VARIATION IN THE BLOOD CHEMICAL CONSTITUENTS OF REINDEER

Significance of season, nutrition and other extrinsic and intrinsic factors

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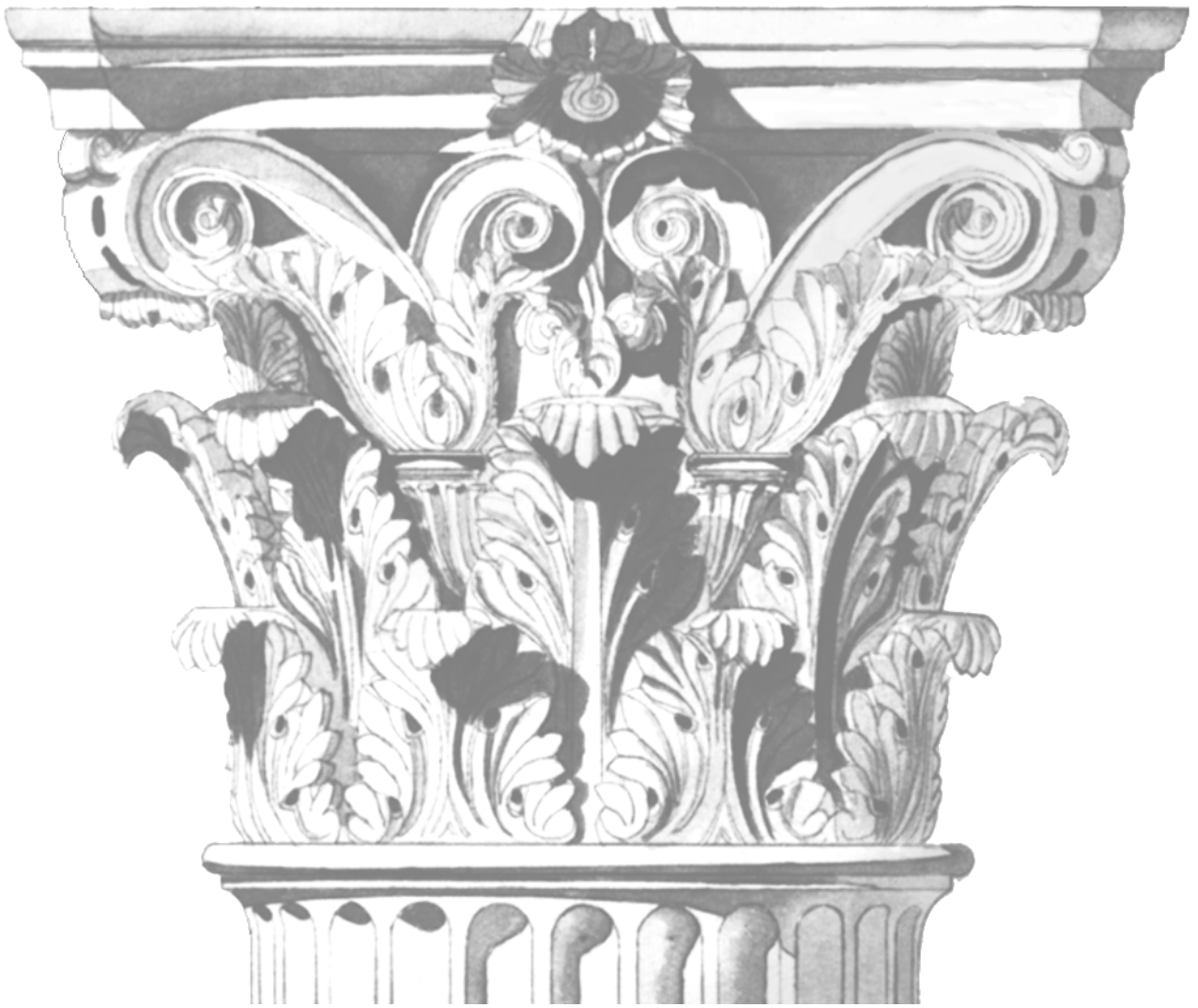
OULU 2005



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Academic Dissertation to be presented with the assent of the Faculty of Science, University of Oulu, for public discussion in Kuusamonsali (Auditorium YB210), Linnanmaa, on August 19th, 2005, at 12 noon

OULUN YLIOPISTO, OULU 2005

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ISBN 951-42-7772-4 (nid.)
ISBN 951-42-7773-2 (PDF) http://herkules.oulu.fi/isbn9514277732/

ISSN 0355-3191 http://herkules.oulu.fi/issn03553191/

OULU UNIVERSITY PRESS
OULU 2005

Säkkinen, Hannele, Variation in the blood chemical constituents of reindeer . Significance of season, nutrition and other extrinsic and intrinsic factors
Faculty of Science, Department of Biology, University of Oulu, P.O.Box 3000, FIN-90014 University of Oulu, Finland
2005
Oulu, Finland

Abstract

Reindeer management in the Fennoscandian area is currently facing challenges such as degradation of winter pastures, which may lead in the most severely affected areas to a concurrent decline in reindeer herd productivity. The use of often expensive supplementary feeding to prevent production losses has increased the demand for studies on the physiological effects of nutritional restriction and supplementary feeding. The knowledge obtained from such studies could be used, for example, to monitor the condition of reindeer in studies assessing herd productivity levels in different pasture conditions and management systems or sustainable use of pasture resources.

In this thesis, the effects of season, year, pasture area, body mass, pregnancy and other extrinsic and intrinsic factors on the variation of blood chemical constituents of reindeer were studied in free-ranging animals under natural foraging conditions. The studied blood chemical constituents covered a wide range of parameters related to protein, carbohydrate, lipid and mineral metabolism. The same blood chemical constituents were studied in captive reindeer under defined feeding conditions, allowing an analysis of the effects of dietary protein, energy and mineral intake on the selected blood constituents and their comparison to a conventional measure of the animals' condition, live body mass.

According to the results, free-ranging reindeer showed great variation in the concentrations of blood chemical constituents compared to the reference values of domesticated ruminants. Intrinsic factors such as body mass, pregnancy and age had only a minor influence on the variation of the studied parameters, whereas extrinsic factors such as season, year and pasture area, which were characterized by marked changes in environmental and nutritional conditions, explained the majority of the variation.

The results obtained from captive animals in defined feeding conditions and from free-ranging animals foraging on natural pastures led to the conclusion that blood total proteins, albumin, urea, creatinine, urea:creatinine ratio, magnesium, inorganic phosphate and, to a lesser extent, globulins and albumin:globulin ratio responded to the changes in feed quality and availability and were the most suitable blood constituents to be used as nutritional biomarkers for reindeer.

Keywords: albumin, body mass, calcium, creatinine, globulins, inorganic phosphate, magnesium, nutrition, Rangifer tarandus tarandus, total proteins, urea

Photo by Petri Kärkkäinen

To my son Dean

Acknowledgements

This thesis was carried out at the Department of Biology, University of Oulu, at the Department of Physiology, University of Oulu, and at the Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science in Oslo. The field studies were conducted in the Finnmark and Filefjell areas in Norway and in Nordenskjøldland in Svalbard. The experimental parts of the thesis were done at the Zoological Gardens, University of Oulu, and at the Reindeer Research Station of Finnish Game and Fisheries Research Institute at Kaamanen.

My most grateful acknowledgements go to my supervisors, docent Seppo Saarela and professor Erik Ropstad, who have both encouraged and supported me in a tremendous way during my studies. Seppo, when I started my Ph.D. studies, you said to me: “Remember, it’s not only about doing research, it’s also about growing as a scientist”. It has been safe to grow under your supervision. Erik, I will never forget the challenges we faced in Finnmark. You were there for me whenever I needed comments on my papers and always gave valuable advice. Thank you also for being a good friend.

Professor emeritus Juhani Leppäluoto is acknowledged for support at the early stages of my studies at the Department of Physiology. Docent Jouni Timisjärvi is warmly remembered for his advice and scientific discussions, which continued until his sudden decease last year. I also express my gratitude to the reviewers of this thesis, professor emeritus Heikki Hyvärinen and docent Birgitta Åhman. Sirkka-Liisa Leinonen is greatly acknowledged for reviewing the language of this thesis.

I want to thank my co-authors Dr. Audun Stien, Dr. Matti Puukka, professor Knut Hove, Dr. Øystein Holand, professor Aage Tverdal, Mrs. Ellen Dahl and docent Mauri Nieminen for their collaboration. Dr. Pete Goddard from the Macaulay Institute, Scotland, is warmly acknowledged for his guidance in the rumen cannula operations and for his kind advice during the writing process. My colleagues in reindeer research, MD Eija Eloranta, DVM Seija Vahtiala, M.Sc.Agr.&For. Ulla Heiskari, DVM, Ph.D. Heli Lindeberg and M.Sc. Harri Norberg, thank you for the numerous pleasant conversations, good advice and great company at the reindeer meetings! You have taught me the most about how to work with reindeer.

I want to warmly acknowledge everyone working at the animal physiology division. I always feel like coming home when I go there. I am grateful to professor Matti Järvilehto

for his help during the final stages of this thesis. I also wish to acknowledge professor Esa Hohtola, whose enthusiastic and innovative attitude towards scientific questions has been a great role model of an outstanding scientist. Tuija, thank you for your friendship and strenuous walks! Kyösti, thank you for being my roommate for so many years. You are not only the nicest person to work with, but also a good friend! Satu, Tuula and Anu, thank you for the good talks and laugher that we shared during our gatherings.

My work would have been impossible to carry out in practice without the excellent staff and facilities of the Zoological Gardens. Jari Y., Jari M., Jari O., Tuula, Jorma, Alpo, Pertti, Saija and everyone who worked at the Zoo: I will be forever grateful for your help with handling the animals and for your great sense of humour that made the work so much fun!

All my friends are warmly acknowledged for caring about me and showing that you accept me as I am. ”Kuhmo girls” Sirpa, Paula and Minna, thank you for being such great friends for most of my life!

I would like to warmly thank professor Juhani Hassi at the Centre for Arctic Medicine for his support towards my career, research and studies. I also want to thank Phil.Lic. Tiina Mäkinen and everyone at the Centre for Arctic Medicine for being so nice to work with. The Graduate School of Circumpolar Wellbeing, Health and Adaptation is warmly acknowledged for supporting me both in my work and in my studies.

Most of all, I want to thank my parents Seija and Martti Säkkinen for their lifelong support and encouragement. Thank you for being the best parents one could ever have. I also want to thank my sister Taina for her unconditional support in my life in general. I dedicate this book to my son Dean, who has taught me that the most important achievements in life are not the accomplishments at work, but the people you love.

This work was supported by the Academy of Finland, the Alfred Kordelin Foundation, the Finnish Cultural Foundation (Kainuu Fund), the Finnish Foundation of Veterinary Medicine, the Finnish Ministry of Agriculture and Forestry, the Natural Environment Research Council (Great Britain), the Norwegian Research Council, the Norwegian School of Veterinary Sciences, Reindriftens utviklingsfond (Norway), the Thule Institute at the University of Oulu, and the University Pharmacy Fund.

Oulu, May 2005 Hannele Säkkinen

Abbreviations

ALB	albumin
AP	alkaline phosphatase
A:G	albumin:globulin ratio
BM	body mass
Ca	calcium
Ca:C	urinary calcium:creatinine ratio
CP	crude protein
DM	dry matter
GLOB	globulins
Mg	magnesium
Mg:C	urinary magnesium:creatinine ratio
MgO	magnesium oxide
P_i	inorganic phosphate
TP	total proteins
U:C	urea:creatinine ratio

List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I 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 Res 32: 211-221.

II Säkkinen H, Stien A, Holand Ø, Hove K, Eloranta E, Saarela S & Ropstad E (2001) Plasma urea, creatinine, and urea:creatinine ratio in reindeer (*Rangifer tarandus tarandus*) and in Svalbard reindeer (*Rangifer tarandus platyrhynchus*) during defined feeding conditions and in the field. Physiol Biochem Zool 74: 907-916.

III Säkkinen H, Eloranta E, Vahtiala S, Puukka M, Timisjärvi J, Saarela S & Ropstad E (2004) Effects of magnesium oxide and magnesium alloy rumen boluses on plasma and urinary magnesium and calcium concentrations in reindeer (*Rangifer tarandus tarandus*). Small Ruminant Res 54: 69-79.

IV Säkkinen H, Tverdal A, Eloranta E, Dahl E, Holand Ø, Saarela S & Ropstad E (2005) Variation of plasma protein parameters in four free-ranging reindeer herds and in captive reindeer under defined feeding conditions. Manuscript (submitted).

Contents

1 Introduction

Reindeer herding has undergone drastic changes during the past decades. In Finland, the management was traditionally based on the sole utilization of natural pasture resources, characterized by fluctuation in animal numbers and calf production as a consequence of variation in winter forage availability and snow conditions (Helle 1980). The introduction of management practices such as calf slaughter, antiparasetic treatments and supplementary feeding, together with favourable weather and snow conditions, initiated an increase in the number of reindeer (*Rangifer tarandus tarandus*) from 1970's to early 1990's (Helle *et al.* 1990). Between these dates, the number of reindeer kept over winter increased from about 120 000 to 260 000 (Statistics of the Finnish Reindeer Herders' Association). After this, the number of reindeer on winter pasture has declined to approximately 200 000 animals due to state restrictions that limit the permitted number of animals on winter pasture.

The quality of the lichen pastures that provide the main winter forage for reindeer (Helle 1980, Kojola *et al.* 1993), declined simultaneously with the increase in the number of reindeer (Helle 1980, Kautto *et al.* 1986, Kumpula *et al.* 1997). Forestry, habitation and other forms of land-use have affected the winter pasture condition and further reduced available pasture areas (Nieminen 1988), but also the reindeer density has shown to largely explain the condition of the lichen range (Helle *et al.* 1990, Kumpula *et al.* 2002).

Similar long-term degradation in lichen pasture conditions has also been reported in other parts of the Fennoscandian reindeer herding areas, including Finnmark in northern Norway (Evans 1996, Johansen *et al.* 1996, Riseth *et al.* 2004). Despite overgrazing, the regulation of winter stocks has not been very successful in Norway, and the number of reindeer increased during 2000-2004 from 65 500 to over 100 000 in the most severely affected county of West-Finnmark (Reindriftsforvaltningen 2003). A large number of reindeer starved to death during the winters of 1997 and 2000 in this area due to unfavourable pasture and weather conditions, and the average slaughter weights are the lowest among the Norwegian reindeer herding area.

A high proportion of slaughter income in reindeer management comes from calf harvest. The calves born in spring are culled for meat production the following autumn (Kojola & Helle 1993). Consequently, the over-winter survival and good condition of

female reindeer before calving in spring is crucial for the productivity of management. Reindeer management in Finland has become more and more dependent on the provision of supplementary feeds to improve the over-winter survival of reindeer herds in unfavourable weather conditions and to compensate for the reduced quality of the lichen range. The use of supplementary feeding has been increasing steadily since it was first introduced in mid-1960's (Helle 1980, Helle & Saastamoinen 1980, Nieminen & Autto 1989, also reviewed by Nieminen *et al.* 1998 and Maijala & Nieminen 2004). At present, supplementary feeding is a regular practice in almost all parts of the Finnish reindeer herding area, and its yearly costs amount to 25-50% of the slaughter income, depending on the district (Nieminen *et al.* 1998, Maijala & Nieminen 2004). In Norway, supplementary feeding of reindeer is not as commonly used, even though winter pasture degradation has increased the demand for it to prevent potential production losses.

The chemical composition of supplementary feeds, e.g., hay or commercial grain-based reindeer feeds, differs largely from the dominant winter forage of lichens, which are rich in digestible carbohydrates but poor in protein and minerals (Nieminen & Heiskari 1989, Nieminen & Risto 1990, Nieminen *et al.* 1998). Especially at times when the animal is in a very poor condition, supplementary feeding may cause health problems, and special attention should be given to the change of the feeding regimen and especially to emergency feeding of reindeer (Åhman *et al.* 2002, Nilsson 2003).

The decline in winter pasture conditions and the use of supplementary feeding with great economical costs have increased the demand for studies on the physiological effects of nutritional restriction and supplementary feeding. The results of such physiological studies would increase our knowledge about how the condition of reindeer is affected by factors related to pasture degradation and different managed systems. From the biological point of view, free-ranging semi-domesticated reindeer - similarly to the other northern deer species - are adapted to the extreme changes in their physical environment. In winter, even in their normal pasture conditions, reindeer are predisposed to undernutrition, defined as insufficient nutritional intake to maintain body mass (BM) (Verme & Ullrey 1984). The yearly cycles of antler growth, mating, calving and lactation also represent significant homeostatic challenges that are of interest for physiological research. For this reason, reindeer can serve as a model species in studies where the physiological indicators of nutritional status and nutritional adaptation mechanisms are observed either under field conditions or in experimental situations, where a range of interventions can be tested.

This study was undertaken in order to increase our knowledge about the sources of variation in blood chemical constituents of reindeer that could potentially be used as indicators of reindeer nutritional condition. Such knowledge could be used to monitor the nutritional condition and welfare of animals in studies where herd productivity levels in different pasture conditions and management systems or the sustainable use of pasture resources are assessed.

2 Review of the literature

The following review of the literature defines some basic concepts related to nutrition, reindeer as a species and assessment of nutritional condition. It also summarises what is currently known about the blood chemical constituents related to protein, carbohydrate, lipid and mineral metabolism, which were of interest in this thesis, and what is known about the effects of extrinsic and intrinsic factors such as season, nutrition, age, and pregnancy status on them.

2.1 Definition of the nutrition, nutritional condition and nutritional status of animals

Nutrition can be defined as the relationship between the body's requirements for different nutrients and energy and the supply of these nutrients available for metabolic processes (McLaren 1988). The nutritional condition of an animal has been considered to represent the effects that nutritional restriction has on body composition as well as its effects on health, growth and productivity (Forbes 1988). Reliable measures of body components (i.e., fat, protein and mineral stores) are therefore valuable both for biological research and as information for the management of reindeer, where animals' nutritional condition is likely to influence their reproductive capacity.

Nutritional restriction may thus result from a deficiency of nutrient availability or intake or ineffective use of nutrients. Undernutrition caused by nutritional restriction has, in several studies on deer species, turned out to be related to BM loss and the mobilization of fat and protein reserves for energy supply (Reimers *et al.* 1982, Reimers & Ringberg 1983, Reimers 1984, Verme & Ullrey 1984, Parker *et al.* 1993, Gerhart *et al.* 1996a), but many of these and other studies (Torbit *et al.* 1985, Adamczewski *et al.* 1987) have also pointed out that BM alone can be a misleading indicator of nutritional status, which is a more general term used either as a synonym of nutritional condition or as a way to define the degree of nutritional restriction.

2.2 Seasonal changes of the nutrition, nutritive requirements and metabolism of reindeer

Reindeer undergo extreme nutritional changes during the year. During summer and autumn, their diet consists of various highly nutritious plants and mushrooms, and their nutrition is characterized by high overall intake of proteins, minerals and energy (Nieminen & Heiskari 1989). Winter and early spring are the most challenging periods for survival due to the poor availability and/or quality of food. In winter, the diet of free-ranging reindeer is dominated by ground lichens (*Cladonia spp.*) (Kojola *et al.* 1993), which are rich in digestible carbohydrates but low in protein (1.4-4.5 % crude protein, CP, in dry matter, DM) and several minerals (Nieminen & Heiskari 1989). Other main sources of winter forage (Kojola *et al.* 1993), such as leaves of wintergreen grass (*Deschampsia flexuosa*) and different shrubs, have a higher CP content (up to 10 % of DM) than lichens (Valmari 1993a). The winter diet of wild Svalbard reindeer (*Rangifer tarandus platyrhynchus*) facing extreme climatic conditions during winter consists primarily of mosses and plants of even lower digestibility and nutritive value than the winter diet of mainland reindeer (*Rangifer tarandus tarandus)* (Staaland *et al.* 1983, Staaland 1986).

The commercial reindeer feeds provided to semi-domesticated reindeer in winter at times when food availability or quality is low differ greatly from the composition of the natural winter diet because of their high overall protein (8-17 % CP in DM), fat and mineral contents (Nieminen & Heiskari 1989, Nieminen & Risto 1990). The nutritive value of hay provided as supplementary feed depends on the time of harvest and some other factors, but CP contents as high as 12-13 % of DM can be reached following early harvesting (Valmari 1993b).

The dietary fats in the ruminant diet consist mainly of triglycerides, formed from glycerol and three fatty acids joined together by an ester bond, and of glycolipids that have one of the three fatty acids replaced by a sugar (Bauman *et al.* 2003). The natural diet of reindeer has a low fat content (Nieminen & Heiskari 1989). Even though fats are needed to increase the energy content of the commercial rations, large amounts of fat are known to depress rumen function (Bartley 1989, McDonald *et al.* 1995) and thus their proportion in the concentrates does not usually exceed 6-7 % of DM (Nieminen & Risto 1990).

The energy requirement of reindeer increases with body size, but varies notably with the conditions encountered by the animals. During winter, the metabolic rate of reindeer slows down compared to the summertime, as indicated by the cessation of growth (McEwan & Whitehead 1970, Nilssen *et al.* 1984). According to the estimates presented by Boertje (1985), a mature female caribou (*Rangifer tarandus granti*) requires daily about 29-38 MJ of metabolizable energy in summer, whereas in winter it needs about 21 MJ. The daily amount of metabolizable energy required for foetal growth (winter) and milk production (summer) are 2.4 and 6.4 MJ, respectively. Because the energy requirement for foetal growth increases mostly during the last trimester of gestation (by about 15 %; McEwan & Whitehead 1972), unfavourable weather or snow conditions that limit forage availability may become critical factors for calving success, which is affected by the maternal condition during late gestation (Cameron *et al.* 1993). The estimated

daily requirement of female reindeer for nitrogen equilibrium is 0.462 g digestible nitrogen per kg of metabolic weight (McEwan & Whitehead 1970). For a 70-kg reindeer, this would equal for about 70 g of digestible protein per day.

Despite the reduced metabolic needs in winter, it is clear that the lower nutritive value of the natural winter forage and its sometimes limited availability cause reindeer to experience a negative energy and protein balance and mineral deficiency in winter (Hyvärinen *et al.* 1977, Reimers & Ringberg 1983, Reimers 1984, Hoff *et al.* 1993). As a consequence, nutrients must be gained and stored during the summer and autumn.

2.3 Changes in body mass related to season and nutrition and body mass as an index of nutritional status

Reindeer exhibit a seasonal cycle of BM, characterized by increasing BM throughout the summer and autumn and a BM loss of about 20 % over the winter. In female reindeer, calving in late spring and lactation cause about 10 % additional loss in BM (Nieminen 1980). This seasonal BM cycle, which is also seen in the other northern deer species, such as caribou and, even more clearly, Svalbard reindeer, has been related to a negative energy and protein balance when feeding on natural winter pastures, manifested as mobilization of the fat and protein reserves (Reimers *et al.* 1982, Reimers 1983, Nieminen & Timisjärvi 1983, Larsen *et al.* 1985, Adamczewski *et al.* 1987, DelGiudice *et al.* 1992, Parker *et al.* 1993, Gerhart *et al.* 1996a). Especially in unfavourable weather conditions, reindeer easily starve if a frozen crust on snow or ice on the ground prevent digging for forage through the snow cover.

Due to the low protein content of lichens and winter forage in general, reindeer often have a negative nitrogen balance in winter (Ryg & Jacobsen 1982). The low nitrogen intake in the form of dietary protein, however, can be partly compensated for by recycling urea from blood back to the rumen, where the rumen microflora is able to synthesize protein for nutritional use (Hove & Jacobsen 1975, Valtonen 1979). The northern deer species rely on fat deposits for winter survival (Reimers 1984, Larsen & Blix 1985, Parker *et al.* 1993), but body proteins are increasingly used as an energy source when fat deposits approach exhaustion (Torbit *et al.* 1985). For example, black-tailed deer (*Odocoileus hemionus sitkensis*) may lose 70-80 % of their body fat and 10-15 % of their protein reserves during winter (Parker *et al.* 1993). Therefore, changes in BM and consequent changes in body composition (i.e., protein and fat reserves) have been used as an index of nutritional status in deer species (Torbit *et al.* 1985, Adamczewski *et al.* 1987, Gerhart *et al.* 1996a).

Besides being a commonly used indicator of body condition and nutritional status, BM has been shown to be a good predictor of reproductive performance in deer species. In female reindeer and caribou, the probability of pregnancy and the time of conception are influenced by the female's condition and BM in the autumn, whereas the time of calving and early calf survival are also affected by the maternal condition during late gestation in spring (Reimers 1983, Skogland 1984, Cameron *et al.* 1993, Reimers 2002).

Besides the direct effects of nutrition on BM, there are intrinsic factors that regulate feed intake and may consequently be involved in the seasonal BM cycle of reindeer. Deer

species decrease their feed intake with a parallel decrease in BM during winter even when they are given high-quality rations without limitation, a phenomenon that is suggested to be controlled by the photoperiod (Ryg & Jacobsen 1982, Suttie & Webster 1995).

One reason for why BM may be a poor predictor of body condition and nutritional status in ruminants is the variability in their rumen fill (Adamczevski *et al.* 1987). Secondly, body mass is dependent on the animal's overall size, causing confusion between large skeleton size and good condition. Precise measurements of body fat and protein content of live animals require complicated methodology, including the use of radioisotopes in captive animals (Larsen & Blix 1985, Parker *et al.* 1993), whereas both fat and protein content can be easily measured from carcasses at slaughter (Reimers & Ringberg 1983, Reimers 1984). However, neither of these methods is practical when the condition of live animals needs to be assessed in field conditions.

Body condition scores, based on assessment of the relative amount of subcutaneous tissue by manual palpation of back fat, have been used for the estimation of the condition and reproductive success of domestic ruminants (Wildman *et al.* 1982), caribou (Gerhart *et al.* 1996b) and other wild herbivores (Franzmann 1985, Cook *et al.* 2001). The benefit of this method is that it does not require special equipment and can be performed under field conditions. The drawback of the method is the subjective differences between the people performing the palpation, because of the relative scale used. Ultrasonographic measurement of back fat thickness has proved an accurate predictor of total body fat and is therefore a useful measure of body condition that can be applied under field conditions (Stien *et al.* 2003), though it requires an initial investment to purchase the equipment.

2.4 Blood chemical constituents as indicators of the nutrition and nutritional condition of ruminants

The parameters used for analysis of nutritional condition should be not only reliable, but also easy to record in field conditions and relatively inexpensive to analyze in sufficient numbers to be of practical value for management purposes. Blood samples are easy to obtain when animals are gathered together for different management practices, and most clinical laboratories employ automated techniques for the analysis of a large range of blood chemical constituents.

Because variation in blood composition often reflects recent changes in the quality and quantity of the diet, understanding of nutrient metabolism is essential for the interpretation of the findings (Guthrie 1971). In domestic ruminant management, "metabolic profiling", where several blood constituents reflecting changes in protein, energy and mineral intake are used to assess the nutritional status of animals for efficient production, was introduced over three decades ago (Payne *et al.* 1970). Assessment of the nutritional status of wild ruminants with blood chemical constituents started to attract interest at the same time. Changes in the quality and/or quantity of the diet have been shown to affect the blood constituents related to protein, carbohydrate, lipid and mineral metabolism in studies on several domesticated ruminant species (cattle; Ide *et al.* 1966, Payne *et al.* 1970, Reid *et al.* 1977, Ropstad *et al.* 1989, Chester-Jones *et al.* 1990, sheep;

Leibholtz 1970, Baker *et al.* 1979, goat; Hussain *et al.* 1996, Beynen *et al.* 2000) and in wild ruminants (caribou; McEwan 1968, reindeer; Bjarghov *et al.* 1976, Hyvärinen *et al.* 1975, 1977, Halse *et al.* 1976, Valtonen 1979, Nieminen & Timisjärvi 1983, Soveri *et al.* 1992, Soppela *et al.* 2000, Nilsson 2003, white-tailed deer (*Odocoileus virginianus*); DelGiudice *et al.* 1987, 1990, 1992) under both experimental and field conditions.

Despite the large number of studies conducted on northern deer species, the variety of environmental and physiological factors has limited the establishment of the kind of standard reference values available for domestic ruminant species and commonly used in veterinary medicine (Kaneko 1989). In addition, studies on northern deer species have given less attention to other potential sources of variation in blood constituents, such as age and pregnancy (McEwan & Whitehead 1969, Nieminen 1980, Ropstad *et al.* 1997), which may confound their use as indicators of nutritional condition. The idea of using blood chemical constituents as predictors of animal survival and calving success was introduced even more recently (Milner *et al.* 2003).

2.4.1 Blood chemical constituents related to protein metabolism

2.4.1.1 Total proteins, albumin, globulins and albumin:globulin ratio

The total protein concentration represents all proteins dissolved in blood plasma or serum. Albumin, synthesized by the liver from amino acid derivatives, constitutes about 35-50 % of total plasma proteins and is responsible for the colloid-osmotic pressure that prevents leakage of blood plasma from capillaries into tissues (Kaneko 1989). In domestic ruminants, blood total protein (TP) and albumin (ALB) concentrations have been long used for the assessment of nutritional condition (Payne *et al.* 1970, Kaneko 1989), and low levels of ALB and TP in plasma have been taken as measures of protein deficiency and undernutrition. In captive reindeer, an increase in plasma TP concentrations following high protein intake and a corresponding decline following low protein intake have been described in several studies (Bjarghov *et al.* 1976, Soppela *et al.* 1992, Soveri *et al.* 1992).

In sheep, the reference values reported for normal plasma TP and plasma ALB concentrations are 60-79 g/l and 24-30 g/l, and the corresponding values in cattle are 67-75 g/l and 30-36 g/l, respectively (Kaneko 1989). In domestic ruminant management, plasma ALB has long been considered a general indicator of nutritional status, related to both feed intake and body weight (Roil *et al.* 1974). Low ALB concentrations of dairy herds have been related to low protein intake rates, whereas animals grazing on highly fertilized pastures have had the highest levels of ALB (Payne *et al.* 1970). Due to the long half-life of ALB (Kaneko 1989), inadequate dietary protein intake leads to a slow, gradual decrease in the blood ALB concentration when not enough amino acids are supplied to the liver cells for ALB synthesis. Thus, the plasma ALB concentration provides a long-term indicator of protein intake in ruminants (Payne 1987).

Under field conditions, the seasonal variation in blood TP and ALB, with high concentrations in autumn and a decrease over winter, has been described in many

northern deer species (reindeer; Hyvärinen *et al.* 1975, Nieminen 1980, Nieminen & Timisjärvi 1983, Soveri *et al.* 1992, white-tailed deer; DelGiudice *et al.* 1992). According to Nieminen and Timisjärvi (1983), and the ranges of mean blood TP and ALB concentrations in free-ranging adult female reindeer are 63-87 g/l and 39-43 g/l, respectively. The seasonal variation in TP and ALB has been suggested to be caused by the seasonal changes in the quality and quantity of diet (Nieminen & Timisjärvi 1983, DelGiudice *et al.* 1992).

In captive reindeer, higher blood TP concentrations have been reported in animals fed medium- or high-protein diets compared to animals fed lichens low in CP (Bjarghov *et al.* 1976, Soppela *et al.* 1992), thus relating the changes in blood TP and ALB concentrations to dietary CP intake. However, studies where varying ratios of dietary protein and energy have been fed to captive white-tailed deer (Seal *et al.* 1978, Warren *et al.* 1982) have reported highly variable effects on blood ALB and TP concentrations.

Together with ALB, globulins (GLOB) account for most TP in blood, acting as antibodies, enzymes and carrier proteins. Plasma GLOB concentration may increase in chronic infections caused by, for example, parasites. At the same time, parasites can cause a notable reduction in plasma ALB concentration due to a loss of ALB from the host animal (Yakoob *et al.* 1983, Kaneko 1989), leading to a reduction in the albumin:globulin ratio (A:G ratio). On a larger scale, parasites have an adverse effect on the population dynamics, host condition and fecundity of free-ranging ruminants, such as indicated during intestinal nematode infections in reindeer (Albon *et al.* 2002, Stien *et al.* 2002). The A:G ratio also serves as a diagnostic tool for several other conditions. A low A:G ratio may indicate an increase in the blood GLOB concentration caused by chronic parasitism or compensation for the ALB loss present in protein malnutrition (Payne 1987). High ratios may occur in response to hyperalbuminaemia caused by dehydration. Because the A:G ratio is affected by such a variety of conditions, it should always be interpreted with care by taking into account the concurrent changes in TP concentration. In reindeer, the blood A:G ratio shows seasonal variation, with higher ratios in winter compared to summer and autumn, mainly as a consequence of changes in GLOB concentration (Hyvärinen *et al.* 1975, Nieminen & Timisjärvi 1983).

Besides nutrition, age and pregnancy also affect the variation in the blood TP, ALB and GLOB concentrations of domestic ruminants, as defined by lower concentrations in newborn and pregnant animals (Kaneko 1989). In reindeer and caribou, TP and ALB concentrations progressively increase after birth, reaching adult levels during the first year of life, and decrease again in old animals (McEwan & Whitehead 1969, Nieminen 1980, Milner *et al.* 2003). The kind of decrease in plasma proteins commonly associated with pregnancy was not observed in a recent study on wild Svalbard reideer (Milner *et al.* 2003).

2.4.1.2 Urea, creatinine and urea:creatinine ratio

Urea is a nitrogenous compound synthesized in the liver and excreted into urine in order to prevent the excess nitrogen released in protein metabolism from becoming toxic (Huntington & Archibeque 1999). Urea production and its concentration in blood respond

to immediate changes in dietary protein intake, thereby reflecting short-term changes in protein metabolism and complementing the information provided by the analysis of blood TP and ALB concentrations (Payne 1987). As in domestic ruminants (Ide *et al.* 1966, Leibholtz 1970), a decrease in the blood urea concentration has been related to low dietary intake of protein in deer species (Bjarghov *et al.* 1976, Valtonen 1979) due to the recycling of urea from blood back to the rumen when the dietary protein intake is low (Robbins *et al.* 1974, Hove & Jacobsen 1975). Inversely, an increase in the plasma urea concentration during high-protein feeding is well known both in domestic ruminants (Ide *et al.* 1966, Leibholtz 1970, Ropstad *et al.* 1989, Gonda & Lindberg 1994) and in deer species (Hove & Jacobsen 1975, Bjarghov *et al.* 1976, Seal *et al.* 1978), when the liver increasingly synthesizes urea from ammonia. The plasma urea concentration may also increase despite low-protein feeding if energy intake is restricted, which is thought to reflect increased breakdown of endogenous proteins for energy production, a decrease in renal reabsorption of urea and/or haemoconcentration (Valtonen 1979, Warren *et al.* 1982, Wolkers *et al.* 1994a,b).

Creatinine is a compound produced in muscle tissue metabolism, released into circulation and filtered freely through the kidneys into urine. Nutrition-related changes in the blood creatinine concentrations of captive deer include elevated levels during fasting (Halse *et al.* 1976) and food restriction (Wolkers *et al.* 1994a), which have been related to reduced filtration in the kidneys and increased production due to muscle catabolism.

Studies on free-ranging deer have described seasonal patterns in blood urea and creatinine concentrations (Hyvärinen *et al.* 1975, Nieminen & Timisjärvi 1983, DelGiudice *et al.* 1992). According to Nieminen and Timisjärvi (1983), the range of mean blood urea concentrations of adult free-ranging female reindeer is 5.7-9 mmol/l, with the highest levels in summer and autumn and a decrease over winter. The seasonal changes in blood creatinine concentration are generally opposite in direction, being characterized by a decrease from late winter to summer and an increase through autumn (DelGiudice *et al.* 1992). The reported mean blood creatinine concentrations of free-ranging reindeer have been above 200 µmol/l in winter, declining to around 170 µmol/l in spring and summer (Nieminen & Timisjärvi 1983). As a result of an increase in blood creatinine and a decrease in urea, a reduction in the serum urea:creatinine ratio through autumn from above 50 down to 10 has been reported in free-ranging white-tailed deer (DelGiudice *et al.* 1992).

As the preceding review of the literature suggests, possibilities of assessing the nutritional status of deer based on plasma or serum urea and creatinine concentrations has attracted considerable interest. The changes in the U:C ratio and the effects of factors other than nutrition and season that potentially influence these parameters have attracted less attention. A current study on Svalbard reindeer (Milner *et al.* 2003) indicated that pregnancy status does not significantly explain the variation in plasma urea or creatinine concentrations, whereas calves had higher plasma urea concentrations and lower creatinine concentrations than yearlings and adult animals 2-7 years of age.

2.4.2 Blood chemical constituents related to carbohydrate and lipid metabolism

2.4.2.1 Glucose

The energy metabolism of ruminants, such as reindeer, is mainly dependent on the utilization of volatile fatty acids (mainly acetate, propionate and butyrate produced by the rumen microflora through rumen fermentation) as an energy source, rather than the straight breakdown of carbohydrates into glucose (White & Gau 1975, Kaneko 1989, Aagnes *et al.* 1995). The reference values for normal blood glucose levels (cattle; 2.5-4.2 mmol/l, sheep; 2.8-4.4 mmol/l) are lower than those for non-ruminants (Kaneko 1989). According to Nieminen and Timisjärvi (1983), the mean plasma glucose concentrations of free-ranging adult females varied between 3.4 and 4.6 mmol/l, with the highest levels occurring in summer and autumn. The studies on changes in the blood glucose concentrations of deer species in relation to changes in nutrition have varied in their outcome. For example, Luick and co-authors. (1973), Bjarghov and co-authors (1976) and Soveri and co-authors (1992) found no systematic changes in the blood glucose level related to either season or nutrition.

Stability of the blood glucose level is essential for the energy supply of several vital organs, including the brain. Therefore, it is not surprising that low or declining blood glucose levels have been mainly reported in fasted or severely nutritionally deprived deer (DeCalesta *et al.* 1975, 1977, Nieminen & Timisjärvi 1983, DelGiudice *et al.* 1987, Wolkers *et al.* 1994a). It should be noted, furthermore, that the blood glucose concentration of deer may also increase markedly in response to physical stress, for example, during round-ups or handling (Hyvärinen *et al.* 1976) or due to the effect of pharmacological sedatives (Mautz *et al.* 1980, Wolkers *et al.* 1994a). This may confound the interpretation of blood glucose concentration as a nutritional biomarker.

2.4.2.2 Triglycerides and cholesterol

Dietary triglycerides and glycolipids are hydrolyzed into glycerol and fatty acids by the ruminal bacteria (reviewed by Bauman 2003 and Funston 2004). Released unsaturated fatty acids have some of their double bonds reduced in a process called biohydrogenation. A portion of the fatty acids found in the rumen are phospholipids mainly of microbial origin. Glycerol is readily turned into volatile fatty acids, mainly to propionic acid, by microbial fermentation and used as an energy source.

Majority of the lipids enter the small intestine as free fatty acids. The triglycerides, glycolipids and microbial phospholipids that pass into the small intestine are hydrolyzed by intestinal and pancreatic lipases. After that, the fatty acids are transported as micelles into the cells of intestinal epithelium, reconstructed into triglycerides, incorporated into chylomicrons together with cholesterol and other lipid-like substances and released into

lymphatic circulation (Bartley 1989). Triglycerides can then be either readily used for metabolic processes or stored in adipose tissue through lipogenesis (Vernon *et al.* 1999).

Cholesterol is a major constituent of animal cell membranes and an important precursor of steroid hormones. Ruminants derive most of their cholesterol by endogenous synthesis from acetate. The major sites of endogenous synthesis of cholesterol and other ruminant lipids are adipose tissue and small intestine, instead of the liver as in non-ruminants (Liepa *et al.* 1978, Nestel *et al.* 1978, Vernon *et al.* 1999). Dietary fat supplementation increases the concentration of blood cholesterol in ruminants by increasing the amount of lipid precursors produced by the rumen and the endogenous cholesterol synthesis (Nestel *et al.* 1978).

Adipose tissue provides a vital storage of energy for over-winter survival in free-ranging deer (Reimers *et al.* 1982, Reimers 1983, Torbit *et al.* 1985, Adamczewski *et al.* 1987, Parker *et al.* 1993). The mobilization of lipids from the adipose tissue called lipolysis is catalyzed by a hormone-sensitive lipase and stimulated by catecholamines and fall in circulating insulin (Hales *et al.* 1978), indicated e.g. by increased concentrations of circulating free fatty acids and glycerol that can be further utilized (Larsen *et al.* 1985).

The mean serum triglyceride and cholesterol concentrations of free-ranging adult female reindeer vary within 0.17-0.33 mmol/l and 1.56-1.85 mmol/l, respectively, with the highest levels occurring in summer and autumn and a decrease over winter. (Nieminen & Timisjärvi 1983). Studies on domestic ruminants and captive deer have reported variable results in relation to the changes in the blood concentrations of cholesterol, triglycerides and other indicators of fat metabolism during fasting or undernutrition as body fat stores are mobilized for energy. In cattle, fasting increased plasma free fatty acid but did not have an effect of blood cholesterol (DiMarco *et al.* 1981), whereas Reid (1977) reported an increase of plasma cholesterol related to fasting.

According to Larsen and co-authors (1985) and Soveri and co-authors (1992), the concentrations of long-chain free fatty acids and glycerol increase in reindeer plasma as the body fat stores are mobilized for energy. Blood cholesterol increased in calves fed commercial reindeer feed (Bjarghov *et al.* 1976), whereas no change was observed in animals fed lichens and no change was observed in blood triglyceride concentrations in relation to either diet. Soppela and co-authors (2000) reported a decrease in serum cholesterol of reindeer calves during *ad libitum* and restricted lichen feeding, whereas triglyceride concentrations decreased during *ad libitum* feeding and increased during feed restriction. In white-tailed deer, both blood cholesterol and triglyceride concentration were found to increase along with progressive undernutrition, indicative of lipolysis when the endogenous fat reserves were not yet exhausted (DelGiudice *et al.* 1990).

2.4.3 Blood chemical constituents related to mineral metabolism

2.4.3.1 Alkaline phosphatase

The enzyme alkaline phosphatase (AP) present in circulation is produced by several tissues, especially liver, bone and placenta. Its activity in both animals and humans

increases in several bone and liver diseases and, for physiological reasons, in juveniles and during pregnancy (Capen & Rosol 1989, Ellonen 1995). Alkaline phosphatase activity has been shown to decrease both in adult and in young deer over winter (Hyvärinen *et al.* 1977, Nieminen 1980, Klinger *et al.* 1986), presumably due to the cessation of growth during winter and nutritional constraints. In free-ranging female reindeer, the highest serum AP activities with a mean value of 523 U/l have been reported to occur in summer, with a fall to a mean value of 129 U/l during the winter months (Nieminen & Timisjärvi 1983).

In humans, dietary protein deficiency has been linked to a decrease in AP activity (Guthrie 1971), whereas the energy content of the diet was found to have the most significant effect on AP activity in white-tailed deer (Seal *et al.* 1978). A decrease in AP activity can also be indicative of a deficiency of phosphorus or vitamin D (Capen & Rosol 1989). Several experimental studies have reported high or increasing AP activities in deer on highly nutritive diets (Bjarghov *et al.* 1976, Seal *et al.* 1978) and decreasing or low activities in animals fed either restricted or nutritionally inadequate diets (Seal *et al.* 1978, Wolkers *et al.* 1994a). However, elevated activities have also been reported in severely undernourished reindeer, reflecting the use of bone minerals to compensate for dietary mineral insufficiency (Hyvärinen *et al.* 1977, Nieminen & Timisjärvi 1983).

A pregnancy-related increase in blood AP activity, which is thought to be due to increased absorption and mobilization of Ca and phosphorus from bone reserves, has been reported in white-tailed deer (Chao *et al.* 1985). Another likely reason for this increase could be, as in humans, placental release of AP into circulation (Guthrie 1971, Ellonen 1995).

2.4.3.2 Calcium, magnesium and inorganic phosphate

Compared to commercial reindeer feeds, the supplemented winter diet of reindeer is deficient in several minerals, including magnesium (Mg) and calcium (Ca) (Nieminen & Heiskari 1989, Nieminen & Risto 1990). The overall ranges of the mean Mg, Ca and inorganic phosphate (P_i) concentrations in the blood of adult free-ranging reindeer are 0.8-1.2 mmol/l for Mg, 2.2-2.6 mmol/l for Ca and 1.6-2.2 mmol/l for Pi (Nieminen & Timisjärvi 1983), with the highest levels occurring in summer and autumn and a decrease over the winter. These seasonal changes in blood mineral concentrations have been related to seasonal changes in feed quality or quantity (Nieminen 1980, Nieminen & Timisjärvi 1983), supported by experimental studies that have reported higher blood levels of these minerals in animals on a commercial diet of high nutritive value compared to animals on a diet with low mineral content (Bjarghov *et al.* 1976). The interpretation of the effects of nutrition (i.e., protein, energy and mineral intake) on the concentrations of Ca, Mg or P_i in blood is confounded by the fact that fasted or undernourished deer may have either very low or elevated concentrations of Ca, Mg or P compared to animals on adequate or sub-maintenance diets, as observed both in the field and in experimental studies (Hyvärinen *et al.* 1977, Seal *et al.* 1978, Nieminen 1980, DelGiudice *et al.* 1987).

In addition to significant effects related to year, season and herd differences, a recent study on free-ranging reindeer reported no significant effect of the animal's age on the

plasma Mg or P_i concentrations, whereas plasma Ca was lower in yearlings and old animals compared to prime-aged animals (Ropstad *et al.* 1997). In the same study, pregnant females had significantly higher plasma Mg and Ca concentrations compared to barren females, whereas pregnancy did not have effect on the plasma P_i concentration or the pregnancy rate.

The decline in winter pasture quality in several parts of the Fennoscandian reindeer herding area (Helle 1980, Kautto *et al.* 1986, Evans 1996, Johansen *et al.* 1996, Kumpula *et al.* 1997) and/or the insufficient availability of feed due to unfavourable weather and snow conditions have concomitantly given rise to reports indicating mineral inadequacies in reindeer herds (Hyvärinen *et al.* 1977, Åhman *et al.* 1986, Hoff *et al.* 1993, Ropstad *et al.* 1997). Since there are no established reference values for plasma Mg and Ca concentrations in reindeer, values of 0.7 mmol/l and 2.2 mmol/l, respectively, have been used as cut-off values for the lower normal limits. These values are based on the renal threshold estimates for Mg in reindeer (Halse *et al.*1976) and for Ca in cattle (Halse *et al.* 1984). Plasma Mg concentrations above 1.0 mmol/l for cattle and 1.15 mmol/l for sheep have been proposed as the upper normal limits (Goff 1999). Based on the aforementioned reference values, Ropstad and co-authors (1997) reported that the frequency of subnormal plasma Mg and Ca concentrations of reindeer grazing on Norwegian winter pasture areas were 0-62% and 1-45 %, depending on the herd and the year of sampling.

In ruminants, the clinical symptoms of hypomagnesemia include muscular excitability, grinding of the teeth, salivation, ataxia, recumbency and tetanic muscle spasms (Goff 1999, Martens & Schweigel 2000). Hypomagnesemia indicated by low blood Mg concentration may also be present for months without apparent signs of disease (Allcroft & Green 1938). Reindeer in the Finnmark area, Norway, with symptoms such as ataxia and paresis (Hoff *et al.* 1993) had extremely low mean serum Mg concentrations (0.19 mmol/l) and reduced levels of serum Ca (2.03 mmol/l). Hyvärinen and co-authors (1977) reported that a Finnish reindeer herd with deaths from malnutrition had a reduced mean serum Mg level and a low mean serum Ca level (1.7 mmol/l). In contrast, the mean serum Mg was low (0.4 mmol/l) without a marked decrease in Ca level in Swedish reindeer in poor nutritional condition during the winter (Åhman *et al.* 1986). The latter findings were in agreement with experimental results (Bjarghov *et al.* 1976).

Magnesium oxide (MgO) in various forms and sustain-release Mg alloy rumen boluses are commonly used for the prevention of hypomagnesemia in domestic ruminants (Ritchie & Hemingway 1968, Hemingway & Ritchie 1969, Chicco *et al.* 1972, Smith *et al.* 1974, House & Mayland 1976, Stuedemann *et al.* 1984, Chester-Jones *et al.* 1990). In Finland, the use of mineral supplements in reindeer management is limited to concentrated feeds, lick stones or mineral mixtures with high mineral content (Nieminen & Risto 1990, Nieminen *et al.* 1998). So far, no studies are available providing information about the physiological effects of Mg or other mineral supplements in reindeer otherwise feeding on a low-mineral diet.

3 Aims of the study

Several studies have described the seasonal or nutritional variation in the blood chemical constituents of reindeer. Less attention has been paid to the effects of other intrinsic or extrinsic factors, such as year, herd, age and pregnancy status, which may confound the use of blood constituents as indicators of nutritional condition. The main objective of this thesis was to study how the blood chemical constituents indicative of energy, protein and mineral homeostasis vary in relation to several intrinsic and extrinsic factors and thereby to clarify how much they actually reflect the nutrition of reindeer. In addition, the variation in selected blood constituents was related to live body mass, which is a conventional measure of animals' condition.

A comparison of free-ranging animals to captive animals in defined feeding conditions, allowed an analysis of the effects of protein, energy and mineral intake on certain selected blood constituents. Such knowledge is of importance if the nutritional condition of the animals needs to be assessed on a herd basis in different management systems. The specific objectives were to:

1. study how feed quality and quantity are reflected in a range of blood chemical constituents related to the energy, lipid, protein and mineral metabolism of reindeer,
2. describe the sources of variation in plasma urea, creatinine and urea:creatinine ratio in free-ranging reindeer and reindeer under defined feeding conditions,
3. study how low and high dietary Mg intake and Mg supplements are reflected in the plasma Mg and Ca concentrations of reindeer, and
4. describe the sources of variation in plasma total proteins, albumin, globulins and the albumin:globulin ratio both in free-ranging reindeer in varying pasture conditions and in reindeer under defined feeding conditions.

4 Material and methods

4.1 Animals

Table 1 summarizes the number, origin, age and dietary regimen or pasture conditions of the reindeer studied in the original papers. A detailed description of the animals used in the studies is given in the original papers. The experimental study presented in paper I was conducted at the Reindeer Research Station at Kaamanen, Finland, and the experimental parts described in the papers II, III and IV were conducted at the Zoological Gardens of the University of Oulu, Finland. The protocols of the experimental studies were approved by the Committee on Animal Experimentation at the University of Oulu.

The data on free-ranging semi-domesticated reindeer were obtained from three herds on winter pastures in the Finnmark area in northern Norway (Seiland, Magerøy, Sørøy) and from one herd in the mountainous district of central southern Norway (Filefjell) (II, IV). In addition, data were obtained from wild Svalbard reindeer on winter pasture at Nordenskjøldland during three consecutive years (II).

The animals used in the studies were adult females, with the exception of the six female calves (<1 year) in the study described in paper III and the calves, yearlings and adults in the free-ranging herds described in paper IV. The classification of the Seiland animals into age classes was based on their general appearance, whereas the other free-ranging animals were aged by their known identity. The pregnancy status of adult captive animals was verified by transrectal ultrasound, whereas a plasma progesterone concentration above 7 nmol/l was used to indicate pregnancy in free-ranging animals (Ropstad *et al.* 1999). In the Magerøy, Sørøy and Filefjell herds, progesterone was analysed by ELISA kits utilising an enhanced chemiluminescence technique (Amerlite, Kodak Clinical Diagnostics, Amersham, UK), while in the Seiland herd it was analysed by Spectria Progesterone ^{125}I Coated Tube RIA kits (Orion Diagnostica, Espoo, Finland).

4.2 Description of the experimental feeding regimens

Detailed descriptions of the experimental feeding regimens and nutrient intake values of the animals studied in defined feeding conditions are given in the original papers I-IV.

In paper I, twenty-four mature pregnant female reindeer were allocated into three groups of eight animals. Group 1 was provided a supplemented winter diet consisting of 0.2-0.5 kg of commercial reindeer feed (Poron Herkku, Raisio Group, Finland) per day per animal in addition to lichens (*Cladonia spp.*), hay (*Deschampsia flexuosa*) and blueberry leaves and twigs that the animals were able to find from their 15 km^2 corral during the 110-day study period. Group 2 was fed lichens (*Cladonia spp.*) *ad libitum* for three weeks, followed by a gradual restriction of their lichen intake to 30 % of the *ad libitum* level over nine weeks (I, Fig. 1). After this, combined commercial reindeer feed and lichen feeding was used for the last three weeks of the study. This feeding was regulated to keep the energy intake of the animals below the levels of *ad libitum* lichen feeding at the beginning of the study. This group of animals is referred to as ”restricted lichen feeding” group. Group 3 was fed commercial reindeer feed *ad libitum*.

In the papers II-IV, nine mature female reindeer were allocated into three groups (groups 1, 2a and 2b), each consisting of two pregnant and one barren female. Varying amounts of lichens (*Cladonia spp.*) or commercial reindeer feed were given to the animals during four successive feeding periods that lasted for 116 days. Lichens were given *ad libitum* for 6 d to all groups, beginning on 4 February (period 1). Next, Group 1 continued to receive lichens *ad libitum*, while lichens were given at 80 % of the *ad libitum* level to the groups 2a and 2b for 51 d. In addition, Group 2a was supplemented daily from this period onwards by 10 g/day/animal of powdered reagent grade MgO (Tamro, Finland) mixed thoroughly into lichens (period 2). The groups 2a and 2b were again given lichens *ad libitum*, beginning on day 58, and Group 1 continued with lichens *ad libitum* (period 3). After this, the groups 2a and 2b continued to receive lichens *ad libitum*, while Group 1 was gradually switched from lichens *ad libitum* to commercial pelleted reindeer feed *ad libitum*, beginning on day 77 (period 4).

In paper III, the six reindeer calves used to study Mg alloy rumen bolus supplementation were fed lichens (*Cladonia spp.*) *ad libitum* from day –18 before the Mg bolus insertion to day 14 after the Mg bolus insertion. From day 15 onwards, the calves received a combination of commercial pelleted reindeer feed (Poron Herkku, Raisio Group, Finland) and lichens. The amount of commercial feed was gradually increased to reach *ad libitum* intake at the end of the study.

4.3 Feed analyses

The feed analyses performed in the experimental studies are described in detail in the original papers (I-IV). Briefly, feed samples of lichens and the commercial reindeer feed (Poron Herkku, Raisio Group, Finland) provided to the animals were taken daily, frozen and combined for chemical analyses at Soil Analysis Service Viljavuuspalvelu Oy, Mikkeli, Finland. The feeds were analysed for CP, crude fat, crude fibre, nitrogen free extracts and minerals, such as Ca, Mg and P.

Daily measurements of the feeds given and the orts left by the animals were used to calculate the daily DM intakes of the groups. The DM contents of the consumed feeds and leftovers were determined daily by drying samples of 100 g at 95 °C to a constant weight. The *ad libitum* feeding in the experiments was adjusted so that there was still some edible material left in the orts.

In paper I, the ME content of the commercial reindeer feed was calculated using the MAFF recommendations (MAFF 1975, 1984). Digestibility tables for ruminants (Tuori *et al.* 1995) were used to estimate the digestibility of the different compounds of the commercial feed. In the studies II-IV, the ME content of the commercial feed used in the experimental setup was based on the manufacturer's information, whereas the ME content of lichens was calculated according to Tuori and co-authors (1995) based on the chemical composition and digestibility estimates of lichens (Jacobsen & Skjenneberg 1977).

In paper I, the energy, protein and mineral intakes were presented for each week of the study as group averages (Fig. 1a, b, c), whereas in the experimental regimens described in the papers II-IV, the same parameters were calculated as group averages over the different periods of feeding. In paper III, individual feeding was used for the calves, but their intake values are presented as averages over the different stages of the study for simplicity.

The daily DM intakes, the ME contents of the feeds, and the results of the chemical analyses of the feeds were used to calculate the intakes of CP, ME and minerals during the feeding regimens presented in detail in the separate papers and as a summary representing the different planes of feeding in Table 2.

4.4 Blood and urine samples and measurements of live body mass and rumen content pH

The blood sample collection and other experimental protocols are described in detail in the original papers (I-IV). The blood sampling frequencies of the animals used in the original papers are shown summarised in Table 1. In the experimental parts of the papers I-IV, the body mass values were recorded with electronic livestock weighing scales. In the field studies (II, IV), the live body masses of the Finnmark animals were recorded with a portable electronic livestock weighing scale (Farmer Tronics, Give, Denmark). The Svalbard reindeer were weighed with a spring scale (Salter Industries, West Bromwich, UK).

No sedatives or medication were used for blood sampling. In the experimental studies, the animals were restrained by hand for blood sampling in a small crate, whereas in the field studies, restraining was done by hand on the ground with the animal lying on its side. Blood samples were collected by jugular venepuncture into either heparinized or plain (serum) tubes. In the experimental studies, plasma or serum was separated by centrifugation within 2 h and stored at -20°C until analysed. In the field studies, blood samples were centrifuged within 6 h and then stored at -20°C or -70°C until analysed.

The excretion of Mg and Ca into urine and the rumen content pH were followed in the reindeer calves (III) as additional information about the physiological changes in the

plasma Mg and Ca concentrations. Urine samples were obtained by catheterization of the bladder with a disposable dog catheter (Buster®, 2.0x500 mm, Jørgen Kruuse, Denmark) after opening of the urethra locally anesthetized with lidocain (Xylocain-gel 2 %, Astra, Sweden), frozen and stored at -20°C until analysed. Samples of the rumen content were obtained through rumen cannulae. The cannulae were fitted caudally on the left side of the abdomen. The calves were anaesthetized (Domitor® 1 mg/ml, Orion Group, Finland) for the operation. Local anaesthetic (Xylocain 20 mg/ml, Astra, Sweden) was used where appropriate. After the surgery, anaesthesia was reversed with Antisedan® (5 mg/ml, Orion Group, Finland). The calves were given analgesic (Romefen® 100 mg/ml, Rhône-Merieux, France) and antibiotic (Penovet® 300 mg/ml, Boehringer Ingelheim, Denmark) post-operatively once per day for five days. The animals were allowed to recover for at least two weeks after the operation. Rumen content pH was measured from the digesta with a pH meter (AP15, Denver Instrument Company, USA). The loss in Mg bolus weights was recorded at the times of rumen content pH measurements.

4.5 Chemical analyses of blood and urine

Table 3 shows a summary of the analytical methods used for the analyses of the chemical constituents of serum or plasma. Blood constituents related to protein metabolism (TP, ALB, GLOB, A:G ratio, urea, creatinine, U:C ratio), carbohydrate and lipid metabolism (glucose, triglycerides and cholesterol) and mineral metabolism (Ca, Mg, P_i, AP) were selected for the analysis. The analyses were performed with appropriate use of control sera, (for example Seronorm, Sero A/S, Billingstad, Norway) and in line with the laboratory standards employed in the laboratories where the analyses were performed. Instead of a direct laboratory analysis, as for other parameters, the plasma GLOB concentration was obtained by subtracting the plasma ALB concentration from the plasma TP concentration. Detailed descriptions of the analytical methods are given in the original papers I-IV.

Urine analyses are explained in detail in paper III. In brief, the urinary Ca and Mg concentrations were analysed with a PU 7000 ICP Spectrometer (Philips Analytical, Cambridge, UK), whereas the urinary creatinine concentration was analysed with HiCo Creatinine (BM/Hitachi 717/911, reagent kit BM 1040847, Boehringer Mannheim, Germany) using the Jaffe method with a sample blank. To correct for dilution, the urinary Mg and Ca concentrations are expressed as ratios of Mg and Ca to creatinine (Mg:C and Ca:C ratio).

4.6 Statistical analyses

Detailed descriptions of the statistical analyses are given in the original papers I-IV. In paper I, the differences between the feeding groups at different time points were assessed by one-way analysis of variance (ANOVA). If ANOVA showed differences at the level of $P<0.05$, multiple comparisons between the groups were performed *post hoc* with Tukey's test.

In paper II, BM, plasma creatinine, plasma urea and the plasma U:C ratio for free-ranging animals were analysed by generalised linear models (GLM) according to McCullagh and Nelder (1989). The experimental data consisted of replicated measurements from the same animals, and the focus of the analysis was on whether individuals showed the same pattern in the response variables. To control for between-individual variation, linear mixed models were fitted with the identity of the reindeer as a random effect. Time since the baseline of the experiment, status with respect to pregnancy and experimental group were fitted as fixed effects. The slope parameter for other continuous variables, including body mass, were fitted as random coefficients with reindeer identity as the grouping variable, to investigate whether there was a consistent positive or negative relationship between the response variable and the predictor across individuals (Davidian & Giltinan 1995). Pearson's correlation coefficient was used for simple correlations.

In paper III, the statistical analysis was done according to the guidelines proposed by Matthews and co-authors (1990). Kruskall-Wallis one-way analysis of variance on ranks was used to detect statistical differences in the plasma Mg and Ca concentrations between the groups during the different feeding periods (experiment 1, MgO supplementation). If the test showed differences at the level of $P<0.05$, multiple comparisons between the groups were performed *post hoc* with Tukey's test. The correlations between the plasma Mg and Ca concentrations within different plasma Mg ranges were tested with Pearson's correlation coefficient test. The differences in the studied parameters between the maximum response and the level at bolus insertion were detected with Student's t-test for paired samples (experiment 2, Mg alloy rumen bolus supplementation).

In paper IV, the dependent variables were analysed for normality by the Shapiro-Wilks method. In captive animals, the effects of group, time and BM on plasma protein parameters were studied by repeated measures mixed model analyses (PROC MIXED of SAS, Littell *et al.* 1996). The dependent variables were plasma TP, ALB, GLOB and A:G ratio. The fixed effects in the models were the time of sampling [samples grouped in weeks], feeding group and the interaction between feeding group and time of sampling. Compared to paper III, which reports results from the same experimental set-up, only the blood samples collected in the periods 2 and 4 were included in the statistical analyses, to focus on these periods representing starvation and re-feeding. Both periods were analysed separately. The analyses of the groups 2a and 2b were combined for paper IV, because no significant differences in the response variables were found between these groups following the same dietary regimen, except for the daily oral MgO supplement received by group 2a (results published in paper III). In period 4, the individual baseline level of each dependent variable was included as a covariate in the analyses. A random effect was included for between-animal variation, assuming a negative exponential temporal autocorrelation between the measurements from the same individual. The effects of year, month of sampling, BM and pregnancy status on the plasma TP, ALB, GLOB and A:G ratio of the free-ranging Seiland animals were studied by multiple regression analyses (PROC REG of SAS, Littell *et al.* 1996). The effects of year, age, pregnancy status and body mass on the plasma TP concentrations of the Magerøy, Sørøy and Filefjell herds were analysed by the same method as in the Seiland herd. The samples from the Magerøy herd collected in March 1992 instead of January, as in 1993-1995, were excluded from the regression analysis.

Table 1. Summary of the number, location, blood sampling procedure and feeding regimen or pasture conditions of the female reindeer studied in the original papers I-IV.

Animals	Paper	Location	n	Age class	Blood sampling	Feeding regimen / Pasture conditions
Free-ranging						
Magerøy herd	IV	Finnmark (71.03 N, 25.45 E)	107-175[a]	A	Mar 1992, Jan 1993-1995	Winter pasture quality moderate, mostly lichen. Summer pasture limited in spring and early summer. Animal density 10 / km^2.
Sørøy herd	IV	Finnmark (70.36 N, 22.46 E)	92, 127[a]	A	Jan 1992, 1994	As in Magerøy herd. Animal density 4 / km^2.
Filefjell herd	IV	Central Norway (61.09 N, 8.15 E)	286[a]	Y, A	Jan 1995	Quality of both winter and summer pasture good.
Seiland herd	II, IV	Finnmark (70.25 N, 23.15 E)	10-140[a]	C, Y, A	Mar, May, Jun, Oct, Nov 1997 Mar, May, Jul, Oct 1998	Lichen reserves on winter pasture small. Summer pasture limited in spring and early summer.
Svalbard	II	Nordenskjøldland (78.2 N, 17.3 E)	74, 81 144	A	April 1996, 1997 April-May 1998	Starvation in 1996 due to ice crusting and difficult snow conditions. In 1997 and 1998, normal snow conditions with access to winter forage.
Captive	I	Kaamanen (69.10 N, 27.20 E)	24	A	Jan, Feb, Mar, Apr, May 1993	Three groups: supplemented winter pasture, restricted lichen feeding, commercial ration *ad lib.*.
	II-IV	Oulu (65.01 N, 25.30 E)	9	A	Three times per week 4 Feb-30 May 1997	Three groups: lichens restricted or a*d lib.*, lichens restricted or *ad lib.* with daily MgO supplement, commercial ration *ad lib.* with some lichens.
	III	Oulu (65.01 N, 25.30 E)	6	C	Once per week 20 Apr-7 May, daily 8 May-14 May, twice a week 15 May-2 June 1998.	Lichens *ad lib.,* followed by commercial ration with some lichens. Mg alloy rumen bolus supplementation.

Symbols: [a] = total number of animals analysed for the chemical constituents studied in the original papers. Small blood sample volume occasionally excluded certain chemical analyses. The n used in each statistical analysis is indicated separately in the original papers and varied depending on the inclusion conditions and the studied parameter. C= calf < 1 yr, Y= yearling, A= adult animal ≥ 2 years.

Table 2. Summary of crude protein (CP), metabolizable energy (ME), magnesium (Mg), calcium (Ca) and phosphorus (P) intake per day per captive animal as studied in the original papers I-IV. The selected feeding regimens represent lichen-based feeding with restricted feed intake (low protein, low energy and low mineral; LP-LE-LM), lichen ad lib. or lichen-based feeding with low protein and mineral intake but medium energy intake (low protein, medium energy, low mineral; LP-ME-LM) and feeding a commercial ration of high nutritive value (high protein, high energy, high mineral; HP-HE-HM). Paper I: n=8 per group, Papers II-IV: n=3 per group, Paper III, calves: n=6.

Nutrient	LP-LE-LM			LP-ME-LM				HP-HE-HM	
(intake per animal)	I	II-IV: Period 2		II-IV: Period 3			III: calves	I	II-IV: Period 4
	Group 2 weeks 4→12	Group2a	Group 2b	Group 1	Group 2a	Group 2b	days 0-14	Group 3	Group 1 days 95-116
CP (g/d)	55→12	30	27	35	38	37	18	230-350	355
ME (MJ/d)	18→6	12	11	14	15	15	6	17-25	27
Mg (g/d)		0.2[a]	0.2	0.2	0.3	0.3	0.2[b]		8
Ca (g/d)	2.3→0.6	0.6	0.5	0.7	0.7	0.7	0.5	14-21	23
P (g/d)	0.6→0.1							11-17	

Symbols: [a]= In addition to Mg received from feeds, animals received a daily oral supplement of 10 g powdered MgO (Tamro, Finland), equal to 6 g of Mg per animal. [b]= In addition to Mg from the diet, the calves were supplemented with two sustain-release Mg alloy rumen boluses (Rumbul, Agrimin Ltd, Brigg, UK) on day 0. The calculated release of Mg from the two rumen boluses varied between 0.34 and 2.24 g per day.

Table 3. Analytical methods used for the determination of chemical constituents from serum or plasma in the original papers I-IV.

Parameter	Paper	Method principle	Analysis method	Reference
Total proteins	I	Biuret	Photometric	Lowry *et al.* (1951)
	IV	Biuret	Photometric (Technicon Axon, method SM4-2147E94)	Weichselbaum (1946)
Albumin	I	Bromcresol green	Photometric	Doumas *et al.* (1971)
	IV	Bromcresol green	Photometric (Technicon Axon, method SM4-2131E94)	”
Urea	I	Glutamate dehydrogenase	Photometric	Guttman & Bergmeyer (1974)
	II	Urease	Photometric (Technicon Axon, method SM4-2150E94)	Tiffany *et al.* (1972)
Creatinine	I	Jaffe	Photometric	Fabiny & Ertingshausen (1971)
	II	Jaffe	Photometric (Technicon Axon, method SM4-2141E94)	Chasson *et al.* (1961), Rossignol *et al.* (1984)
Triglycerides	I	Enzymatic hydrolysis	Photometric	Wahlefeld (1974)
Cholesterol	I	Enzymatic hydrolysis	Photometric	Allain *et al.* (1974)
Glucose	I	4-amino-phenazone	Photometric	Trinder (1969a, b)
Magnesium	III	Xylidyl -blue	Photometric (Kone Optima, reagent 981385)	Mann & Yoe (1956)
	III, C	Calmagite	Photometric (Cobas Fara, reagent 61411 Bio-Merieux)	Gindler & Heth (1971)
Calcium	I	Atomic absorbance	Flame photometer (EFOX 5053)	
	III	o-Cresolphtalein complexone,	Photometric (Technicon Axon, method SM4-2138K94)	Gitelman (1967)
	III, C	Atomic absorbance	Flame photometer (EFOX 5053)	
Inorganic phosphate	I	Fosfomolybdate	Photometric	Daly & Ertingshausen (1972)
Alkaline phosphatase	I	Orthophosphoric-monoester phosphohydrolase	Photometric	The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974)

Symbols: C=calves

5 Results

5.1 Blood constituents related to protein metabolism

5.1.1 Total proteins, albumin, globulins and albumin:globulin ratio

In free-ranging reindeer, the blood constituents related to protein intake and metabolism showed large concentration ranges with clear seasonal patterns, as observed in the two-year follow-up of the Seiland herd. The overall ranges for TP, ALB, GLOB and A:G ratio were 36-110 g/l, 18-59 g/l, 17-59 g/l and 0.5-2.1, respectively (IV, Table 2). The mean plasma TP and ALB concentrations declined during spring, being lowest in May and highest in October (IV, Fig. 1). The differences in TP, ALB and GLOB and A:G ratio were significant between months and years, with the exception that there was no effect of year on the A:G ratio (IV, Table 3). There was a significant effect of month*year interaction, indicating that the effect of month differed between years. The year and month effects were illustrated by the fact that, in March 1997, 36 % of the blood samples from the Seiland herd had plasma TP concentrations below 60 g/l, increasing to 97 % by May, whereas in March and May 1998, the respective percentages were as low as 3 and 21 %.

In the other free-ranging mainland reindeer herds, the between-year variation in TP was analysed for the Magerøy and Sørøy herds, from which samples were collected over several years (IV). The mean plasma TP concentration in the Sørøy herd was significantly lower in 1992 compared to 1994 (mean 67.2 g/l, range 54-78 g/l *vs.* mean 71.7 g/l, range 47-91 g/l, respectively), whereas no significant effect of year was found in the Magerøy herd. There was also notable variation in the mean plasma TP concentrations between the herds from different pasture areas (IV, Fig. 2). The mean plasma TP concentration was lowest in the Magerøy herd in 1992 (58.2 g/l, range 28-80 g/l), when 58 % of the sampled animals had plasma TP concentrations below 60 g/l. For comparison, the proportion of animals with plasma TP concentrations below 60 g/l was under 4% in both the Sørøy and

the Filefjell herds. The overall mean concentration of plasma TP in samples collected on winter pasture was 68.5 (range 28-91 g/l).

Different intrinsic and extrinsic factors were found to explain the variation in TP, ALB, GLOB and A:G ratio. An increase in BM was associated with a small, but significant increase in all of these parameters, except in the A:G ratio (IV, Seiland herd). As an example, a BM increase of 10 kg increased plasma TP by 1.1 g/l. The late stages of gestation in March and May significantly reduced the plasma TP, ALB and GLOB concentrations by 3.0, 1.9, and 1.2 g/l, respectively, but did not have an effect on the A:G ratio (IV, Seiland herd). Animal's age did not have a significant effect on any of the studied variables when BM was included in the same model.

As in the Seiland herd, pregnancy significantly reduced plasma TP concentration by 2.2 g/l in the other free-ranging mainland herds, and a 10 kg increase in BM was associated with a 0.9 g/l increase in TP concentration. When the effect of age was included in the analysis, pregnancy was no longer significantly related to TP variation. The relationship between age and TP was curvilinear, the young and old animals having lower concentrations than prime-aged animals.

The studies on captive animals revealed some common effects of feed quality and quantity in the changes of plasma TP, ALB, GLOB and A:G ratio. The mean serum TP and ALB concentrations were significantly higher in the reindeer receiving commercial reindeer feed compared to the animals on a supplemented winter diet or a restricted lichen diet, indicating that feed quality and quantity had significant effects on these blood constituents (I). During the four-month study, the mean serum TP concentration decreased by 10 % in the animals on a supplemented winter diet and by 20 % in the animals with restricted lichen feeding compared to their respective initial mean concentrations of 75 and 77 g/l (I, Fig. 2b), whereas it increased from 73 g/l to 83 g/l within three weeks in the animals receiving commercial reindeer feed. In paper IV, where GLOB and A:G ratio were also included as plasma protein parameters, there was a significant decline in the mean plasma TP, ALB and GLOB concentrations, but not in the A:G ratio, when lichens with low protein content were given as the only feed (IV, Fig. 3, period 2). When commercial reindeer feed was given *ad libitum* to one group of animals, their plasma TP, ALB and GLOB significantly increased and A:G ratio significantly decreased (IV, Fig. 3). The animals that continued to receive lichens *ad libitum* showed a significant decrease in plasma TP and ALB concentrations and in the A:G ratio, whereas their plasma GLOB concentration significantly increased (IV, Fig. 3). The group differences were characterized by significantly higher TP and ALB in the animals receiving commercial reindeer feed compared to the lichen-fed animals (IV, Table 4).

5.1.2 Urea, creatinine and urea:creatinine ratio

Both blood urea and creatinine concentrations, which were discussed in more detail in the papers I and II showed large concentration ranges with clear seasonal patterns, as observed in the free-ranging Seiland herd. The overall range of mean plasma urea concentrations was from 2.5 to 17.4 mmol/l, with lower concentrations during the winter (Nov, March) and the highest concentrations in the summer and autumn months (May-

Oct) (II, Fig. 1c). The range of mean plasma creatinine concentrations was from 90 to 280 µmol/l with an increase over winter and a decrease by two thirds to the lowest concentrations in June-July (II, Fig. 1b). There was significant between-month and between-year variation in the mean plasma urea and creatinine concentrations, with significant month-year interaction. The month of sampling accounted for almost 90 % of the variation in the plasma urea concentrations and 65 % of the variation in the creatinine concentrations. The variation between the years and the year-month interaction explained 7 and 3 %, respectively, of the concentration of creatinine. In the Svalbard reindeer population, the mean plasma urea concentration was highest in 1996 and lowest in 1998 (II, Fig. 1c). Similarly to the findings from the mainland reindeer, the year of sampling had a significant effect on both plasma urea and creatinine concentrations, accounting for 11 % and 7 % of the variation in these parameters.

Of the explanatory factors studied, BM had only a minor effect on the plasma urea concentration. It explained 0.8 % of the variation in the Seiland herd and did not have a significant effect on the variation in the Svalbard reindeer, despite the fact that the mean BM of this population was more than 20 % lower in 1996 than in 1997 and 1998 (II, Fig. 1a). The relationship between BM and plasma creatinine was generally positive, but explained only 9 % of the variation in the plasma creatinine concentrations of the Seiland herd. Interestingly, the plasma creatinine levels of the Svalbard animals showed a decrease over the sampling periods from mid-April to mid-May, which was dependent on neither the year of sampling nor the decrease in BM (II, Fig. 2).

The mean plasma U:C ratio of the Seiland herd ranged from 8.9 to 120.8 (II, Fig. 1d), with the highest values occurring in June-October and the lowest in November-March. Similarly to plasma urea and creatinine, it showed significant variation between the months of sampling and between years. The changes in the U:C ratio were mainly determined by the plasma urea concentration, since it varied by a factor of 7, whereas plasma creatinine varied by a factor of 3. In the Svalbard population, the plasma U:C ratio varied between 30 and 34. Nevertheless, it was significantly higher in 1996 than in 1998.

The studies on captive animals (I, II) revealed some common effects of feed quality and quantity on the blood urea and creatinine concentrations. In general, the mean blood urea concentrations were significantly lower and the creatinine concentrations higher in the lichen-fed animals compared to the animals on a supplemented winter diet or commercial reindeer feed (I: Fig. 3a, 3b and II: Fig. 3b and 3c, period 4).

Despite the differences between the groups in the concentrations of plasma creatinine related to the feed quality and/or quantity, the temporal changes of these parameters during the 4-month study showed similarities between the lichen-fed animals and the animals on a supplemented winter diet (I). For example, the mean serum creatinine concentrations of these two groups of animals increased from the initial concentrations between 199 µmol/l and 213 µmol/l in January, reaching mean concentrations of 316 µmol/l and 270 µmol/l, respectively, in March (I, Fig. 3a). Thereafter, the mean concentrations declined in both groups towards the end of the study in May.

The captive animals studied in paper II (Fig. 3b) showed similar changes in their plasma creatinine concentrations, with an increase to 250 µmol/l during lichen feeding *ad libitum* and a decrease below 100 µmol/l when the lichen diet was gradually replaced by commercial reindeer feed (Group 1). Interestingly, there was also a significant decrease in

the mean plasma creatinine concentration of the animals that continued on lichen feeding (Group 2, period 4), which was not related to the BM change and occurred at the same time of the year as in the wild Svalbard reindeer, i.e., in April-May.

The changes in plasma urea concentrations were parallel to the changes in the feeding regimen. Feeding on lichens with a low protein content as the only feed decreased the mean plasma urea concentration from 5-8 mmol/l to around 2 mmol/l (II, Fig. 3c). A 20 % restriction in lichen intake increased the mean plasma urea concentration by 3.8 mmol/l compared to the animals receiving lichens *ad libitum*. When the same animals where again given lichens *ad libitum*, their mean plasma urea concentration declined within a few days to the same level as in the animals on *ad libitum* feeding. Feeding on a commercial ration with a high protein content compared to lichens increased the mean plasma urea concentration above 10 mmol/l (II, Group 1, Period 4*)*. As in the free-ranging Seiland herd, the pattern of plasma U:C ratio in the captive animals closely resembled the changes in plasma urea concentration (II, Fig. 3d). Thus, low ratios were observed when the animals received lichens *ad libitum* and 15-fold ratios were achieved by the end of the study in the reindeer whose diet was gradually replaced by commercial reindeer feed *ad libitum*.

5.2 Blood constituents related to carbohydrate and lipid metabolism

The blood constituents related to carbohydrate and lipid metabolism were followed in the first feeding experiment that lasted from January until May (I). At the beginning of the study, the mean serum glucose concentration was 3.16 mmol/l in the reindeer on a supplemented winter diet, 2.61 mmol/l in the group fed commercial reindeer feed and 2.78 mmol/l in the animals fed lichen followed by restricted feed intake (I, Fig. 5a). The animals on a supplemented winter diet had a higher mean serum glucose level compared to the other groups, even though this difference was significant only in February and in May. Also, the changes in blood glucose levels between the sampling months were more pronounced in the supplemented winter diet group, where the mean values varied between 3.53 mmol/l and 2.86 mmol/l from February to May, whereas more stable mean concentrations varying between 3.06 mmol/l and 2.54 mmol/l were measured in the other groups.

The mean serum cholesterol concentration was around 1.7 mmol/l in all groups at the start of the experiment (I, Fig. 5b). In the animals with restricted lichen feeding, the mean serum cholesterol decreased during the first weeks of the study to 1 mmol/l, increased to 2.0 mmol/l in April after seven weeks of restricted lichen feeding, and decreased again by May when the animals' nutrient intake was again increased after nine weeks of restriction. In the groups provided either commercial reindeer feed or a supplemented winter diet, the mean serum cholesterol concentrations varied within 1.3-2.0 mmol/l, the animals on supplemented winter diet showing increasing concentrations from March onwards.

Serum triglycerides (I, Fig. 5c) showed little variation regardless of the feeding group, with the exception of the significantly higher mean concentration in the lichen-fed animals in April (0.8 mmol/l *vs.* 0.3 mmol/l in the other groups). However, there was

considerable between-animal variation in this group in April. The group on a supplemented winter diet and the group fed the commercial ration showed a slight increase in triglyceride concentrations during the last two months of the study from the beginning of March until the beginning of May, but there were no significant differences in serum triglyceride concentrations between these groups.

5.3 Blood chemical constituents related to mineral metabolism

The serum AP activity and P_i concentrations were followed in the first feeding experiment from January until May (I). The overall mean serum AP activity varied between 46 and 137 U/l (I, Fig. 3c). At the beginning of the study, the mean activities were between 55 and 79 U/l, but increased to between 88 and 137 U/l in early May. Serum AP activities increased towards the end of the study in all groups, regardless of the differences in feeding. The increase in serum AP activity during the study was 94 % in the supplemented winter diet group, 30 % in the group fed lichens in restricted amounts and 74 % in the group on commercial reindeer feed. The animals fed lichens in restricted amounts had the lowest serum AP activities throughout the study compared to the other groups, but these differences were not significant.

At the beginning of the study in mid-January, the mean serum P_i concentration ranged between 2.62 and 2.76 mmol/l (I, Fig. 4b). Thereafter, the mean value was highest in the group fed commercial reindeer feed (max. 3.31 mmol/l in February) and lowest in the animals on lichen feeding with restricted feed intake (min. 1.58 mmol/l in February). The difference between these two groups was significant from February until the end of the study in May. The mean serum P_i concentrations of the animals on a supplemented winter diet were significantly lower compared to the animals on the commercial ration and significantly higher compared to the animals on lichen feeding with restricted feed intake. The mean P_i concentration of the supplemented winter diet group decreased during the study to 2.22 mmol/l in May.

The changes in the mean serum Ca concentrations of the groups were inconsistent (I, Fig. 4a). The lowest mean concentrations were observed in the group fed lichens in restricted amounts, where the mean serum total Ca concentration decreased gradually from 2.48 mmol/l to 2.29 mmol/l between January and April. However, the difference compared to the other groups was only significant in April. In the other groups, the mean serum Ca concentrations varied between 2.33 and 2.52 mmol/l, without a consistent direction of change.

The effects of Mg supplements either in the form of powdered MgO or Mg alloy rumen boluses were studied in the animals receiving lichens with low mineral content by comparing them to the animals receiving a commercial ration with high mineral content (III). The mean plasma Mg concentration increased rapidly to above 1 mmol/l in the animals given supplemental MgO, whereas in the group where lichens were given as the only feed in restricted amounts, the mean plasma Mg concentration declined below the level of 0.7 mmol/l, which was considered hypomagnesemic, in 18 days (Fig. 1). After 76 days of lichen feeding, the mean plasma Mg concentration of the two groups where lichens had been given as the only feed, first in restricted amounts and then *ad libitum*,

had declined to 0.60 mmol/l and to 0.48 mmol/l (Period 3). The lowest measured individual plasma Mg concentration was only 0.31 mmol/l after 84 days on lichen feeding. However, none of the animals showed clinical signs of Mg deficiency. When commercial reindeer feed was given to one of the groups, its mean plasma Mg concentration increased to 1 mmol/l within five days. The animals supplemented with MgO had significantly higher plasma Mg concentrations than the animals on lichen feeding without the supplementation, but this difference disappeared after commercial reindeer feed was given to one of the non-supplemented groups (III, Fig. 1, Table 3).

The mean plasma Ca concentration decreased gradually during the experiment in all groups. The mean plasma Ca concentration was lowest in the MgO-supplemented group (III, Fig. 1), but the difference compared to the other groups was significant only during period 3, when lichen feeding *ad libitum* followed the 20 % restricted lichen intake in period 2 (III, Table 3). There was a significant positive correlation between the plasma Mg and Ca concentrations in the plasma Mg range ≤0.7 mmol/l (r=0.42) and a negative correlation in the plasma Mg range of 0.71-1.15 mmol/l (r=-0.37). At plasma Mg concentrations >1.15 mmol/l, there was no significant correlation between plasma Mg and Ca.

The effects of sustain-release Mg alloy rumen boluses on plasma Mg and Ca concentrations were similar to the effects of MgO supplementation, but as expected, the difference in the methods of administration (continuous *vs.* single administration) caused a difference in the response profiles. The mean plasma Mg concentration of reindeer calves was 0.82 mmol/l immediately prior to the insertion of Mg rumen boluses (III, Fig. 2a), and the mean maximum plasma Mg concentration of 1.13 mmol/l (range 1.05-1.17 mmol/l) was reached 2-6 days after the insertion of the boluses into the rumen. The increase from the pre-bolus and to the peak plasma Mg concentration was significant. After this, the mean plasma Mg concentration gradually declined, but was still higher at the end of the study 25 days after the bolus insertion than before the bolus insertion.

The mean plasma Ca level decreased after the insertion of the rumen boluses (III, Fig. 2a). The lowest plasma Ca concentrations were reached 1-5 days after the boluses had been placed into the rumen. The mean minimum Ca concentration was 2.36 mmol/l. The difference between the level before the bolus insertion and the minimum level was statistically significant. Thereafter, the mean plasma Ca concentration gradually increased and was 2.43 mmol/l at the end of the study.

5.4 Urinary Mg and Ca, rumen content pH and Mg bolus weights

Urinary Mg:C and Ca:C ratios, rumen content pH values and bolus weights were followed as sources of additional information about the physiological effects and the efficacy of the sustain-release Mg boluses as a potential method of mineral supplementation for reindeer (III). The urinary Mg:C and Ca:C ratios were 0.14 and 0.95 mmol/mmol prior to the bolus insertion (III, Fig. 2b). After the bolus insertion, both ratios increased. The mean peak urinary Mg:C ratio of 2.25 mmol/mmol (range: 0.89-6.18 mmol/mmol) occurred within the days 18-25 after the bolus insertion, while the peak urinary Ca:C ratio of 2.10 mmol/mmol (range: 1.58-2.52 mmol/mmol) occurred within

the days 10-21 after the bolus insertion. The difference between the maximum urinary Mg:C ratios and the ratios before the bolus insertion was not statistically significant, whereas the difference in urinary Ca:C between the peak ratios and the ratios before the bolus insertion was.

The overall range of the rumen content pH was 5.2-7.0. The rumen pH significantly decreased when the animals received lichens as the only feed and increased when they were give commercial reindeer feed (mean increase 0.71 units). The increase was significant between the pH values recorded prior to giving commercial feed (on day 33) and at the end of the study on day 44.

The initial bolus weights were between 39.5 and 41.4 g/bolus. The loss in bolus weights during the study was 10.8-24.6 g/bolus. The loss in bolus weights and, thus, also the decomposition rates (g/d) were slower during lichen feeding (0.2-1.1 g/day/bolus) compared to feeding with commercial ration (0.7-1.3 g/d/bolus). The calculated release of Mg from the two rumen boluses varied between 0.34 and 2.24 g per day.

5.5 Changes in BM in relation to feeding, season, age and pregnancy

Similar patterns in BM gain and loss, related to the feeding regimen (lichens *ad libitum*, restricted lichen feeding or commercial reindeer feed) were observed in all studies (I-IV). In general, the animals on lichen feeding showed a decrease of live BM, whereas the BM of the animals receiving commercial reindeer feed increased. The BM loss and gain took place irrespective of the animals' age (III) or pregnancy status (II, IV), but were modulated by them and the by the plane of feeding.

For example, the loss of mean BM was 15 % in pregnant females with restricted lichen feeding, whereas otherwise BM was either maintained, as in the animals feeding on a supplemented winter diet, or increased, as in the animals fed commercial reindeer feed *ad libitum* (I, Fig. 2a). In paper II, the BM loss of captive animals varied from 4 % to 25 %. The reduction in BM was smaller in pregnant females than in non-pregnant ones during the periods when lichens were given as the only feed to all groups either *ad libitum* or at 80 % of *ad libitum* intake (II, Periods 2 and 3), whereas the overall BM loss was similar between the feeding groups. When commercial reindeer feed was provided to one group after this, the animals showed a marked increase in BM, whereas the BM of the animals that remained on a lichen diet continued to decline (II, Fig. 3a). Pregnant females increased their weight just before calving and lost weight after parturition, but the weight gain was more pronounced in the animals receiving commercial reindeer feed.

As expected, there was large variation in BM in the field conditions. For example, in the Seiland herd, where data were collected from some calves and yearlings in addition to adult females, the BM range was from 23 to 84 kg (mean 62.9 kg, IV, Table 2). There was also significant between-month and between-year variation in BM, illustrated by a 10 % mean BM loss over the winter months and a 4.2 kg higher mean BM in October 1998 than in October 1997 in the Seiland herd (II, Fig. 1a).

6 Discussion

The main seasonal changes in the studied blood constituents and BM in free-ranging reindeer are shown summarised in Fig. 1, whereas the changes in captive animals in relation to their protein, energy and mineral intake are presented in Fig. 2 and discussed separately below.

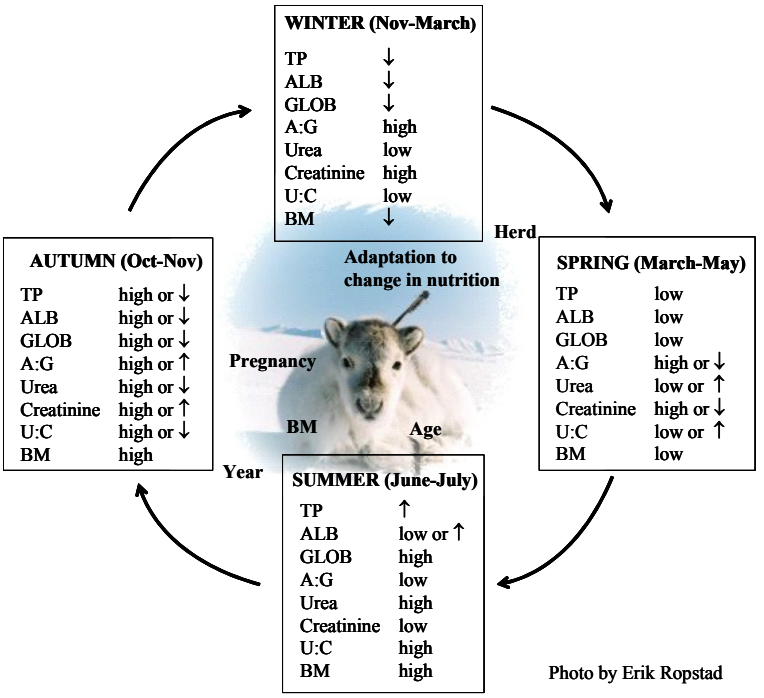

Fig. 1. Summary of the seasonal changes observed in the blood chemical constituents and BM of free-ranging reindeer. Other intrinsic and extrinsic factors studied as potential explanatory factors for the variation of the blood chemical constituents are represented in the centre of the figure. Symbols: ↑=increasing concentrations, ↓= decreasing concentrations.

In general, the feeding regimens used in the experimental studies were suitable for a comparison of the effects of nutritional constraints and feeding with a commercial ration on blood chemical constituents. As shown in Table 2, the CP and energy intakes of adult captive reindeer on restricted lichen feeding were markedly lower compared to the requirements presented for female reindeer or caribou in previous studies, whereas the protein and energy intake of the animals receiving commercial reindeer feed exceeded these estimates (McEwan & Whitehead 1970, Boertje 1985). As a consequence, the animals on restricted feeding were unable to maintain their BM, whereas the animals receiving commercial feed gained BM. The energy and CP intakes of the animals feeding lichens *ad libitum*, defined by their low protein, medium energy and low mineral intake, were intermediate compared to the animals on restricted feeding and the ones receiving commercial reindeer feed.

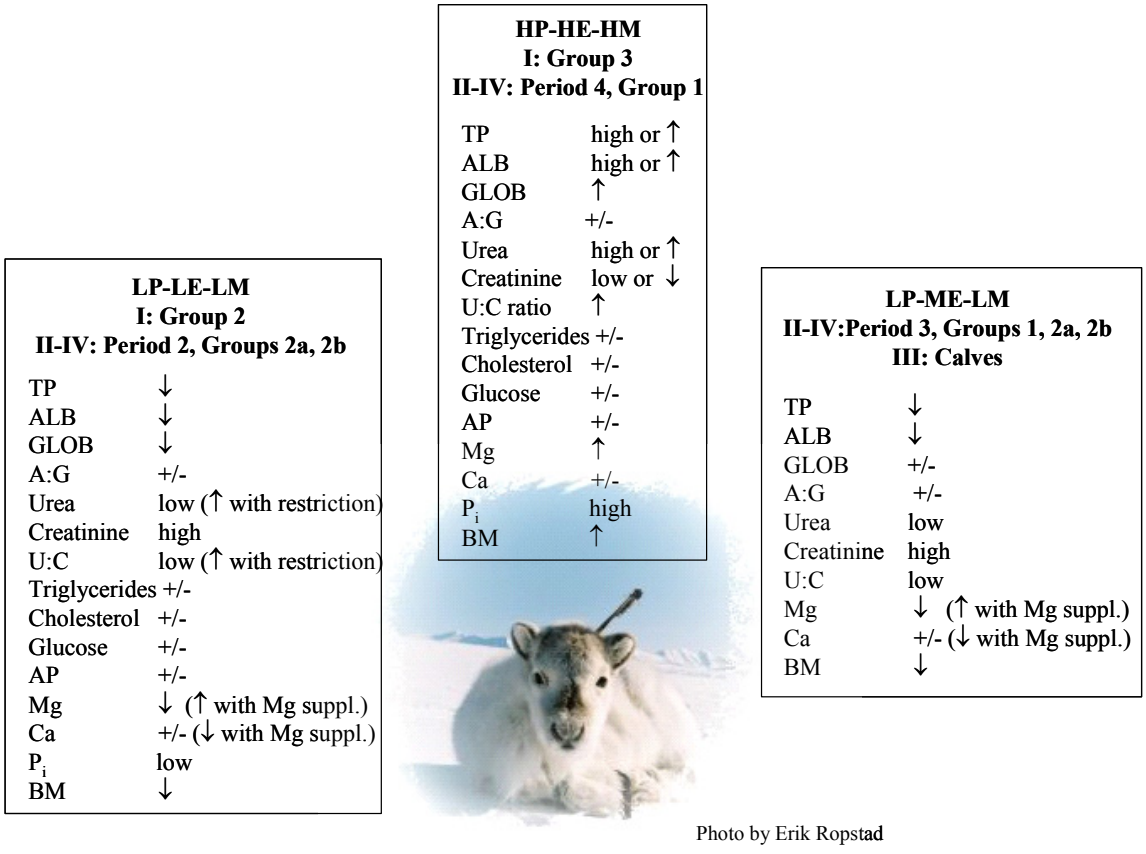

Photo by Erik Ropstad

Fig. 2. Summary of the changes observed in the blood chemical constituents and BM of captive reindeer in relation to feeding regimen. HP-HE-HM: high protein, high energy, high mineral intake, LP-ME-LM: low protein, medium energy, low mineral intake, LP-LE-LM: low protein, low energy, low mineral intake. Symbols: ↑=increasing concentrations, ↓= decreasing concentrations, ±= inconsistent changes.

6.1 Blood constituents related to protein metabolism

In free-ranging animals, the seasonal changes in blood constituents related to protein metabolism were characterized by a decrease in the plasma mean TP, ALB, GLOB and urea concentrations and in the plasma mean U:C ratio and a decrease in the plasma creatinine concentration and the mean BM over winter (Fig. 1). The seasonal changes and concentration ranges resembled those reported in earlier studies on reindeer (Hyvärinen *et al.* 1975, Nieminen 1980, Nieminen & Timisjärvi 1983), and the magnitude of variation in the plasma GLOB and A:G ratio also largely agreed with these and other previous studies (Nieminen *et al.* 1979). As expected, the concentration ranges of the studied blood constituents were wider than reported for domestic ruminants with, for example, a large proportion of the TP and ALB concentrations measured on the winter pasture (IV) falling below the lower normal limits of 60 and 24 g/l established for sheep (Kaneko 1989). Because free-ranging reindeer encounter a large variety of environmental, nutritional and physiological challenges during their seasonal cycle, it is more difficult to define the limits of "normal" variation in plasma protein parameters for reindeer than for domestic ruminant species with their less variable living conditions.

Season and year were the most important sources of variation in the plasma TP, ALB, GLOB, urea and creatinine concentrations and in the U:C ratio of free-ranging reindeer (II, IV). For example, the month of sampling accounted for 90 % of the variation in plasma urea concentrations and 65 % of the variation in plasma creatinine concentrations. Part of the variation in the plasma TP concentrations of free-ranging animals was also caused by the differences in pasture conditions between the herds, as shown by the fact that the Filefjell herd with good winter pasture conditions and the Sørøy herd with a lower animal density had higher mean plasma TP concentrations than the Magerøy herd in comparable years.

The results from captive animals in defined feeding conditions showed that both forage quality (protein intake; lichen feeding *vs.* commercial reindeer feed) and, to a lesser extent, forage availability (energy intake; *ad libitum vs.* reduced feed intake) explain the seasonal and between-year variation in blood constituents related to protein metabolism.

The concentrations of TP, ALB, GLOB and urea and the plasma U:C ratios were either significantly lower or decreasing, and the creatinine concentrations were either significantly higher or increasing in animals on lichen feeding compared to animals receiving commercial reindeer feed (Fig. 2). A decrease in the blood TP and ALB concentrations together with declining BM have been reported in previous studies on captive deer on lichen-based or other low-protein diets (Bjarghov *et al.* 1976, Soppela *et al.* 1992, Soveri *et al.* 1992, Wolkers *et al.* 1994, Nilsson 2003), which reflects the gradual loss of endogenous proteins when not enough amino acids are available for protein synthesis (Payne 1987). Plasma TP and ALB are considered long-term indicators of protein status compared to, for example, plasma urea, which responds to the immediate intake of protein (Payne 1987). As an example of this, a significant increase in the plasma TP and ALB concentrations of animals on commercial reindeer feed (IV) took place over a time period of 3-4 weeks, reflecting the long half-life of ALB and its relatively slow synthesis by the liver. Globulin concentrations were less affected by a low protein intake

than TP and ALB (IV), which could be partially attributable to a tendency to compensate for the decrease in ALB to maintain osmotic blood pressure (Payne 1987). It is also likely that immunological functions are maintained as long as possible, and that globulins are therefore better conserved during nutritional constraints than ALB. This may also explain the lack of interrelationship between TP, ALB and survival and fitness estimates (Milner *et al.* 2003).

Blood urea was chosen as the blood constituent reflecting short-term changes in protein metabolism and thus complementing the information provided by TP and ALB analyses (Payne 1987). The low blood urea and high creatinine concentrations, with the consequent reduction in the blood U:C ratio, which were recorded during lichen feeding (Fig. 2: LP-LE-LM and LP-ME-LM feeding regimen) can be related to physiological adaptation of the renal function in order to save protein through recycling of urea when dietary protein intake is low (Hove & Jacobsen 1975, Valtonen 1979). According to Hove and Jacobsen (1975), reindeer may reabsorb over 90 % of urea filtered in the kidneys when their CP intake is at a comparable level as in adult captive animals fed lichens *ad libitum* (Table 2: LP-ME-LM), with a concurrent decline in blood urea concentration close to 1 mmol/l. Consistent with earlier findings on both domesticated ruminants (Ide *et al.* 1966, Gonda & Lindberg 1994) and reindeer (Bjarghov *et al.* 1976), the mean blood urea concentrations were high or significantly increased in animals receiving commercial reindeer feed with high protein content (Fig. 2; HP-HE-HM), which can be attributed to a decrease in urea recycling when urea is increasingly excreted into urine (Robbins *et al.* 1974, Hove & Jacobsen 1975, Valtonen 1979).

The effect of protein intake on the blood urea concentration was confounded when a low protein intake was combined with a restricted feed intake. Restriction in feed intake by 20 % increased the mean plasma urea concentration by about 3.8 mmol/l compared to the animals receiving the same feed *ad libitum* (II). Warren and co-authors (1982) reported a similar increment in the blood urea concentration of white-tailed deer on low-energy forage, which can be explained by a decrease in urea recycling when the energy intake is insufficient (Valtonen 1979) or by increased production of urea due to muscle catabolism (Wolkers *et al.* 1994a,b). Analysis of creatinine kinase or some other indicator of muscle catabolism might have given additional information as to which of these processes explain the increase in urea concentration in the present study.

Because creatinine is freely filtered into urine by the kidneys, the significant increase in plasma creatinine concentration during lichen feeding with low protein content and the decrease on a high-protein diet (Fig. 2; LP-ME-LM *vs.* HP-HE-HM) may take place through the same renal mechanisms, which favours the recycling of urea when the dietary protein intake is low and increases its excretion into urine when the protein intake is high. The reindeer kidney has a thick cortex relative to the medulla and therefore a limited capacity to concentrate urine (Valtonen & Eriksson 1977). To excrete protein metabolites, such as urea, reindeer on high-protein diets have to increase their glomerular filtration rate and urine production compared to reindeer on low-protein diets (Valtonen 1979). The excretion of creatinine to urine increases in the presence of high protein intake (DelGiudice *et al.* 1995), and the increase in plasma creatinine concentration on a low-protein diet could therefore,result from the decrease in the glomerular filtration rate and the decrease in the excretion of creatinine into urine. The plasma creatinine concentration has also been suggested to increase when protein derived from muscle is used as an

energy source at times of nutritional deprivation (Nieminen & Timisjärvi 1983). However, our results indicate that insufficient energy intake affects plasma creatinine concentration only to a minor extent compared to the effect of protein intake, since no significant difference in the plasma creatinine level was observed between restricted and *ad libitum* lichen-fed reindeer (II).

BM had a significant, but small effect on plasma TP, ALB, GLOB, urea and creatinine concentrations compared to the effect of season. A 10 kg increase in BM was associated with around a 1 g/l increase in TP (IV), whereas BM accounted for 0.8 % of the variation in the plasma urea concentrations and 9 % of the variation in the plasma creatinine concentrations in free-ranging animals (II). In Svalbard reindeer, BM has been reported to account for 5.9 %, 2.6 % and 2.5 % of the variation in the plasma TP, ALB and GLOB concentrations (Milner *et al.* 2003), which is very similar to our findings. The minor effect of BM on creatinine concentration was surprising, because the amount of muscle mass is considered one of the most important factors affecting the production rate and concentration of blood creatinine (Rodwell 2000), but was not the only peculiarity found in free-ranging reindeer in respect to this blood constituent. Interestingly, the decrease in plasma creatinine concentrations in the captive animals (II, group 2, period 4) and in the wild Svalbard reindeer in April-May was unrelated both to feeding and to the BM change, which indicates that part of the variation in plasma creatinine concentrations is related neither to changes in feed quality and/or availability nor to BM change. One possible explanation could be innate seasonality in the renal function of reindeer unrelated to the quality of diet.

The late stages of gestation (March-May) were associated with a small but significant decrease in TP, ALB and GLOB (IV), which agrees with the findings on domestic ruminants indicating an increasing demand for amino acids for foetal growth closer to term (Kaneko 1989). Age also had a small but significant effect on TP, but eliminated pregnancy as a significant explanatory factor. This was expected, because younger, most likely non-pregnant animals were also included in this analysis. The relationship between age and TP concentration was curvilinear, with young and old animals having lower plasma TP concentrations than the other animals. This agrees with the recent findings on Svalbard reindeer (Milner *et al.* 2003) and is probably related to the poorer ability of young and old animals to compete for feed, dental wear by age, and the more catabolic metabolism of old animals.

The pattern of plasma U:C ratios was mainly determined by the plasma urea concentration. In captive reindeer, U:C ratios above 20 appeared either when a low protein intake was combined with a low energy intake and declining BM, or when both protein intake and energy intake were high and BM was increasing (Fig. 2 and II, Fig. 2d). A reduction in the plasma U:C ratio was observed when a low protein intake was combined with a medium energy intake accompanied by a slowdown in the BM loss rate. The reduction in the plasma U:C ratio has been interpreted to reflect a shift in metabolism towards conserved endogenous protein (Ramsay *et al.* 1991). Previous studies have reported a reduction in the urea nitrogen:creatinine ratio in both white-tailed deer (DelGiudice *et al.* 1992) and red deer (*Cervus elaphus*; Wolkers *et al.* 1994a) during nutritional restriction. However, the variation in the plasma U:C ratio in relation to protein and feed intake irrespective of BM changes indicates that the plasma U:C ratio is not solely useful as an index of nutritional condition.

Unlike the other blood constituents related to protein metabolism, the variation in the A:G ratio was not significantly explained by year, BM or pregnancy and was only slightly affected by feeding (IV). This could be partially explained by the method of determining the plasma GLOB concentration. Plasma GLOB was obtained by subtracting the measured ALB concentration from TP, and the resulting A:G ratio is less definitive than that obtained by direct analysis of GLOB. Therefore, when the concentration of plasma ALB changes parallel to the TP concentration, the A:G ratio remains unaltered. The reduction in the plasma A:G ratio observed in captive animals receiving lichens (IV, Group 2) resulted from a decline in plasma ALB with a consequent increase in GLOB towards the end of the study. The increase in GLOB can take place in compensation of the ALB loss (Payne 1987), and it was not at least caused by parasite infection because these animals had been previously given anti-parasitic treatment.

Altogether, especially the TP, ALB, urea and creatinine concentrations and the U:C ratio showed significant seasonal and between-year variation that could be related to physiological adaptation to changes in nutrition and therefore appear as useful indicators of the protein metabolism of reindeer. The use of creatinine concentration and U:C ratio may have additional value, as the former has been shown to significantly predict calving success in Svalbard reindeer, while the latter may improve adult survival models in ecological studies (Milner *et al.* 2003).

6.2 Blood constituents related to carbohydrate and lipid metabolism

The mean serum glucose concentrations were of the same order of magnitude as reported in free-ranging reindeer (Nieminen 1980, Nieminen & Timisjärvi 1983) and in domestic ruminants (Kaneko 1989). There was no significant difference in blood glucose concentrations between the animals on a restricted lichen diet and those fed a commercial ration (I). As in the present study, Luick and co-authors (1973), Bjarghov and co-authors (1976) and Soveri and co-authors (1992) did not find systematic changes in the blood glucose level related either to season or to nutrition. Low or declining blood glucose levels have been reported in fasted or nutritionally deprived deer (DeCalesta 1975, 1977, DelGiudice *et al.* 1987, Nieminen & Timisjärvi 1983, Wolkers *et al.* 1994a, Nilsson *et al.* 2000, Milner *et al.* 2003). However, the lack of group differences despite a 70 % reduction in the feed intake of the lichen-fed animals is not surprising in the light of the fact that the maintenance of a stable blood glucose level is essential for energy supply to he vital organs and thus for survival.

Because the changes in blood glucose level may be very rapid and are affected by numerous external and internal factors (Berne & Levy 1993), it is difficult to differentiate between the effects of nutrition and season and the potential effects of animal handling from each other. The blood glucose concentration of reindeer has been shown to increase as a physiological response to excitement (Hyvärinen *et al.* 1976). The blood glucose level of the supplemented winter diet group was higher compared to both the animals on commercial feed and those fed lichens (I). This group of animals was gathered together from a larger pen, and blood sampling may therefore have been more time-consuming and stressful for these animals, additionally confounding the interpretation of blood

glucose as a nutritional biomarker. Altogether, these findings suggest that the use of blood glucose as a nutritional indicator may be limited to occasions of severe nutritional deprivation, and that other blood constituents or parameters related to, for example, volatile fatty acid production and utilization could have shown more nutrition-related effects.

The mean serum cholesterol and triglyceride concentrations were within the range reported earlier in free-ranging reindeer (Nieminen & Timisjärvi 1983). Adipose tissue provides a vital store and endogenous source of energy for free-ranging deer for over-winter survival (Ringberg *et al.* 1981, Reimers *et al.* 1982, Torbit *et al.* 1985, Parker *et al.* 1993). As an indication of lipolysis and utilization of body fat for energy, the serum cholesterol concentration of the reindeer fed lichens with gradually restricted feed intake increased in March and April following a decrease during the first three weeks of the study (I), while the concentrations of serum TP, ALB, urea and creatinine simultaneously indicated insufficient protein and energy intake. In the groups fed either commercial reindeer feed or a supplemented winter diet, the mean serum cholesterol concentration varied between 1.3 and 2.0 mmol/l, and the animals on the supplemented winter diet showed increasing cholesterol concentrations from March onwards.

Serum triglycerides generally showed less variation in relation to feeding than serum cholesterol. However, the mean serum triglyceride concentration was significantly higher in April in the animals fed a restricted lichen diet compared to the other groups. Together with the simultaneous increase in serum cholesterol, this indicates that the animals were utilising their body fat reserves to compensate for the restricted dietary energy supply, as also reported in undernourished white-tailed deer (DelGiudice *et al.* 1990).

Commercial concentrates and other feedstuffs rich in lipids have been shown to increase plasma cholesterol and triglyceride concentrations in domestic ruminants (Beynen *et al.* 2000), but in this study, neither the cholesterol nor the triglyceride concentrations of the animals fed commercial reindeer feed were different from the other groups (I). It should also be noted that the feeding of supplemental fat does not only have the positive effect of increasing the energy density of the diet, but may also suppress rumen function if given in large amounts (Bartley 1989, McDonald *et al.* 1995). In addition, the feeding of supplemental fat has given variable results when used to improve productivity in domestic ruminant management (Funston 2004).

The results of this study and the previous studies about the nutrition-related changes in the blood triglyceride and cholesterol concentrations of domestic ruminants and captive deer show that these parameters may either decrease or increase as a consequence of nutritional constraints, thus confounding their interpretation as nutritional biomarkers (Bjarghov *et al.* 1976, Reid *et al.* 1977, Seal *et al.* 1978, DiMarco *et al.* 1981, DelGiudice *et al.* 1990, Soveri *et al.* 1992). Other blood constituents related to lipid metabolism and energy balance, such as free fatty acids and glycerol (Larsen *et al.* 1985), polyunsaturated fatty acids (Soppela *et al.* 2000) or β-hydroxybutyric acid (Milner *et al.* 2003) may be useful as additional or alternative indicators of lipolysis during nutritional constraints in reindeer.

6.3 Blood chemical constituents related to mineral metabolism

Several studies on free-ranging reindeer on winter pasture have reported low plasma concentrations of Mg, Ca and P_i compared to the normal reference values for domestic ruminants or clinical symptoms related to disturbances in Mg and Ca homeostasis (Hyvärinen *et al.* 1977, Åhman *et al.* 1986, Hoff *et al.* 1993, Ropstad *et al.* 1997).

Based on the low mineral content in the winter forage of reindeer and the progressive degradation of winter pasture areas, it can be expected that the occurrence of such disturbances in mineral homeostasis will increase, unless prevented by mineral supplements or supplementary feeds with high mineral content. Therefore, the main focus of this thesis was to study how low or high mineral intake and Mg supplementation were reflected in the selected blood constituents related to mineral metabolism (I, III).

Paper I focused on studying the effects of nutrition on serum AP activities and P_i concentrations in addition to serum Ca concentration, which is discussed in more detail in association with the findings of paper III. The daily P_i and Ca intakes of the lichen-fed group with restricted feed intake were only about 2.6% and 7.3 % of the corresponding values of the group fed commercial reindeer feed (I). This was reflected as significant group differences in the serum concentrations of P_i but not of Ca. Ca homeostasis, unlike P_i, is under strict hormonal control (Capen & Rosol 1989), which enables mobilization of Ca from bone reserves when the dietary Ca intake is low. The reported serum P_i concentrations were of the same magnitude as in the earlier studies on captive reindeer fed either lichens or concentrates (Bjarghov *et al.* 1976, Soveri *et al.* 1992). Low energy intake has also been reported to increase serum P_i concentration (Seal *et al.* 1978), but the present results do not confirm this finding. Even though commercial reindeer feed with a high phosphorus content had a positive effect on the serum P_i concentration, feeding of phosphorus in excess to the dietary requirements has not been shown to further improve the health, body condition or reproductive performance of domestic ruminants (Lopez *et al.* 2004a,b). It also increases fecal phosphorus excretion and hence has harmful effects on environmental eutrophication (Chapuis-Lardy *et al.* 2004). Physiologically, excessive P_i in blood has the ability to lower the amount of Ca in blood, resulting in increased mobilization of Ca from bone reserves (Capen & Rosol 1989).

The mean serum AP activities were generally lower in the animals fed lichens (Table 2, LP-LE-LM, Group 1) compared to the animals on a supplemented winter diet and to the animals fed a commercial ration of high nutritional value (Table 2, HP-HE-HM, Group 3), but the differences were not significant (I). The reported activities were within the same range as reported for free-ranging reindeer in winter (Nieminen & Timisjärvi 1983), but even lower activities were observed in the lichen-fed animals when their feed intake was gradually restricted to 30 % of the *ad libitum* level.

Parallel to the present findings, high AP activities have been reported in deer on rations of high nutritive value, whereas decreasing or low activities have been reported in both captive and free-ranging deer on nutritionally inadequate diets and during the winter (Hyvärinen *et al.* 1977, Seal *et al.* 1978, Nieminen 1980, Klinger *et al.* 1986, Soveri *et al.* 1992, Wolkers *et al.* 1994a). As AP is a non-specific marker of growth and metabolic activity, these findings have been concluded to reflect the cessation of growth and the slow-down of general metabolic activity during nutritional constraints.

Several dietary factors can mediate the low AP activity seen in the lichen-fed reindeer. In humans, dietary protein deficiency has been linked to a decrease in AP activity (Guthrie 1971), whereas the energy content of the diet was found to have the main effect on AP activity in white-tailed deer (Seal *et al.* 1978). In the lichen-fed reindeer, both protein and energy intakes were lower compared to the reindeer on a supplemented winter diet and to those receiving the commercial ration. Secondly, Mg acts as a cofactor for AP, increasing the activity of the apoenzyme (Bosron *et al.* 1977). Lichens contain 20 times less Mg than commercial reindeer feed (Nieminen & Heiskari 1989), and one explanation for the low AP activities of the lichen-fed animals may thus be their low Mg intake. The decrease in AP activity could also be related to a phosphorus deficiency (Capen & Rosol 1989), as the phosphorus intake of the lichen-fed animals was less than 3 % of the level of the animals feeding on the commercial ration, and their mean serum P_i concentration gradually decreased during the experiment.

Serum AP activities increased with advancing pregnancy independently of feeding (I), related to the increase in Ca absorption and mobilization for the needs of the growing fetus in white-tailed deer (Chao *et al.* 1985). Another likely reason for this increase taking place during gestation could be, as in humans, the release of AP into circulation by the placenta (Guthrie 1971, Ellonen 1995).

The effects of lichen feeding with low dietary Mg intake on plasma Mg concentration were clear. Blood Ca concentration, on the other hand, only slightly decreased (III) or varied inconsistently and was not significantly different in the reindeer fed lichens compared to those fed concentrates with high overall nutrient content (III).

When lichens were given as the only feed, either restricted or *ad libitum* (III, Groups 1 and 2b), the mean plasma Mg concentrations dropped below the concentration of 0.7 mmol/l considered hypomagnesemic in about three weeks. The lowest measured mean and individual plasma Mg concentrations were 0.48 and 0.31 mmol/l, but none of the animals showed clinical signs of Mg deficiency. This may be expected, since reindeer with clinical symptoms of Mg –defieciency, such as ataxia and paresis, have had extremely low blood Mg concentrations (Hoff *et al.* 1993), and the low Mg concentrations have been combined with low blood Ca levels and exhausted bone reserves of Ca in affected animals (Hyvärinen *et al.* 1977). In addition, the decrease of urinary Mg:C ratios in the rumen-cannulated reindeer calves given lichens as the only feed indicates effective renal conservation of this element when the dietary Mg intake is low. This supports the evidence of a potent renal control mechanism between Mg and Ca (Halse 1984).

Both of the tested Mg supplements significantly increased plasma Mg and decreased plasma Ca on an otherwise low-mineral diet (Table 2, Fig. 2). Due to the different supplement formulations and dosage schedules (daily *vs.* single dose), the response profiles of plasma Mg and Ca were different. Mg alloy rumen boluses increased plasma Mg by an average of 0.3 mmol/l within 2-6 days, after which the plasma Mg concentration gradually declined. This transient increase in plasma Mg after the insertion of the rumen boluses resembled the response in sheep supplemented with similar boluses (House & Mayland 1976) and was accompanied by a temporary, but significant decrease in plasma Ca. During MgO supplementation, the plasma Mg concentration increased above 1 mmol/l within a few days, accompanied by a simultaneous decrease in plasma Ca, and both changes persisted throughout the supplementation.

According to the results of the previous studies with various Mg supplements, several physiological control mechanisms can mediate the plasma Ca-decreasing effect of Mg supplementation. The present results from rumen-cannulated reindeer calves with sustain-release Mg alloy rumen boluses (III) indicate that this effect could take place through increased urinary Ca excretion following the Mg bolus insertion and a concurrent increase in urinary Mg excretion. House and Mayland (1976) reported a similar increase in the urinary excretion of Mg during Mg alloy rumen bolus supplementation, accompanied by an increase in urinary Ca excretion. In bovine steers, a decrease in blood Ca concentration concurrent with an increase of dietary Mg intake and blood Mg concentration has been related to a decrease in Ca absorption from the alimentary tract Chester-Jones *et al.* (1990).

The correlation between plasma Mg and Ca varied, depending on the concentration range of plasma Mg. The previous results regarding the correlation between plasma Mg and Ca concentrations have included both a positive correlation in reindeer (Ropstad *et al.* 1997) and a negative correlation in hypocalcemic periparturient dairy cows (Riond *et al.* 1985). The controversy in these findings may be caused by the complexity and large number of regulation mechanisms that control the concentration of these minerals in blood, including the hormonal regulation of plasma Ca concentration in addition to the previously discussed renal and intestinal mechanisms.

In reindeer management, sustain-release Mg alloy rumen boluses that require only a single dose over several weeks would be beneficial over Mg supplementation methods that require more frequent handling of the animals. However, the efficacy of rumen boluses was slightly questionable based on the considerable between-individual variation in the bolus weight loss, which makes the duration of supplementation unpredictable.

Rumen content pH seemed to affect the rate of bolus decomposition, being one determinant of the rumen bolus efficacy as a Mg supplement. In general, the rumen content pH decreased and the rumen decomposition rate was slower during lichen feeding, whereas the rumen content pH and the bolus decomposition rate increased when commercial reindeer feed was given to the animals at the later stages of the experiment (III). In further studies, a closer look could be taken on other factors, such as the composition of the feed, which could potentially affect the decomposition rate of the rumen boluses.

The fact that the plasma Mg concentration of the animals given commercial reindeer feed increased to the same level as that in the MgO-supplemented animals indicates that no other form of Mg supplementation is required when commercial feed containing an adequate amount of Mg is provided to reindeer. However, not all individuals consume the feeds in equal amounts, and some animals might therefore still be susceptible to Mg deficiency.

In summary, blood Mg and P_i concentrations responded significantly to changes in their intake, whereas Ca concentration showed variable, often not significant responses. However, Mg supplementation significantly decreased plasma Ca concentration, possibly through increased urinary Ca excretion, indicating a common renal control mechanism between these minerals. As for P_i, serum AP activities were lower in the lichen-fed animals compared to the animals on commercial reindeer feed, but the lack of significant differences between the groups and the unspecific nature of this enzyme does not recommend its use as a nutritional biomarker for reindeer.

7 Conclusions

The present findings showed that both free-ranging and captive female reindeer under defined feeding conditions show marked variation in the concentrations of blood chemical constituents related to protein, energy and mineral metabolism. Intrinsic factors, such as BM, pregnancy and age, had only a minor influence on the variation of blood constituents, whereas extrinsic factors, such as season and year, which were characterized by large differences in environmental and nutritional conditions, explained a majority of the variation.

The results on both captive animals in defined feeding conditions and free-ranging animals led to the conclusion that TP, ALB, urea, creatinine, U:C ratio, Mg and Pi and, to a lesser extent, GLOB and A:G ratio respond to changes in feed quality and availability and are the most suitable blood constituents to be used as nutritional biomarkers for reindeer. A suitably selected range of blood constituents reflecting different aspects of nutrition is likely to give a reliable picture of the animal's nutritional condition, especially when combined with measurements of body mass.

Further studies are needed to establish reference values for reindeer blood chemical constituents at different seasons and different levels of nutrition, to further investigate blood chemical constituents as predictors of animal fitness and survival and to take a closer look on the relationship between blood chemical constituents, BM change and other indicators of animal condition, such as bone marrow fat or bone density.

Modern reindeer management is approaching a crossroad. If management is to be based on the use of natural pasture resources, blood chemical constituents could be used to monitor the condition of reindeer in studies where herd productivity levels in different pasture conditions or the sustainable use of pasture resources are assessed. If supplementary feeding is increasingly employed, the potential implications could include development of feeding recommendations for different production levels and monitoring of animal nutrition and welfare in different management systems, feeding areas and enclosures and during the transportation to slaughterhouses.

References

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

Adamczewski JZ, Gates CC, Hudson RJ & Price MA (1987) Seasonal changes in body composition of mature female caribou and calves (*Rangifer tarandus groenlandicus*) on an arctic island with limited winter resources. Can J Zool 65: 1149-1157.

Albon SD, Stien A, Irvine RJ, Langvatn R, Ropstad E & Halvorsen O (2002) The role of parasites in the dynamics of a reindeer population. Proc R Soc Lond B 269: 1625-1632.

Allain CC, Poon LS, Chan CSG, Richmond W & Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20: 470-475.

Allcroft WM & Green HM (1938) Seasonal hypomagnesemia of the bovine, without clinical symptoms. J Comp Pathol Therap 51: 176-191.

Baker RM, Boston RC, Boyes TE & Leaver DD (1979) Variations in the response of sheep to experimental magnesium deficiency. Res Vet Sci 26: 129-133.

Bartley JC (1989) Lipid metabolism and its diseases. In: Kaneko JJ (ed) Clinical Biochemistry of Domestic Animals. 4th Edition. Academic Press, New York, p 106-141.

Bauman DE, Perfield JW, de Veth MJ & Lock AL (2003) New perspectives on lipid digestion and metabolism in ruminants. Proceedings Cornell Nutrition Conference for Feed Manufacturers, October 20-23, Syracuse, NY, p 175-189.

Berne RM & Levy MN (1993) Physiology. 3rd Edition. Mosby Year Book, St. Louis.

Beynen AC, Schonewille JTh & Terpstra AHM (2000) Influence of amount and type of dietary fat on plasma cholesterol concentrations in goats. Small Ruminant Res 35: 141-147.

Bjarghov RS, Fjellheim P, Hove K, Jacobsen E, Skjenneberg S & Try K (1976) Nutritional effects on serum enzymes and other blood constituents in reindeer calves (*Rangifer tarandus tarandus*). Comp Biochem Physiol 55A: 187-193.

Boertje RD (1985) An energy model for adult female caribou of the Denali herd, Alaska. J Range Manage 38: 468-473.

Bosron WF, Anderson RA, Falk MC, Kennedy FS &Vallee BL (1977) Effect of magnesium on the properties of zinc alkaline phosphatase. Biochemistry 16: 610-614.

Cameron RD, Smith WT, Fancy ST, Gerhart KL & White RG (1993) Calving success of female caribou in relation to body weight. Can J Zool 71: 480-486.

Capen CC & Rosol TJ (1989) Calcium-regulating hormones and diseases of abnormal mineral (calcium, phosphorus, magnesium) metabolism. In: Kaneko JJ (ed) Clinical Biochemistry of Domestic Animals. 4th Edition. Academic Press, New York, p 678-752.

Chao CC, Brown RD & Deftos LJ (1985) Metabolism of calcium and phosphorus during pregnangy and lactation in white-tailed deer. Acta Endocr 109: 269-275.

Chapuis-Lardy L, Fiorini J, Toth J & Dou Z (2004) Phosphorus concentration and solubility in dairy feces: variability and affecting factors. J Dairy Sci 87: 4334-4341.

Chasson AL, Grady HJ & Stanley MA (1961) Determination of creatinine by means of automatic chemical analysis. Am J Clin Pathol 35: 83-88.

Chester-Jones H, Fontenot JP & Veit HP (1990) Physiological and pathological effects of feeding high levels of magnesium to steers. J Anim Sci 68: 4400-4413.

Chicco CF, Ammerman CB, Hillis WG & Arrington LR (1972) Utilization of dietary magnesium by sheep. Am J Physiol 222: 1469-1472.

The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974) Recommended methods for the determination of four enzymes in blood. Scand J Clin Lab Invest 33:291-306.

Cook RC, Cook JG, Murray DL, Zager P, Johnson BK & Gratson MW (2001) Development of predictive models of nutritional condition for Rocky Mountain elk. J Wildl Manage 65: 973-987.

Daly JA & Ertingshausen G (1972) Direct method for determining inorganic phosphate in serum with the "CentrifiChem". Clin Chem 18: 263-265.

Davidian M & Giltinan DM (1995) Nonlinear Models for Repeated Measurement Data. Chapman & Hall, London.

DeCalesta DS, Nagy JG & Bailey JA (1975) Starving and refeeding mule deer. J Wildl Manage 39: 663-669.

DeCalesta DS, Nagy JG & Bailey JA (1977) Experiments on starvation and recovery of mule deer does. J Wildl Manage 41: 81-86.

DelGiudice GD, Asleson MA, Varner LW & Hellgren EC (1995) Twenty-four-hour urinary creatinine and urea nitrogen excretion in male white-tailed deer. Can J Zool 73: 493-501.

DelGiudice GD, Mech LD, Kunkel KE, Gese EM & Seal US (1992) Seasonal patterns of weight, hematology, and serum characteristics of free-ranging female white-tailed deer in Minnesota. Can J Zool 70: 974-983.

DelGiudice GD, Mech LD & Seal US (1990) Effects of winter undernutrition on body composition and physiological profiles of white-tailed deer. J Wildl Manage 54: 539-550.

DelGiudice GD, Mech LD, Seal US & Karns PD (1987) Effects of winter fasting and refeeding on white-tailed deer blood profiles. J Wildl Manage 51: 865-873.

DiMarco NM, Beitz DC & Whitehurst GB (1981) Effect of fasting on free fatty acid, glycerol and cholesterol concentrations in blood plasma and lipoprotein lipase activity in adipose tissue of cattle. J Anim Sci 52: 75-82.

Doumas BT, Watson WA & Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31: 87-96.

Ellonen M (1995) Alkalinen fosfataasi. In: Kunnamo I, Nyberg P, Alenius H, Ellonen M, Eskola K, Jousimaa J, Keinänen-Kiukaanniemi S, Mäkelä M, Pitkälä K, Saarelainen M, Varonen H & Voutilainen S (eds) Yleislääkärin käsikirja. Duodecim, Helsinki, p 303-304.

Evans R (1996) Some impacts of overgrazing by reindeer in Finnmark, Norway. Rangifer 16(1): 3-19.

Fabiny DL & Ertingshausen G (1971) Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. Clin Chem 17: 696-700.

Forbes GB (1988) Body composition: influence of nutrition, disease, growth and ageing. In: Shils, ME & Young VR (eds) Modern Nutrition in Health and Disease. Lea and Febiger, Philadelphia, p 533-556.

Franzmann AW (1985) Assessment of nutritional status. In: Hudson RJ & White RG (eds) Bioenergetics of Wild Herbivores. CRC Press, Boca Raton, p 239-259.

Funston RN (2004) Fat supplementation and reproduction in beef females. J Anim Sci 82: E154-161.

Gerhart KL, White RG, Cameron RD & Russell DE (1996a) Body composition and nutrient reserves of arctic caribou. Can J Zool 74: 136-146.

Gerhart KL, White RG, Cameron RD & Russell DE (1996b) Estimating fat content of caribou from body condition scores. J Wildl Manage 60: 713-718.

Gindler EM & Heth DA (1971) Colorimetric determination with bound ”calmagite” of magnesium in human blood serum. Clin Chem 17: 662.

Gitelman HJ (1967) An improved automated procedure for the determination of calcium in biochemical specimen. Anal Biochem 18: 521-531.

Goff JP (1999) Treatment of calcium, phosphorus, and magnesium balance disorders. Vet Clin N Am -Food Anim Pract 15: 619-639.

Gonda HL & Lindberg JE (1994) Evaluation of dietary nitrogen utilization in dairy cows based on urea concentrations in blood, urine and milk, and on urinary concentration of purine derivatives. Acta Agric Scand Sect A -Anim Sci 44: 236-245.

Guthrie HA (1971) Introductory Nutrition. 2nd Edition. The C.V. Mosby Company, St Louis.

Guttman I & Bergmeyer HU (1974) Determination of urea with glutamate dehydrogenase as indicator enzyme. In: Bergmeyer HU (ed) Methods of Enzymatic Analysis. Academic Press, New York, p 1794-1798.

Halse K (1984) Calcium effects on renal conservation of magnesium in cows. Acta Vet Scand 25: 213-228.

Halse K, Skjenneberg S, Jacobsen E & Bjarghov RS (1976) Blood magnesium and the renal magnesium treshold in lichen fed and fasted reindeer. Nord Vetmed 28: 529-538.

Helle T (1980) Laiduntilanteen muutokset ja riskinotto Suomen poronhoidossa. Lapin tutkimusseuran vuosikirja XXI: 13-22.

Helle T, Kilpelä S-S & Aikio P (1990) Lichen ranges, animal densities and production in Finnish reindeer management. Rangifer, Special Issue No. 3: 115-121.

Helle T & Saastamoinen O (1980) Porojen laitumet ja ruokintatavat talvina 1975 ja 1977. Poromies 1/1980: 6-8.

Hemingway RG & Ritchie NS (1969) Magnesium alloy bullets for suckling calves. Vet Rec 83: 465-467.

Hoff B, Rognmo A, Havre G & Morberg H (1993) Seasonal hypomagnesemia in reindeer on Kautokeino winter pasture in Finnmark County, Norway. Rangifer 13(3): 133-136.

House WA & Mayland HF (1976) Magnesium and calcium utilization in sheep treated with magnesium alloy rumen bullets or fed magnesium sulfate. J Anim Sci 42: 506.

Hove K & Jacobsen E (1975) Renal excretion of urea in reindeer. Effect of nutrition. Acta Vet Scand 16: 513-519.

Huntington GB & Archibeque SL (1999) Practical aspects of urea and ammonia metabolism in ruminants. Proceedings of the American Society of Animal Science, Annual Meeting 1999, http://www.asas.org/JAS/symposia/proceedings/0939.pdf. Accessed 10.2.2005.

Hussain Q, Havrevoll Ø, Eik LO & Ropstad E (1996) Effects of energy intake on plasma glucose, non-esterified fatty acids and acetoacetate concentration in pregnant goats. Small Ruminant Res 21: 89-96.

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

Hyvärinen H, Helle T, Nieminen M, Väyrynen P & Väyrynen R (1977) The influence of nutrition and seasonal conditions on mineral status in the reindeer. Can J Zool 55: 648-655.

Hyvärinen H, Helle T, Väyrynen R & Väyrynen P (1975) Seasonal and nutritional effects on serum protein and urea concentration in the reindeer (*Rangifer tarandus tarandus* L.). Br J Nutr 33: 63-72.

Ide Y, Shimbayashi K & Yonemura T (1966) Effect of dietary conditions upon serum and milk-urea nitrogen in cows. I. Serum and milk-urea nitrogen as affected by protein intake. Jap J Vet Sci 28: 321-327.

Jacobsen E & Skjenneberg S (1977) Fordøyelighet av lav til rein. Forskn og Forsøk i Landbr 28: 63-67.

Johansen B, Tømmervik H & Karlsen SR (1996) Reinbeitene sett fra satellitt. Ottar 211(3): 17-24.

Kaneko JJ (1989) Clinical Biochemistry of Domestic Animals, 4th Edition. Academic Press, New York.

Kautto A, Kärenlampi L & Nieminen M (1986) Jäkäläisten talvilaidunten kunnon muutos Suomen poronhoitoalueella vuosina 1972-1983. Poromies 3/1986: 28-34.

Klinger SR, Robel RJ, Brown BA & Brent BE (1986) Blood characteristics of white-tailed deer from northeastern Kansas. J Wildl Dis 22: 385-388.

Kojola I & Helle T (1993) Calf harvest and reproductive rate of reindeer in Finland. J Wildl Manage 57: 451-453.

Kojola I, Helle T, Aikio P & Niskanen M (1993) Talvilaitumet ja porojen talviravinto Pohjois-Lapissa. Poromies 2/1993: 34-37.

Kumpula J, Colpaert A, Kumpula T & Nieminen M (1997) Suomen poronhoitoalueen talvilaidunvarat. Kala- ja riistaraportteja nro 93, Helsinki.

Kumpula J, Colpaert A & Nieminen M (2002) Productivity factors of the Finnish semi-domesticated reindeer (*Rangifer t. tarandus*) stock during the 1990s. Rangifer 22(1): 3-12.

Larsen TS & Blix AS (1985) Seasonal changes in total body water, body composition and water turnover in reindeer. Rangifer 5(1): 2-9.

Larsen TS, Nilsson NÖ & Blix AS (1985) Effects of prolonged food restriction on some aspects of lipid metabolism in Norwegian and Svalbard reindeer. Acta Physiol Scand 124: 173-180.

Leibholtz J (1970) The effect of starvation and low nitrogen intake on the concentration of free amino-acids in the blood plasma and on the nitrogen metabolism in sheep. Aust J Agric Res 21: 723-734.

Liepa GU, Beitz DC & Linder JR (1978) Cholesterol synthesis in ruminating and nonruminating goats. J Nutr 108: 535-543.

Littell RC, Milliken GA, Stroup WW & Wolfinger RD (1996) SAS System for Mixed Models. SAS Inst, Cary, NC.

Lopez H, Kanitz FD, Moreira VR, Wiltbank MC & Satter LD (2004a) Effect of dietary phosphorus on performance of lactating dairy cows: milk production and cow health. J Dairy Sci 87: 139-145.

Lopez H, Kanitz FD, Moreira VR, Satter LD & Wiltbank MC (2004b) Reproductive performance of dairy cows fed two concentrations of phosphorus. J Dairy Sci 87: 146-157.

Lowry OH, Rosenbrough NJ, Farr L & Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193: 265.

Luick JR, Person SJ, Cameron RD & White RG (1973) Seasonal variations in glucose metabolism of reindeer (*Rangifer tarandus*) estimated with [U-14C] glucose and [3-3H] glucose. Br J Nutr 29: 245-259.

MAFF (1975) Energy allowances and feeding systems for ruminants. Tech Bull 33. Her Majesty's Stationery Office, London.

MAFF (1984) Energy allowances and feeding systems for ruminants. Reference Book 433. Her Majesty's Stationary Office, London.

Maijala V & Niminen M (2004) Poron ympärivuotinen ruokinta ja sen kannattavuus. Kala- ja riistaraportteja nro 304, Helsinki.

Mann CK & Yoe JH (1956) Spectrophotometric determination of magnesium with sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)naphtalene-1'(2-hydroxy-benzene-5-sulfonate). Anal Chem 28: 202-205.

Martens H & Schweigel M (2000) Pathophysiology of grass tetany and other hypomagnesemias. Implications for clinical management. In: Metabolic Disorders of Ruminants. Vet Clin N Am - Food Anim Pract 16: 339-368.

Matthews JNS, Altman DG, Campbell MJ & Royston P (1990) Analysis of serial measurements in medical research. BMJ 300: 230-235.

Mautz WW, Seal US & Boardman CB (1980) Blood serum analyses of chemically and physically restrained white-tailed deer. J Wildl Manage 44: 343-351.

McCullagh P & Nelder JA (1989) Generalized Linear Models. 2nd Edition. Chapman & Hall, London.

McDonald P, Edwards RA, Greenhalgh JFD & Morgan CA (1995) Animal Nutrition. 5th Edition. Longman Scientific & Techical, Harlow.

McEwan EH (1968) Hematological studies of barren-ground caribou. Can J Zool 46: 1031-1036.

McEwan EH & Whitehead PE (1969) Changes in the blood constituents of reindeer and caribou occurring with age. Can J Zool 47: 557-562.

McEwan EH & Whitehead PE (1970) Seasonal changes in the energy and nitrogen intake in reindeer and caribou. Can J Zool 48: 905-913.

McEwan EH & Whitehead PE (1972) Reproduction in female reindeer and caribou. Can J Zool 50: 43-46.

McLaren DS (1988) Clinical manifestations of nutritional disorders. In: Shils ME & Young VR (eds) Modern Nutrition in Health and Disease. Lea and Febiger, Philadelphia, p 733-745.

Milner JM, Stien A, Irvine RJ, Albon SD, Langvatn R & Ropstad E (2003) Body condition in Svalbard reindeer and the use of blood parameters as indicators of condition and fitness. Can J Zool 81: 1566-1578.

Nestel PJ, Poyser A, Hood RL, Mills SC, Willis MR, Cook LJ & Scott TW (1978) The effect of dietary fat supplements on cholesterol metabolism in ruminants. J Lipid Res 19: 899-909.

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

Nieminen M (1988) Porojen laitumet ja ruokinta poronhoitovuonna 1986-1987. I. Laitumet ja niiden kunto. Poromies 6/1989: 8-19.

Nieminen M & Autto P (1989) Porojen laitumet ja ruokinta poronhoitovuonna 1986-1987. II. Rehut ja ruokinta. Poromies 2/1989: 38-43.

Nieminen M & Heiskari U (1989) Diets of freely grazing and captive reindeer during summer and winter. Rangifer 9(1): 17-34.

Nieminen M, Koskela M, Leinonen M & Timisjärvi J (1979) Electrophoretical and immunoelectrophoretical studies on serum proteins in growing and fully-grown reindeer (*Rangifer tarandus tarandus* L.). Comp Biochem Physiol 65B: 35-44.

Nieminen M, Maijala V & Soveri T (1998) Poron ruokinta. Riista- ja kalatalouden tutkimuslaitos, Painorauma, Rauma, 142 p.

Nieminen M & Risto A (1990) Teolliset poronrehut. Poromies 3/1990: 40-46.

Nieminen M & Timisjärvi J (1983) Blood composition of the reindeer. II. Blood chemistry. Rangifer 3(1): 16-32.

Nilssen KJ, Sundsfjord JA & Blix AS (1984) Regulation of metabolic rate in Svalbard and Norwegian reindeer. Am J Physiol 247: R837-R841.

Nilsson A (2003) Adaptation of semi-domesticated reindeer to emergency feeding. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala. Acta Universitatis Agriculturae Sueciae, Agraria 399.

Nilsson A, Danell Ö, Murphy M, Olsson K & Åhman B (2000) Health, body condition and blood metabolites in reindeer after submaintenance feed intake and subsequent feeding. Rangifer 20(4): 187-200.

Parker KL, Gillingham MP, Hanley TA & Robbins CT (1993) Seasonal patterns in body mass, body composition, and water transfer rates of free-ranging and captive black-tailed deer (*Odocoileus hemionus sitkensis*) in Alaska. Can J Zool 71: 1397-1404.

Payne JM (1987) Indicators of protein status. In: Payne JM & Payne S (eds) The Metabolic Profile Test. Oxford University Press, New York, p 27-35.

Payne JM, Dew SM, Manston R & Faulks M (1970) The use of a metabolic profile test in dairy herds. Vet Rec 87: 150-158.

Ramsay MA, Nelson RA & Stirling I (1991) Seasonal changes in the ratio of serum urea to creatinine in feeding and fasting polar bears. Can J Zool 69: 298-302.

Reid IM, Stark AJ & Isenor RN (1977) Fasting and refeeding in the lactating dairy cow. 1. The recovery of milk yield and blood chemistry following a six-day fast. J Comp Path: 87: 241-251.

Reimers E (1983) Reproduction in wild reindeer in Norway. Can J Zool 61: 211-217.

Reimers E (1984) Body composition and population regulation of Svalbard reindeer. Rangifer 4(2):16-21.

Reimers E (2002) Calving time and foetus growth among wild reindeer in Norway. Rangifer 22(1): 61-66.

Reimers E & Ringberg T (1983) Seasonal changes in body weights of Svalbard reindeer from birth to maturity. Acta Zool Fennica 175: 69-72.

Reimers E, Ringberg T & Sørumgård R (1982) Body composition of Svalbard reindeer. Can J Zool 60: 1812-1821.

Reindriftsforvaltningen (2003) Totalregnskap 2003 og budsjett 2004. Alta, Norway.
Ringberg T, White RG, Holleman DF & Luick JR (1981) Body growth and carcass composition of lean reindeer (*Rangifer tarandus tarandus*) from birth to maturity. Can J Zool 59: 1040-1044.
Riond J-L, Kocabagli N, Spichiger UE & Wanner M (1995) Postparturient hypocalcemia of dairy cows: a model for the study of the interdependence of Ca, P_i and Mg homeostasis. Bone 17: 429S-434S.
Riseth JÅ, Johansen B & Vatn A (2004) Aspects of a two-pasture -herbivore model. Rangifer, Special Issue No. 15: 65-81.
Ritchie NS & Hemingway RG (1968) Magnesium alloy bullets for dairy cattle. Vet Rec 82: 87-89.
Robbins CT, Prior RL, Moen AN & Visek WJ (1974) Nitrogen metabolism of white-tailed deer. J Anim Sci 38: 186-191.
Rodwell VW (2000) Conversion of amino acids to specialized products. In: Murray RK, Granner DK, Mayes PA & Rodwell VW (2000) Harper's Biochemistry. 25th Edition. Appleton & Lange, Stamford, p 347-358.
Roil MR, Suckling GW & Mattingley J (1974) Serum total protein and albumin levels in grazing sheep. N Z Vet J 22: 232-236.
Ropstad E, Johansen O, Halse K, Morberg H & Dahl E (1997) Plasma magnesium, calcium and inorganic phosphate in Norwegian semi-domestic female reindeer (*Rangifer tarandus tarandus*) on winter pastures. Acta Vet Scand 38: 299-313.
Ropstad E, Johansen O, King C, Dahl E, Albon SD, Langvatn RL, Irvine RJ, Halvorsen O & Sasser G (1999) Comparison of plasma progesterone, transrectal ultrasound and pregnancy specific proteins (PSPB) used for pregnancy diagnosis in reindeer. Acta Vet Scand 40: 151-162.
Ropstad E, Vik-Mo L & Refsdal AO (1989) Levels of milk urea, plasma constituents and rumen liquid ammonia in relation to the feeding of dairy cows during early lactation. Acta Vet Scand 30: 199-208.
Rossignol B, Rossignol D & PetitClerc C (1984) Improvement of creatinine measurement on RA-1000. Clin Biochem 17: 203-204.
Ryg M & Jacobsen E (1982) Seasonal changes in growth rate, feed intake, growth hormone, and thyroid hormones in young male reindeer (*Rangifer tarandus tarandus*). Can J Zool 60: 15-23.
Seal US, Verme LJ & Ozoga JJ (1978) Dietary protein and energy effects on deer fawn metabolic patterns. J Wildl Manage 42: 776-790.
Skogland T (1984) The effects of food and maternal conditions on foetal growth and size in wild reindeer. Rangifer 4(2): 39-46.
Sletten H & Hove K (1990) Digestive studies with a feed developed for realimentation of starving reindeer. Rangifer 10(1): 31-37.
Smith WH, Lechtenberg VL & Hodges JR (1974) Grass tetany –Incidence and methods of prevention. J Anim Sci 39: 1001.
Soppela P, Heiskari U, Nieminen M, Salminen I, Sankari S & Kindahl H (2000) The effects of a prolonged undernutrition on serum lipids and fatty acid composition of reindeer calves during winter and spring. Acta Physiol Scand 168: 337-350.
Soppela P, Nieminen M & Saarela S (1992) Water intake and its thermal energy cost in reindeer fed lichen or various protein rations during winter. Acta Physiol Scand 145: 65-73.
Soveri T, Sankari S & Nieminen M (1992) Blood chemistry of reindeer calves (*Rangifer tarandus*) during the winter season. Comp Biochem Physiol 102A:191-196.
Staaland H (1986) Svalbardreinens ernæring. In: Øritsland NA (ed) Svalbardreinen og dens livsgrunnlag. Universitetsforlaget, Oslo, p 72-91.
Staaland H, Brattbakk I, Ekern K & Kildemo K (1983) Chemical composition of reindeer forage plants in Svalbard and Norway. Holarctic Ecology 6: 109-122.
Statistics of the Finnish Reindeer Herders' Association. Published each year in the second number of the journal *Poromies*.
Stien A, Irvine RJ, Langvatn R & Ropstad E (2003) Evaluation of ultrasound scanning as a method for measuring subcutaneous fat in Svalbard reindeer. Rangifer 23(2): 71-74.
Stien A, Irvine RJ, Ropstad E, Halvorsen O, Langvatn R & Albon SD (2002) The impact of gastrointestinal nematodes on wild reindeer: experimental and cross-sectional studies. J Anim Ecol 71: 937-945.

Stuedemann JA, Wilkinson SR & Lowrey RS (1984) Efficacy of a large magnesium alloy rumen bolus in the prevention of hypomagnesemic tetany in cows. Am J Vet Res 45: 698-702.

Suttie JM & Webster JR (1995) Extreme seasonal growth in arctic deer: comparisons and control mechanisms. Amer Zool 35: 215-221.

Tiffany TO, Jansen JM, Burtis CA, Overton JB & Scott CD (1972) Enzymatic kinetic rate and end-point analyze of substrate, by use of a GeMSAEC fast analyzer. Clin Chem 18: 829.

Torbit SC, Carpenter LH, Swift DM & Alldredge AW (1985) Differential loss of fat and protein by mule deer during winter. J Wildl Manage: 49: 80-85.

Trinder P (1969a) Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Path 22: 246.

Trinder P (1969b) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 6:24-27.

Tuori M, Kaustell K, Valaja J, Aimonen E, Saarisalo E & Huhtanen P (1995) Rehutaulukot ja ruokintasuositukset. Yliopistopaino, Helsinki.

Valmari A (1993a) Poronhoitoalueen luonnonolot. In: Huttu-Hiltunen V, Nieminen M, Valmari A & Westerling B (eds) Porotalous. Painatuskeskus, Helsinki, p 57-75.

Valmari A (1993b) Porojen lisäruokinta. In: Huttu-Hiltunen V, Nieminen M, Valmari A & Westerling B (eds) Porotalous. Painatuskeskus, Helsinki, p 91-106.

Valtonen M (1979) Renal responses of reindeer to high and low protein diet and sodium supplement. J Scient Agrig Soc Finl 51: 381-419.

Valtonen M & Eriksson L (1977) Responses of reindeer to water loading, water restriction and ADH. Acta Physiol Scand 100: 340-346.

Verme LJ & Ullrey DE (1984) Physiology and nutrition. In: Halls LK (ed) White-tailed Deer: Ecology and Management. Stackpole Books, Harrisburg, p 91-118.

Vernon RG, Barber MC, Travers MT (1999) Present and future studies on lipogenesis in animals and human subjects. P Nutr Soc 58: 541-549.

Wahlefeld AW (1974) Triglycerides. Determination after enzymatic hydrolysis. In: Bergmeyer HU (ed) Methods of Enzymatic Analysis. Academic Press, New York, p 1831-1835

Warren RJ, Kirkpatrick RL, Oelschlaeger A, Scanlon PF, Webb KE Jr & Whelan JB (1982) Energy, protein, and seasonal influences on white-tailed deer fawn nutritional indices. J Wildl Manage 46: 302-312.

Weichselbaum TE (1946) An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Pathol 16: 40.

White RG & Gau AM (1975) Volatile fatty acid (VFA) production in the rumen and cecum of reindeer. Proceedings of the 1st International Reindeer/Caribou Symposium, Fairbanks, 1972. Biological Papers of the University of Alaska, Special Report No. 1 September 1975, p 284-289.

Wildman EE, Jones GM, Wagner PE, Boman RL, Troutt HF & Lesch TN (1982) A dairy cow body condition scoring system and its relationship to selected production characteristics. J Dairy Sci 65:495-501.

Wolkers H, Wensing T & Schonewille JT (1994a) Effect of undernutrition on haematological and serum biochemical characteristics in red deer (*Cervus elaphus*). Can J Zool 72: 1291-1296.

Wolkers H, Wensing T, Schonewille JT & Van't Klooster ATh (1994b) Undernutrition in relation to changed tissue composition in red deer (*Cervus elaphus*). Can J Zool 72: 1837-1840.

Yakoob AY, Holmes PH, Parkins JJ & Armour J (1983) Plasma protein loss associated with gastrointestinal parasitism in grazing sheep. Res Vet Sci 34: 58-63.

Åhman B, Nilsson A, Eloranta E & Olsson K (2002) Wet belly in reindeer (*Rangifer tarandus tarandus*) in relation to body condition, body temperature and blood constituents. Acta Vet Scand 43: 85-97.

Åhman B, Rydberg A & Åhman G (1986) Macrominerals in free-ranging Swedish reindeer during winter. Rangifer, Special Issue No. 1: 31-38.