Digestion of timothy silage and hay in reindeer

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Abstract: Leafy timothy (*Phleum pratense*) silage (S), silage mixed with molasses (SM) and hay (H) were fed to nine male reindeer (*Rangifer tarandus tarandus*) calves in winter to investigate rumen function and digestion. Three calves were given S with 18.5% dry matter (DM), three were given SM (21.9% DM) and three were given H (85.0% DM). The content of water soluble carbohydrates (in % of DM) was 8.2% in S, 16.0% in SM and 8.5% in H. Median (range) daily DM food intake per kg BM was 12.9 (9.2-14.4) g in calves fed S, 19.0 (19.0-21.9) g in calves fed SM and 21.0 (19.2-21.1) g in calves fed H. *In vivo* digestion of S and SM DM ranged from 78.5-83.1% compared to only 69.9-72.9% in calves fed H. *In vitro* DM digestion (IVDMD) of cellulose (median) incubated for 48 hours in rumen fluid was, however, significantly (P=0.05) lower in calves fed S (24.4%) compared to calves fed SM (42.2%). Median IVDMD of cellulose (48 hours) in calves fed H was 36.4%. Total concentration of VFA (range) in the rumen fluid from reindeer fed H (99.7-113.6 mM) and was significantly (P<0.05) higher compared to animals fed S (57.7-85.9 mM) or SM (51.4-72.0 mM). Likewise, the pH of the rumen fluid (range) was significantly (P<0.05) lower in reindeer fed H (6.40-6.78) compared to animals fed S (6.97-7.30) or SM (6.79-7.27). Based on this study it is concluded that leafy timothy preserved as hay seems to be more suitable as emergency feed compared to silage. Supplementation of molasses to silage seems to stimulate food intake and ruminal cellulose digestion in reindeer. The lower intake of S compared to SM or H by reindeer may be explained by ruminal energy deficiency.

Key words: *Rangifer tarandus tarandus,* grass, molasses, food intake, cellulolysis, rumen fill.

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Introduction

Reindeer (*Rangifer tarandus tarandus*) are ruminants and digest plant cell wall carbohydrates by a complex symbiotic microbial system (Hobson *et al.,* 1976; Syrjälä *et al.,* 1973; Aagnes *et al.,* 1995; Olsen *et al* , 1997) producing volatile fatty acids (VFA) rich in energy. These intermediate feeders are able to adapt to large seasonal changes in both quality and availability of pasture plants. It is generally known that during summer reindeer eat grass, herbs and leaves, while in winter they feed mostly on lichen, but also small quantities of vascular plants (e. g. Gaare & Skogland, 1975; Staaland *et al.,* 1995). The lichen (*Cladonia stellaris*) eaten by reindeer contains mostly hemicellulose and very little cellulose (Person *et al* , 1980), and is as such an uni-

que substrate for a herbivorous animal. During winter, however, changes in temperature can result in ice and crusts of ice which covers the pasture, and therefore expose the reindeer to acute starvation particularly in northern parts of Norway. Emergency food could prevent loss of animals due to starvation. Aagnes & Mathiesen (1995) used round baled silage of mixed grasses as emergency food for reindeer in winter. The palatability of this grass silage was high, but low digestibility and ruminal concentrations of VFA indicated that the silage was marginal for use as sole food for reindeer. However, a low stem to leaf ratio and a high concentration of water soluble carbohydrates seems to have a positive influence on food intake and ruminal digestion in reindeer (Aagnes *et al.,* 1996). The present work was conducted to investigate the effect of leafy timothy silage and hay, and addition of molasses to the silage, on rumen function and digestion in reindeer.

Materials and methods

Experimental design

Nine male reindeer calves (age 6-7 months) were taken from a free ranging herd in northern Norway (68°N, 17°E) and brought to Department of Arctic Biology, University of Tromsø 24 November 1993. The calves were orally treated with Panacur® and Valbazen vet.® with doses scaled to the body mass of the calves. Three calves were offered timothy silage with molasses (SM) for 46, 47 and 48 days, respectively, three were offered timothy silage (S) and three timothy hay (H) for 50 days. Calves fed S and H were kept in metabolism cages (60 cm wide, 140 cm long, 97 cm high) (Aagnes *et al.,* 1996) during the whole experiment. Calves fed SM were kept outdoors for 35 days in individual semi-outdoors graveled paddocks (84 m^2) with the food and water kept under roof, and then 11, 12, and 13 days respectively indoors in metabolic cages. These calves were initially fed silage without addition of molasses for 12 days outdoors prior to the feeding experiment. Indoors the calves were housed at 4 °C and exposed to a light regime simulating the natural photoperiod in Tromsø. The calves were given *ad lib.* access to food in plastic tubs, and water in plastic buckets, and they were fed every day at 10:30 a. m. and 04:30 p.m. Food and water intake were calculated based on recordings from 5 days (day 41-46) in animals fed SM and 11 days (day 39-50) in animals fed S and H. During this same period of time, *in vivo* digestibility of the different diets and the nitrogen balance were also recorded. The animals were then slaughtered and *in vitro* digestion of cellulose, changes in pH and concentration of VFA and lactate in fresh rumen fluid were recorded. The body composition of the animals was also investigated.

Food

The timothy used as food in this experiment was a regrowth harvested on the 11 August 1993 at The Norwegian Crop Research Institute, Holt Research Centre in Tromsø. The meadow used to produce the timothy hay and silage was fertilised using a complex fertilizer from Hydro Landbruk, Norway, in spring (50 kg containing 7 kg N, 3 kg P and 8 kg K/1000 m^2) and after the first cut (25 kg containing 4.5 kg N, 0.75 kg P and 3.75 kg K/1000 m^2) 12 July 1993. The timothy was cut using a rotor reaper JF 190 (Denmark) with stem breaker. The hay was produced by drying on hay rack for two weeks. The grass for ensiling was predried in the field for three hours and thereafter round baled with Serigstad 135 MK 1340 (Bryne, Norway) which cut the grass in 3-7 cm and pressed with Orkel GP 1200 (Orkdalen, Norway). To each bale (approximately 600-700 kg) 3.1 litre Natuferm solution (homofermentative lactic acid bacteria, Norsk Hydro; Norway) was added. The bales were mechanically wrapped with six layers of plastic. After fermentation (6 Oktober) the silage was re-packed in plastic bags (30 kg) and stored at -20 °C. The silage was thawed before being offered to the animals. The SM diet used was made by addition of aqueous molasses syrup to the silage to a final concentration of 16% water soluble carbohydrates of dry matter and mixed thoroughly into the silage just before it was fed to the reindeer. All three diets were collected from the same meadow, and the stem to leaf ratio in the diets was determined in a randomly selected sub-sample of the timothy silage. It was separated into leaf (blade and sheath above the first stem node) and stem (below the first stem node), before drying at < 60 °C.

In vivo *digestibility and nitrogen balance*

Apparent digestibility coefficients of dry matter, crude protein, ether extract, cellulose, hemicellulose and water soluble carbohydrates in the animals were determined according to Aagnes *et al* (1996). The N-balance was determined from food intake, urine- and feces production. Urine and feces were collected at 10:00 a. m. for 11 days (day 39-50) from calves fed S and H and for 5 days (day 41-46) from calves

feed SM. The daily production of feces was wrapped up in plastic bags and 10% of the daily urine production was collected in a plastic bottle and stored at -20 °C, before a subsample for each animals was analysed.

In vitro *digestibility of cellulose*

In vitro digestion of cellulose incubated in rumen fluid started within 9-18 min after death, and were performed according to Tilley & Terry (1963), with modifications described by Aagnes & Mathiesen (1995) and Olsen *et al.* (1995). To each Hungate anaerobic culture tube 100 mg cellulose (Whatman filter paper no.1, Whatman International Ltd., Maidstone, England), 9 ml artificial saliva (McDougall, 1948; Aagnes & Mathiesen, 1995) and 1 ml rumen fluid were added. The rumen fluid was obtained by straining the rumen contents through two layers of muslin.

Ruminal NH_4-N, pH, volatile fatty acids and lactate concentration

Ammonia and other volatile nitrogen components (NH_4-N) in the reticulo-rumen digesta were liberated from an aqueous extract by addition of magnesium oxide to give pH<7.5 when boiled, the distillate was allowed to react with H_2SO_4, and exess acid was titrated with NaOH solution (Horwitz, 1980). The zero-time *in vitro* technique (Carroll & Hungate, 1954; Hungate *et al*, 1961; Hungate, 1966; Olsen & Mathiesen, 1996) was used to calculate rumen pH, VFA (acetate, propionate and butyrate) and lactate concentration at the time of death. A sample of rumen contents (1-2 liter) was incubated in sealed thermos flasks and subsamples taken at 10-15 minutes intervals for 60 minutes after death. A linear regression was calculated for the pH, VFA and lactate data. Values for pH, VFA and lactate concentration at the time of death was obtained by extrapolating the regression-line back to time zero. The pH in the rumen fluid was recorded with a calibrated portable pH meter (PHM 80, Radiometer®, Copenhagen) with a combined pH electrode (GK 2501C, Radiometer®, Copenhagen). The rumen contents was mixed thoroughly between each sampling, and sub-samples from the incubated rumen contents were filtered through two layers of muslin, and two samples of 10 ml of the filtrate fixed in 2.5 ml 0.5 M HCl and frozen (-20 °C). Acidic fermentation products (VFA and lactate) were determined by gas liquid chromatography (Chrompack CP 9000; Chrompack, Bergen op Zoom, Holland) as described by Sørmo *et al* (1994) and Olsen & Mathiesen (1996).

Body mass and anatomy

The body mass was recorded using an automatic scale (Farmer Tonic Alpha 100, Denmark, sensitivity 0.5 kg) when the calves arrived at Department of Arctic Biology and thereafter weekly during the trial. The animals were slaughtered at 11:00 a. m., and the gastrointestinal tracts removed and emptied by hand. The carcass weight was measured on a spring balance (Salter weight, England, sensitivity 0.5 kg). The weights of the reticulo-rumen and the total gastrointestinal tract (wall and contents) were measured on an electronic balance (Sartorius GMBH Göttingen, Germany, sensitivity 1 g). The dry matter of the reticulo-rumen digesta was determined by drying at 100 °C.

Chemical analysis

Random samples from the S, SM and H were used for chemical analysis. Mixed samples from the collected feces and urine from each animal were analysed. Dry samples were ground in a mill with 1 mm mesh before analysis. Wet samples were predried at 60 °C to 90-95% dry matter and then treated as dry samples. Wet samples were used in cases where the drying-process influenced analysis, e. g. carbohydrates, amino-acids and ammonia. Analyses of dry matter, ash, nitrogen, water soluble carbohydrates, ammonia and other volatile nitrogen components were conducted as described by Olsen *et al.* (1994) and analyses of ether extract and fibre according to Aagnes & Mathiesen (1995). The dry matter was determined after preheating for 24 hours at 80 °C and heated at 104-105 °C until constant weight (Horwitz, 1980). The contents of ash was found after treatment for 24 hours in 550 °C (Horwitz, 1980). Kjeldahl nitrogen was determined using the Kjeldahl analysis (Horwitz, 1980). Crude protein was defined by multiplying Kjeldahl-N by 6.25. Amides, amines and free amino-acids were liberated by boiling silage in aluminium and copper solution (Barnstein-Stutzermethod) and true protein was determined with the Kjeldahl method (Horwitz, 1980). Ammonia and other volatile N components (NH_4-N) were determined as described for the rumen content. Ether extract was determined by the Soxhlet method (Horwitz, 1980). The water soluble carbohydrates was determined after extraction with water, filtered, hydrolyzed with H_2SO_4 in a water bath, neutralized with NaOH (Smith &

Grotelueschen, 1966; Grotelueschen & Smith, 1968), and deprotenized with zinc sulphide-barium hydroxide, and the carbohydrate contents were assayed by the ferricyanine method (Furuholmen *et al.*, 1964). The contents of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to Van Soest (1963 a, b); Van Soest & Wine (1967), and Goering & Van Soest (1970). The fibre analyses gave values for determining cellulose, hemicellulose and lignin. The acids were determined in frozen, chopped and water extracted silage. Formic acid concentration was found after concentrating the water extract and transferring it to ether solution for analysis by gas-chromatography (Carlo Erba Strumentazione, Fractovap 4200/42, Milano Italy; integrator: Perkin-Elmer, LCI-100) with SP1220/H_3PO_4 as stationary phase, and detected with a thermal conductivity detector (TCD) (Hauser & Zabransky, 1975; Supelco, 1975). Acetic, propionic, butyric, and lactic acids concentration in the water extract was determined with gas-chromatography (Carlo Erba Strumentazione, Fractovap 4200/42, Milano, Italy; integrator: Perkin-Elmer, LCI-100) with Carbopack/Carbowax stationary phase and detected with a flame-ionisation detector (FID) (Fussel & McCalley, 1987; Supelco, 1985).

Statistics

Food and water intake are presented as mean ± standard deviation (SD) of means, and compared using the Kruskal Wallis test (Johnson & Battacharyya, 1992). Since the groups were found to be significantly different (P=0.05) by use of the Kruskal Wallis test, P ($H \geq 53.8 \mid H_0$) = 5.99 and P ($H \geq 59.7 \mid H_0$) = 5.99 for food and water intake, respectively, the Wilcoxon rank-sum test (Johnson & Bhattacharyya, 1992) (H_0 rejected at P = 0.05, n_1=3, n_2 = 3, W_S=6) was used to compare the relative values.

In vivo digestibility coefficients and nitrogen balance as mean, and in vitro digestion of cellulose incubated in rumen fluid were expressed as median values. Also differences in body composition, total ruminal VFA concentrations and pH were tested using Wilcoxon rank-sum test (Johnson & Bhattacharyya, 1992) comparing two and two groups. H_0 was rejected at $P \leq 0.05$, n_1=3, n_2 = 3, W_S=6.

Linear regression analysis of the data from the *in vitro* dry matter digestion of cellulose were made using all data (four parallels) from all three animals in each plot (24-72 hours incubation periods), and likewise for the VFA consentrations as a function of time (two parallels). Statistical analysis comparing the b[1] in the regression line (y=b[1] x + b[0]) for each plot were performed using the General Linear Models procedure described by SAS Institute Inc. (1989). Statistical significance level was assumed to be at α=0.05, $F_{(1, 69)}$ = 3.96 for the cellulose digestibility plots and $F_{(1, 57)}$ = 4.00 for the VFA plots (F-values from Table A4 in Kleinbaum *et al.* (1988)).

Results

Food

Botanical analyses of the timothy silage showed that the diets consisted of 79.3% leaves and 20.7% stem. Weed constituted less than 1% of the grass harvested. The chemical analyses of timothy silage (S), silage mixed with molasses (SM) and hay (H) are presented in Table 1. High lactic acid concentrations and low pH, ammonia, acetic, propionic and butyric acid concentrations indicated good fermen-

Table 1. Chemical composition of regrowth timothy (*Phleum pratense*) silage (S), silage mixed with molasses (SM) and hay (H)

	S	SM	H
Dry matter, (DM) %	18.5	21.9	85.0
In % of DM:			
Ash	8.0	8.7	9.0
Crude protein	14.0	12.3	15.7
True protein	7.4	-[a]	12.5
Ether extract	4.6	3.6	3.2
Cellulose	27.7	24.2	28.6
Hemicellulose	22.3	21.5	27.3
Lignin	2.1	1.8	1.9
CWC[b]	52.1	47.5	57.8
WSC[c]	8.2	16.0	8.5
Fermenting products			
Formic acid	0.58	-	-
Acetic acid	1.2	-	-
Propionic acid	< 0.05	-	-
Butyric acid	< 0.05	-	-
Lactic acid	13.2	-	-
NH_4-N (% of tot. N)	3.7	-	-
pH	3.7	3.8	-

[a] not determined; [b] CWC = plant cell wall carbohydrates (cellulose + hemicellulose + lignin); [c] WSC = water soluble carbohydrates.

Table 2. Food and water intake (mean ± SD), mean apparent digestibility coefficients (DC) and nitrogen balance in reindeer fed regrowth timothy (*Phleum pratense*) silage (S), silage mixed with molasse (SM) and hay (H).

	S			SM			H		
Animal no.	1	2	3	1	2	3	1	2	3
Intake DM (kg/d)[a]	0.68±0.10	0.50±0.09	0.64±0.09	1.08±0.09	1.03±0.06	1.00±0.07	1.04±0.14	1.15±0.13	0.92±0.10
Intake DM (g/kg BM/d)[a]	14.4±2.1	9.2±1.7	12.9±1.7	21.9±1.8	19.0±1.1	19.0±1.4	21.0±2.8	21.1±2.4	19.2±1.9
Water intake (kg/d)[a]	0.09±0.08	0.32±0.24	0.19±0.30	0.68±0.29	0.41±0.25	0.12±0.07	4.36±0.67[c]	5.09±0.56	3.90±0.49
DC of:									
DM	81.1	82.6	78.5	79.1	81.6	83.1	72.9	70.8	69.9
Crude protein	72.7	75.5	67.6	61.8	64.4	69.6	65.4	64.6	60.4
Ether extract	63.2	69.1	64.3	56.2	64.3	63.6	44.2	37.9	38.0
Cellulose	97.0	94.2	91.6	90.1	93.9	93.8	84.5	80.4	84.8
Hemicellulose	84.5	88.2	80.3	79.5	83.1	85.3	80.1	75.8	76.6
WSC[b]	97.0	96.7	96.2	97.2	97.5	97.7	96.8	95.8	95.8
N balance (g/d)	+1.27	-1.80	-0.35	+0.68	+3.03	+3.59	+1.58	-0.08	-1.33

[a] n=11 days for animals fed S or H and n=5 days for animals fed SM; [b] WSC = water soluble carbohydrates; [c] n=10.

tation of the grass silage. The contents of true protein and hemicellulose was higher in hay compared to the grass silages, while addition of molasses gave higher content of water soluble carbohydrates in SM compared to S and H (Table 1).

Food and water intake

Food intake (dry matter; mean±SD) of S by calves (n=6) was 108.0 ± 111.7 g the first day, compared to 134.0 ± 77.4 g by calves (n=3) fed H. Food intake increased, and stabilized after 5-10 days. During the period of the *in vivo* digestibility experiment, daily DM intake ((median (range) g/kg BM) (median (range)) by calves fed SM (19.0 (19.0-21.9)) and H (21.0 (19.2-21.1)) was significantly (P=0.05) higher than in calves fed S (12.9 (9.2-14.4)) (Table 2). Intake of water was significantly (P= 0.05) higher in calves fed H compared to calves fed S or SM (Table 2).

In vivo *digestibility and nitrogen balance*

The median (range) apparent digestibility coefficient of dry matter in the animals fed S (82.6% (78.5-81.1%)) and SM (81.6% (79.1-83.1%)) was higher than in the animals fed H (70.8% (69.9-72.9%)). *In vivo* digestion of cellulose was also significantly (P=0.05) higher in the animals fed S or SM compared to animals fed H (Table 2). Calves fed S demonstrated significantly (P=0.05) higher digestion of crude protein compared to calves fed H (Table 2). The N-balance in calves fed SM ranged from + 2.68 to +3.59 g/d being higher than in animals fed S and H which had a N-balance ranging from -1.80 to +1.27 g/d and -1.33 to +1.58 g/d, respectively (Table 2).

In vitro *digestibility of cellulose*

Median (range) *in vitro* dry matter digestibility (IVDMD) of cellulose incubated in rumen fluid from reindeer fed S was 24.4% (14.7-33.9%) after 48 hours, significantly (P=0.05) lower than in animals fed timothy SM (42.2% (41.5-44.2%)). The IVDMD of cellulose in animals fed H was 36.4% (30.2-57.8%) (Fig. 1). The slope of the regression line through all the data in the plot for IVDMD of cellulose in rumen fluid from reindeer fed S was 0.83, compared to 0.98 for reindeer fed SM and 1.2 for reindeer fed H (Fig. 1). The slope of the regression line for IVDMD of cellulose in rumen fluid from S-fed

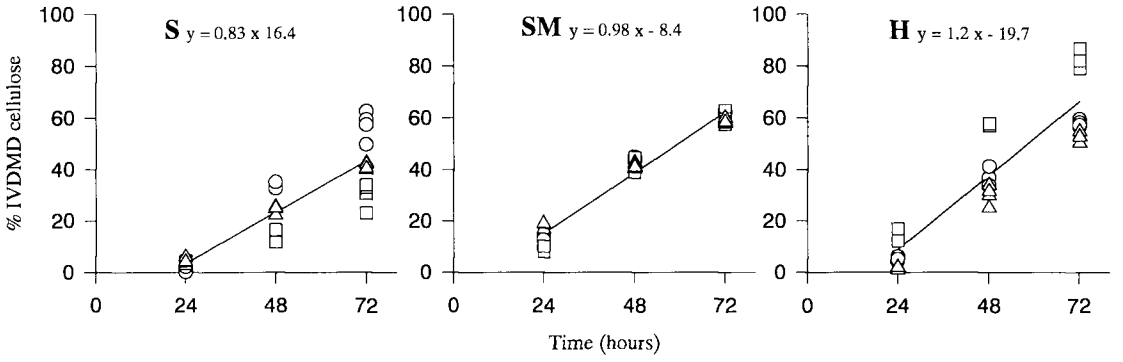

Fig. 1. Regression lines plot for the *in vitro* dry matter digestibility (IVDMD) of cellulose incubated in rumen fluid from reindeer fed regrowth timothy (*Phleum pratense*) silage (S) (Animal S1=○, S2=□, S3=△), silage mixed with molasses (SM) (Animal SM1=○, SM2=□, SM3=△) and hay (H) (Animal H1=○, H2=□, H3=△) as a funtion of time.

animals was significantly lower compared to both SM-fed animals ($P(F_{(1, 69)} \geq 90.50 \mid H_0)=3.96$, $\alpha=0.05$) and H-fed animals ($P(F_{(1, 69)} \geq 32.11 \mid H_0)=3.96$, $=0.05$). The slope of the regression line for IVDMD of cellulose was, however, not significantly different ($P(F_{(1, 69)} \geq 0.15 \mid H_0)=3.96$, $\alpha=0.05$) comparing SM- and H-fed animals.

Ruminal NH_4-N, pH, volatile fatty acids and lactate concentration

The ruminal contents of NH_4-N (median (range), % dry matter) was significantly ($P=0.05$) higher in calves fed H (0.38 (0.38-0.39)) than in calves fed S (0.15 (0.14-0.30)). The ruminal pH was highest in animals fed S and SM compared to animals fed H, reflecting the VFA concentration in the rumen fluid of the animals (Table 3). Significantly higher ($P=0.05$) concentrations of VFA were found in the rumen fluid of reindeer fed H compared to animals fed S or SM (Table 3). Regression lines plot for the concentration of total ruminal VFA concentration as a function of time, gave slopes of 0.019 for reindeer fed S, 0.187 for reindeer fed SM and 0.002 for reindeer fed H (Fig. 2). Slopes for VFA-production rates were significantly higher in both S-fed ($P(F_{(1, 57)} \geq 69.57 \mid H_0)=4.00$, $\alpha=0.05$) and SM-fed ($P(F_{(1, 57)} \geq 201.90 \mid H_0)=4.00$, $\alpha=0.05$) animals compared to H. No significant differences were found comparing the slope for VFA production in rumen fluid from animals fed S and SM ($P(F_{(1, 57)} \geq 0.82 \mid H_0)=4.00$, $\alpha=0.05$), this was probably due to the large differences in VFA-concentrations between animals fed S (Fig. 2).

Body mass and anatomy

Total body mass of the reindeer in the three groups did not differ significantly ($P>0.05$) although the carcass weights of the reindeer fed SM were signifi-

Table 3. Ruminal pH, concentration of volatile fatty acids (VFA) and lactic acid, and the relative proportion (%) of acetate, propionate and butyrate, and the acetate/propionate ratio in reindeer offered regrowth timothy (*Phleum pratense*) silage (S), timothy silage mixed with molasse (SM) and hay (H).

	S			SM			H		
Animal no.	1	2	3	1	2	3	1	2	3
pH	6.97	7.30	6.99	7.27	6.79	7.05	6.40	6.78	6.72
Total VFA (mM)	81.8	57.7	85.9	51.4	72.0	68.6	113.6	99.7	108.0
Lactic acid (mM)	0.31	0.65	0.78	-[a]	-	-	0.50	0.23	0.26
% of total VFA									
Acetate	77.7	76.1	73.9	75.9	75.8	74.4	85.7	77.1	79.0
Propionate	14.9	19.9	19.9	18.4	18.4	19.1	10.6	17.5	15.9
Butyrate	7.5	4.0	6.3	5.7	5.8	6.4	3.7	5.4	5.0
Acetate/propionate	5.2	3.8	3.7	4.1	4.1	3.9	8.1	4.4	5.0

[a] Not detectable amounts

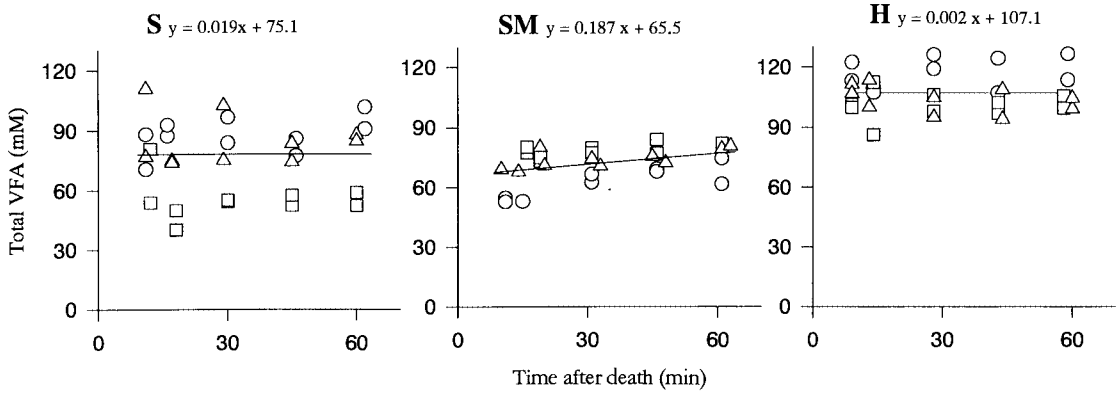

Fig. 2. Regression lines plot for the concentration of the main volatile fatty acids in the rumen of reindeer fed timothy (*Phleum pratense*) silage (S) (Animal S1=○, S2=□, S3=△), silage mixed with molasses (SM) (Animal SM1=○, SM2=□, SM3=△) and hay (H) (Animal H1=○, H2=□, H3=△) as a funtion of time.

Table 4. Body mass at the start and at the end of the feeding experimenr, carcass weight, weight of total gastrointestinal (GI) tract and the reticulo-rumen in reindeer fed regrowth timothy (*Phleum pratense*) silage (S), silage mixed with molasses (SM) and hay (H).

	S			SM			H		
Animal no.	1	2	3	1	2	3	1	2	3
Body mass (kg), start	43.0	44.0	46.0	45.0	45.5	45.5	46.0	44.5	43.5
Body mass (kg), end	47.0	54.5	49.5	49.5	54.0	52.0	49.5	54.5	48.0
Carcass weight (kg)	20.5	19.0	21.0	23.5	24.5	24.0	22.5	25.0	20.5
Total GI-tract:									
Contents (kg wet weight)	12.45	21.32	13.75	11.3	16.5	13.5	12.91	12.57	13.34
In % of body mass	26.5	39.1	27.8	22.8	30.6	26.0	26.1	23.1	27.8
Reticulo-rumen:									
Contents (kg wet weight)	11.12	19.57	12.34	8.40	13.10	10.71	11.13	10.88	11.86
In % of body mass	23.7	35.9	24.9	17.0	24.3	20.6	22.5	20.0	24.7
Contents (kg dry weight)	0.73	1.31	0.91	0.65	0.96	0.87	0.87	0.87	0.91

cantly higher compared to the that of reindeer fed S (Table 4). The median (range) total wet weight of reticulo-rumen digesta constituted 24.9% (23.7-35.9%), 20.6% (17.0-24.3%) and 22.5% (20.0-24.7%) of body mass in calves fed S, SM and H, respectively (Table 4).

Discussion

Intake of grass by reindeer could be affected by several factors, such as the structure of the grass, the dry matter content of the grass and the chemical composition of the grass. The intake of highly matured grass, with a low leaf/stem ratio and much cell wall carbohydrates, is limited in reindeer (Aagnes *et al.*, 1996). The silage and hay offered to the three groups of animals in this experiment were harvested from the same crop of timothy, having the same phenological growth stage. Structural differences in the grass harvested could therefore not explain the significantly ($P<0.05$) lower intake observed in animals fed S compared to animals fed SM and H (Table 2). The structure of the grass is therefore not the only factor influencing intake.

It is believed that predried and well prepared grass silage is palatable and stimulates food intake in ruminants (Van Soest, 1994). Low dry matter content of the grass eaten could therefore be another factor limiting intake in reindeer. In fact, Murdoch (1964) found a significant increase in food intake in sheep with increased dry matter content in silage harvested from the same crop. Reindeer fed H ate

more compared to reindeer fed S (Table 2), confirming data on cattle fed hay and silage from the same crop of ryegrass (Thiago *et al.,* 1992). The intake of H was, however, not different from that of SM. Furthermore, the dry matter content in S and SM were approximately at the same level (Table 1), but still the dry matter intake was up to 50% higher in calves fed SM (Table 2). Hence, the dry matter contents of the diet can only in part explain the differences in intake.

In the current study reindeer fed a leafy timothy silage (S) had 50% lower intake compared to another group of reindeer fed timothy silage with 89% leaves (Aagnes *et al.,* 1996). The difference between these two leafy qualities of timothy silage was found in the chemical composition, the latter quality having a higher content of water soluble carbohydrates. High levels of water soluble carbohydrates seems to influence intake of timothy silage positively in reindeer (Aagnes *et al.,* 1996), contradicting earlier studies on young cattle in which supplementation with sucrose to silage had no effect on food intake (England & Gill, 1985). Due to addition of molasses, the SM contained approximately twice as much water soluble carbohydrates as S (Table 1). This may explain the significantly higher ($P<0.05$) intake of SM compared with S (Table 2). Furthermore, timothy silage (Olsen *et al.,* 1995) resembling S (Table 1) with respect to cell wall carbohydrates, but with twice as much water soluble carbohydrates (19.6% of dry matter) was fed to reindeer, giving a mean intake (median (range)) of 17.6 (15.9-21.1) g/kg BM/d (M. A. Olsen, unpubl. data). The low intake observed in animals fed S (Table 2) may therefore be explained by lack of available energy for rumen digestion. Readily fermentable carbohydrates support microbial growth in the rumen when supplied in moderate amounts, and can therefore increase plant cell wall digestion as long as ruminal acidosis does not occur. Even though H contained only 8.5% water soluble carbohydrates (dry matter) (Table 1) the food intake by animals fed H was twice that of animals fed S (Table 2). Compared to S and SM, H is rich in true protein (Table 1), which is important for maintaining a high concentation of N available for microbial synthesis. The difference in NH_4-N in the rumen contents of H and S fed animals were confirmed by chemical analysis. Hence, the low nitrogen level in the rumen of calves fed S could suppress ruminal microbial synthesis particularly when intake of readily available energy is low.

Our data showed that *in vitro* digestion of cellulose (24-72 hours incubation) occurred at a significantly higer rate in rumen fluid from reindeer fed SM compared to S ($P(F_{(1,\ 69)} \geq 1.69 \mid H_0) = 3.96$, $\alpha=0.05$) (Fig. 1). Similarly, Borroughs *et al.* (1950) also showed that addition of molasses influences *in vitro* rumen cellulolysis positively. Hence, addition of small amounts of molasses to regrowth timothy silage seems to increase cellulolytic activity in the rumen of reindeer (Fig. 1) and also ruminal VFA production rates (Fig. 2). However, maximum *in vitro* cellulolysis (during 72 hours incubation) in rumen fluid from H-fed reindeer was higher than in animals fed SM (Fig. 1), which may be due to a combined effect of both water soluble carbohydrates and N for microbial synthesis in H-fed animals compared to SM-fed reindeer.

In reindeer calves fed rough timothy silage mean ruminal pH and VFA concentrations were 7.14 and 51 mM, while in animals fed leafy timothy silage ruminal pH and VFA were 6.58 and 85 mM (Aagnes *et al.,* 1996) reflecting the differences in chemical composition of the two substrates. The SM-diet with 16% water soluble carbohydrates (dry matter) did not give a detectable effect on the ruminal VFA concentration and pH compared to the S-diet with 8.2% water soluble carbohydrates (Table 3). The concentration and pool size of VFA seems to be correlated with VFA production rates in domestic ruminants (Weston & Hogan, 1968). However, Lechner-Doll *et al.* (1991) observed that rumen dilution rates, fluid volume and rate of absorption influenced ruminal VFA concentration far more than production rates in African ruminants. Sørmo *et al.* (1997) also found that in Svalbard reindeer (*Rangifer t. platyrhynchus*) ruminal concentration of VFA was not nessecarily related to VFA production. Josefsen *et al.* (1996) have shown that the surface area of the rumen decreases with poor quality food. Ruminal concentration of volatile fatty acids is influenced by eating, drinking, salivary secretion and absorption accross the rumen wall. We assume that salivary production is higher in SM-fed reindeer compared to S-fed reindeer since food intake is 50% higher, and this again could influence buffer capacity (Table 2). These factors could explain that no differences were observed in VFA concentration between the SM- and S-fed animals (Table 3).

Reindeer are classified as intermediate mixed feeders (Hofmann, 1985), and they are recognised to have a highly adaptable digestive system, which is able to adjust to substrates of different chemistries.

The *in vitro* cellulase assay shows the efficiency of the rumen fluid to digest cellulose. In animals where rumen cellulolysis occurs slowly (Fig. 1), the rumen increase in size (Table 4), ruminal VFA concentration is low (Table 3) and in extreme cases food intake is also depressed (Table 2) (Olsen *et al.*, 1995, 1997). Reduced ruminal cellulolysis does, however, not seem to influence *in vivo* digestion of cellulose (Table 2) which is probably explained by reduced turnover of food in the rumen and compensatory cellulolysis in the distal fermentation chamber in reindeer (R. Moen, unpubl. data; Sørmo *et al.*, 1998).

High intake measured in reindeer fed SM was not associated with a reduction of *in vivo* dry matter digestibility (Table 2). This high digestibility could be explained by increased ruminal efficiency. Our results indicate that the rumen of reindeer fed timothy was large compared to reindeer fed a pure diet of lichen with reticulo-rumen wet weight digesta comprising 8.4-14.8% of BM (H. Øksendal, unpubl. data). In reindeer fed rough timothy silage the reticulo-rumen wet weight digesta constituted as much as 25.4-33.4% of the total BM (Aagnes & Mathiesen, 1996), resembling that of reindeer fed S (26.5-39.1%) in the current study (Table 4). While in reindeer fed leafy timothy silage the reticulo-rumen contents constituted only 10.4-18.3% of the BM (Aagnes & Mathiesen, 1996). We found no significant ($P>0.05$) difference in the relative size of the reticulo-rumen between reindeer fed S and SM, but there was a general trend that S-fed animals had larger rumen (mean: 28.2%) compared to SM-fed animals (mean: 20.6%) (Table 4). The relatively smaller rumen, faster rumen cellulolysis, higher production rates of VFA and higher intake found in SM-fed animals compared to S-fed animals (Fig. 1, Table 2 and 4) indicated that molasses could stimulate to a higher ruminal passage rate. Syrjälä (1972) investigated the effect of adding sucrose (15% and 30% of dry matter) on utilization of grass silage, but found no positive effect of this supplemental energy on *in vivo* digestibility of organic matter or crude fibre in sheep. Similarly, in our study on reindeer, addition of molasses to silage did not give any significant ($P>0.05$) effect on the *in vivo* digestion of the food (Table 2). The lower *in vivo* digestibility of H dry matter compared to SM (Table 2) may be explained by the different chemistry of silage and hay, since grass silage is being partly predigested during the fermentation of the silage (Table 1).

In conclusion, this study has shown that leafy, high quality hay of timothy seems to be the best suitable emergency food for reindeer. Addition of molasses to grass silage seems to stimulate ruminal fermentation in reindeer and this could prevent failure of rumen digestion. A higher intake of food by reindeer calves fed SM and H compared to animals fed S, may indicate ruminal energy deficiency in the latter group.

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References

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

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

Aagnes, T. H., Blix, A. S. & Mathiesen, S. D. 1996. Food intake, digestibility and rumen fermentation in reindeer fed baled timothy silage in summer and winter. – *J. Agric. Sci., Cambridge.* 127: 517–523.

Aagnes, T. H. & Mathiesen, S. D. 1996. Gross anatomy of the gastrointestinal tract in reindeer, free-living and fed baled timothy silage in summer and winter. – *Rangifer* 16 (1): 31–39.

Burroughs, W., Long, J., Gerlaugh, P. & Bethke, R. M. 1950. Cellulose digestion by rumen microorganisms as influenced by cereal grains and protein-rich feeds commonly fed to cattle using an artificial rumen. – *J. Anim. Sci.* 9: 523–530.

Carroll, E. J. & Hungate, R. E. 1954. The magnitude of the microbial fermentation in the bovine rumen. – *Appl. Microbiol.* 2: 205–214.

England, P. & Gill, M. 1985. The effect of fish meal and sucrose supplementation on the voluntary intake of grass silage and hve-weight gain of young cattle. – *Anim. Prod.* 40: 259–265.

Furuholmen, A. M., Winefordner, J. D., Knapp, F. W. & Dennison, R. A. 1964. The quantitative analysis of glucose and fructose in potatoes. – *Agric. Food Chemistry* 12 (2): 109–112.

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

Gaare, E. & Skogland, T. 1975. Wild reindeer food habits and range use at Hardangervidda. – *In:* Wielgolaski, F. E. (ed.). *Ecological studies. Analysis and synthesis. Fennoscandian Tundra Ecosystems.* Berlin: Springer-Verlag, 17 (2): 195–205.

Goering, H. K. & Van Soest, P. J. 1970. *Forage fiber analyses (Apparatus, Reagents, Procedure, and Some Applications).* Agriculture Handbook 379, Agricultural Research Service, United States Department of Agriculture, Washington, D. C.

Grotelueschen, R. D. & Smith, D. 1968. Charbohydrates in grasses. III. Estimation of the degree of polymerization of the fructosans in the stem bases of timothy and bromegrass near seed maturity. – *Crop Sci.* 8: 210–212.

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

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

Hofmann, R. R. 1985. Digestive physiology of the deer – Their morphophysiological specialisation and adaptation. *In: Biology of Deer Production. – Roy. Soc. New Zealand, Bulletin.* 22: 393–407.

Horwitz, W. 1980. Official methods of analysis of the Association of Official Analytical Chemists, 13th ed. Association of Offical Analytical Chemists, Washington, D. C.

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

Hungate, R. E., Mah, R. A. & Simesen, M. 1961. Rates of production of individual volarile fatty acids in the rumen of lactating cows. – *Appl. Microbiol.* 9: 554–561.

Josefsen, T. D., Aagnes, T. H. & Mathiesen, S. D. 1996. Influence of diet on the morphology of the ruminal papillae in reindeer calves (*Rangifer tarandus tarandus* L.). – *Rangifer* 16 (3): 119–128.

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

Kleinbaum, D. G., Kupper, L. L. & Muller, K. E. 1988. Applied regression analysis and other multivariable methods. Second edition. Duxbury Press, Belmont California.

Lechner-Doll, M., Becker, G. & Engelhardt, W. v. 1991. Short chain fatty acids in the forestomach of camels and indigenous cattle, sheep and goats and in the caecum of donkeys grazing a thornbush savannah pasture.– *In:* Isotope and related techniques in animals production and health, International Atomic Energy Agency, Vienna, (IAEA-SM-318/21), pp. 253–268.

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

Murdoch, J. C. 1964. Some factors affecting the intake of roughage by sheep. – *Br. grassl. Soc.* 19: 316–320.

Olsen, M. A., Aagnes, T. H. & Mathiesen, S. D. 1994. Digestion of herring by indigenous bacteria in the minke whale forestomach. – *Appl. Environ. Microbiol.* 60 (12): 4445–4455.

Olsen, M. A., Aagnes, T. H. & Mathiesen, S. D. 1995. Failure of cellulolysis in the rumen of reindeer fed rimorhy silage. – *Rangifer* 15 (2): 79–86.

Olsen, M. A. & Mathiesen, S. D. 1996. Production rates of volatile fatty acids in the minke whale (*Balaenoptera acutorostra*) forestomach. – *Brit. J. Nutr.* 75: 21–31.

Olsen, M. A., Aagnes, T. H. & Mathiesen, S. D. 1997. The effect of timothy silage on the bacterial population in rumen fluid of reindeer (*Rangifer tarandus tarandus*) from natural summer and winter pasture. – *FEMS Microbiol. Ecol.* 24: 127–136.

Person, S. J., White, R. G. & Luick, J. R. 1980. Determination of nutritive value of reindeer-caribou range. – *In:* Reimers, E., Gaare, E. & Skjenneberg, S., (eds.). *Proceedings of the 2nd. International Reindeer/Caribou Symposium, Røros.* Direktoraret for vilt og ferskvannsfisk, Trondheim, pp. 224–239.

SAS Institute Inc. 1989. *SAS/STAT Users Guide,* Version 6, vol. 1-2, Cary, NC.

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

Staaland, H., Garmo, T. H., Hove, K. & Pedersen, Ø. 1995. Feed selection and radiocaesium intake by reindeer, sheep and goats grazing alpine summer habitats in southern Norway. – *J. Environ. Radioactivity,* 29 (1): 39–56.

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

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

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

Syrjälä, L. 1972. Effect of different sucrose, starch and cellulose supplements on the utilization of grass silages by ruminants. – *Ann. Agric. Fenn.* 11: 199–276.

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

Sørmo, W., Haga, Ø. E. & Mathiesen, S. D. 1997. Comparative aspects of volatile fatty acid production in the rumen and distal fermentation chamber in Svalbard reindeer. – *Rangifer* 17 (2): 81–95.

Sørmo, W., Haga, Ø. E. & Mathiesen, S. D. 1998. Cellulolysis in the rumen and distal fermentation chamber in Svalbard reindeer. – *Rangifer* 18 (1): 47–50.

Thiago, L. R. L., Gill, M. & Dhanoa, M. S. 1992. Studies of method of conserving grass herbage and frequency of feeding in cattle. 1. Voluntary feed intake, digestion and rate of passage. – *Brit. J. Nutr.* 67: 305–318.

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

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

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

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

Van Soest, P. J. 1994. Nutrirional ecology of the ruminant. 2nd ed. Cornell University Press, Ithaca, New York.

Weston, R. H. & Hogan, J. P. 1968. The digestion of pasture plants by sheep. I. Ruminal production of volatile fatty acids by sheep offered diets of ryegrass and forage oats. – *Australian J. Agric. Res.* 19: 419–432.

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