Rumen function in reindeer *(Rangifer tarandus tarandus)* after sub-maintenance feed intake and subsequent feeding

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Abstract: The aim of this experiment was to ascertain how different feeding strategies affect the rumen function of reindeer after nutritional deprivation. Rumen adaptation to various diets, after restricted feeding, was studied in 44 eight-month-old semi-domesticated female reindeer (*Rangifer tarandus tarandus*). All animals were initially fed a simulated winter diet based on lichens (lichen diet). A control group, continuously offered the lichen diet *ad libitum*, was compared to four groups of reindeer that were first restrictively fed (half the *ad libitum* ration) for eight days followed by one day without feed. The rumen content of restrictively fed animals had higher pH, lower dry matter content and volatile fatty acid (VFA) concentration, a changed composition of VFAs, and lower counts of bacteria compared to that of the control group. The effect was less dramatic than previously reported for reindeer starved for several days. On day 10, the four restrictively fed groups were introduced to different diets. One group was re-fed the lichen diet *ad libitum* and did not differ from the control group when the experiment ended after five weeks of feeding. Two groups were fed grain-based reindeer feed (pellets) combined with either lichens or grass silage, and one group was fed silage with a gradually increased addition of pellets. Diarrhoea and so called "wet belly" occurred initially in the three latter groups. After five weeks of feeding, the reindeer in the three pellet-fed groups had an altered composition of VFAs and higher counts of protozoa, and also tended to have higher total VFA concentration in the rumen, compared to the control animals and those re-fed the lichen diet. Only small changes were observed in the size of rumen papillae and these could be associated with energy intake. Protozoa decreased over time on the lichen diet. This study confirmed that rumen function was significantly affected by a relatively short period of restricted feed intake. The experiment also revealed a clear difference in rumen function between reindeer adapted to a lichen-based diet and those adjusted to basically grain-based diets. Bacteria that were utilising lichens were drastically reduced when the diet lacked lichens; consequently these bacteria may be regarded as a substrate-specific group.

Key words: bacteria, ciliate protozoa, diet, forage, lichen, papillae, volatile fatty acids.

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Introduction

Semi-domesticated reindeer (*Rangifer tarandus tarandus*) usually feed all year from natural habitats and are well adapted to the seasonal differences that occur in forage availability and quality (White, 1975). The winter diet of reindeer normally consists of lichens (mainly *Cladina*/*Cladonia* spp.), mixed with shrubs (e.g. *Empetrum* spp., *Vaccinium* spp.), grasses (e.g. *Deschampsia flexuosa*) and sedges (*Carex* spp.) (Gaare & Skogland, 1975; Mathiesen *et al*., 2000a; Heggberget *et al*., 2002). Lichens have a unique chemical composition, rich in energy but poor in protein. The hemicellulose fraction contains characteristic carbohydrates, such as lichenin and isolichenin (Llano, 1956). Reindeer have been shown to digest from 40 to 90 per cent of the organic matter in lichens, depending on the lichen species (Jacobsen & Skjenneberg, 1975; Danell *et al*., 1994; Storeheier *et al*., 2002; Wallsten, 2003), whilst a considerably smaller fraction of the lichens is digested by sheep and cattle (Presthegge, 1954; Garmo, 1986; Wallsten, 2003).

Emergency feeding of reindeer occasionally becomes necessary, due to deep or ice-crusted snow that prevents

the animals from reaching the ground vegetation. Regular supplementary feeding has become more common in some areas, especially in Finland, due to decreased availability or quality of winter pasture (Kumpula *et al*., 2002). Feeding usually involves a rapid switch to a diet consisting of grain-based pellets, often combined with hay or grass silage, which differs considerably from the natural diet. The adaptation to a new diet may be problematic, especially if the animals are already in a poor condition, and digestive problems are common (Josefsen, 1997).

This study was conducted as part of a more comprehensive investigation, in which we also studied blood metabolites, gains and losses of body mass and tissue depots (Nilsson *et al*., 2000; Wiklund *et al*., 2000), health (Åhman *et al*., 2002), heart rate and behaviour (Nilsson *et al*., 2006). In the present part of the study, we have tested the effect on rumen function of four feeding strategies based on common feeds available for reindeer. Procedures including confining groups of reindeer in outdoor enclosures and introducing the different diets after a period of sub-maintenance feed intake were used to create a situation resembling the practical circumstances of reindeer husbandry. The study was done with the objective of ascertaining how the different feeding strategies affected the rumen function of reindeer recovering from nutritional deprivation. Particular attention was paid to finding possible rumen bacteria adapted to the digestion of lichens.

Material and methods

Experimental design, diets, and animal health

The experiment was conducted at the research station of the Department of Biology, University of Oulu, Finland (65°N, 25°E), in the winter of 1996–1997. The larger investigation, of which this is a part, included altogether 69 eight-month-old female reindeer, divided into five groups (Nilsson *et al*., 2000). The present study includes 44 of these reindeer. The reindeer were gathered from natural pasture within the southern part of the Finnish reindeer herding area. They were brought to the research station between 12 November and 7 January, and then kept in outdoor pens throughout the experiment. The ground was covered with snow during the experiment and the outdoor temperature varied between –28 °C and +6 °C.

From arrival until 27 January (pre-experimental period), all reindeer were offered a simulated winter diet (lichen diet) *ad libitum*, consisting of 80% *Cladina* spp. lichens and 20% of a mix of *Vaccinium myrtillus* shrubs and *Salix* spp. leaves (on a dry matter, DM, basis). A control group (group C) was offered the lichen diet *ad libitum* throughout the experiment. The average intake in this group was 1.3 kg DM and 64 g crude protein (CP) per animal and day. From day 1 to day 8 of the experiment, the other four groups were given half of the previous *ad libitum* ration (0.63 kg DM per animal and day) followed by one day (day 9) of total feed deprivation (days 1-9 are referred to as the restriction period).

From day 10 to day 44 (the feeding period) four feeding strategies were used on the previously feed-restricted animals. One group (L) was re-fed the lichen diet *ad libitum*. Two groups were fed diets of 80% (DM basis) commercial reindeer feed (pellets) combined with either 20% lichen (group PL) or 20% grass silage (group PS). The offered amount was gradually increased during the first week of feeding from 0.6 kg DM up to *ad libitum*, 1.8 kg DM per animal and day. Group PL then received 174 g CP and group PS 231 g CP per animal and day. The remaining group (group SP) was fed silage *ad libitum* (0.9 kg DM per animal and day) for five days and thereafter adapted gradually, during two weeks, to a diet of 80% pellets and 20% silage (1.9 kg DM and 246 g CP per animal and day). The average daily intake of metabolisable energy (ME) on the different diets, when given *ad libitum*, was calculated as 13 MJ ME for the lichen diet and 18 MJ ME for the pellet-based diets (Nilsson *et al*., 2000).

The animals were fed twice daily with one-third of the ration in the morning and the rest at noon. The feedstuffs were mixed manually and offered in cribs. The *ad libitum* ration was set so that there was always some edible material (at least 10% of the given feed) left in the cribs the next day. The reindeer were not offered any new feed on the day of slaughter. Mineral blocks (Natura Slicksten®, Suomen Rehu, Helsinki, Finland) were available in all pens during the experiment and the animals had free access to water (at about 10 °C).

The chemical composition and energy content of the different feeds are given in Table 1. Shrubs and leaves were harvested in July–August and lichens in September–October. Lichens and leaves were stored dry while the shrubs were stored frozen. Lichens were soaked in water to obtain 25-35% DM before feeding. The pellets (Renfor Bas®, Lantmännen Fori Holmsund, Sweden) consisted of oats and wheat and their bran products, sugar beet pulp and soybean meal. The silage (Table 1) contained predominantly timothy (*Phleum pratense*), ensiled in plastic-wrapped bales. The ammonia-N content of the silage was 10% of total nitrogen and the pH was 4.65.

During the restriction period and first week of the feeding period some reindeer were affected by the "wet belly" disorder (Åhman *et al*., 2002). Five affected animals died, but the others recovered before

Table 1. Chemical composition and calculated metabolisable energy (ME) of various feedstuffs (from Nilsson *et al.,* 2000).

	Lichens	Shrubs	Leaves	Silage	Pellets
Number of samples	5	5	5	5	1
Dry matter (DM), per cent	-	55.1	72.2	56.6	88.9
Composition, per cent of DM:					
Crude protein (CP)	3.3	7.7	15.8	19.8	11.2
Ash	1.6	3.6	4.9	10.2	7.6
Water-soluble carbohydrates	0.39	7.1	4.6	1.82	9.4
Neutral detergent fibre	75.7	48.3	37.1	52.5	35.8
Acid detergent fibre	13.1	43.9	31.4	31.3	17.4
Lignin	6.6	19.7	15.5	3.8	3.3
Calculated ME, MJ/kg DM	10.1	9.5	10.4	10.1	11.4

the end of the experiment. All the reindeer in groups PL and PS had diarrhoea during the first week of the feeding period. Two reindeer included in this study were treated with antibiotics (dihydrostreptomycin) and recovered within two or three days. Within two weeks of the feeding period all reindeer had recovered from diarrhoea.

Reindeer, randomly allocated at the start of the experiment, were slaughtered on three occasions. Eight reindeer were slaughtered after the adaptation period, before the start of the actual experiment (day 0; slaughter I). After the restriction period, six restrictively fed reindeer and five control animals were slaughtered (day 10; slaughter II). Five reindeer from each group were slaughtered at the end of the feeding period (days 43 and 44; slaughter III). Three animals per slaughter and treatment (altogether 24 reindeer) were randomly chosen for determining the number of rumen bacteria and protozoa.

Slaughter, sampling, and assessment of rumen weight, DM, pH, and volatile fatty acids (VFAs)

Live body mass was monitored in the morning before slaughter I and at slaughters II and III, on the preceding day. The reindeer were taken out of their pens on the morning of the slaughter day. They were then transported for about 30 min on a lorry to the abattoir and slaughtered within 90 min of arrival using a captive bolt, starting at 11 a.m. on all occasions.

The abdomen was cut open and the reticulo-rumen (referred to as rumen) was removed and weighed. Within 30 min of death, the dorsal wall of the rumen was cut open. The pH was measured with a portable pH-meter (Knick Portamess 654, electrode from Mettler Toledo Inlab® 427) placed in the middle of the opened section 4-5 cm deep into the rumen content. The rumen content was gently mixed and about 300 ml was sampled by filling a plastic container to capacity. The container was sealed immediately and stored in cool and anaerobic conditions before processing in the laboratory for protozoa count and bacteria growth. The processing was made as soon as possible, normally within 2-4 hours from death (in some single case up to five hours). There were no systematic differences between groups in the time between sampling and processing. Samples of rumen content for the determination of DM and analyses of VFAs were collected in 50 ml plastic tubes that were filled to capacity and sealed. The samples were cooled directly and then frozen at -20 °C within three hours after slaughter. After sampling, the rumen was weighed empty to calculate the weight of the rumen content.

Samples of the rumen wall (10 x 10 mm^2) for measuring papillae were taken from four points in the rumen (atrium, dorsal and ventral walls, and ventral caudal blind sac) within one hour of death and stored in buffered 10% formalin.

To determine DM, the rumen content was dried for 48 h at 90 °C, followed by 110 °C for 6 h. The concentration of VFA was determined by gas chromatography (HP5880), with a flame ionisation detector (FID). The individual acids were separated in a three-metre glass column packed with 80/120 Carbopack™ B-DA/4% Carbowax® 20M (Supelco Inc., USA).

Rumen papillae

The lengths of 20 papillae per sample were measured with a Cal Comp 2200 digitiser, and the number of papillae per cm^2 of rumen wall was calculated. Rumen-wall samples were embedded in plastic (Historesin®, LKB-Produkter AB, Sweden), and 5-µm cross-sections of papillae were cut and stained

with haematoxylin and eosin. The cross-sectional area and perimeter of sections cut from the middle of each of 20 papillae were determined by digitiser and light microscope. The following values were calculated: mean papillar volume (length of papilla x cross-sectional area), areal papillar volume (mean papillar volume x number of papillae per cm^2 of rumen wall), mean papillar (epithelial) surface area (length of papilla x perimeter), and areal papillar surface (mean papillar surface area x number of papillae per cm^2 of rumen wall).

Protozoa count and growth and count of rumen bacteria

One gram of fresh rumen content for ciliate protozoa count was gently mixed into 9 ml of a solution of 5% formaldehyde and 45% glycerol in water, followed by tenfold dilutions with the same solution in three steps. A sample was taken from the 10^{-3} dilution and the protozoa were counted in a MacMaster counting chamber (Hawksley, England).

Preparation of samples for bacteria growth was made according to van Gylswyk (1990). Ten grams of rumen content was diluted 1:10 with an anaerobic diluent (described below). The sample was then homogenised with an Ultra-Turrax homogeniser at 20 000 r.p.m. for 30 s, while cooling the container in ice water. Samples were subsequently serially diluted. Aliquots of 0.5 ml for culturing on the TC medium and LU medium below were taken from the 10^{-8} dilution and 10^{-7} dilution, respectively (five replicates per animal), using sterile, O_2-free and CO_2-purged syringes. The aliquots were added to roll tubes with appropriate molten (48 °C) agar medium (see below). The roll tubes were then spun while cooling with ice water and incubated at 39 °C for a minimum of three days before final colony counting.

Preparation of diluents and media for bacteria growth

A basal medium was prepared as described by van Gylswyk (1990), containing the following (per litre): K_2HPO_4, 0.23 g; KH_2PO_4, 0.23 g; NaCl, 0.46 g; $(NH_4)_2SO_4$, 0.46 g; $CaCl_2$ (anhydrous), 0.05 g; $MgSO_4{\cdot}7H2O$, 0.10 g; $NaHCO_3$, 6.4 g; agar, 10 g; L-cysteine·HCl·H_2O, 0.25 g; $Na_2S{\cdot}9H_2O$, 0.25 g; indigo carmine, 0.005 g; reindeer rumen fluid, 400 ml, and distilled water up to 1 litre. The rumen fluid donor was a slaughtered adult reindeer that had been fed a mixture of pellets, lichens (*Cladina* spp.) and leaves (*Salix* spp.). The rumen content was collected within 30 min of death, strained through four layers of cheesecloth, and centrifuged at 1500 g for 30 min, before the obtained fluid was frozen and stored until use. The gas phase consisted of O_2-free CO_2, prepared by passing high purity CO_2 through heated copper tubing. The medium for total culturable counts of bacteria (TC) was the basal medium supplemented with 0.5 g each of glucose, starch, cellobiose and xylan (from oat spelt) per litre.

To support growth of lichen-utilising (LU) bacteria, the basal medium was supplemented with 10 g of ball-milled (Ogimoto & Imai, 1981) dry lichens (*Cladina* spp.) per litre. Culture media (4 ml) were distributed into anaerobic roll tubes under O_2-free conditions, sealed with oxygen-impermeable rubber stoppers and aluminium crimps.

An anaerobic diluent for the rumen content was prepared with the same concentrations of minerals, bicarbonate, and indigo carmine as the basal medium. O_2-free CO_2 was used as the gas phase. L-cysteine·HCl·H_2 (0.5 g per litre) was used as the reducing agent (van Gylswyk, 1990). All roll tubes were heat sterilised and sterile anaerobic techniques were used throughout.

Statistical analyses

To test the effect of treatment, data from slaughter II and III, respectively, were analysed by fitting fixed linear models, using the different feeding regimes as independent variables. For slaughter II, ration (restricted vs. *ad libitum* feeding) was used as the independent variable. For slaughter III, group (C, L, PL, PS and SP) was used as the independent variable. Change over time in the control group was analysed separately, using slaughter occasion (I, II and III) as independent variable. All statistical analyses were performed using the SAS GLM procedure (SAS Inst. Inc., Cary, NC, USA). The significance level was set at $P<0.05$.

Results

Change in reindeer body mass

All restrictively fed reindeer lost live body mass during the restriction period (an average of 1.8 kg), whilst the controls did not significantly change their body mass. During the feeding period all reindeer in the lichen-fed groups (C and L) lost body mass (1.4 kg on average), whilst reindeer in the pellet-fed groups (PL, PS, and SP) all gained body mass (on average 4.0, 3.7 and 2.6 kg, respectively).

Rumen weight, DM, pH and VFA

In controls that were fed the lichen diet *ad libitum* (group C), there was no significant difference between the three slaughter occasions, neither in the weight of the rumen contents nor in rumen DM, pH or VFA (Table 2). The acetic acid concentration also remained stabile, whilst the propionic acid concentration increased ($P<0.002$) and the other acids decreased ($P<0.01$-0.001) in this group from slaughter I to slaughter II or III (Table 3).

Table 2. Carcass weight, rumen content, rumen dry matter (DM), pH and total volatile fatty acid (VFA) concentration in female reindeer calves fed various diets (Group C, controls, fed a lichen based diet *ad libitum*, Group L, PL, PS and SP, first fed a restricted amount of the lichen-based diet, half the *ad libitum* amount, followed by one day without feed and then fed different diets *ad libitum*), and slaughtered on three occasions (Slaughter I, at the start of the experiment, Slaughter II after nine days restriction period and Slaughter III, at the end of the experiment) (least square means ± standard errors). Means within row and slaughter occasion with different superscripts are significantly different. Significant changes in controls with time are specified in the text. LD = lichen-based diet, Pe = pellets, Li = lichens, Si = silage.

Slaughter:	I	II	II	III	III	III	III	III	
Group:	All	C	L, PL, PS, SP	C	L	PL	PS	SP	SE of group means
Diet:	LD	LD	LD restricted	LD	LD	Pe+Li	Pe+Si	Si / Pe+Si	
Number of animals	8	5	6	5	5	5	5	5	
Carcass weight (kg)	20.2 ± 0.9	20.8 ± 1.1	19.2 ± 1.4	19.5	17.8	23.3	20.3	22.8	1.0
Rumen content (per cent of body mass)	15.4 ± 1.4	17.8 ± 1.8	15.3 ± 1.6	15.2	16.2	10.8	15.0	10.0	2.1
Rumen DM (per cent)	16.2 ± 1.0	16.9^{a} ± 1.3	13.2^{b} ± 1.2	13.9	14.9	16.9	14.2	17.4	1.7
Rumen pH	6.36 ± 0.07	6.07^{a} ± 0.09	7.00^{b} ± 0.08	6.20^{a}	6.09^{a}	6.23^{ab}	6.29^{ab}	6.51^{b}	0.10
VFA total (mmol l^{-1})	110 ± 4.9	112^{a} ± 6.3	48^{b} ± 5.7	116^{a}	117^{a}	140^{b}	129^{ab}	123^{ab}	7.5
VFA total (mol)	0.76 ± 0.06	0.83^{a} ± 0.08	0.28^{b} ± 0.07	0.72	0.75	0.67	0.76	0.56	0.08

Table 3. Relative molar proportion of individual volatile fatty acids (molar per cent of total VFA) in rumen content of female reindeer calves fed various diets (Group C, controls, fed a lichen based diet *ad libitum*, Group L, PL, PS and SP, first fed a restricted amount of the lichen-based diet, half the *ad libitum* amount, followed by one day without feed and then fed different diets *ad libitum*), and slaughtered on three occasions (Slaughter I, at the start of the experiment, Slaughter II after nine days restriction period and Slaughter III, at the end of the experiment) (least square means ± standard errors). Means within row and slaughter occasion with different superscripts are significantly different. Significant changes in controls with time are specified in the text. LD = lichen-based diet, Pe = pellets, Li = lichens, Si = silage.

Slaughter:	I	II	II	III	III	III	III	III	
Group:	All	C	L, PL, PS, SP	C	L	PL	PS	SP	SE of group means
Diet:	LD	LD	LD restricted	LD	LD	Pe+Li	Pe+Si	Si / Pe+Si	
Number of animals	8	5	6	5	5	5	5	5	
Acetic acid	67.2 ± 0.8	65.4^{a} ± 1.1	71.0^{b} ± 1.0	67.7	68.0	67.4	68.0	65.4	1.4
Propionic acid	17.8 ± 0.7	20.6^{a} ± 0.8	15.1^{b} ± 0.8	21.1	21.0	18.0	18.3	19.4	1.1
Butyric acid	12.6 ± 0.6	12.4^{a} ± 0.8	8.7^{b} ± 0.7	9.7	9.6	12.1	10.7	11.8	0.9
Isobutyric acid	0.8 ± 0.1	0.5^{a} ± 0.2	1.8^{b} ± 0.1	0.5^{a}	0.4^{a}	0.7^{b}	0.9^{b}	1.0^{b}	0.07
Valeric acid	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0.5^{a}	0.5^{a}	0.9^{b}	1.0^{b}	1.0^{b}	0.09
Isovaleric acid	0.96 ± 0.2	0.5^{a} ± 0.2	2.5^{b} ± 0.2	0.5^{a}	0.4^{a}	0.9^{b}	1.1^{b}	1.4^{b}	0.1

At slaughter II, the rumen content of animals fed the restricted ration was not significantly different in weight than that of controls but had lower DM ($P<0.04$), higher pH ($P<0.001$) and lower concentrations of total VFA ($P<0.001$). The composition of VFAs was also affected by feed restriction (Table 3). Propionic and butyric acid concentrations were lower ($P<0.001$) in the restrictively fed animals, whilst acetic, isobutyric and isovaleric acid concentrations were all higher ($P<0.001$) than in the controls.

At slaughter III, neither the body mass, the total rumen content, nor the rumen DM differed significantly between any of the groups, irrespective of their diet (Table 2). The rumen pH, total VFA (Table 2) and the molar proportions of the individual VFAs (Table 3) were similar in the two lichen-fed groups (L and C). The rumen pH was highest in group SP, differing significantly from groups C and L ($P<0.04$ and $P<0.009$, respectively). The average concentrations of total VFA were higher in the pellet-fed groups (PL, PS, and SP) than in the lichen-fed groups, but the difference was significant ($P<0.04$) only for group PL. The proportions of acetic, propionic and butyric acids were similar for all groups, whilst the proportions of isobutyric, valeric and isovaleric acids were all higher ($P<0.04$-0.001) in reindeer fed the pellet-based diets (groups PL, PS, and SP) compared to the lichen-fed animals (groups C and L).

Rumen papillae

The papillae in the atrium part of the rumen of reindeer from the control group were generally larger (length, volume, areal volume, surface area and areal surface) at slaughter II than at slaughters I and III. The surface area, but not the other measures, was larger also in the papillae of the dorsal wall. The same trends ($0.05<P<0.1$) could be seen in length and volume of papillae in the dorsal wall and blind sac. At slaughter II, the papillar volume and surface area in the atrium were larger in group C than in the restrictively fed groups. There were, however, no significant differences between the reindeer from the restrictively fed groups and the control animals from slaughter I.

At slaughter III, significant differences between groups were found only in the atrium part of the rumen. Mean values of all measures which describe the size of papillae were highest in group SP and lowest in group C. Animals in group SP had statistically significantly longer papillae and larger areal papillar volume ($P<0.01$) than those in group C, and larger papillar surface area and areal papillar surface than animals in groups C and PL. Reindeer in group L had larger areal volume and areal surface of the papillae than animals in group C.

Counts of rumen bacteria and protozoa

The number of total culturable bacteria (TC) in the rumen of reindeer from the control group decreased ($P<0.003$) from slaughter I to slaughter II, but then increased somewhat until slaughter III (Table 4). The number of lichen-utilising (LU) bacteria in the control animals continuously increased and was higher at slaughter III than at slaughter I ($P<0.001$), whilst the number of protozoa declined from slaughters I and II until slaughter III ($P<0.001$).

Table 4. Counts of total culturable (TC) bacteria, lichen-utilizing (LU) bacteria and protozoa per g of rumen content in female reindeer calves fed various diets (Group C, controls, fed a lichen based diet *ad libitum*, Group L, PL, PS and SP, first fed a restricted amount of the lichen-based diet, half the *ad libitum* amount, followed by one day without feed and then fed different diets *ad libitum*), and slaughtered on three occasions (Slaughter I, at the start of the experiment, Slaughter II after nine days restriction period and Slaughter III, at the end of the experiment) (least square means ± standard errors). Means within row and slaughter occasion with different superscripts are significantly different. Significant changes in controls with time are specified in the text. LD = lichen-based diet, , Pe = pellets, Li = lichens, Si = silage.

Slaughter:	I	II	II	III	III	III	III	III	
Group:	All	C	L, PL, PS, SP	C	L	PL	PS	SP	SE of group means
Diet:	LD	LD	LD restricted	LD	LD	Pe+Li	Pe+Si	Si / Pe+Si	
Number of animals	3	3	3	3	3	3	3	3	
TC (10^8)	40 ± 13	19^a ± 4	3^b ± 4	28^a	28^a	35^a	11^b	32^a	4.4
LU (10^7)	18 ± 5.1	35^a ± 5	8^b ± 5	86^a	90^a	59^a	0.07^b	2.5^b	4.7
Protozoa (10^4)	24 ± 10	25 ± 11	18 ±11	10^a	14^a	70^b	49^b	79^b	14

Reindeer fed restricted rations showed lower mean counts of TC and LU bacteria at slaughter II than did the control group ($P<0.003$ and $P<0.001$, respectively), whilst the number of protozoa did not differ significantly from that of the control group.

TC counts were similar for all groups at slaughter III, except for group PS, which had lower counts ($P<0.009$-0.001) than the others (Table 4). At slaughter III, reindeer receiving diets containing lichens (groups C, L, and PL) had considerably higher counts of LU bacteria ($P<0.001$) than reindeer in groups PS and SP, which had no lichens in their diets. At slaughter III, groups C and L had similar numbers of protozoa. The number of protozoa was higher ($P<0.02$-0.007), but also more variable, in reindeer fed the pellet-based diets (groups PL, PS, and SP).

Discussion

When the experiment started, the rumen function of the reindeer seemed to be representative of animals on good winter pasture, as shown by pH, total VFA, molar proportions of individual acids and counts of TC bacteria corresponding with levels measured in free-living reindeer grazing on natural forage in winter (Åhman & Åhman, 1980; Aagnes *et al.*, 1995; Olsen *et al.*, 1997; Mathiesen *et al.*, 2000b). A normal winter condition in the reindeer at the experimental start was confirmed also by the values obtained for different blood metabolites (Nilsson *et al.*, 2000).

It is possible that the time delay between the death of the animal and the preparation of samples for VFA and bacteria had some effect on the results. Immediate preparation of the samples would have been preferred. However, with the large number of animals and the variety of samples and measurements which we believe is a main strength of this investigation, it was not possible to do all preparations immediately. Our purpose was to compare the different groups, and the handling of samples was the same in all groups. As stated above, the obtained values on VFA and bacteria in the control animals seem to be well within the range of what has been observed by others.

The number of ciliate protozoa found in the rumen at the start of the experiment (on average 24×10^4 per ml) was lower than the counts around 200×10^4 per ml previously observed in reindeer from good winter pastures (Westerling, 1970) or reindeer on summer pastures (Westerling, 1970; Dehority, 1975; Imai *et al.*, 2004). The delay between death and preparation of the samples may have reduced the number of protozoa in the present material. Protozoa counts more similar to ours were, however, found by Westerling in reindeer from a herd that had been grazing on winter ranges with very deep snow (56×10^4 per ml) and by Syrjälä *et al.* (1973) in reindeer on natural pasture in December (19×10^4 per ml).

The restricted feed intake affected most of the analysed indicators of rumen function. As expected, the VFA concentrations declined and pH increased. The observed changes were similar to those observed after one day without access to feed by Aagnes *et al.* (1995), but less dramatic than for reindeer starved for 2-4 days (Mathiesen *et al.*, 1984; Sletten & Hove, 1990; Aagnes *et al.*, 1995). The increased molar proportions of valeric acid, isobutyric and isovaleric acids was in accordance to what has previously been observed in connection with low feed intake (Åhman & Åhman, 1980). A negative effect of restricted feed intake on the area volume and surface of rumen papillae may be explained by a decline in the production of VFA, since VFA is a main growth factor for rumen papillae (Warner *et al.*, 1956; Sander *et al.*, 1959; Sakata & Tamate, 1978; 1979) although the time was rather short and adaptation has been reported to take 2-3 weeks with a low-energy diet (Dirksen *et al.*, 1984). Poor access to food generally reduces the number of bacteria (Mathiesen *et al.*, 1984; Aagnes *et al.*, 1995) and protozoa (Westerling, 1970; Syrjälä *et al.*, 1973; Mathiesen *et al.*, 1984) in the reindeer rumen. In this experiment, both the TC and LU bacteria declined significantly as an effect of the restricted feed intake, whilst no significant decline could be detected in the number of protozoa. Due to the large variation between individual reindeer and the low number of samples (three animals from each group) we might however have missed a possible effect on protozoa.

Although there were clear negative effects of feed restriction on rumen function, no problems were observed in Group L when re-feeding reindeer with the lichen diet after feed restriction (Nilsson *et al.*, 2000). Apparently, the restriction did not cause lasting effects on rumen function when the composition of the diet was not changed.

By the end of the experiment, after five weeks of *ad libitum* feeding, Group L was similar to the control group in all the measured traits. It is not possible to say at what time the rumen ecology had recovered, since no rumen samples were taken between slaughters II and III. The plasma concentrations of urea and glucose were, however, comparable to those of the controls within two to three weeks (Nilsson *et al.*, 2000), which indicates that the diet was satisfactorily utilised at this time.

The reindeer fed the lichen diet, even when fed *ad libitum*, apparently consumed less feed than they needed to maintain body mass and fat reserves (Nilsson *et al.*, 2000). This could explain why the number of protozoa declined in these reindeer, since low energy

intake has been shown to affect the number of rumen protozoa negatively (Dehority, 2003). The gradual increase in the number of LU bacteria in reindeer that were continuously fed the lichen diet shows that the rumen microbes of these reindeer were still adapting to the diet after the time of slaughter II, even though the reindeer had been fed this diet for at least four weeks. The provided lichen diet, although regarded as natural, most likely contained substantially more lichens than the natural diet that these reindeer had obtained on pasture, before being brought to the experimental station. This could be expected, since lichen biomasses have been reported to be low within the southern part of the Finnish reindeer herding area (Väre *et al*., 1996), which was the region from where the reindeer were taken.

The rapid shift of diet at the start of the feeding period resulted in obvious problems for the reindeer in groups PL, PS and SP. This was demonstrated by initial diarrhoea in group PL and PS and in severe cases of wet belly and death of some animals in group SP (Nilsson *et al*., 2000; Åhman *et al*., 2002). The addition of lichens in the diet of the animals in group PL did not prevent diarrhoea in this group.

Apart from the reindeer that died, the animals in the pellet-fed groups recovered and no lasting effects on rumen function or body condition could be observed at the end of the experiment. After the initial problems the reindeer seem to have adapted well to the diets. A satisfactory adaptation and an adequate feed intake was demonstrated by improved body condition during the feeding period in terms of body mass and fat deposition (Nilsson *et al*., 2000; Wiklund *et al*., 2000). This was supported also by an increase in the levels of plasma protein and insulin (Nilsson *et al*., 2000; Åhman *et al*., 2002), which was not observed in the reindeer fed the lichen diet.

The VFA concentrations in groups PL, PS, and SP at the end of the experiment varied more but were generally higher than for groups L and C, indicating a higher fermentation rate in the pellet-fed groups. This difference was not accompanied by a lower pH for the pellet-fed groups, which could have been expected. Instead, reindeer in group SP had a higher pH than the lichen-fed animals. On the other hand, the pH in all groups was well within the normal range for healthy reindeer (Åhman & Åhman, 1980; Sletten & Hove, 1990) and is affected also by the production of saliva, which may have differed between the groups, depending on the structure of the feed. It could be concluded that, despite the increase in fermentable organic matter with the pellet-based diets, the amount of fibre in these diets was sufficient to maintain enough flow of saliva and a normal rumen pH.

High-cereal diets, such as the pellet-based diets, have been found to stimulate the production of propionate rather than acetate (Beever, 1993), whilst high-fibre diets stimulate the production of acetate and reduce the production of butyrate. Lichens contain a large hemicellulose fraction (Person *et al*., 1980), although with specific carbohydrates (Llano, 1956), and would therefore be expected to act like the high-fibre diet. However, in spite of the difference in carbohydrate source between the lichen-based and the pellet-based diets, we found no significant effects of diet on the molar proportions of the main VFAs. Isobutyric, valeric and isovaleric acids are formed in the rumen through the deamination of amino acids (McDonald *et al*., 1995). The observed higher concentrations of these acids in animals on the pellet-based feeding regimes, compared to those on the lichen diet, could thus be expected due to the higher protein content in pellets compared to lichens.

The time taken for the papillar morphology of ruminants to adapt to changes in diet can be from three weeks (Hofmann, 1973; Dirksen *et al*., 1984; Ortega-Reyes *et al*., 1992) up to 8-9 weeks (Liebich *et al*., 1987). As the main factor for papillar growth seems to be the production of ruminal VFA (Warner *et al*., 1956; Sander *et al*., 1959; Sakata & Tamate, 1978, 1979) we can interpret that the size of ruminal papillae reflects the amount of VFA in rumen in preceding 3-8 weeks. The changes in this study were, however, small and could be found only in the atrium. They are not comparable to large changes reported by Josefsen *et al*. (1996), who found papillae to be larger in reindeer fed on a diet poor in cellulose compared to animals fed on a cellulose-rich diet over 6 weeks. In many ruminants, the dorsal wall of the rumen is regarded to be the most sensitive to dietary changes (Hofmann & Schnorr, 1982). Josefsen *et al*. (1996), however, found the atrium to be the area most sensitive to dietary changes in reindeer calves, which is in agreement with the present results.

The total number of culturable bacteria (TC) seems not to have been affected by the different feeding regimens, except for slightly lower counts for the PS group. The observed counts were similar to what has previously been reported for free-living and silage-fed reindeer (Aagnes *et al*., 1995; Olsen *et al*., 1997).

A dramatic effect was observed for the LU bacteria, which were considerably lower by the end of the feeding period for reindeer on the lichen-deficient diets (groups PS and SP) compared to the diets that contained lichens. Aagnes *et al*. (1995) failed to cultivate any specific lichen group of bacteria when using purified lichenin as a substrate. We used a method where the bacteria grew on a medium containing milled lichens of the same species as those in the reindeer diet,

thereby assuring that essential growth factors, besides just the carbohydrate fraction, were included. The large number of LU bacteria, even when lichens were only a minor part of the diet, and the almost total loss of these bacteria on the lichen-deficient diets, demonstrated that the bacteria were sensitive to lack of lichens. This group of bacteria could therefore be regarded as a substrate-specific group for lichen degradation. LU bacteria were present at levels of up to almost 10^9 per g of rumen content in the lichen-fed reindeer and most likely made a significant contribution to rumen fermentation. The identification and further investigation of the growth requirements of these bacteria remain to be done.

There is a clear relationship between diet and amount of protozoa in reindeer (Westerling, 1970; Dehority, 1975; Syrjälä-Qvist, 1982). It has also been shown in several experiments on sheep and cattle (summarised by Dehority, 2003) that feeding concentrates increases the number of protozoa substantially, compared to feeding a roughage diet. This was confirmed by our results, with larger protozoa populations in reindeer fed the pellet-based diets (groups PL, PS, and SP). The change in protozoa composition was probably due to the increased fermentable carbohydrate and protein content in the pellet-based diets compared to the lichen diet.

Conclusions

This study confirmed that rumen function in reindeer is significantly affected by a relatively short period of restricted feed intake. The rumen functions can be rapidly restored through re-feeding a lichen-based diet to which the animals are adapted, whilst feeding a new diet containing mainly grain-based pellets cause initial digestive problems. After five weeks of feeding, the experiment revealed a clear difference in rumen function between reindeer adapted to a lichen-based diet and those adjusted to basically grain-based diets. The higher energy and protein intake associated with the feeding of pellets seemed to stimulate VFA production, development of rumen papillae and growth of rumen protozoa. Bacteria growing on the lichen media were drastically reduced when the diet lacked lichens; consequently, these bacteria may be regarded as a substrate-specific group. More detailed studies are needed to identify and find out more about the growth requirements of these bacteria. The drastic reduction of lichen-utilising bacteria in reindeer on grain- and silage-based diets suggests that some lichens should be present in the diet of artificially fed reindeer if the intention is that they should return to lichen-dominated winter pasture.

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Vomfunktionen hos ren (*Rangifer tarandus tarandus*) efter begränsat födointag och påföljande utfodring

Abstract in Swedish / Sammanfattning: Ett försök utfördes med syfte att ta reda på hur olika utfodringsstrategier påverkar vomfunktionerna hos ren (*Rangifer t. tarandus*) efter en period med lågt näringsintag. Vommens anpassning till olika dieter studerades hos 44 åtta månader gamla honkalvar. Alla renarna gavs initialt en simulerad vinterdiet baserad på lav (lavdiet). En kontrollgrupp, som fick äta fritt av lavdieten under hela försöket, jämfördes med fyra grupper renar, som först utfodrades restriktivt med lavdieten (halva fodergivan jämfört med fri utfodring) under åtta dagar och sedan var helt utan foder under en dag. Vominnehåll från de restriktivt utfodrade renarna hade högre pH, lägre torrsubstans, lägre koncentration av flyktiga fettsyror (VFA), förändrad sammansättning av VFA och mindre mängd bakterier än vad som uppmättes i kontrollgruppen. Effekten var inte så dramatisk som den som tidigare rapporterats för renar som svultit flera dagar. Dag 10 sattes de fyra restriktivt utfodrade grupperna på olika dieter. En grupp utfodrades åter med lavdieten i fri mängd, och skiljde sig inte från kontrollgruppen när försöket avslutades efter fem veckors utfodring. Två grupper gavs spannmålsbaserat renfoder (pellets) kombinerat med antingen lav eller ensilage gjort på gräs, och en grupp gavs först enbart ensilage och därefter pellets i gradvis ökande mängd. Flera renar i de tre senare grupperna drabbades av diarré och så kallad ”blöt buk”. Efter fem veckors utfodring hade renarna i de tre pelletsutfodrade grupperna en ändrad sammansättning av VFA och mer protozooer, och tenderade även att ha högre total VFA-koncentration, än de två grupper som fick lavdiet. Endast små förändringar observerades i storleken på vompapiller, och dessa kunde kopplas till energiintag. Protozooerna minskade med tiden på lavdieten. Denna undersökning bekräftar att vomfunktionerna påverkas signifikant av en relativt kort period med minskat födointag. Undersökningen visar också en klar skillnad i vomfunktion mellan renar anpassade till lavdiet och dem som är anpassade till en i huvudsak spannmålsbaserad diet. Bakterier som växte på lav minskade drastiskt när laven uteslöts ur dieten; dessa bakterier kunde följaktligen betraktas som en substrat-specifik grupp.