurocraea* 47 (41), and *Stereocaulon paschale* 47 (36) (Table 3). However, the IVDMD values for the 60-h fermentation period using warm fluids were somewhat high in relation to the linear trend of values for all fermentation periods. In contrast, the 90-h values were low. The expected values after 60 h of fermentation for the three species were 49, 37 and 36% (same order), as calculated from regression equations for fermentation periods of 30-180 h. Substitution of the values for 60-h fermentation with the values for cold ruminal fluids increased the correlation coefficients in two of three cases (Table 3). The results suggest that cooling the ruminal fluids to 20°C may not impair digestibilities significantly, but further tests are needed.

### DISCUSSION

Results obtained in 1981 were consistent in many respects with previous results (Thomas and Kroeger, 1980, 1981): (1) there was no apparent difference in the digestive capacities of ruminal fluids obtained from different groups of caribou; (2) fructose lichens were more digestible in Erlenmeyer flasks than in test tubes; (3) digestibilities of lichens with low protein content increased with increasing fermentation times, limited to 120 h in 1980 and to 180 h in 1981, and near-maximum DMDs were attained at shorter fermentation times for lichen species with relatively high protein contents.

Therefore, source of donor animals is not critical; Erlenmeyer flasks should be used for IVDMD tests on lichen species and any others that form air-tight plugs in test tubes; and 60 mg of urea should be added to the flasks to speed the digestion of species containing little protein. Erlenmeyer flasks are preferred for digesting lichens because contact is maintained between the rumen mixture and the forage. Gas causes a plug of plant material to be forced above the fluid in test tubes containing fructose lichen species, green parts of sedges, and some mosses (*Dicranum* spp. and *Pleurozium* spp.). This problem is only partly overcome by frequent stirring and shaking of the tubes. Use of Erlenmeyer flasks instead of test tubes in an earlier study (Thomas and Kroeger,

1980) resulted in a significant increase in the DMD for the leaves of *Saxifraga oppositifolia* and no changes for stems of *Salix arctica* and cured leaves of *Carex stans*. The added urea probably simulates more closely what occurs in the rumen. Caribou recycle urea from the digestive tract via the blood stream and salivary glands to the rumen. Urea is routinely added to the rumen fluid mixture in many laboratories. In an earlier study (Thomas and Kroeger, 1981), addition of 63 mg urea to each tube resulted in no consistent changes to the DMD of leaves and stems of four species of shrubs.

The rates of digestion of lichens *in vitro* seemed to be linked to their nitrogen contents. The IVDMD of *Peltigera* spp. was about maximal after 30 h; their protein contents are 18-22% (Scotter, 1965; Kelsall, 1968; Skunke, 1969; Miller, 1976). The IVDMD of *Stereocaulon paschale* (protein level 7-10%) was nearly maximal after 60 h of fermentation. The IVDMD of genera with low (generally <3%) protein content, i.e., *Cladina*, *Cladonia*, and *Cetraria*, continued to increase with fermentation times to 180 h. The same trends were found in March 1980 (Thomas and Kroeger, 1981). Those slow rates of digestion were caused by N deficiencies and loss of fragment contact with the inoculum-buffer mixture in test tubes. The evidence advanced by Trudell *et al.* (1980) for the negative effects of inhibitors or end products may simply reflect N or trace element deficiencies in some plant species.

The higher IVDMDs in March 1981 compared with those of one year earlier were related to the condition of the donor caribou from which the ruminal fluids were obtained. Further studies should show whether the correlation was spurious. The kidney fat index of females over three years old increased ( $P < 0.01$ ) from 46.7  $\pm$  1.8 ( $n = 13$ ) in 1980 to 75.4  $\pm$  7.5 ( $n = 13$ ) in 1981; whole body weight increased from 80.7  $\pm$  1.7 kg ( $n = 13$ ) to 84.4  $\pm$  3.0 kg ( $n = 5$ ) (Thomas and Kiliaan, 1982). The kidney fat index (100  $\times$  weight of fat around the kidneys/weight of kidneys) is a commonly-used index to the degree of fatness in wild mammals. The lesser stimulatory effect of added urea in the 1981 trials compared with the 1980 tests suggests that protein deficiencies were greater in 1980. In Norway, addition of an unspecified amount of urea increased the DMD of mixed *Cladina stellaris* (*alpestris*) (70%) and *C. mitis* from 37 and 38% to 54 and 56% (46-47% increase) (Trudell *et al.*, 1980). The quantity and composition of micro-organisms in the ruminal fluids are affected by the nutritional history of donor caribou (Nieminen *et al.*, 1980). Thus, some of the annual differences in digestibilities could relate to nutritional differences weeks and months before the ruminal fluids were obtained. The general body condition of captive reindeer in Alaska, or losses of rumen fluids around a cannula, influenced forage DMDs (Trudell *et al.*, 1980).

The IVDMDs of lichens fermented for 60 h in Erlenmeyer flasks with 60 mg of exogenous urea probably approximates digestibilities in the caribou if nitrogen is not limiting. DMD in flasks without exogenous urea may approximate protein-limited digestibilities in the caribou at the time of collection. More likely, however, the recycling of urea by caribou may result in digestibilities close to those achieved in the laboratory

by use of Erlenmeyer flasks and exogenous urea. An interesting experiment would be periodic addition of small amounts of urea during fermentation to simulate natural conditions. The average DMDs for seven species of terricolous lichens was 71% in flasks with added urea, 62% in flasks, and 43% in tubes. These values compare with *in vivo* and IVDMD averages of 58 and 34%, respectively, for 11 species of terricolous lichens tested in Alaska using reindeer rumen fluids (Person *et al.*, 1980b). Thus, 60 h of fermentation in Erlenmeyer flasks produces DMDs comparable to those achieved *in vivo* after 48 h, and addition of exogenous urea produces even higher values.

The feeding strategy of barren-ground caribou in north-central Canada, based on the composition of rumen samples (Scotter, 1967; Kelsall, 1968; Miller, 1976) and on field observations, appears to be selection of forages with high digestibilities (lichens and green parts of sedges), forages with high protein contents (*Peltigera* spp., *Stereocaulon* spp., and green parts of sedges), species that are generally readily available (terricolous lichens), and species that are rapidly digested (lichens) if N levels are adequate. There appears to be selection against aromatic plants (needles of evergreens and leaves of *Ledum* spp. and *Empetrum nigrum*), forages with low digestibilities (mosses, liverworts, and bark), woody tissues (stems of most species), leaves that are "waxy" (*Vaccinium vitis-idaea* and *Arctostaphylos* spp.), dead leaves of grasses and sedges, and arboreal lichens that are closely bound to twigs of evergreen trees. The results from IVDMD trials provide many of the clues that explain the feeding strategies of barren-ground caribou.

#### ACKNOWLEDGEMENTS

We thank the Fort Smith Hunters and Trappers Association and the Northwest Territories Wildlife Service for their help and cooperation. D. Vitt identified the bryophytes. We thank W.E. Stevens, G.W. Scotter, M. Kingsley, and two referees for their comments.

#### REFERENCES

- KELSALL, J.P. 1968. The Migratory Barren-Ground Caribou in Canada. Canadian Wildlife Service Monograph No. 3. Ottawa: Queen's Printer. 340 p.
- MILLER, D.R. 1976. Biology of the Kaminuriak Population of Barren-Ground Caribou. Part 3: Taiga winter range relationships and diet. Canadian Wildlife Service Report Series No. 36. 42 p.
- NIEMINEN, M., KELLOKUMPU, S., VAYRYNEN, P. and HYVARINEN, H. 1980. Rumen function of the reindeer. In: Reimers, E., Gaare, E. and Skjenneberg, S. (eds.). Proceedings, Second International Reindeer/Caribou Symposium, Røros, Norway, 1979. Trondheim: Direktoratet for vilt og ferskvannsfisk. 213-223.
- PARKER, G.R. 1975. An Investigation of Caribou Range on Southampton Island, N.W.T. Canadian Wildlife Service Report Series No. 33. 82 p.
- PERSON, S.J., WHITE, R.G. and LUICK, J.R. 1980a. Determination of nutritive value of reindeer-caribou range. In: Reimers, E., Gaare, E. and Skjenneberg, S. (eds.). Proceedings, Second International Reindeer/Caribou Symposium, Røros, Norway, 1979. Trondheim: Direktoratet for vilt og ferskvannsfisk. 224-239.
- PERSON, S.J., PEGAU, R.E., WHITE, R.G. and LUICK, J.R. 1980b. *In vitro* and nylon-bag digestibilities of reindeer and caribou forages. Journal of Wildlife Management 44:613-622.
- SCOTTER, G.W. 1965. Chemical composition of forage lichens from northern Saskatchewan as related to use by barren-ground caribou. Canadian Journal of Plant Science 45:246-250.
- \_\_\_\_\_. 1967. The winter diet of barren-ground caribou in northern Canada. Canadian Field-Naturalist 81:33-39.
- \_\_\_\_\_. 1972. Chemical composition of forage plants from the Reindeer Preserve, Northwest Territories. Arctic 25:21-27.
- SKUNKE, F. 1969. Reindeer Ecology and Management in Sweden. University of Alaska Biological Paper No. 8. 82 p.
- THOMAS, D.C. and KILJAAN, H.P.L. 1982. A Brief Report on the March 1982 Sample of Barren-Ground Caribou from the Beverly Herd. Canadian Wildlife Service Report. 15 p.
- THOMAS, D.C. and KROEGER, P. 1980. *In vitro* digestibilities of plants in rumen fluids of Peary caribou. Arctic 33:757-767.
- \_\_\_\_\_. 1981. Digestibility of plants in ruminal fluids of barren-ground caribou. Arctic 34:321-325.
- TILLEY, J.M.A. and TERRY, R.A. 1963. A two-stage technique for *in vitro* digestion of forage crops. Journal of the British Grasslands Society 18:104-111.
- TRUDELL, J., WHITE, R.G., JACOBSEN, E., STAALAND, H., EKERN, K., KILDEMO, K. and GAARE, E. 1980. Comparison of some factors affecting the *in vitro* digestibility estimate of reindeer forages. In: Reimers, E., Gaare, E. and Skjenneberg, S. (eds.). Proceedings, Second International Reindeer/Caribou Symposium, Røros, Norway, 1979. Trondheim: Direktoratet for vilt og ferskvannsfisk. 262-273.