Health, body condition and blood metabolites in reindeer after submaintenance feed intake and subsequent feeding

Nilsson, A.[1*], Danell, Ö.[1], Murphy, M.[2], Olsson, K.[3] & Åhman, B.[1]

[1] Dept. of Animal Breeding and Genetics, Reindeer Husbandry Unit, SLU, P.O. Box 7023, S-750 07 Uppsala, Sweden.
[2] Dept. of Animal Nutrition and Management, SLU, Knngsängen Research Centre, S-753 23 Uppsala, Sweden.
[3] Dept. of Animal Physiology, SLU, P.O. Box 7045, S-750 07 Uppsala, Sweden.
[*] corresponding author (anna.nilsson@huv.slu.se).

Abstract: The transition from experimentally induced poor nutritional conditions to feeding was studied with 69 eight-month-old female reindeer (*Rangifer tarandus tarandus*). During a pre-experimental period, all reindeer were fed a simulated winter diet with 80% lichens *Cladina* spp. and 20% *Vaccinum myrtillus* shrubs and *Salix* spp. leaves (lichen diet) *ad lib.* The reindeer were divided into five groups. A control group (group C) was fed the lichen diet *ad lib.* throughout the experiment. Four groups were fed half of that ration for eight days and were then totally deprived of feed for one day (restriction period). During the following 34 days (feeding period) the groups were re-fed the lichen diet (group L), fed pelleted reindeer feed combined with either lichen (group PL) or grass silage (group PS), or fed silage with a gradually increasing addition of pellets (group SP). Weekly measurements of blood samples and body weighr showed that the control group remained clinically healthy and had stable blood plasma concentrations of protein, urea, glucose and insulin throughout the experiment, but they lost weight. At slaughter, before and after the restriction period, all animals had lost rumen-free body weight, but the reindeer fed a restricted amount of feed lost more than the control group. Also the plasma metabolites were affected by the restricted feeding, with increased concentrations of urea and decreased concentrations of glucose. Group L responded immediately to the *ad lib.* feeding with blood metabolite levels rapidly approaching those of group C. The body weight developments were similar in groups L and C. Although the feed rations were increased gradually, diarrhoea occurred in some animals belonging to groups PL and PS within the first week of the feeding period. All reindeer recovered, after antibiotic treatment of the worst affected animals. The PL and PS groups, which had high contents of metabolisable energy and crude protein in their diets, showed increased concentrations of plasma protein, urea and insulin. At the end of the feeding period, these groups had increased their body and carcass weights and gained fat, whereas reindeer fed the lichen diet had lost weight. Severe health problems (malnutrition and so-called wet belly) occurred in group SP during the first weeks of feeding and led to loss of animals, and consequently the SP group was excluded from the remainder of rhe experiment. The general conclusion is that the lichen diet did not cause any digestive problems, but resulted in a continuous decline in body weight and small or deficient fat reserves. After the initial diarrhoea, feeding with diets comprising pellets from the start resulted in improved condition, expressed as increased body weight, fat gain and higher concentrations of plasma protein, urea and insulin in relation to the control group. The diet initially based on grass in the form of silage of the given quality seemed insufficient as feed to reindeer calves in a poor nutritional state.

Key words: energy, glucose, insulin, lichen, plasma protein, silage, starvation, urea.

Rangifer, 20 (4): 187–200

Introduction

Reindeer are well adapted to seasonal differences in forage availability and quality. Emergency feeding occasionally becomes necessary, however, due to inadequate access to pasture caused by deep snow or ice crust that prevents the reindeer from reaching the vegetation on the ground. When reindeer begin to starve, the condition of the animals deteriorates rapidly unless measures to improve feed accessibility are taken. The only solution is often to feed the animals. A gradual adaptation to a new diet, which is necessary for ruminants in order to avoid metabolic disturbances, is difficult to apply for reindeer in large herds. The feeding may, thus, cause severe problems. Current feeding strategies for reindeer in poor condition have not been sufficiently evaluated and the metabolic characteristics of reindeer during the adaptation period are still poorly understood.

Digestive problems are not known to occur when undernourished or starved reindeer are transferred back to a natural pasture, or to a pure lichen diet (Bøe & Jacobsen, 1981; Sletten & Hove, 1990). Lichens are difficult to obtain in large amounts, however, and a pure lichen diet seems to be nutritionally insufficient during longer periods of feeding, due to the low protein content (Jacobsen & Skjenneberg, 1975).

Variable results have been obtained when using commercial reindeer feed as emergency feed for reindeer (*e.g.* Jacobsen & Skjenneberg, 1979; Bøe & Jacobsen, 1981; Mathiesen *et al*, 1984; Sletten & Hove, 1990). Overeating as well as refusing to eat, leading to problems with acidosis and diarrhoea, have been reported. On the other hand, with animals in good condition, transition from pasture to pellets feeding has worked without problems (Åhman & Åhman, 1980). Mixing an unfamiliar diet with lichens during the first days of feeding may contribute to the adaptation and is commonly used in practice. When lichen was mixed into a pellets and silage diet or into solely silage during the first two-three weeks of feeding, no adaptation problems occurred (Nilsson *et al.*, 1996a; 1996b). Silage alone may work as an initial feed to reindeer in moderate condition, provided its nutritional quality is optimal (Øksendal, 1994; Aagnes, 1998; Olsen *et al*, 1995; Norberg & Mathiesen, 1998). However, feeding with only silage may lead to malnutrition (*e.g.* Nilsson *et al.*, 1996a).

The metabolic state of ruminants may be assessed from blood concentrations of, e.g. urea, plasma protein, glucose and insulin. Urea, plasma protein and glucose have been measured in several field studies (Hyvärinen *et al.*, 1976; Nieminen 1980; Nieminen & Timisjärvi, 1983; Soveri *et al*, 1992) and urea also in basic physiological studies (Wales *et al*, 1972; Hove & Jacobsen, 1975; Valtonen & Eriksson, 1977; Valtonen, 1979). There are some data on single metabolites from feeding experiments with reindeer (Larsen *et al*, 1985; Nieminen *et al*, 1987; Säkkinen *et al.*, 1999).

We have tested four different feeding strategies on reindeer calves in poor condition, obtained by a period of sub-maintenance intake of a simulated winter diet followed by one day of total food deprivation. A control group was continuously fed a simulated winter diet *ad lib.* The aim was to assess how the different feeding strategies affected nutritional state and metabolic characteristics of the reindeer during recovery from nutritional deprivation.

Material and methods

Animals, experimental design and diets

Sixty-nine eight-months-old female reindeer (*Rangifer tarandus tarandus*) were brought to the research station at the Department of Biology at the University of Oulu, Finland in the winter of 1996/97. The reindeer originated from the southern part of the Finnish reindeer herding area. Reindeer from three different deliveries were equally distributed, but randomly allotted from each delivery, into five experimental groups and kept in pens of about 500 m^2. The experiment included three periods: the pre-experimental period, starting November 12, the restriction period, starting January 28, and the feeding period, starting February 6 (Fig. 1). The ground was covered with snow throughout the experiment and the outdoor temperature was, on average, -6 °C (-28 to +6 °C).

From arrival, all reindeer were offered a simulated winter diet (lichen diet) containing 80% of dry matter (DM) of lichens, *Cladina* spp. mixed with *Vaccinum myrtillus* shrubs and *Salix* spp. leaves, *ad lib.* (Fig.1). The shrubs and leaves were harvested in July-August and the lichens in September-October. The lichens and leaves were stored dry while the shrubs were stored frozen. A control group (group C) was continuously offered the lichen diet *ad lib.* throughout the experiment. During the restriction period, four groups were given half of the daily amount of the lichen diet consumed during the *ad lib.* feeding, followed by one day of total feed deprivation. Four feeding strategies were then applied

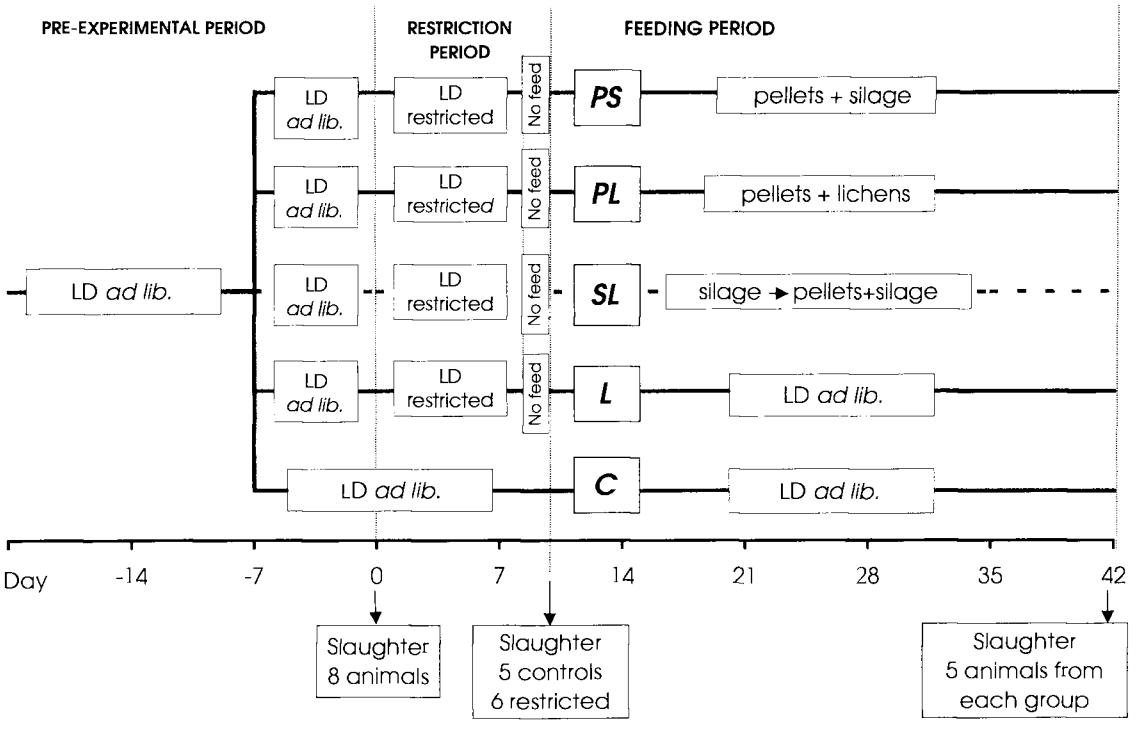


Fig. 1. Design of the experimenr which included initially 69 reindeer calves divided in 5 groups (n=13-15 animals per group). The control group (C) was fed a lichen-based diet (LD) *ad lib.* throughout the experimenr. After a period of restricred intake of the LD, the other groups (PS, PL, SP and L, with 10 animals in each group) were fed different diets. Reindeer were slaughtered on three occasions: before and after the resrriction period, and at the end of the experiment. Group SP was eventually excluded from the experiment because of loss of animals at the beginning of the feeding period

during the feeding period. Group L was re-fed the lichen diet *ad lib.* Two groups were fed diets of 80% (DM basis) commercial pelleted reindeer feed (pellets) combined with either 20% lichen (group PL) or 20% silage (group PS) and the rations were gradually increased from 0.6 kg DM per animal to *ad lib.* during the first week of feeding. Group SP was fed silage *ad lib.* for five days and thereafter, with gradual change, a diet with 80% pellets and 20% silage. The pellets (Renfor Bas, Lantmännen Fori, Holmsund, Sweden) were composed of oat, wheat and their bran products, dried molassed sugar-beet pulp and soya bean meal. The baled grass-silage consisted mainly of timothy (*Phleum pratense*).

All groups of animals were fed twice a day, with one-third of the ration in the morning and the rest at noon. During sampling days, the animals were fed after sampling with the whole daily ration given at noon. The feedstuffs were mixed manually and offered in cribs. The *ad lib.* allotment was set so that there was still some edible material (at least 10%) left in the feed residues. Daily measurements of the allotted feed and feed residues for each group, together with estimated amounts of snow and DM content of the residues, were used to calculate DM intake per animal. Mineral blocks (Natura Slicksten, Suomen Rehu, Helsinki, Finland) were available in all pens and the animals had free access to temperate water (about 10 °C). The lichens were soaked in water to 25-35% DM before feeding.

Sampling and slaughter routines

Five animals per group were randomly allotted for blood sampling. The sampling routines were introduced during the pre-experimental period and conducted in a standardised way throughout the experiment. At sampling, all reindeer were taken out of the pen via a corridor into an enclosure (7.5 m^2). From there the individual animal was caught by hand and led to a balance, where body weight was measured on the unrestrained animal. The animal was then led into a sampling room where the nose,

mouth, eyes and general body condition were inspected while the animal was manually held. Animals not to be blood sampled were then released out into the pen while animals to be sampled were restrained in a treatment crush by means of straps around the belly. The head was manually held while blood samples were taken via puncture of *vena jugularis*. Body weight measuring and blood sampling were conducted once a week with an extra sampling during the restriction period. In addition, the animals were taken into the sampling room once a week to check heart rate monitors that were implanted on three animals per group (the results will be presented separately).

Reindeer, randomly allotted at the start of the experiment, were slaughtered on three occasions. Eight reindeer were slaughtered at the end of the pre-experimental period (day 0). After the restriction period (day 10), six restrictively fed reindeer and five control animals were slaughtered. Five reindeer from each group were slaughtered after the feeding period (days 43 and 44). The reindeer to be slaughtered were not offered any feed at the day of slaughter. They were taken out of their pens in the morning, transported for about 30 min on a lorry to the slaughter house and slaughtered within 90 min after arrival by stunning with a captive bolt. The abdomen was cut open and the stomachs and guts were collected and weighed. The fat in the abdominal cavity (kidney fat and fat in the omentum) was weighed. Kidney fat less than 5 g, and omentum with fat less than 35 g was considered non-measurable and given the value 0 g. The weights of the carcasses were recorded. All slaughtered animals were inspected according to human food regulations and, in addition, stomachs and intestines were checked.

Blood analyses

The blood samples were collected in heparinised tubes (Venoject®, Leuven, Belgium) and were chilled and centrifuged. Total plasma protein concentration was estimated by refractometry (Reichert-Jung, Cambridge Instruments Inc. Buffalo, NY 1415, USA). The plasma samples were frozen immediately and stored at -20 °C. Plasma levels of insulin were analysed by radioimmunoassay (Insulin RIA, Pharmacia, Uppsala, Sweden). Plasma glucose concentration was determined by an enzymatic colorimetric method (Peridochrom Glucose GOD-PAP, Boehringer Mannheim Gmbh Diagnostica, Germany). Plasma urea concentration was determined by using an enzymatic colorimetric method (UREA liquicolor, Human Gesellschaft für Biochemica and Diagnostica mbH, Taunusstein, Germany).

Feed analyses

Feed samples were taken daily, frozen and bulked for periods of one week and the chemical compositions of the silage, shrubs and leaves were determined. The DM content of the frozen samples was determined by drying samples for 16 h at 65 °C, followed by 5 h at 105 °C. Ash was determined by heating 2 g of dried sample for 2 h at 600 °C. Crude protein (CP) was determined by a Kjeldahl technique (Bremner and Breitenbeck, 1983). Ammonia-nitrogen was determined by direct distillation on a Kjeltec Auto System 1030. Water-soluble carbohydrates (WSC) were determined by extracting samples of 3 g dry material in 100 ml boiling water. Extracts were then filtered and hydrolyzed with 0.074 M H_2SO_4 (200 ml extract + 200 ml H_2SO_4, 70 min at 80 °C). Glucose and fructose were determined enzymatically (Larsson & Bengtsson, 1983). Silage pH was measured in silage juice by a Methrom 654 pH-meter. The content of neutral detergent fibre (NDF), acid detergent fibre and lignin was determined as described by Goering & VanSoest (1970) and used to estimate crude fibre. The hygienic quality of the feed samples was analysed at the National Veterinary Institute in Uppsala, Sweden.

Metabolisable energy (ME) for the silage was calculated from *in vitro* digestibility and from the content of DM (Lindgren, 1979), while the ME for the pellets was given by the feed manufacturer. Assessment of *in vitro* digestibility of lichen, shrubs and leaves was made in a semi-continuous in vitro rumen simulation system (Murphy & Lindgren, 1997) using rumen fluid from a cow adapted to hay and concentrates.

Statistical analyses

The effect of delivery (day of arrival) on the initial state of the reindeer was tested using a mixed linear model for the repeated live body weight measures obtained during the pre-experimental period. Independent variables in the model were delivery, group, animal within delivery and group, and quadratic regressions on number of days from delivery day fitted individually within each delivery. Animals were treated as a random effect and the required animal and residual variances were

Table 1. Chemical composition and estimated digestibility coefficients used for calculation of the metabolisable energy (ME) of the feed components of the various diets given to the reindeer calves.

	Silage	Pellets	Lichens	Shrubs	Leaves
Number of samples	5	1	5	5	5
Dry matter, %	56.6	88.9	45.2	55.1	72.2
Composition, % of dry matter					
Crude protein	19.8	11.2	3.3	7.7	15.8
Ash	10.2	7.6	1.6	3.6	4.9
Water soluble carbohydrates	1.82	9.4	0.39	7.1	4.6
Neutral detergent fibre	52.5	35.8	75.7	48.3	37.1
Acid detergent fibre	31.3	7.4	13.1	43.9	31.4
Lignin	3.8	3.3	6.6	19.7	15.5
Ammonia-N, g/kg dry marter	15.1				
pH	4.65				
Digestibility coefficients (estimated)					
Crude Protein			0[3]	75[4]	75[4]
Ether extract			57.5[3]	70[4]	70[4]
N-free extract			70.6[3]	90[4]	90[4]
Crude fibre			80.3[3]	21[4]	26[4]
Calculated ME, MJ/kg DM	10.1[1]	10.0[2]	10.1	9.5	10.4

[1] calculated according to Lindgren (1983).
[2] according to feed manufacrurer.
[3] from Jacobsen & Skjenneberg (1977).
[4] from own *in vitro* analyses.

obtained iteratively by restricted maximum likelihood from the same data.

Observed live body weights were scaled to rumen-free body weight in order to avoid the variation caused by changes in rumen fill, using the ratio of body weight without reticulo-rumen with content and the total body weight of the slaughtered animals within the same treatment and period as scaling factors.

For the restriction and feeding periods, group and treatment were statistically confounded and usually only one measurement per observed variable was available for each animal and period. The changes in rumen-free body, slaughter data and blood plasma parameters during the subsequent periods were, therefore, analysed using fixed linear models including only delivery and treatment as independent variables. Feed and water intakes were analysed according to the same model. All statistical analyses were performed using the SAS/STAT® software package (SAS 1997). The significance level was set at $P<0.05$.

Results

Feed composition

The DM and chemical composition of the silage, shrubs, leaves and pellets are presented in Table 1. The hygienic quality of the silage and pellets was good, while the lichens and shrubs showed growth of *Penicillium spinulosum* and *P. brevicompactum.*

The ME content of the shrubs and leaves was calculated from the chemical composition as described by Axelsson (1941), using digestibility coefficients estimated from the in vitro analyses (Table 1). The observed in vitro digestibility of NDF (for estimation of crude fibre digestibility) for lichens was 38%, which was obviously too low, and probably due to the unadapted rumen fluid. The ME content of the lichens was therefore calculated from the chemical composition and energy factors as

described by Axelsson (1941), using digestibility coefficients according to Jacobsen & Skjenneberg (1977).

Pre-experimental state of the reindeer

All animals appeared healthy during the pre-experimental period and no problems were observed with adapting the reindeer to feeding, handling and sampling. The mean daily feed intake during the last week of the pre-experimental period was 1.2-1.4 kg DM per reindeer. Since the reindeer arrived at varying dates, the delivery groups were not synchronous in their adaptation to feeding and the effect of days spent at the station was significant. However, the mean body weights at day 1 were similar in all groups, 41-44 kg, ranging from 32.6 to 51.6 kg for the individual animals (Fig. 2).

No significant differences were found between the experimental groups in any of the measured blood plasma metabolites at day 1. The animals slaughtered at day 0 had similar carcass and rumen weights (Table 5). Four of the eight animals had measurable kidney fat but none had any fat in the omentum.

Animal health

During the restriction period, seven reindeer, all from groups fed the restricted rations (four from group SP, two from PL and one from PS), developed wet fur with initial signs of so-called wet belly (Åhman *et al.*, 1999). The animals were licking fur, both on themselves and on pen mates. No other signs of digestive disturbances or health problems were observed during the restriction period.

No health problems occurred during the feeding period in groups C and L. One animal from group PS was excluded on day 21 due to an injured eye. Diarrhoea occurred in both groups PL and PS within the first week of the feeding period. The worst affected animals (three from PL and one from PS) were treated with antibiotics (dihydrostreptomycin) and all recovered within two to three days. Animals

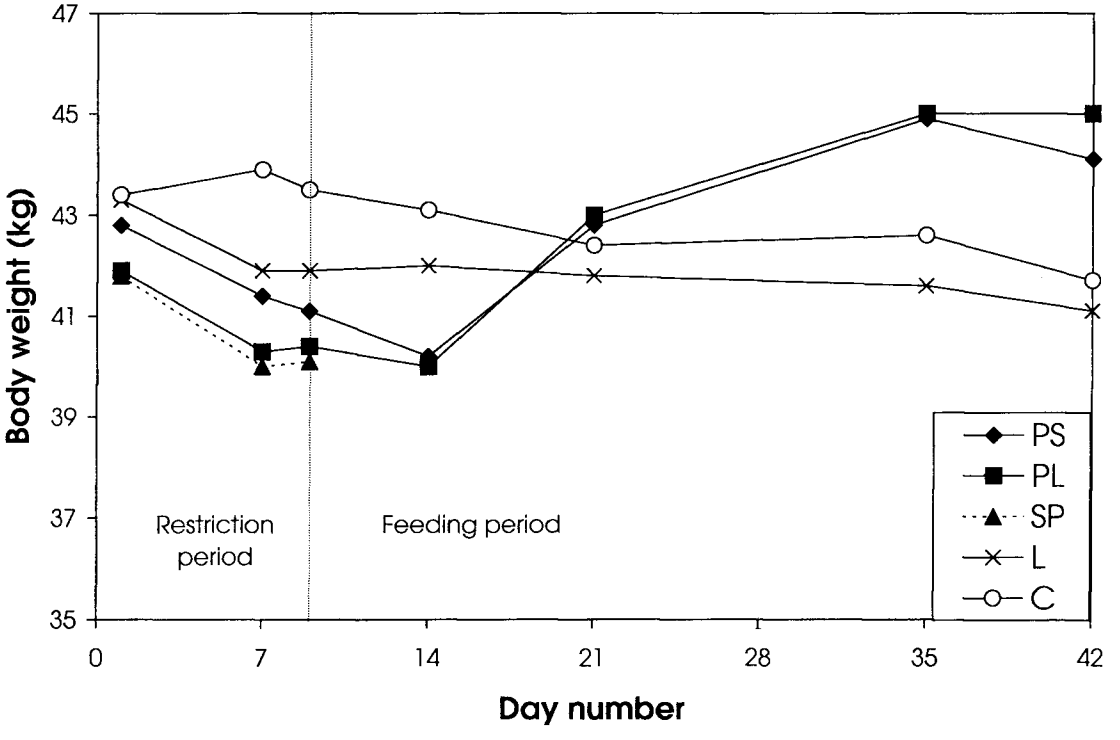


Fig. 2. Body weight development (least square means) during the restriction period (n=13-15 reindeer per group), when controls (group C) were fed a lichen based diet *ad lib.* and the other groups were fed a restricted amount of the same diet, and during the feeding period (n=10 reindeer per group), when groups C and L were fed the lichen diet *ad lib.* and group PS and PL were fed pellets combined with silage and lichens, respectively. Standard errors varied between 1.1 and 1.5. Group SP was excluded after the restriction period, due to loss of animals. Missing values on day 28 were due to a technical error.

Table 2. Average daily intakes of feed, water and crude protein (CP) and calculared intake of merabolisable energy (ME) per animal in each group during rhe last three weeks of the feeding period (least-square means), LD = lichen based diet, Pe = pellets, Li = lichens, Si = silage.

Group:	C	L	PL	PS	
Diet:	LD	LD	Pe + Li	Pe + Si	Standard error
Ration:	*ad lib.*	*ad lib.*	*ad lib.*	*ad lib.*	of group means
No. of animals	10	10	10	9	
Feed, kg DM	1.30	1.28	1.82	1.76	±0.05
Drinking-water, l	0.32	0.14	1.23	2.48	±0.08
Total water, l	3.7	3.5	2.1	2.8	±0.1
CP, g	64.2	63.2	174.1	231.2	±0.24
ME, MJ	13.5	13.3	18.2	17.7	±0.18
ME, $MJ/kg^{0.75}$	0.8	0.7	1.0	1.0	±0.02

with signs of wet belly in groups PL and PS gradually recovered during the feeding period while the problems continued in group SP and more animals got wet. The characteristics of the affected animals were that they had wet fur, were restlessly eating which resulted in increased body weight (due to increased rumen content), seemed apathetic and had increased urea concentrations in the blood. Four reindeer died (on days 11, 13, 21 and 25) and one reindeer was euthanised (on day 23). Autopsies of the animals showed *e.g.* emaciation. Four of the lost animals were intended for blood sampling and further results from the SP group have therefore been excluded in this paper and will be reported separately.

Feed and water intake

During the restriction period the restricted groups ate almost all of the feed provided (0.7 kg DM per reindeer and day) which was half of the amount eaten in group C. Also dead lichens and coarse parts of the shrubs, that were rejected during the *ad lib.* feeding, were eaten. At the start of the feeding period, the feed intake for group L was stable and equal to that of group C within four days. Reindeer in groups PL and PS ate all of the offered feed during the first week of feeding when the rations were gradually increased. During the last three weeks of the feeding period, the mean daily intake of DM, CP and ME was significantly higher in groups PL and PS compared with groups C and L (Table 2). Reindeer in group PS had significantly higher intake of CP than group PL, while estimated ME was similar for the two groups.

The total daily water intake was, on average, 2.0 l per animal in the restricted groups and 3.8 l per animal in group C. During the feeding period, the water consumption was on a similar level for groups C and L. Groups PL and PS consumed significantly more drinking-water than groups C and L during the feeding period, but the total water intake was significantly lower in groups PL and PS. Both drinking-water and total water intake were significantly higher for group PS than for group PL.

Body weight

During restricted feeding, the reindeer lost, on average, 1.6 kg body weight while reindeer in group C, that were fed *ad lib.*, gained on average 0.2 kg body weight during the same period. The weight of the rumen content in group C was, however, higher after than before the restriction period, and there was a decline in the estimated rumen-free body weight. A slightly (not significantly) larger decline in rumen-free body weight was observed in the restricted groups (Table 3).

During the feeding period, groups L and C lost equal amounts of body weight and rumen-free body, while groups PL and PS gained body weight (Fig. 2) and rumen-free body (Table 4). Group PL gained significantly more rumen-free body weight than group PS did.

Slaughter data

Carcass and rumen weights were similar for all slaughtered animals after the restriction period, and no significant differences were found between animals from group C and animals from the restricted groups (Table 5). Only two slaughtered animals, one control and one restricted, had measurable amounts of kidney fat.

After the feeding period, groups L and C did not differ with respect to any of the variables measured at slaughter (Table 5). The carcass weights and

Table 3. Number of reindeer, body weight at the start of the restriction period (day 1) and calculated changes in body weight during the restriction period, days 1- 9 (least-square mean ± standard error). LD = lichen-based diet.

Group:	C	L	PL	PS	SP
Diet:	LD	LD	LD	LD	LD
Ration:	*ad lib.*	restricted	restricted	restricted	restricted
No. of animals	15	11	12	11	12
Body weight at day 1, kg	43.4 ± 1.13	43.3 ± 1.32	41.9 ± 1.26	42.8 ± 1.31	41.8 ± 1.26
Change in body weight, kg/day	0.028 ± 0.02	-0.156 ± 0.03	-0.165 ± 0.02	-0.195 ± 0.03	-0.195 ± 0.02
Change in estimated rumen-free body, kg/day	-0.06 ± 0.02	-0.09 ± 0.02	-0.10 ± 0.02	-0.12 ± 0.02	-0.13 ± 0.02
Total change in estimated rumen-free body, kg	-0.56 ± 0.18	-0.82 ± 0.20	-0.90 ± 0.19	-1.13 ± 0.20	-1.13 ± 0.19

Table 4. Number of reindeer, body weight at the start of the feeding period (day 10) and calculated changes in body weighr during the feeding period, days 10 - 42 (least-square mean ± standard error). LD = lichen-based diet, Pe = pellets, Li = lichens, Si = silage.

Group:	C	L	PL	PS
Diet:	LD	LD	Pe + Li	Pe + Si
Ration:	*ad lib.*	*ad lib.*	*ad lib.*	*ad lib.*
No. of animals	10	10	10	9
Body weight at day 9, kg	43.7 ± 1.27	41.9 ± 1.38	40.4 ± 1.31	41.1 ± 1.37
Change in body weight, kg/day	-0.05 ± 0.02	-0.02 ± 0.02	0.12 ± 0.02	0.11 ± 0.02
Change in estimated rumen-free body, kg/day	-0.009 ± 0.01	-0.042 ± 0.01	0.157 ± 0.01	0.086 ± 0.01
Total change in estimated rumen-free body, kg	-0.30 ± 0.44	-1.33 ± 0.44	5.02 ± 0.44	2.75 ± 0.46

dressing percentages did not differ significantly between groups PL and PS, but were significantly higher in group PL than in group C. Weights of rumen content were similar for all groups after the feeding period, with a tendency to lower weights for group PL. One animal in group PS had a very large rumen content, 750 g per kg carcass weight, which greatly increased the group average. All five slaughtered animals from C and three animals from group L had measurable kidney fat, but only two from each group had measurable fat in the omentum. All five slaughtered animals from group PL and four animals from group PS had measurable kidney fat and four from each group had measurable fat in the omentum. From inspections of the abomasums, few and minor signs of abomasal lesions were found (one slightly red in January; one with black spots in February; two with small red wounds in March). No abnormalities on carcasses were observed on any slaughter occasion.

Blood plasma metabolites

The concentrations of the four plasma metabolites remained stable in group C throughout the experiment (Fig. 3). All groups fed the restricted ration responded similarly to the restriction with a rise in urea concentration and a fall in glucose, with levels significantly differing from group C. Also a tendency to increased concentration of plasma protein and a fall in insulin concentrations were observed in the restricted groups, but the difference compared with the levels in group C was not statistically significant.

Already at the first sampling during the feeding period (day 14), animals in group L had concentrations of blood metabolites similar to those of group

Table 5. Number of slaughtered reindeer, carcass weight (CW), dressing percentage and relative weights of digesta and fat (least square means ± standard errors), LD = lichen-based diet, Pe = pellets, Li = lichens, Si = silage.

Day (period):	Day 0 (before restriction)	Day 10 (after restriction)		Day 43 or 44 (after the feeding period)					
Group:	All	C	L, PL, PS, SP	C	L	PL	PS		Standard
Diet:	LD	LD	LD	LD	LD	Pe + Li	Pe + Si		error of
Ration:	*ad lib.*	*ad lib.*	restricted	*ad lib.*	*ad lib.*	*ad lib.*	*ad lib.*		group means
No. of animals	8	5	6	5	5	5	5		
CW, kg	20.2 ± 0.9	20.8 ± 1.1	19.2 ± 1.4	19.5	17.8	23.3	20.3		±1.0
Dressing percentage	45.1 ± 0.8	47.3 ± 0.9	48.1 ± 1.2	46.6	46.1	51.9	49.4		±1.3
Content in reticulo-rumen, g/kg CW	357 ± 18	362 ± 33	293 ± 42	321	356	208	315		±59
Fat in the abdominal cavity, g/kg CW	0.35 ± 0.16	0.15 ± 0.15	0.10 ± 0.19	1.4	1.2	6.7	3.4		±1.3

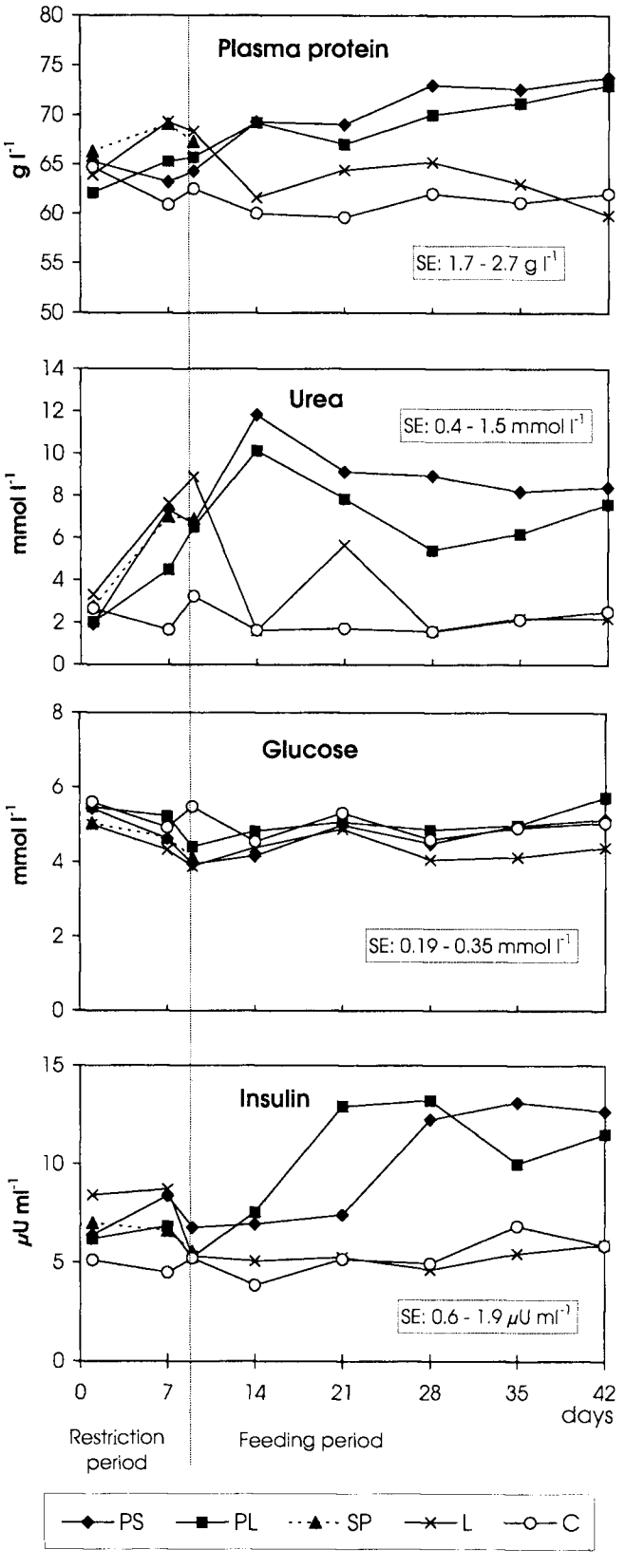
C, and thereafter the concentrations remained rather stable. Urea concentrations continued to increase in groups PL and PS during the first week of feeding. Then they declined somewhat and stabilised at a level that was significantly higher than for groups C and L. Plasma protein and insulin concentrations increased gradually in groups PL and PS from the start of the feeding period and were significantly higher than in group C after one to three weeks of feeding (Fig. 3). The increase in insulin concentrations was slightly slower in group PS than in group PL. The glucose concentrations remained stable in groups C and L during the feeding period. A slight increase was observed in groups PS and PL, but the difference compared to groups C and L was not significant.

Discussion

At the start of the restriction period, the reindeer seemed well adapted to the experimental situation. There were no problems with the feeding and the animals were adjusted to the standardised handling. All reindeer had spent at least three weeks on the lichen diet during the pre-experimental period, and still the body weight development differed between animals from the different deliveries. This was taken into account in the statistical model and all deliveries were represented in all treatment groups.

The initial blood metabolites and slaughter data together with the total results of group C, indicated that the lichen diet mimicked a winter diet of moderate quality, giving a representative winter condition. The glucose concentrations were similar to those found in free-grazing reindeer (Soveri *et al.*, 1992; Nieminen, 1980; Nieminen & Timisjärvi, 1983), and the low plasma protein and urea concentrations indicated that the animals were not degrading much muscle protein. The concentrations of insulin were low compared with those of adult reindeer in experiments by Larsen *et al.* (1985) and Raphaela Stimmelmayr (pers. comm.) and, together with the small or deficient fat reserves, reflected that our animals did not get excess of energy. Reindeer in group C remained healthy with stable blood metabolites throughout the experiment, and had weight losses similar to those observed for reindeer fed a low protein diet (Valtonen, 1979), but less than for reindeer fed a pure lichen diet (Sletten & Hove, 1990; Aagnes & Mathiesen, 1994; Øksendal, 1994). This indicated that the mixing of shrubs and leaves did increase the protein content of the diet, but only marginally.

The daily maintenance requirements during winter conditions for a pen-kept reindeer of 43 kg body weight is probably about 11 MJ ME (Hudson & White, 1985; Fancy, 1986) and around 100 g digestible protein (McEwan & Whitehead, 1970). For the reindeer in our experiment, that were kept outdoors and exposed to normal winter temperatures, the estimated intake of ME was about 13 MJ and the intake of CP was 60 g, when given the lichen diet *ad lib.* This indicated that the protein intake was



below, while the ME intake was likely above, the maintenance requirements. The ability of reindeer to digest shrubs and leaves has been poorly investigated. Our estimates of digestibility were, however, within the same range as those found by Pål Vegar Storeheier (pers. comm.) in an *in vitro* study using reindeer rumen content. The very low digestibility for lichens that was found in our *in vitro* analysis (and that we therefore did not use) was probably due to the use of cow rumen fluid and a limited ability of the microorganisms in the cow rumen to digest the special carbohydrates in the lichens. According to studies on reindeer (Nordfeldt *et al.*, 1961; Jacobsen & Skjenneberg, 1977), the digestibility of organic matter in lichens is at least 70%. According to body weight development and slaughter data, the lichen diet was obviously sufficient, when fed *ad lib.*, to keep the animals healthy although without any fat gain or increase in weight.

A restricted ration of the lichen diet followed by one day of starvation was clearly below the maintenance requirements of the reindeer. The losses of both absolute body weight and estimated rumen-free body together with increased plasma urea concentrations during the restriction period, indicated that the animals degraded muscle tissue. This was in accordance to Valtonen (1979), who found that reindeer fed a low protein diet with insufficient energy intake used body protein as an energy source, which resulted in increased plasma urea values. The drop in glucose concentrations and

Fig. 3. Protein, urea, glucose and insulin concentrations in blood plasma (least square means for five reindeer per group) during the restriction period, when controls (group C) were fed a lichen-based diet *ad lib.* and rhe other groups were fed a restricted amount of the same diet, and during the feeding period, when group C and L were fed the lichen diet *ad lib.* and group PS and PL were fed pellets combined with silage and lichens, respectively. Group SP was excluded after the restriction period, due to loss of animals. The ranges of standard errors are shown in the graphs.

the low insulin concentrations in combination with small or lacking fat reserves also reflected that the animals were in a negative energy balance.

The eating behaviour was affected during the restriction period, whereas very little feed was left in contrast to the *ad lib.* feeding when the animals rejected most of the coarse parts of the shrubs and lichens. The nutrient composition of the diet was thereby affected, since the coarse parts probably contained more NDF and lignin. Malnutrition may occur when diets with high fibre content are given to reindeer (Aagnes, 1998). The changed eating behaviour might also have increased the amount of eaten plant parts with penicillium growth. *P. spinulosum* have not been observed to affect animal health negatively and the influence of *P. brevicompactum* has not been described (Karl-Gustav Jacobsson, pers. comm.). The good health of the animals in group C and the fact that wet belly was not seen in all restricted groups, rather indicated that individual differences may have influenced which animals were affected by health problems. Also the differences in observed fat reserves from the slaughtered animals indicated individual differences. We did not find any significant differences in any measurements between the restrictedly fed groups, although the higher frequency of wet belly symptoms in group SP may indicate that the animals in this group were more negatively affected by the restriction.

At the start of the feeding period, we had a situation mimicking an emergency feeding situation with reindeer in negative energy balance, probably with individual differences, and thus vulnerable to a change in diet. As often has been observed in practice when transferred back to a sufficient natural pasture, group L responded without problems to the *ad lib.* feeding of the lichen diet. The positive response to the re-feeding was seen in an immediate feed intake similar to that of group C and in healthy animals with stable body weights. As the energy intake increased, the glucose concentration rose, and the sufficient energy and protein intake was also reflected in decreased concentrations of urea and plasma protein, indicating less degradation of muscle tissue. Animals in groups SP, PL and PS also started to eat their new diets without hesitation and no suspiciousness or refusals to eat were observed, in contrast to earlier studies (*e.g.* Bøe & Jacobsen, 1981; Mathiesen *et al*., 1984). In contrast to group L, health problems occurred in these groups within the first weeks of feeding. The experimental design with mixed diets, which is commonly used in practice, did not give information about the individual eating behaviour. It is possible that some animals may have preferred a single feed stuff, which could have affected their individual reaction.

High palatability but low digestibility leading to malnutrition are observations from feeding with only silage (*e.g.* Syrjälä-Qvist, 1982; Aagnes & Mathiesen, 1995; Nilsson *et al*., 1996a), but generally after a longer period of feeding. The immediate outbreak of health problems at the start of feeding in the present experiment, and the difference compared with earlier experiments, may be explained by some of our reindeer being in poor condition at that time. The ME content of the silage, which was estimated based on digestibility for cows and most likely overestimated for reindeer, was probably insufficient for our animals in negative energy balance. The silage was of an ordinary quality that may be available in an unplanned feeding situation and was not optimal for reindeer. With silage of better quality for reindeer, *e.g.* high-quality leaf-rich timothy with a high content of WSC (Aagnes, 1998), this feeding strategy might have been more successful.

A rapid change to pellets with low fibre content and high content of WSC may lead to diarrhoea, which was seen in groups PL and PS within the first week of feeding. The negative energy balance of the reindeer after the restriction period might have made the reindeer more vulnerable to change in diet, but the gradual increase in the rations and the combination with lichens or silage might nonetheless have reduced the problem compared with an immediate *ad lib.* feeding with only pellets. The treatment with antibiotics was successful, but may be difficult to apply in a practical situation without other negative effects, considering the stress that may then be involved. The increased plasma protein and urea concentrations together with a tendency for increased concentrations of insulin and glucose illustrated the response to the increased protein and energy content of the pellets-based diets.

As the feeding continued, the groups fed lichen-based diets (C and L) showed a stable or slightly declining body weight trend until the end of the experiment. The blood metabolites and carcass weights for groups C and L ended up similar to those relevant for reindeer on a moderate winter pasture. In contrast to the effect of the lichen diet, the blood metabolites changed and body condition was improved in animals fed the pellets-based diets (group PL and PS). After three weeks of feeding, the

plasma protein, urea and glucose concentrations stabilised on relatively high levels, reflecting a sufficient protein and energy intake. It is well established that ruminant blood urea rises with increasing protein intake (*e.g.* Lewis, 1957) and this positive correlation has also been shown in feeding experiments with reindeer (Valtonen, 1979; Säkkinen *et al.*, 1999; Ulla Heiskari, pers. comm.). Valtonen (1979) concluded that blood urea concentrations reflected the alterations in dietary protein intake, but only when the energy intake was sufficient. The concentration of urea may, thus, be hard to interpret as an indicator of the nutritional state of reindeer. High concentrations may reflect high protein content of the diet as well as muscle degradation caused by a diet with low energy content. Elevated urea levels have also been reported in connection with long and stressful gatherings (*e.g.* Hyvärinen *et al.*, 1976; Rehbinder & Edqvist, 1981) and has been discussed as a consequence of reduced urinary excretion and/or protein catabolism caused by stress. Since group C in the present experiment had low and stable urea concentrations, and no findings (e.g. severe abomasal lesions) indicating a prolonged stress effect were made, the repeated standardised sampling procedures throughout the experiment did not seem to have affected the animals.

The ME and protein intake were clearly above the maintenance requirements for the groups fed the pellets-based diets, although some of the weight gain during the second week of feeding was probably partly due to an increase in rumen content. The increased concentrations of insulin reflected that fat anabolism had started. This was shown at slaughter as gained fat compared with the fat status before the feeding period. The high protein and energy intake had also resulted in gained carcass weights both in absolute weight and relative to the live body weights, which indicated relatively more muscle tissues on the pellets-fed reindeer.

The conclusions of the experiment are that the lichen diet did not cause any digestive problems, but resulted in a continuous decline in body weight and small or lacking fat reserves. After the initial diarrhoea, feeding with diets comprising pellets from the start resulted in improved condition, expressed as increased body weight, fat gain and higher concentrations of plasma protein, urea and insulin in relation to the control group. The diet initially based on grass, in the form of silage of the given quality, seemed insufficient as feed to reindeer calves in a poor nutritional state.

Acknowledgements

The Committee on Animal Experiments of the University of Oulu, Finland, approved the experimenral procedures and the handling of the animals. The authors thank the staff at Oulu University, especially Eija Eloranta, Jari Ylönen, Tuula Pudas and Harri Norberg, for their co-operation in rhe management of the reindeer throughout the experiment and for their assistance in the collection of samples. We also thank Jorma Pudas for continuous veterinary supervision of the animals during the experiment. Financial support for this work was provided by rhe Swedish Council for Forestry and Agricultural Research and from the Saami Fund in Sweden.

References

Aagnes, T. H. & Mathiesen, S. D. 1994. Food and snow intake, body mass and rumen function in reindeer fed lichen and subsequently starved for 4 days. – *Rangifer* 14: 33–37.

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

Aagnes Utsi, T. H. 1998. *Digestive strategies in reindeer in winter.* Doctoral thesis, Department of Arctic Biology and Institure of Medical Biology, University of Tromsø.

Axelsson, J. 1941. Der Gehalt des Futters an umsetzbar Energie. – *Züchtungsk.* 16: 335–347.

Breimner J. M. & Breitenbeck, G. A. 1983. A simple method for derermination of ammonium in semimicro Kjeldahl analysis of soils and plant materials using block digester. – *Communication in Soil and Plant Analysis* 14: 905–913.

Bøe, U. B. & Jacobsen, E. 1981. Fôringsforsøk med olike typer fôr till rein (Trials with different feeds to reindeer. – *Rangifer* 1 (1): 39–43. (In Norwegian with English summary.)

Fancy, S. G. 1986. *Daily energy budgets of Caribou: A simulation approach.* Ph.D. thesis, University of Alaska, Fairbanks.

Goering, H. K. & VanSoest, P. J. 1970. *Forage Fibre Analysis.* USDA Agricultural Research Service Handbook, Nr. 379.

Hove, K. & Jacobsen, E. 1975. Renal excretion of urea in reindeer. Effect of nutrition. – *Acta Vet. Scand.* 16: 513–519.

Hudson, R. J. & White, R. G. (eds.). 1985. *Bioenergetics of wild herbivores,* pp. 1–314. CRC Press, Inc., Boca Raton, Florida.

Hyvärinen, H., Helle, T., Nieminen, M., Väyrynen, R. & Väyrynen, P. 1976. Some effects of handling reindeer during gatherings on the composition of their blood. – *Anim. Prod.* 22: 105–114.

Jacobsen, E. & Skjenneberg, S. 1975. Some results from feeding experiments with reindeer. – *In:* J. R. Luick, P. C. Lent, D. R. Klein & R. G. White (eds.). *Proc. 1st Int. Reindeer/Caribou Symposium, Fairbanks 1972. Biological Papers of the University of Alaska, Special report* 1. University of Alaska, Fairbanks, pp. 95-107.

Jacobsen, E. & Skjenneberg, S. 1977. Digestibility of lichen for reindeer (Fordøyelighet av lav til rein). – *Res. Norw. Agric.* 28 (1): 63–67. (In Norwegian with English summary).

Jacobsen, E. & Skjenneberg, S. 1979. Experiments with different diets to reindeer (Forsøk med ulike forblandinger til rein). – *Res. Norw. Agric.* 58: 1–11. (In Norwegian with English Summary).

Larsen, T. S., Nilsson, N. Ö. & Blix, A. S. 1985. Effects of prolonged food restriction on some aspects of lipid metabolism in Norwegian and Svalbard reindeer. – *Acta Physiol. Scand.* 124: 173–180.

Larsson, K. & Bengtsson, S. 1983. *Determination of non structural carbohydrates in plant material.* Method description no. 22, National Laborarory for Agricultural Chemistry, Uppsala. (In Swedish).

Lewis, D. 1957. Blood-urea concentration in relation to protein utilization in the ruminant. – *J. Agric. Sci.* 48: 438–446.

Lindgren, E. 1979. *The nutritional value of roughages determined in vivo and by laboratory methods.* Report 45, Swedish University of Agricultural Sciences, Department of Animal Nutrition, Uppsala (In Swedish with English Summary).

Mathiesen, S. D., Rognmo, A. & Blix, A. S. 1984. A test of the usefulness of a commercially available mill «waste product» (AB-84) as feed for starving reindeer. – *Rangifer* 4 (1): 28–34.

McEwan, E. H., & Whitehead, P. E. 1970. Seasonal changes in the energy and nitrogen intake in reindeer and caribou. – *Can. J. Zool.* 48: 905–913.

Murphy, M. & Lindgren, E. 1997. A simple *in vitro* rumen simulation system used in evaluating diets with varying composition. – *Swedish J. Agric. Res.* 27: 179-187.

Nieminen, M. 1980. Nutritional and seasonal effects on the haematology and blood chemistry in reindeer calves (*Rangifer tarandus tarandus* L.). – *Comp. Biochem. Physiol.* Vol 66A: 399–413.

Nieminen, M. & Timisjärvi, J. 1983. Blood composirion of the reindeer. II. Blood chemistry. – *Rangifer* 3 (1): 16–32.

Nieminen, M., Pokka, A.-S. & Heiskari, U. 1987. Artificial feeding and nutritional status of semi-domesticated reindeer during winter. – *Rangifer* 7 (2): 51–58.

Nilsson, A., Olsson, I. & Lingvall, P. 1996a. Comparison between grass-silages of different dry matter content fed to reindeer during winrer. – *Rangifer* 16: 21-30.

Nilsson, A., Olsson, I. & Lingvall, P. 1996b. Evaluation of silage diets fed to reindeer calves intended for slaughter. II. Feeding of silage and concenrrate from January ro March. – *Rangifer* 16: 139–146.

Norberg, H. J. & Mathiesen, S. D. 1998. Feed intake, gastrointestinal system and body composition in reindeer calves fed early harvested first cur timothy silage (*Phleum praténse*). – *Rangifer* 18: 65–72.

Nordfeldt, S., Cagell, W. & Nordkvist, M. 1961. Smältbarhetsförsök med renar Öjebyn 1957-60 (Digesribility experiments with reindeer). – *Kungliga Lanthrukshögskolan och Statens Lanthruksförsök - Statens Husdjursförsök - Särtryck och förhandsmeddelanden* nr. 151: 1–16. (In Swedish with English summary.)

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

Rehbinder, C. & Edqvist, L. E. 1981. Influence of stress on some blood consrituents in reindeer (*Rangifer tarandus* L). – *Acta vet. Scand.* 22: 480–492.

SAS Institute. 1997. *SAS System for Windows,* release 6.12. Cary, NC: SAS Institute Inc.

Sletten, H. & Hove, K. 1990. Digestive srudies with a feed developed for realimenrarion of starving reindeer. – *Rangifer* 10: 31–37.

Soveri, T., Sankari, S. & Nieminen, M. 1992. Blood chemistry of reindeer calves (*Rangifer tarandus*) during the winter season. – *Comp. Biochem. Physiol.* Vol 102A, No. 1: 191–196.

Syrjälä-Qvist, L. 1982. Comparison of grass silage utilization by reindeer and sheep. 1. Palatability, feeding values and nutrient supply. – *J. Sci. Agric. Soc. Finl.* 54: 119–126.

Säkkinen, H., Timisjärvi, J., Eloranta, E., Heiskari, U., Nieminen, M. & Puukka, M. 1999. Nutrition-induced changes in blood chemical parameters of pregnant reindeer hinds (*Rangifer tarandus tarandus*). – *Small Ruminant Research* 32: 211–221.

Valtonen, M. & Eriksson, L. 1977. Responses of reindeeer to water loading, water restriction and ADH. – *Acta Physiol. Scand.* 100: 340–346.

Valtonen, M. 1979. Renal responses of reindeer to high and low protein diet and sodium supplement. – *Journal of the Scientific Agricultural Society of Finland.* Vol. 51: 381–419.

Wales, R. A., Milligan, L. P. & McEwan, E. H. 1972. Urea recycling in caribou, cattle and sheep. – *Proc. 1st Int. Reindeer/Caribou Symp. Biol. Paper. Univ. Alaska. Spec. Rep.* 1: 297–307.

Øksendal, H. 1994. *Lav og rundballeensilert engsvingel som krisefôr til reinkalver, innverknad på mage-tarmanatomi og evnen til cellulosegjæring* (Lichen and baled meadow fescue silage as an emergency food to reindeer calves; influence on the gastrointestinal anatomy and the ability to ferment cellulose). Thesis (cand. Agric), University of Tromsø, Tromsø, pp. 1–37. (In Norwegian).

Åhman, B. & Åhman, G. 1980. *Övergång från vinterbete till kraftfoderutfodring av ren* (Changing from winter pasture to concentrate feeding of reindeer). Report 76, Swedish University of Agricultural Sciences, Department of Animal Husbandry. Uppsala, 65pp. (In Swedish with English summary.)

Åhman, B., Nilsson, A. & Eloranta, E. 1999. Clues to the cause of the «wet belly» syndrome in reindeer. – *In:* Z. Zomborsky (ed.). *Advances in Deer Biology. Proceedings of the 4th International Deer Biology Congress, Kaposvár 1998.*, pp. 294-296. Tipo-Express Ltd., Kaposvar, Hungary.

Manuscript received 19 January, 2000
accepted 20 July, 2000