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ADAPTATIONS OF NORTHERN UNGULATES TO SEASONAL CYCLES IN NITROGEN INTAKE

BY

RAYMOND LLOYD CASE

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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The undersigned certify that they have read and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ADAPTATIONS OF NORTHERN UNGULATES TO SEASONAL CYCLES IN NITROGEN INTAKE submitted by RAYMOND LLOYD CASE in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in WILDLIFE PRODUCTIVITY AND MANAGEMENT.

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DEDICATION

To my wife Cheryl. Thank-you for all your support.

Abstract

Adaptations to low nitrogen intake in wapiti (*Cervus elaphus*), reindeer (*Rangifer tarandus tarandus*) and barren-ground caribou (*R.t. groenlandicus*) were investigated by manipulating diets of captive wapiti and reindeer, and by assessing body composition and physiological status in caribou harvested in different seasons. Low nitrogen diets caused both wapiti and reindeer to lose striated muscle mass, as indicated by creatinine excretion. This indicated that both species catabolized endogenous protein reserves to make up for the nitrogen deficient diet. Indirect evidence of higher gut fill in wapiti fed the low nitrogen diet indicated that they attempted to adjust nitrogen intake through increased intake and/or increased retention time. Reindeer reduced feed intake when on the low nitrogen diet, thus relying on endogenous protein reserves for maintenance. Both reindeer and wapiti reduced urea-N excretion when on the low nitrogen diet. Wapiti used a combination of lower water flux and increased reabsorption in the kidney to conserve urea-N. Renal function in reindeer did not change and urea-N conservation was only due to reduced water flux. Changes in protein turnover in response to low nitrogen diets could not be evaluated as excretion of N^{τ}-methylhistidine (N^{τ}-MH) was invalidated as an index of protein turnover in both wapiti and reindeer. Wapiti excreted only minute amounts of N^{τ}-MH despite high levels of free N^{τ}-MH in serum. Reindeer excreted N^{τ}-MH in amounts comparable to cattle on a live weight basis, however, tracer studies indicated that N^{τ}-MH was not quantitatively excreted and was metabolized. Water flux was found to affect N^{τ}-MH excretion in reindeer with reduced water flux increasing the proportion of N^{τ}-MH reabsorbed in the kidney. Comparison of seasonal body composition in caribou and reindeer herds supported the existence of a set point for fall body composition modulated by the magnitude of loss of body reserves over winter. Environmental factors influence the ability of caribou to attain this set point. The urine urea:creatinine ratio was supported as an index of physiological status and an indirect indicator of physical condition. Serum urea-N, urine N^{τ}-MH:creatinine, and serum N^{τ}-MH failed to provide good indicators of physiological status.

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Chapter 1
General Introduction

Wild wapiti (*Cervus elaphus*), reindeer (*Rangifer tarandus tarandus*) and barren-ground caribou (*R.t. groenlandicus*) face an annual cycle in forage quality and availability. During the summer months, high quality forage is abundant and readily available in the form of deciduous leaves, sedges, forbs, flowers and grasses. Animals can select diets of high digestibility, high protein, high energy, and low in plant toxins and anti-nutritive substances (White 1983). These forages provide the necessary digestible energy, nitrogen and minerals for lactation, protein and fat deposition in adult animals and for rapid growth in sub-adult animals during a short summer season.

During winter both the quality and availability of forages decline dramatically. Forage plants, so high in nutrients during the summer, enter dormancy reducing crude protein and increasing lignin contents (Eastman 1983, Renecker and Hudson 1988, Klein 1990). In addition to lower quality, forage plants become less available as they are often covered with deep or hard packed snow (Geist 1982, Brown and Theberge 1990). This may result in wapiti, caribou and reindeer facing periods of low nitrogen and energy intake of up to 7 months (Morgantini and Hudson 1989, Boertje 1990).

The ability of northern ungulates to adapt to these harsh conditions is critical to their survival and subsequent productivity. Studies on reindeer indicate that a low winter plane of nutrition can lead to lower calf birth weights and lower milk production during early lactation which can both result in poor calf survival (White 1983). In Peary caribou (*R.t. pearyi*), there is a close relationship between pregnancy rates and levels of fat reserves and body weight (Thomas 1982). Thorne *et al.* (1976) found that over winter weight loss in wapiti cows is related to lower birth weights of calves and lower survival of calves. Northern ungulates must therefore make behavioural and physiological changes to meet nutritional requirements in winter.

Behavioural Adaptations

Behavioural adaptations to winter and to low forage quality have been documented in wapiti, reindeer and caribou. One adaptation which is very evident is migration. Wapiti in mountainous areas make vertical migrations primarily in response to snow depth and forage availability (Adams 1982). These migrations also permit wapiti access to high quality forages earlier in the spring (Morgantini and Hudson 1989). Caribou and reindeer also migrate to winter ranges. Although the reasons for, and the factors influencing, this migration are still being debated, increased access to lichen provided by softer snow in the boreal forest is likely a factor (Kelsall 1968).

Reduction in activity, and therefore energy requirements during winter, has been well documented in reindeer and caribou (Roby 1980, Boertje 1985, Russell and Martell 1986, Adamczewski *et al.* 1993) and wapiti (Geist 1982) and seems to be typical of all northern ungulates (Moen 1978). Although behavioural adaptations are important it is likely that the most significant adaptive changes to winter are physiological (Tyler and Blix 1990, Klein 1990).

Physiological adaptations

The research described in this thesis focuses on physiological adaptations, and in particular those adaptations which allow the *Cervus* and *Rangifer* to over winter with very low nitrogen intake. Seasonal changes in body composition caused by deposition and catabolism of fat and protein have been well documented in reindeer, caribou, and wapiti (McEwan 1968, Dauphiné 1976, Adamczewski *et al.* 1987, Thomas 1982, Bubenik 1982, Reimers and Ringberg 1983, Morgantini and Hudson 1989, Allaye-Chan 1991) as have the importance of fat deposits to fertility and calf survival (Thorne *et al.* 1976, Thomas 1982, White 1983, Crête *et al.* 1993). In addition to the fat cycle, consideration must be given to how nitrogen requirements are met. Striated muscle mass provides a potential source of amino acids for nitrogen deficient animals. However, significant losses of muscle mass would increase susceptibility to predation. Thus, it would be expected that

nitrogen deficient animals would minimize muscle catabolism and reduce excretion of nitrogen.

While both wapiti and reindeer experience restricted nitrogen intake over winter, the cycle of forage quality and availability is more pronounced in the arctic ranges of the caribou than in the forest dwelling wapiti. The snow free season in the arctic may be as short as two months (Thomas 1982). It is suggested, therefore, that physiological adaptations to low nitrogen intake will be more pronounced in reindeer and caribou and that these animals would be able to maintain themselves on low nitrogen intake better than wapiti. This study tested this hypothesis by putting captive raised members of both species on similar high and low protein diets and monitoring changes in muscle mass. A lower loss of muscle mass in reindeer would be interpreted as support for this hypothesis.

In attempting to maintain endogenous protein reserves, both wapiti and reindeer would benefit by minimizing the source of urinary nitrogen loss, particularly urea-N loss as it is the major nitrogen component of urine (Dukes 1947). This hypothesis was tested in this study by measuring urea-N excretion, and urea-N reabsorption in the kidney, in wapiti and reindeer fed high and low protein diets. Data from reindeer suggest that they can reabsorb over 90% of the urea filtered at the glomerulus when fed a low protein lichen diet (Hove and Jacobsen 1975). Recycling of urea-N into the gut has been documented in both caribou and wapiti (Wales *et al.* 1975, Mould and Robbins 1981). Thus if urea-N loss in urine could be minimized, this urea-N could be recycled back into amino acids by gut microbes.

Another way northern ungulates could reduce nitrogen loss during winter is to decrease the rate of tissue protein turnover. Catabolism and the subsequent anabolism of structural proteins requires a net energy expenditure. If dietary energy is insufficient to support this process along with other metabolic processes, lipolysis would have to make up the deficit. Once fat reserves are depleted, net protein catabolism would be required to provide ATP for anabolic processes. When this occurs insufficient energy would be available to use all available nitrogen and nitrogen would be excreted as a waste product. Reports of lower metabolic rates in winter in white-tailed deer (*Odocoileus virginianus*)

(Silver *et al.* 1969), moose (*Alces alces*) (Renecker and Hudson 1989) and caribou (Fancy 1986) may be indicative of lower protein turnover. However, Tyler and Blix (1990) suggest the observed changes in metabolic rate were due to changes in voluntary food intake and there was no seasonal variation in fasting metabolic rate, thus suggesting there would have been no change in protein turnover.

Urinary excretion of N^{τ}-methylhistidine (N^{τ}-MH) has been validated and used as an index of myofibrillar protein degradation in cattle (Harris and Milne 1981a), rats (Tomas *et al.* 1984) and man (Long *et al.* 1988). However, urinary excretion of N^{τ}-MH was not a valid index of protein degradation in sheep, pigs and goats as less than 60% of a labelled intravenous injection N^{τ}-MH was excreted in urine over 6 days (Harris and Milne 1980, Harris and Milne 1981b, Brown *et al.* 1987). In this study, urinary excretion of N^{τ}-MH was evaluated to determine if it could be used to monitor protein degradation in wapiti and reindeer. Validation was based on whether or not N^{τ}-MH was quantitatively excreted in urine. If urinary excretion of N^{τ}-MH was validated, it could then be used to compare protein turnover in wapiti and reindeer with and without restricted nitrogen intake.

Suggestions that northern ungulates regulate nitrogen losses, metabolic rate, and possibly protein turnover imply that changes in body composition are not just food related ie. body composition is not just a matter of the quality and availability of forage. The validity of this hypothesis was evaluated by determining if there was evidence of set points of body composition in two barren-ground caribou herds. A set point for body fatness in domestic ruminants was suggested by Bailey and Forbes (1974). Ryg (1983) suggested there was also a seasonally varying set point for fatness in reindeer. Observations of caribou on Coats Island by Adamczewski *et al.* (1987) supported this concept.

Steen (1968) and Neiminen and Heiskari (1989) suggested that reindeer fed a lichen diet must catabolize endogenous protein to meet nitrogen requirements and, when fat deposits are depleted, energy requirements. Urinary urea-N to creatinine ratios (UUC) and serum urea-N concentrations (SUN) have been found to reflect net protein catabolism

in ungulates during early to severe undernutrition (Warren *et al.* 1981, DelGiudice and Seal 1988, DelGiudice *et al.* 1987, 1989, 1991a, 1991b, Cool 1992). These authors assumed that the protein catabolism indicated by elevated UUC and SUN would correspond to low fat reserves. However, the relationship between physical condition of animals and elevated UUC and SUN was not documented. The relationship between body composition and these indicators was evaluated in wild caribou harvested in late winter from the Bathurst caribou herd in the Northwest Territories, Canada.

Objectives and thesis organization

The study is summarized in six chapters that were intended to meet the following objectives:

Chapter 2: To determine the effect of consuming low protein forages on body composition in wapiti and reindeer, and to compare the adaptation of the two species to low nitrogen intake by monitoring changes in muscle mass.

Chapter 3: To assess and compare the ability of wapiti and reindeer to reduce urea-N excretion in response to consumption of a low protein diet and to determine whether or not UUC can be used to monitor urea-N excretion.

Chapter 4: To investigate the use of urinary N^{τ}-MH excretion as an indicator of protein turnover in wapiti and reindeer and the relationship between N^{τ}-MH excretion and nitrogen intake in the diet.

Chapter 5: To determine if seasonal changes in body composition were affected by more than just the quality and availability of forage by testing for evidence of set points and by evaluating factors affecting set points in caribou and reindeer.

Chapter 6: To determine if urinary N^{τ}-MH:creatinine and UUC, serum N^{τ}-MH and SUN reflect muscle mass and fat reserves in caribou.

Chapter 7: To provide a synthesis of the ecological implications of the findings and conclusions and recommendations resulting from the study.

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Chapter 2

Effect of restricted nitrogen intake on body composition as estimated from creatinine excretion

Introduction

Wapiti (*Cervus elaphus*) and caribou (*Rangifer tarandus* spp.) face an annual cycle in the nitrogen content of the forage they consume. For example, in summer, wapiti on the eastern slopes of the Rocky Mountains select for high crude protein forages such as willow (*Salix* spp.) leaves and current year twigs resulting in a summer diet containing over 13% crude protein (Morgantini and Hudson 1989). In the winter, rough fescue (*Festuca scabrella*), which is highly digestible but low in crude protein (2.6% Morgantini and Hudson 1985) (4.2% Johnston and Beveau 1962) makes up a large portion of the diet. Hobbs *et al.* (1981) and Nelson and Leege (1982) found that the winter dietary protein requirement for wapiti is around 5-7% crude protein. Free roaming wapiti, therefore, consume forages with low nitrogen concentrations for up to 7 months a year (Morgantini and Hudson 1989).

In summer, barren-ground caribou (*R.t. groenlandicus*) select plants of high digestibility, high protein, and high energy. This includes leaves and new growth of willow, birch (*Betula nana*), shrubs (*Vaccinium vitis-idaea, Dryas integrifolia*) and graminoids (*Eriophorum vaginatum, Carex aquatilus/stans*) which range from 10 - 30% crude protein and are highly digestible (Thomas *et al.* 1984). The winter diet of barren-ground caribou, consisting primarily of lichen, is high in energy but very low in crude protein (2 - 3%) (Scotter 1965, Scotter 1967, Thomas and Hervieux 1986). This may result in caribou having a negative nitrogen balance for over 7 months (Boertje 1990).

The ability of wapiti and caribou to adapt to these low nitrogen winter diets is critical to their survival and subsequent productivity (Thorne *et al.* 1976, White 1983). Restricted nitrogen intake during winter would be reflected in muscle mass as animals in a negative nitrogen balance will use endogenous protein to provide a source of amino

acids for essential metabolic events. Weight loss, including fat, muscle and water losses, of over 40% of peak summer weight generally result in the death of wild cervids (DelGiudice and Seal 1988, Reimers *et al.* 1982). These animals therefore need to adjust their feeding strategy and adapt physiologically to maintain themselves over winter.

This study examines how wapiti and the caribou's domesticated relative, the reindeer (*R.t. tarandus*), adjust to a low protein diet and how this diet affects body composition. Both species are classed as mixed feeders (Hofmann 1989) which can adjust to changes in forage quality. However, as reindeer typically winter on lower crude protein forages than wapiti and have restricted nitrogen intake for a longer period of time, it would be expected that reindeer would be better able to maintain their body composition on a low protein diet. This was tested by restricting nitrogen intake in both species and monitoring muscle mass as estimated from creatinine excretion.

Materials and Methods

The experimental procedures in this study met the requirements of the Canadian Council on Animal Care and approved by the Animal Policy Committee, Faculty of Agriculture and Forestry, University of Alberta (wapiti) and were approved by the Animal Care Committee, Department of Biology, University of Alaska, Fairbanks (reindeer).

Experimental animals

This study used ten adult female wapiti aged 4-6 years at the University of Alberta's Ministik Wildlife Research Station (MINISTIK) near Edmonton and six adult female reindeer aged 5-8 years at the Large Animal Research Station (LARS) at the University of Alaska, Fairbanks. All wapiti and reindeer were pregnant during the winter and successfully calved in the spring following the treatments.

Dietary treatments

Two diets were formulated to provide a wide divergence in protein intake. The high protein diet was based on a textured diet developed for reindeer at LARS (Q-Tex). For reindeer Q-Tex was ground and pelleted with a molasses based binder. For wapiti, some ingredients of Q-Tex were not readily available (wheat and cracked corn) so a similar diet was formulated with a higher barley content (Table 2-1). This resulted in the wapiti high protein diet being marginally lower in nitrogen and gross energy content. The low protein diet for both reindeer and wapiti had oat hulls as the major ingredient. Both formulations had similar nitrogen and gross energy content. The nitrogen content of the low protein diet (11.7-14.8 g/kg) was closer to that characteristic of the winter diet of wapiti (8.0-11.2 g/kg) (Nelson and Leege 1982) than the low nitrogen (5.0 g/kg) lichen winter diet consumed by wild caribou and reindeer (Scotter 1965).

To investigate the influence of diet, five wapiti and three reindeer, selected at random, were placed on the high protein diet while the remaining animals were placed on the low protein diet. Reindeer were fed the pelleted diets *ad libitum* from November 1991 to April 1992. Wapiti were fed the pellets *ad libitum* from December 1990 to April 1991 and from November 1991 to April 1992.

Nitrogen balance trials

Nitrogen balance trials were conducted for wapiti in January, April and November 1991 and in April 1992. Animals were adapted to the experimental diet before all trials. Animals were placed in 2.4 x 1.2 m metabolic crates equipped with vinyl coated grated floors which allowed urine to be separated from feces and collected in underlying trays. The metabolic crates were outside at ambient temperature but were protected from wind and precipitation. Three 24 hour collections were made per animal to compensate for daily fluctuations. The trials were separated by one day to minimize the stress involved in being restrained in the metabolic crates for longer periods and to allow the animals to exercise. Between time in the crates the animals were held in individual 3 x 5 m pens.

Nitrogen balance trials for reindeer were conducted in November 1991 and January 1992. Animals were placed in 0.6 x 1.2 m metabolic crates equipped with vinyl

coated grated floors and plastic fecal separation screens which allowed urine and feces to be separated with little opportunity for cross contamination. The metabolic crates were outside at ambient temperature but were protected from wind and precipitation. Three 24 hour collections were made per animal to compensate for daily fluctuations. As the crates did not allow the animals to turn around or lie down, trials were separated by two days thereby minimizing stress and allowing the animals to exercise. Between collections the animals were held in individual 4 x 5 m pens.

Weights were recorded for both the wapiti and reindeer prior to being placed in the metabolic crates. When the animals were removed from the metabolic crates they were reweighed and a blood sample was obtained via venipuncture, centrifuged and serum was collected. Total fecal weight was recorded and a 250-500 g subsample was collected. Total urine volume was also recorded, the urine was thoroughly mixed and a 50 ml subsample was collected. Samples were frozen at -20°C until analyzed.

Feed consumption was estimated using acid insoluble ash (AIA) as an internal marker (van Keulen and Young 1977). Nitrogen intake was then calculated from the estimated feed consumption and composition. Total nitrogen excretion was calculated from the nitrogen content of feces and urea-N and creatinine-N in the urine. Nitrogen balance was estimated by subtracting nitrogen excreted in urine and feces from total nitrogen intake. Daily digestible energy (DE) intake was calculated from the estimated daily feed intake less the gross energy content of the feces.

Body composition

The proportion of body weight which was muscle mass was estimated in reindeer and wapiti from creatinine excretion (Talbot 1938, Boileau *et al.* 1972, Forbes and Bruining 1976). Average daily creatinine excretion was determined from the three 24 hour urine collections made during the nitrogen balance trials. In order to put the data into terms of body composition the relationship between striated muscle mass (SMM, kg) and creatinine excretion found by Talbot (1938) was used (estimated SMM = total creatinine excreted (mg)/17.9mg/kg). Estimated percent striated muscle mass (%SMM) was obtained by dividing estimated SMM by body weight (kg) and multiplying by 100.

A third trial to evaluate muscle mass was planned for reindeer in April 1992, however calving started earlier than expected from the estimated breeding date. This prevented the use of total urine collections as both the risk of calving in the metabolic crates and the risk of neonate abandonment were unacceptable. Body composition was therefore estimated after calving in late May 1992 using the tritiated water (TOH) technique (Holleman *et al.* 1982).

On day 0 pre-injection blood samples were collected from the jugular vein using 10 ml heparin coated Vacutainer tubes (Becton-Dickinson, Rutherford, NJ). Approximately 1 ml of TOH at a concentration of 1 mCi/ml was injected into the five lactating females. The one non-lactating female, which lost her calf post partum, was injected with approximately 0.25 ml of the same solution. The exact amount injected was determined by weighing the syringe before and after injection. Post injection blood samples were collected after 1, 3, 5, and 8 days. All blood samples were centrifuged on a TRIAC centrifuge (Becton, Dickinson, Rutherford, NJ) for 5 minutes and the plasma separated. Samples were stored at -70°C until subsequent analysis.

Water was separated from plasma by lyophilization. A standard made from the injected solution (0.2769 g HTO/1 l distilled water) (1 ml) and each water sample (1 ml) were mixed with Biosafe II Scintillation fluid (7 ml) and counted for three five-minute periods on a Beckman LS-7500 Liquid Scintillation System. The equilibrium specific radioactivity of plasma TOH was estimated by extrapolation to time zero using least squares regression of the specific radioactivity from post injection samples versus time (Holleman *et al.* 1982). The total body water space was calculated using the equation:

$$TBWS = (D/[SA_0-SA_i])*1000$$

Where TBWS = total body water space (L), D = Dose (nCi), SA_0= estimated equilibrium specific radioactivity (nCi/ml) and SA_i= pre-injection specific radioactivity (nCi/ml). TBWS was reduced by 5% to adjust for overestimation of total body water caused by isotopic hydrogen exchanging with labile hydrogen atoms in the non-water constituents of the body (Sheng and Huggins 1979, Holleman *et al.* 1982, Fancy *et al.* 1986).

Ingesta free body weight (IFBW, kg) and %Fat in IFBW were calculated from body weight (BW, kg) using the equations developed by Reimers *et al.* (1982):

log IFBW = -0.2583+(1.0032*log(BW))

%FAT in IFBW = 103.316-(1.500*(%WATER in IFBW))

where %WATER in IFBW = ((TBW-(BW-IFBW))*0.81)/IFBW

Fat weight was then calculated (%FAT in IFBW/100*IFBW) and subtracted from IFBW to give ingesta free fat free body weight (IF-FF-BW) which was then expressed as a percentage of total body weight (%IF-FF-BW).

Chemical analyses

Urinary concentrations of urea-N (mg/dl) were determined using a colorimetric urea assay kit based on the diacetyl monoxime reaction (Sigma Diagnostics, St. Louis MO). Creatinine concentrations (mg/dl) were determined using a colorimetric method based on the Jaffé reaction (Sigma Diagnostics, St. Louis MO). Percent dry matter (%DM) was determined by drying samples at 110°C. Nitrogen (g/kg) content of feeds and feces was determined using the macro-Kjeldahl procedure, gross energy (kJ/g) was determined via bomb calorimetry with an adiabatic calorimeter (Parr Instrument Company Inc., Moline IL).

Statistical analyses

Differences between trials were determined using repeated measures analysis of variance (PROC GLM, SAS 1988). Pairwise comparisons between trial means were quantified using paired sample t-tests. Comparisions between groups within trials were analyzed using t-tests (PROC TTEST, SAS 1988).

Results

Feed intake

Individual feed intake could not be monitored between trials as animals were grouped in large pens. During the first treatment period (December 1990 to April 1991)

wapiti fed the low protein diet (LOW group) had significantly higher average feed intake than those fed the high protein diet (HIGH group) (4.6 kg/animal/day vs 3.2 kg/animal/day $P<0.05$). This difference was not as large during the second treatment period (November 1991 to April 1992) (3.8 kg/animal/day for LOW vs 3.3 kg/animal/day for HIGH $P<0.20$). The high protein diet occasionally resulted in soft feces from wapiti and fecal pellets were not as firm as is typical of winter feces.

During the January trial, reindeer on the low protein diet had a slightly lower average intake than animals on the high protein diet (1.62 kg/animal/day vs 1.93 kg/animal/day $p=P<0.10$). As with wapiti, the high protein diet resulted in soft feces.

Body weight

Prior to placing animals on the experimental diets, there was no difference in mean weights between wapiti selected for the HIGH and LOW treatment groups (Figure 2-1). The average weight in the HIGH group dropped quickly after they were put on the experimental diets in December 1990 and remained lower than the LOW group until April 1991. When both groups were released to pasture and supplemented with hay in early May the HIGH group regained the weight difference quickly. Weights remained very similar over summer but diverged when the animals were fed the experimental diets in November 1991. Weights continued to diverge, with the LOW group increasing weight and the HIGH group remaining constant, until both groups were returned to pasture in April 1992. A similar divergence in weight was observed during the second winter.

Mean weights of reindeer selected for the HIGH and LOW groups were similar in June 1991 although there was a wide range of weights within each group (Figure 2-1). Body weights in both groups increased rapidly in July then remained constant through to November and the first trial. After being placed on the experimental diets in early November, body weight in both groups increased slightly. In mid-December animals in both groups began to lose weight. In mid-January the HIGH group stopped losing weight while the LOW group continued to lose weight through to mid-February. From mid-February through to the end of the experiment the LOW group remained approximately

10% lighter than their counterparts in the HIGH group. Both groups lost weight at the end of the trial due to calving.

Nitrogen balance

Nitrogen balance and DE intake during the four wapiti trials are shown in Figure 2-2. There were significant differences in nitrogen balance in January and April 1991 ($P<0.05$). In January 1991 the LOW group had significantly lower DE intake ($P<0.05$). In January 1991, four of the five animals in the LOW group had a negative nitrogen balance while the fifth animal was only slightly positive. No differences in nitrogen balance or DE intake were observed in the November 1991 trial when all animals were on the same diet or in the April 1992 trial when animals in the LOW group improved their nitrogen and energy status.

There were no significant differences in nitrogen balance between groups in the two reindeer trials. Mean nitrogen balance in the LOW group was 1/3 that of the HIGH group (Figure 2-3). Both nitrogen balance and DE intake dropped significantly in the LOW group from November 1991 to January 1992.

Striated muscle mass

There were significant time effects for body weight, estimated SMM and estimated %SMM in the wapiti trials ($P<0.05$). Pairwise comparisons of the means indicated that body weight did not vary significantly ($P>0.05$) between trials in the HIGH wapiti group (Table 2-2). Estimated SMM increased from January 1991 to April 1991, but not significantly. Estimated SMM increased significantly ($P<0.01$) from November 1991 to April 1992. For SMM corrected to weight (%SMM), there was a significant ($P<0.05$) difference between April 1991 and November 1991. The HIGH group tended to become leaner (increased %SMM) during both winters, however, the changes were not significant ($P>0.05$).

Significant increases in body weight were observed in the LOW wapiti group from January to April 1991 ($P<0.05$) and from November 1991 to April 1992 ($P<0.01$) while the animals were on the experimental diet (Table 2-2). Estimated SMM declined

slightly from January to April 1991. In contrast, during the second trial estimated SMM increased significantly ($P<0.05$). The proportion of the body that was striated muscle remained essentially unchanged through the experiment.

Significant ($P<0.05$) differences between groups were found for estimated SMM and %SMM during January and April 1991. The LOW group was also significantly ($P<0.05$) heavier than the HIGH group in April 1992.

Body weight of reindeer in the HIGH group remained unchanged from November 1991 to May 1992 (Figure 2-1, Table 2-3). Reindeer in the LOW group lost some weight from November 1991 to January 1992 and continued to lose weight through to May 1992. Large variations in weight within the groups resulted in there being no significant ($P>0.05$) differences between the groups. This same variation resulted in there being no significant differences between groups in estimated SMM and %SMM. From November 1991 to January 1992, estimated SMM declined significantly ($P<0.05$) in both groups. When estimated SMM was corrected for weight (%SMM), the change in the HIGH group was no longer significant.

During the May 1992 TOH trials, there were no significant differences between the groups in %IF-FF-BW (Table 2-3).

Discussion

Body weight

Body weight has often been used as an indicator of condition in wild ungulates (Huot 1988). This assumes that heavier animals have greater fat reserves as ruminants will preferentially catabolize fat reserves during early undernutrition (Berg and Butterfield 1976, Torbit *et al.* 1985). When energy intake is insufficient to meet metabolic requirements this model holds. However, in some situations, including this study, nitrogen intake is limiting, not energy, so changes in body weight involves other factors including gut fill, water volume, and muscle mass.

Weight changes in wapiti during this study did not correspond to nitrogen balances and DE intake. The rapid loss in body weight in the HIGH group when put on

experimental diet in December 1991 suggests a reduction in gut fill. The weight increases observed in the LOW group while on a negative nitrogen balance may also relate to gut fill. While other researchers have observed that captive red deer and wapiti reduce feed intake during the winter (Suttie *et al.* 1983, Renecker and Hudson 1990), in this study the LOW group either did not reduce intake or did not reduce intake to the same degree as the HIGH group. As gut fill is related to feed intake, fermentation and passage rate, and because fermentation capacity and passage rates are similar between most ruminants (Baker and Hansen 1985, Renecker and Hudson 1990) the LOW group would be expected to have had a greater gut fill. This strategy is similar to that observed for roe deer and moose which increase gut fill in response to low quality winter forages (Gasaway and Coady 1974, Renecker and Hudson 1990, Holand 1992). This conclusion is supported by the observation that the HIGH group increased weight quickly after being placed on hay diets in May while the LOW group did not gain weight.

It is possible that the entire weight difference between the two groups could be accounted for by gut fill. The weight difference in April 1991 was 24 kg and was nearly 30 kg in April 1992. At 23 percent of live weight (Bubenik 1982) or 60 kg in a 260 kg animal, the gut volume would have to increase by 50% to make up the weight difference observed. Holand (1992) found that roe deer increased gut content by 50% from summer to winter.

It is also possible that other factors contributed to the weight difference. Dehydration, or alternately lack of water retention, in the HIGH group may also have been important, as feces from the HIGH group were moist and average urine volumes were significantly higher (8.9 vs 3.1 l and 4.9 vs 2.2 l in January and April 1991 respectively $P<0.01$). Retention of body water and lower water flux have been documented in reindeer with low nitrogen intake (Cameron and Luick 1972, Cameron *et al.* 1982) and water intake was strongly correlated to crude protein intake Syrjälä *et al.* (1980). A similar situation in wapiti would result in a larger body water volume in the LOW wapiti.

In contrast to wapiti, reindeer in the LOW group reduced feed intake and slowly lost weight over the trial. Reduction in voluntary feed intake by caribou in response to

low quality diets has previously been reported by McEwan and Whitehead (1970) and more recently by Crête *et al.* (1993). With the lower intake and the resultant lower nitrogen balance, the LOW group may have been expected to lose even more weight. The difference may have been made up by water retention in the LOW group which had reduced average urine excretion in January 1992 (2.5 l for HIGH vs 1.5 l for LOW).

Striated muscle mass

Measurement of 24-hour urinary creatinine excretion is considered a reliable index of total striated muscle mass (Talbot 1938, Schutte *et al.* 1981). It has been consistently demonstrated that animals with higher rates of creatinine excretion by body weight are leaner than those with lower creatinine excretion rates (Boileau *et al.* 1972). Talbot (1938) calculated that 1 kg of striated muscle is associated with the excretion of 17.8 mg creatinine/day. Schutte *et al.* (1981) found Talbot's equation calculated SMM within 4% of actual SMM in dogs.

A recognized weakness in the use of creatinine excretion to estimate muscle mass is the lack of studies correlating creatinine excretion with in vitro measurements of striated muscle. This relationship has not been determined in wapiti or reindeer. The SMM values in this study can therefore only be used as an estimate of SMM. The results of research on other species and observations that creatinine excretion is not affected by nutrition suggests that it is a valid indicator and permits comparisons between animals on different diets.

As expected from the very low nitrogen balance, wapiti in the LOW group had significantly lower estimated SMM than the HIGH group in January and April 1991. The figures for nitrogen balance are overestimates as gaseous nitrogen losses are not considered. Also, the urine nitrogen calculation only included urea-N and creatinine nitrogen, not total nitrogen, and losses to hair were not included. Therefore, the LOW wapiti were unlikely to have met their nitrogen requirements with intake and had a net catabolism of striated muscle. Similar losses of muscle have been reported in cattle on restricted diets (Butterfield 1966, Price 1977) although these losses were generally observed in conjunction with catabolism of fat reserves.

Other than visual assessments, no measurements of fat content were made. However, given that the LOW wapiti did not lose weight it is possible that fat reserves were maintained. Visual comparison of the two groups, and other wapiti at MINISTIK suggested that the LOW group had not lost significant levels of fat. If fat was lost then increased gut fill and water retention would have made even greater contributions to body weight.

A similar difference in estimated SMM was not observed in April 1992, reflecting better nitrogen balance. The improved nitrogen status was likely a result of higher nitrogen content in the pellets the second winter and much lower ash content.

Both reindeer groups had a significant decrease in estimated SMM from November to January suggesting the LOW group lost significantly more SMM (21.5 vs 7.4 kg $P<0.05$). As in the LOW wapiti, the nitrogen balance data suggest it is unlikely that the LOW reindeer were able to meet their nitrogen requirement with dietary sources and therefore had a net catabolism of muscle protein. The small change in estimated %SMM in the HIGH group suggests that loss of SMM was related to total weight loss while in the LOW group loss of SMM was proportionally greater than total weight loss.

The TOH data from May indicate that the LOW reindeer had also catabolized more fat than the HIGH group. This was also evident from a visual comparison of the two groups.

Unfortunately, the two body composition indices (%SMM and %IF-FF-BW) are not directly comparable so it is not possible to determine if additional SMM loss occurred from January to May 1992. Creatinine excretion is related directly to muscle mass while calculation of %IF-FF-BW relies on the TOH technique which was designed to determine total body water and is generally used to estimate body fat content. As a result, %IF-FF-BW does not allow one to determine the proportion of body weight that is muscle from that which is other non-fat soft tissues.

Other factors also complicate comparisons. While creatinine excretion is independent of fluctuations in body water volume and water turnover (Vestergaard and Leverett 1958), the TOH technique determines fat reserves based on body water content. Cameron and Luick (1972) found seasonal variations in total body water, water flux and

extracellular fluid volume in reindeer. They attributed the increases in extracellular fluid volume during winter to catabolism of protein reserves. The highest total body water volumes were found in May and June when catabolism was likely to be the greatest. It is possible that the high %IF-FF-BW figures for the LOW group in this study also reflect higher proportion of water in lean tissue.

Conclusions

Although both wapiti and reindeer are classed as mixed feeders (Hofmann 1989) they had different approaches to adapting to the low protein diet. Wapiti increased feed intake and likely gut fill while reindeer slightly decreased feed intake. If one considers the energetics of these strategies in the wild they make sense. Winter forage for wapiti, although of poor quality, is generally not restricted in abundance or availability. If deep snow restricts availability, wapiti will often migrate to areas with higher forage accessibility (Adams 1982). For reindeer, winter forages are energetically expensive as they are covered with either deep or hard packed snow and body heat must be used to bring them from ambient to body temperature. Migration below treeline may give some relief from wind packed snow but these are often long migrations. In addition, lowering intake reduces water flux when replenishment of water is energetically expensive (Valtonen 1980, Cameron *et al.* 1982). Thus in reindeer it may not be prudent to attempt to adapt to low nitrogen in the diet by increasing intake as increased energy costs may outweigh any advantages.

Changes in SMM in animals fed the LOW diets suggest that both species needed to catabolize endogenous sources of protein. By increasing intake and perhaps retention time and gut fill, wapiti appear to have been able to meet DE intake requirements but not nitrogen intake requirements thus maintaining fat reserves but not protein reserves. By decreasing feed intake on the LOW protein diet, reindeer "chose" to catabolized both fat and protein reserves.

In comparison with wild diets, the low protein diet had a higher nitrogen content than that recorded for wapiti by Morgantini and Hudson (1985) and much higher than that

recorded for caribou by Scotter (1965, 1967). The results of this study would therefore suggest that in the wild both species would use endogenous protein as a source of nitrogen. However, any comparisons with wild diets would need to give consideration to the different composition of the diets. For example the lichen diet of wild caribou and reindeer may allow them to maintain fat reserves while using protein reserves (Steen 1968, Nieminen and Heiskari 1989).

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Table 2-1: Dry matter (DM), acid insoluble ash (AIA), nitrogen (N), gross energy (NRG) content and ingredients, of diets fed to adult female wapiti and reindeer from December 1990 to May 1992.

		ANALYSIS ON "AS FED" BASIS		
WAPITI FEEDS	%DM	AIA (g/kg)	N (g/kg)	NRG (kJ/g)
December 1990 - February 1991				
Low Protein[a]	80.9	24.28	11.92	15.22
High Protein[b]	77.7	6.63	25.08	14.66
March 1991 - April 1991				
Low Protein[a]	84.7	26.67	11.74	14.24
High Protein[b]	85.1	5.63	24.40	13.15
November 1991				
Alfalfa Pellets[c]	86.8	8.33	22.71	15.42
November 1991 - March 1992				
Low Protein[a]	77.9	6.32	14.80	13.85
High Protein[b]	77.9	4.05	23.34	13.02
REINDEER FEEDS				
November 1991				
High Protein[d]	85.6	10.01	26.51	15.04
November 1991 - May 1992				
Low Protein[e]	87.3	25.36	12.29	15.00
High Protein[d]	85.0	7.54	27.56	15.12

Ingredients in pelleted diets:

[a] Oat hulls, beet pulp, barley, binding agent, vitamin/mineral mix and salt

[b] Barley, soyameal, binding agent, vitamin/mineral mix and salt

[c] Chopped alfalfa, binding agent, vitamin/mineral mix and salt

[d] Wheat millrun, rolled barley, cracked corn, soyameal, binding agent, vitamin/mineral mix and salt

[e] Oat hulls, barley, binding agent, vitamin/mineral mix and salt

Table 2-2: Changes in body weight and body composition of wapiti as indicated by estimated striated muscle mass (SMM) and percent striated muscle mass (%SMM).

	January 1991		April 1991		November 1991		April 1992
Weight (kg)							
HIGH n=5	255.6±7.6	ns	258.6±11.3	ns	270.33±3.13	ns	281.1±8.0
	ns		ns		ns		*
LOW n=5	265.4±2.9	*	282.4±7.9	ns	268.40±3.21	**	310.0±3.5
SMM (kg)							
HIGH n=5	118.0±10.2	ns	147.0±17.3	ns	100.0±4.8	*	144.6±15.8
	*		*		ns		ns
LOW n=5	91.8±4.6	ns	84.0±6.2	ns	96.8±3.1	*	126.4±11.1
%SMM (%)							
HIGH n=5	46.09±3.54	ns	56.90±6.24	*	36.97±1.68	ns	51.71±6.23
	*		*		ns		ns
LOW n=5	34.58±1.55	ns	29.78±2.08	ns	36.09±1.37	ns	40.76±3.59

Note: Significant pairwise comparisions are indicated by * = P<0.05 ** = P<0.01.
Data are expressed as mean±SE.

Table 2-3: Changes in body weight and body composition of reindeer as indicated by estimated striated muscle mass (SMM), percent striated muscle mass (%SMM), and percent ingesta free, fat free body weight (%IF-FF-BW).

	November 1991		January 1992		May 1992
Weight (kg)					
HIGH n=3	102.1±7.5	ns	101.1±9.5	ns	99.3±6.3
LOW n=3	102.7±10.9	ns	96.1±8.2	*	88.9±6.7
SMM (kg)					
HIGH n=3	47.0±2.9	*	39.6±2.9		
LOW n=3	49.7±6.6	*	28.2±4.9		
%SMM (%)					
HIGH n=3	46.16±1.50	ns	39.20±4.15		
LOW n=3	48.05±1.74	**	28.94±2.78		
%IF-FF-BW (%)					
HIGH n=3					59.47±4.99
LOW n=3					68.60±4.93

Note: There were no significant differences between groups (P<0.05). Significant pairwise comparisions are indicated by * = P<0.05 ** = P<0.01.
Data are expressed as mean±SE.

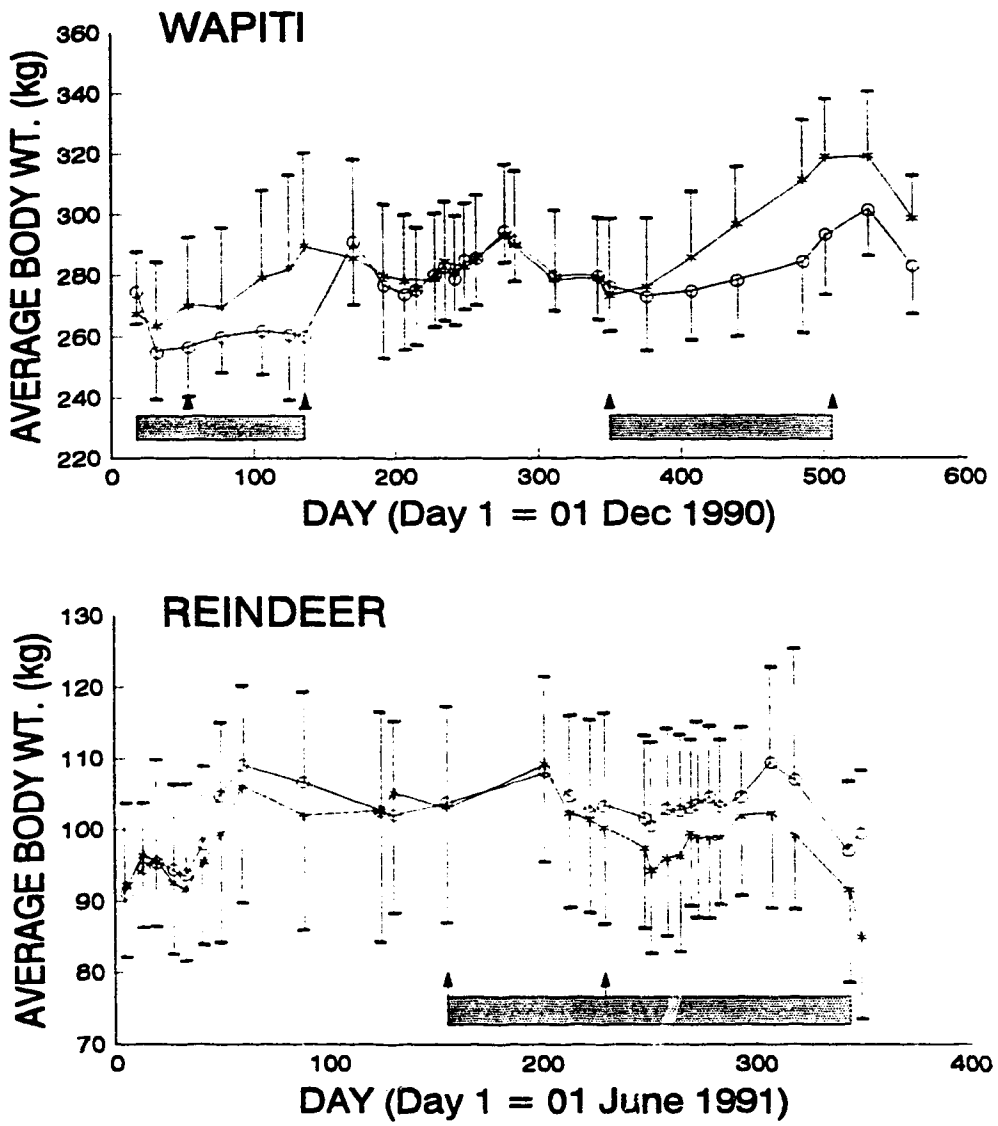

Figure 2-1: Body weight of wapiti (n=5 per group) and reindeer (n=3 per group) fed high ○ and low × crude protein diets (mean±SE). Periods when animals were on experimental diets are indicated by the bar. Nitrogen balance trials are indicated by the arrows.

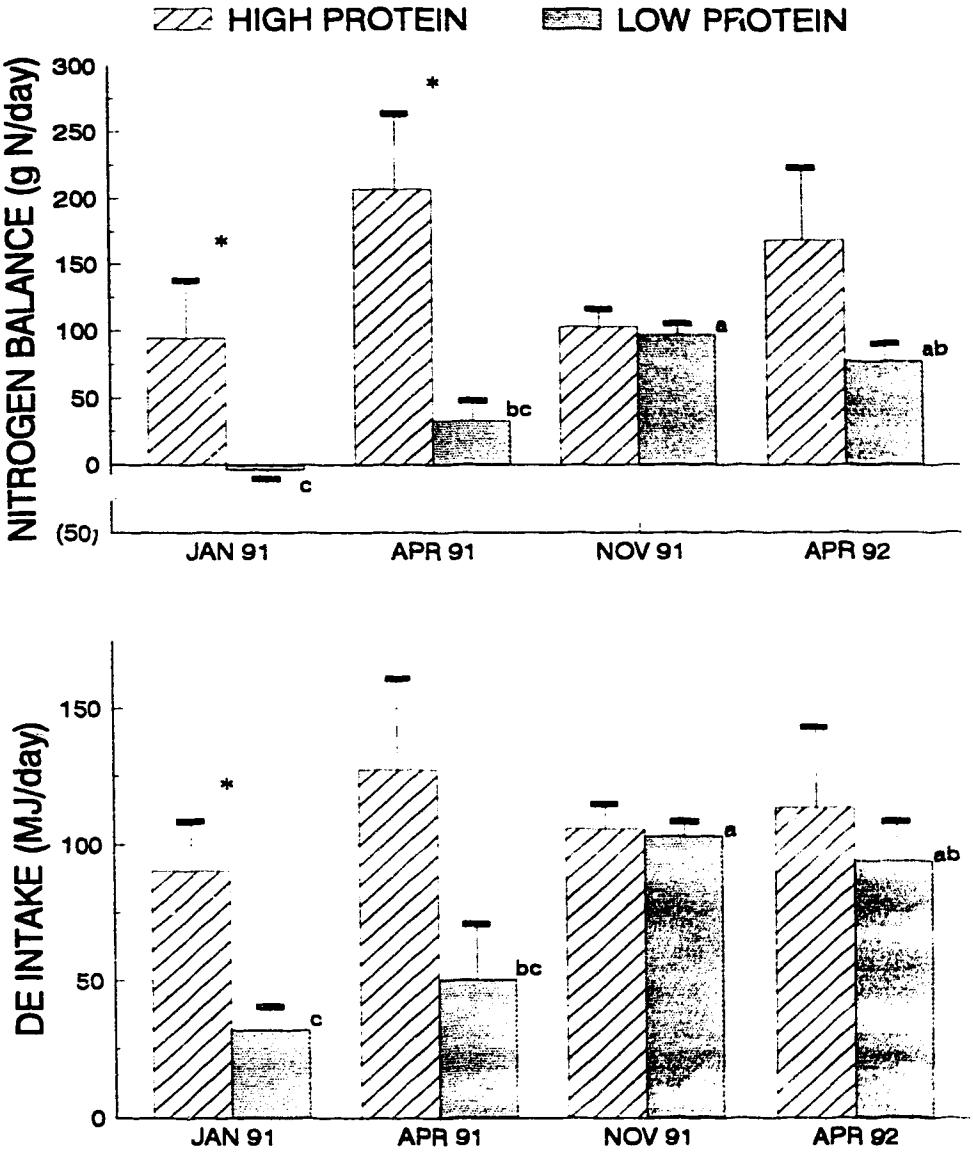

Figure 2-2: Nitrogen balance and DE intake in wapiti (mean ± SE n=5/group). There are no significant differences between trials for the high protein group. The low protein group bars with the same letter are not significantly different ($P<0.05$). Significant differences between groups are marked by * ($P<0.05$).

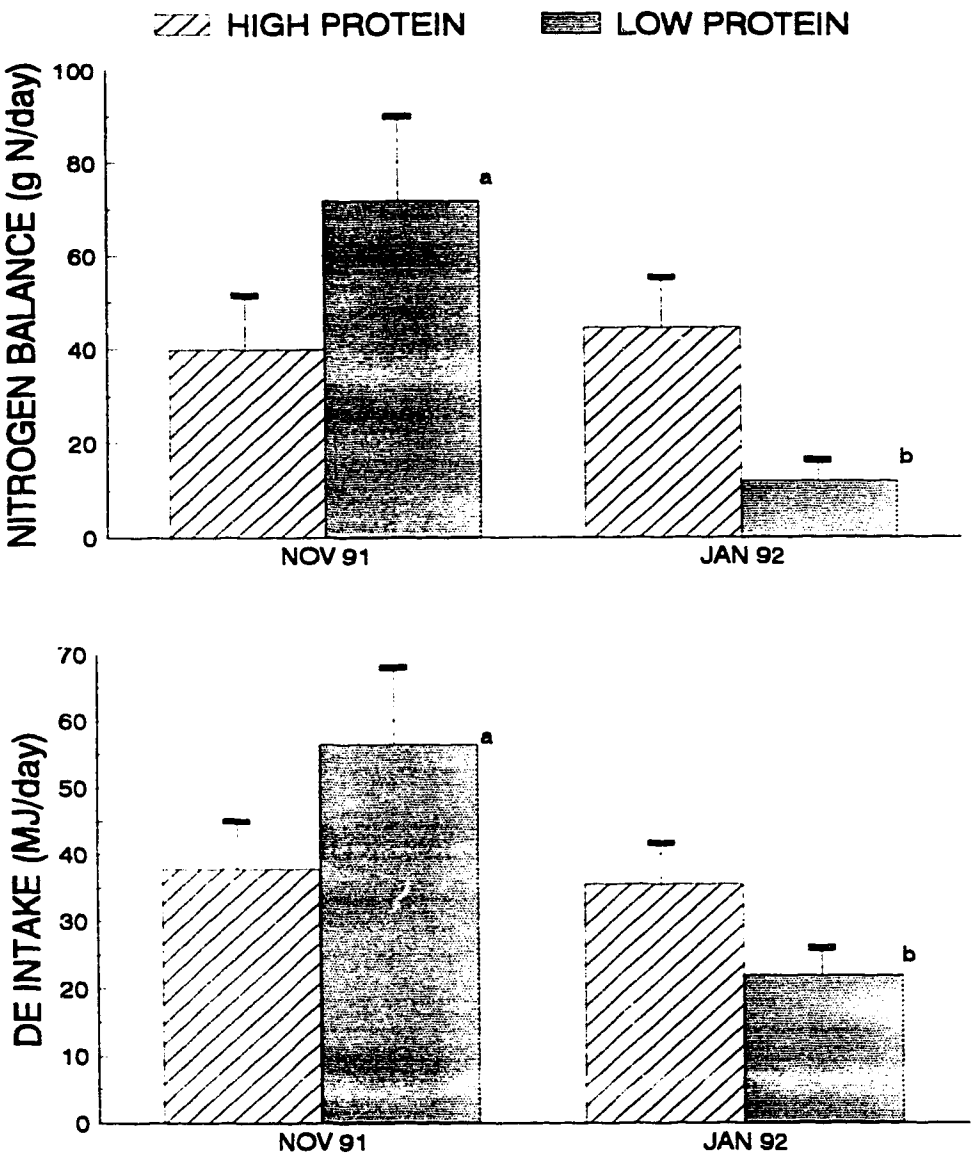

Figure 2-3: Nitrogen balance and DE intake in reindeer (mean±SE n=3/group). There are no significant differences between trials for the high protein group. For the low protein group both nitrogen and energy balance were significantly different between Nov 91 and Jan 92 ($P<0.05$). Differences between groups are not significant ($P>0.05$).

Chapter 3
Urea-N excretion in reindeer and wapiti

Introduction

As a result of both low nitrogen content in forages consumed and reduced accessibility of forages, wapiti (*Cervus elaphus*) and reindeer (*Rangifer tarandus*) are subject to lengthy periods of low nitrogen intake during the winter (Morgantini and Hudson 1989, Boertje 1990). In periods of dietary nitrogen deficiency the rumen microbial environment needs to be buffered by recycling of urea-N into the rumen (Houpt 1959). Synthesis of microbial proteins is needed for a supply of amino acids for endogenous protein synthesis necessary for tissue maintenance.

Urea can enter the rumen either via the saliva or diffuse directly across the rumen wall (Houpt 1959, Somers 1961). Once in the rumen, urea is readily hydrolysed by bacterial urease to produce carbon dioxide and ammonia. The ammonia can then be directly reabsorbed or used for bacterial protein synthesis. Recycling of urea into the rumen has been documented in both wapiti and caribou. Caribou on simulated winter diets recycled approximately 58% of urea into the rumen (Wales *et al.* 1975). In wapiti urea recycling as high as 85% has been documented (Mould and Robbins 1981).

Given that urea can be recycled in the rumen, it seems reasonable to predict that both wapiti and reindeer will attempt to minimize urea-N losses in the urine, by increasing tubular transport capacity for urea-N reabsorption (Leng *et al.* 1985). Decreases in urinary excretion of urea-N in response to low protein diets have been well documented in domestic ruminants (Schmidt-Nielsen *et al.* 1958, Schmidt-Nielsen and Osaki 1958, Houpt 1959, Cocimano and Leng 1967, Houpt and Houpt 1968, Ford and Milligan 1970), white-tailed deer (Robbins *et al.* 1974, DelGiudice *et al.* 1989), and in reindeer and caribou (Hove and Jacobsen 1975, Wales *et al.* 1975). However, it is not clear if these changes are due to changes in renal function.

Urinary urea-N to creatinine ratios (UUC) have been used as an index of urea-N excretion in wild ruminants (DelGiudice and Seal 1988). However, Chetal *et al.* (1975) suggested that variations in creatinine excretion would affect UUC, independent of urea-N excretion. Investigations of the constancy of creatinine excretion in humans (Vestergaard and Leverett 1958, Forbes and Bruining 1976), sheep (van Niekerk *et al.* 1963, Hodgen *et al.* 1967), beef steers (Dinning *et al.* 1949), buffalo calves (Chetal *et al.* 1975), moose and wapiti (Cool 1992) have given mixed results. The constancy of creatinine excretion and the effects of energy and nitrogen balance on creatinine excretion have not been well studied in wild ungulates.

The objectives of this study were to determine if urea-N excretion was minimized by wapiti and reindeer on low protein diets and to determine if any changes in reabsorption in the kidney were made. The constancy of creatinine excretion and therefore the use of UUC to monitor urea-N excretion was also evaluated.

Methods

The experimental procedures in this study met the requirements of the Canadian Council on Animal Care and were approved by the Animal Policy Committee, Faculty of Agriculture and Forestry, University of Alberta (wapiti) and by the Animal Care Committee, Department of Biology, University of Alaska, Fairbanks (reindeer).

Experimental animals

This study used ten adult female wapiti aged 4-6 years at the University of Alberta's Ministik Wildlife Research Station (MINISTIK) near Edmonton and ten adult female reindeer aged 5-8 years at the Large Animal Research Station (LARS) at the University of Alaska, Fairbanks. All wapiti were pregnant during the winter and successfully calved in the spring following the treatments. Six of the ten reindeer were used to evaluate the effect of dietary treatments. All six animals were pregnant. Four reindeer were used to evaluate daily fluctuations in creatinine and urea-N excretion. Two of these animals were pregnant and two were not.

Dietary treatments

Two diets were formulated to provide a wide divergence in protein intake. The high protein ration was based on a textured ration developed for reindeer at LARS (Q-Tex). For reindeer, Q-Tex was ground and pelleted with a molasses based binder. For wapiti, some ingredients of Q-Tex were not readily available so a similar diet was formulated (see Chapter 2 for ingredients). This resulted in the wapiti high protein diet being marginally lower in nitrogen and gross energy content. The low protein diet for both reindeer and wapiti had oat hulls as the major ingredient and had similar crude protein content (7.3 - 9.3%).

To investigate the influence of diet, five wapiti and three reindeer, selected at random, were placed on the high protein diet and five wapiti and three reindeer were placed on the low protein diet. Reindeer were fed the pelleted diets *ad libitum* from November 1991 to April 1992. Wapiti were fed the pellets *ad libitum* from December 1990 to April 1991 and from November 1991 to April 1992.

Sampling protocol

Total daily urine collections were made during the nitrogen balance trials described in Chapter 2. Trials for wapiti were conducted in January, April and November 1991 and in April 1992. Three urine collections were made per animal, and parameter means were used to compensate for daily fluctuations in urine volume and urea-N and creatinine excretion. It was not possible to ensure that animals had cleared their bladders before, and at the end of, the trials. However, animals usually urinated while being weighed prior to going into the crates, and animals were roused for several minutes prior to removal from the crates to allow time for urination and defecation. Blood was collected via venipuncture at the end of the trial and serum was separated and saved. Urine, blood and serum samples were collected opportunistically between trials. Urine and serum samples were frozen at -20C until analyzed.

Trials for reindeer were conducted in November 1991 and January 1992. Each trial consisted of three collections per animal. Data from each animal were pooled to compensate for daily fluctuations. Blood samples were taken via venipuncture after each

24 hour urine collection and serum was separated and collected. Urine, blood and serum were also collected monthly.

Total urine collections were also made from four reindeer (one animal was tested twice for a total of five collections) held in 2.4 x 1.2 m metabolic crates for 5-7 days to evaluate daily and diurnal variations in urea-N excretion, creatinine excretion and urea-N reabsorption. Animals in these trials were placed on the high protein pelleted ration five days before the trials. All three animals in the January trial and one animal in the May trial refused the high protein ration when place in the metabolic crates even though they consumed it when out of the crates. These four animals were supplemented with lichen after 55 hours and 72 hours in January and May respectively.

Urine output was recorded, thoroughly mixed and a subsample taken every twelve hours (0900 and 2100). Serum was separated and saved from blood samples collected via venous catheter at the same intervals.

Chemical analyses

Urine and serum samples were analyzed for creatinine and urea-N content. Creatinine concentrations (mg/dl) were determined using a colorimetric method based on the Jaffé reaction (Sigma Diagnostics, St. Louis MO). Concentrations of urea-N (mg/dl) were determined using a colorimetric urea-N assay kit based on the diacetyl monoxime reaction (Sigma Diagnostics, St. Louis MO). The glomerular filtration rate (GFR) was estimated using the creatinine clearance technique as creatinine is freely filtered at the glomerulus, is not reabsorbed by the tubules and only small amounts are secreted into the tubules (Kaplan and Pesce 1989, p. 354). Urea is also freely filtered at the glomerulus so the amount filtered can be estimated from the GFR and serum concentration of urea-N. The percentage of urea-N reabsorbed was determined by comparing the amount of urea-N filtered at the glomerulus with the amount of urea-N excreted in urine.

Statistical analyses

Differences between trials were analyzed using repeated measures analysis of variance (PROC GLM, SAS 1988). Pairwise comparisons between trial means were

quantified using paired sample t-tests. Comparisons between group means within trials were analyzed using t-tests (PROC TTEST, SAS 1988). Regression equations were determined using least squares regression (PROC REG, SAS 1988).

Results

Wapiti

In wapiti, urea-N excretion was significantly ($P<0.05$) higher in the high protein group (HIGH) than in the low protein group (LOW) during the January 1991 and April 1991 trials (Table 3-1). Both treatment groups reduced urea-N excretion significantly from January to April 1991 and increased excretion significantly from April 1991 to November 1991. Only the LOW group significantly reduced urea-N excretion from November 1991 to April 1992.

Reabsorption of urea-N at the tubular level in the kidney was low, but highly variable, in both treatment groups during January 1991. Reabsorption increased significantly ($P<0.05$) in the LOW group by April 1991. The HIGH group also increased reabsorption in April but with high variation in both January and April the difference was not significant ($P=0.08$). In November 1991, when both groups were on the high protein diet, reabsorption was very low but it increased to over 50% in both groups in April 1992.

The coefficients of variation (CV) in 24 hour creatinine excretion ranged from 1.07 to 67.44% in wapiti (n=3 for each sample). The average of the CV's from five animals was similar in January 91, April 1991 and November 1991 (15.47, 15.23 and 12.08 respectively) but was very high in April 1992 (32.61). Creatinine excretion in the HIGH group increased slightly from January to April 1991 and significantly ($P<0.05$) from November 1991 to April 1992. In the LOW group creatinine excretion decreased slightly from January to April 1991 but increased significantly ($P<0.05$) from November 1991 to April 1992. Excretion in the LOW group was significantly lower than the HIGH group in January and April 1991. There was no difference between groups in November 1991 and April 1992 ($P>0.05$).

During January and April 1991, and April 1992 UUC were significantly ($P<0.05$) lower in the LOW group (Table 3-1). These differences were not observed in November 1991, when animals were on the same feed. UUC declined significantly in both treatment groups through both winters (Table 3-1, Figure 3-1). The highest UUC were observed in June 1991 when animals were returned to pasture (Figure 3-1). Very low UUC were recorded in July 1991 and October 1991 while wapiti were still on pasture even though they were supplemented with alfalfa pellets. UUC increased in November when animals were placed on the high protein experimental pellets then declined through the following winter in both treatment groups. Changes in UUC were generally parallelled by changes in serum urea-N concentration (Figure 3-1), however, only the LOW group had significant differences between trials.

Reindeer

In reindeer, only the LOW group significantly reduced urea-N excretion from November 1991 to January 1992 which resulted in a significant difference between groups in January 1992 (Table 3-2). This pattern was not observed in urea-N reabsorption.

The CV's for 24 hour urinary creatinine excretions in reindeer ranged from 2.27 to 29.62% (n=3 for each sample). The average of the CV's from the three reindeer was similar between the two trials (Nov 91=11.41 and Jan 92=11.52). Creatinine excretion declined in both groups with the LOW group having a larger decrease. UUC reflected urea excretion with the LOW group declining from November 1991 to January 1992 and being significantly lower than the HIGH group in January 1992.

Differences in UUC between groups were observed from December 1991, shortly after animals were placed on the experimental rations, through to the end of the trial in May 1992 (Figure 3-2). The higher UUC values in the HIGH group were reflected in serum urea-N concentrations.

During the 5-7 day experiments, excretion of urea-N gradually declined through the experiment in the four animals which fasted or consumed only lichen (Figure 3-3). The animal which consumed the high protein ration had consistently higher urea-N excretion averaging over twice that of the other four animals.

The slow decline in urea-N excretion was not reflected in reabsorption of urea-N. Reabsorption remained near 50% in the animals fed lichen through 120 hours (Figure 3-2). The animal fed lichen through to 156 hours showed a slight rise in reabsorption near the end of the experiment. The animal on high protein pellets started with high reabsorption but this declined for the first 48 hours before increasing to levels similar to the lichen group near the end of the trial.

Urinary creatinine excretion did not differ between the animals fed lichen and the animal fed pellets and remained consistent through the trial (Figure 3-4). As a result, UUC reflected urea-N excretion, declining slowly in the lichen fed animals and staying significantly higher in the pellet fed animal.

Serum urea-N concentrations declined in both fasting and pellet fed reindeer over the first 52 hours, after which the HIGH group increased and the LOW group continued to decline (Figure 3-5).

Discussion

Urea-N excretion

Urea-N excretion, as indicated by both total urea-N excretion and UUC, was lower in both wapiti and reindeer fed the low protein diet. This agrees with observations by Westra and Hudson (1979) for wapiti and Eriksson and Valtonen (1974) and Hove and Jacobsen (1975) for reindeer. These authors observed that animals with reduced urea-N excretion also had lower plasma urea-N concentrations. In wapiti (Mould and Robbins 1981) and white-tailed deer (Robbins *et al.* 1974) urea-N excretion was shown to be a function of plasma urea-N concentration.

Plotting the UUC and serum urea-N data from all the daily collections (Figure 3-6) illustrates that although the trend in both reindeer and wapiti is towards higher urea-N excretion with higher serum urea-N concentrations the relationship is not as tight as that observed by Robbins *et al.* (1974) and Mould and Robbins (1981). Schmidt-Nielsen and Osaki (1958) found that as long as tubular transport of urea-N in the kidney remains constant then plasma urea-N concentration is a determinant of urea-N excretion. These

results suggest that there could be considerable variation in tubular transport, particularly in wapiti.

Urea-N reabsorption

Robbins *et al.* (1974) and Mould and Robbins (1981), found that the proportion of urea-N recycled into the rumen increased with lower plasma urea-N concentrations. If urea-N, not excreted in urine, is recycled into the gastrointestinal tract, and serum urea-N concentrations and volume remain constant, then the percent reabsorption of urea-N calculated in this study would be an indicator of recycling. Given this assumption, several of the results from this study do not conform to the above mentioned relationship.

During the wapiti trial in January 1991, reabsorption of urea-N in both treatment groups was lower than what would be predicted by serum urea-N concentrations and dietary nitrogen intake. These results can be explained by the phenomenon called exaltation. During rapidly rising urine flows urea-N clearance is usually high relative to the filtration rate due to a flushing of urea-N from tissue water at the tip of the renal papilla (Schmidt-Nielsen *et al.* 1958). In January 1991, the wapiti were taken from pens where snow was the primary water source to the metabolic crates where water was available *ad libitum* and water intake increased rapidly. During all the rest of the trials animals had access to water prior to the trial.

Exaltation was also observed in the 5-7 day reindeer trials when the urine flow in the animal fed high protein pellets increased rapidly during the first 48 hours. This resulted in very low urea-N reabsorption rates. After urine flow stabilized, reabsorption rates increased and stabilized.

There was no change in urea-N reabsorption in the LOW reindeer group in January 1992 despite a significant decrease in serum urea-N. In wapiti in April 1991 the opposite was observed, with no change in serum urea-N with increased reabsorption. The differences in reabsorption between April 1991 and 1992 and November 1991 in wapiti were not reflected by differences in serum urea-N.

Schmidt-Nielsen *et al.* (1958) used the urea-N/inulin clearance ratio to determine the fraction of urea-N filtered at the glomerulus that was excreted (ie 1 - fraction

reabsorbed). As creatinine clearance was found to be within 3% of inulin clearance (Schmidt-Nielsen and Osaki 1958), the urea-N/creatinine clearance ratio was used in this study. This ratio is calculated by dividing urine urea-N/serum urea-N (urea U/S) by urine creatinine/serum creatinine (creat U/S).

In sheep on normal protein intake, between 40 and 50% of the urea-N filtered is reabsorbed through a wide range of urine flows while sheep on a low protein diet reduced the fraction excreted to less than 10% with low urine flows (Schmidt-Nielsen *et al.* (1958). In reindeer, urea-N/creatinine clearance ratios remained near 50% and were similar for all animals, on both diets (Figure 3-7). The rate of urine flow (creat U/S) had little effect on the fraction of urea-N reabsorbed. Thus, with decreased urine flow (increasing creat U/S) excretion of urea-N decreased proportionally.

In contrast, the LOW wapiti in April 1991 had lower urea-N/creatinine clearance ratios than animals on the high protein diet (Figure 3-7). Data from January 1991 were not included due to rapidly changing urine volumes. Thus, with decreased urine flow the proportion of urea-N filtered which is excreted decreases. This indicates that the LOW wapiti had increased tubular reabsorption of urea-N. Several of the urea-N/creatinine clearance ratios for HIGH wapiti were greater than unity suggesting that these animals may have been actively transporting urea-N into urine.

Reindeer with restricted nitrogen intake should maximize urea reabsorption in the kidney (minimize secretion) thereby minimizing nitrogen losses. However, this did not seem to happen, even in reindeer fed only lichen. Reabsorption of urea-N remained in the 40-50% range for all the diets used. Hove and Jacobsen (1975) have documented higher reabsorption in reindeer. In a study of renal reabsorption of urea-N in 9 month old reindeer calves, they found that animals fed a low protein diet (mainly lichens) had average urea-N reabsorption rates of up to 97% while average reabsorption on high protein diets (lichen and soyabean meal) was only 50%. The experimental animals in their study had been on the low protein diet for 2.5-3 months prior to the experiment and had very low serum urea-N (5 mg/dl).

UUC index

The use of UUC as an index of urea-N excretion allows for the analysis of a single sample, as opposed to a 24 hour sample, and it allows for analysis of samples collected from snow, with unknown dilutions. To be an appropriate denominator creatinine must be excreted consistently and not affected by nutrition.

The variation in creatinine excretion observed in individual wapiti and reindeer was slightly higher than that observed in humans (Vestergaard and Leverett 1958, and Forbes and Bruining 1976), and sheep (van Niekerk *et al.* 1963, Hodgen *et al.* 1967) while Dinning *et al.* (1949) found higher variation in beef steers. The observed variation may, in part, have been caused by the collection technique as it was not possible to ensure the collections were complete, despite efforts to ensure animals urinated prior to being placed in the metabolic crates and prior to being removed from the crates.

The excretion of creatinine observed during the 5-7 day trials demonstrated that creatinine is excreted consistently and is not affected by nutrition. Diurnal fluctuations have been observed in some studies (Boileau *et al.* 1972) but none were observed in this experiment. Reindeer fed lichen had similar creatinine excretion rates as the reindeer fed a high protein diet, supporting the observations of van Niekerk *et al.* (1963) that, over the short term, nutrition does not affect creatinine excretion. Only over the longer term, when nutrition affects body composition, are changes in creatinine excretion observed.

Although there was variation in daily creatinine excretion observed in both wapiti and reindeer it appears that UUC would still be a valid index of urea-N excretion. Even with changes in creatinine excretion due to changes in body composition, UUC reflected changes in urea-N excretion.

Conclusions

Both wapiti and reindeer reduced excretion of urea-N significantly when fed a low nitrogen diet. Urea-N conservation in wapiti appeared, in part, to be due to a change in the reabsorption of urea in the tubules of the kidney. Reindeer, despite their nitrogen deficiency, did not attempt to conserve urea-N by increasing reabsorption. Reindeer reduced urea-N loss by reducing urine volume. This is in accordance with the conclusions of Eriksson and Valtonen (1974) who, based on the concentration gradient through the cortex and medulla of the kidney, concluded that reindeer do not conserve urea-N by decreasing its concentration in urine.

Data obtained during this study also support the contention that UUC can be used as an index of urea-N excretion. This conclusion is important to the use of UUC as a nutritional index as proposed by DelGiudice *et al.* (1989).

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Table 3-1: Urea-N excretion (UREA EXCR), proportion of urea-N filtered in the glomerulus reabsorbed in tubules (% UREA REABS), creatinine excretion (CREAT EXCR), urinary urea-N to creatinine ratios (UUC), and serum urea-N concentrations (SERUM UREA) in wapiti fed high and low protein diets.

	January 1991		April 1991		November 1991		April 1992
UREA EXCR (g/day)							
HIGH n=5	65.89±15.0	*	31.11±4.39	*	56.43±2.53	ns	33.12±4.72
	*		*		ns		ns
LOW n=5	31.81±2.80	**	9.71±1.20	**	59.60±2.51	*	24.30±3.31
% UREA REABS (%)							
HIGH n=5	14.81±14.8	ns	52.20±8.89	*	3.77±4.68	*	53.60±7.32
	ns		*		ns		ns
LOW n=5	7.38±12.5	*	77.78±3.38	*	8.20±10.8	*	54.31±2.89
CREAT EXCR (g/day)							
HIGH n=5	6.59±0.57	ns	8.21±0.96	ns	5.59±0.27	*	8.07±0.88
	*		*		ns		ns
LOW n=5	5.13±0.26	ns	4.96±0.34	ns	5.41±0.18	*	7.06±0.62
UUC (mg/dl:mg/dl)							
HIGH n=5	9.62±1.66	*	3.79±0.27	**	10.12±0.39	**	4.22±0.27
	*		*		ns		*
LOW n=5	6.14±0.36	**	2.10±0.27	**	11.02±0.35	**	3.34±0.31
SERUM UREA (mg/dl)							
HIGH n=5	21.14±2.11	ns	19.82±1.23	ns	19.97±1.06	ns	19.88±0.89
	ns		ns		ns		ns
LOW n=5	19.83±1.67	ns	18.32±1.10	*	23.53±1.64	*	16.96±1.11

Note: Significant pairwise comparisons are indicated by * = P<0.05 ** = P<0.01.
Data are expressed as mean±SE

Table 3-2: Urea-N excretion (UREA EXCR), proportion of urea-N filtered in the glomerulus reabsorbed in tubules (% UREA REABS), creatinine excretion (CREAT EXCR), urinary urea-N to creatinine ratios (UUC) and serum urea-N concentrations (SERUM UREA) in reindeer fed high and low protein diets.

	November 1991		January 1992
UREA EXCR (g/day)			
HIGH n=3	27.19±1.49	ns	21.97±1.46
	ns		*
LOW n=3	27.61±2.98	*	8.06±1.80
% UREA REABS (%)			
HIGH n=3	44.36±4.77	ns	42.42±1.46
	ns		ns
LOW n=3	38.42±1.62	ns	47.63±3.13
CREAT EXCR (g/day)			
HIGH n=3	2.62±0.16	*	2.21±0.16
	ns		ns
LOW n=3	2.78±0.37	*	1.58±0.27
UUC (mg/dl:mg/dl)			
HIGH n=3	10.41±0.38	ns	9.66±0.42
	ns		*
LOW n=3	10.12±0.45	*	5.09±0.51
SERUM UREA (mg/dl)			
HIGH n=3	28.12±2.24	ns	26.96±1.31
	ns		*
LOW n=3	24.77±1.31	*	18.30±0.47

Note: Significant pairwise comparisons are indicated by * = P<0.05 ** = P<0.01.
Data are expressed as mean±SE.

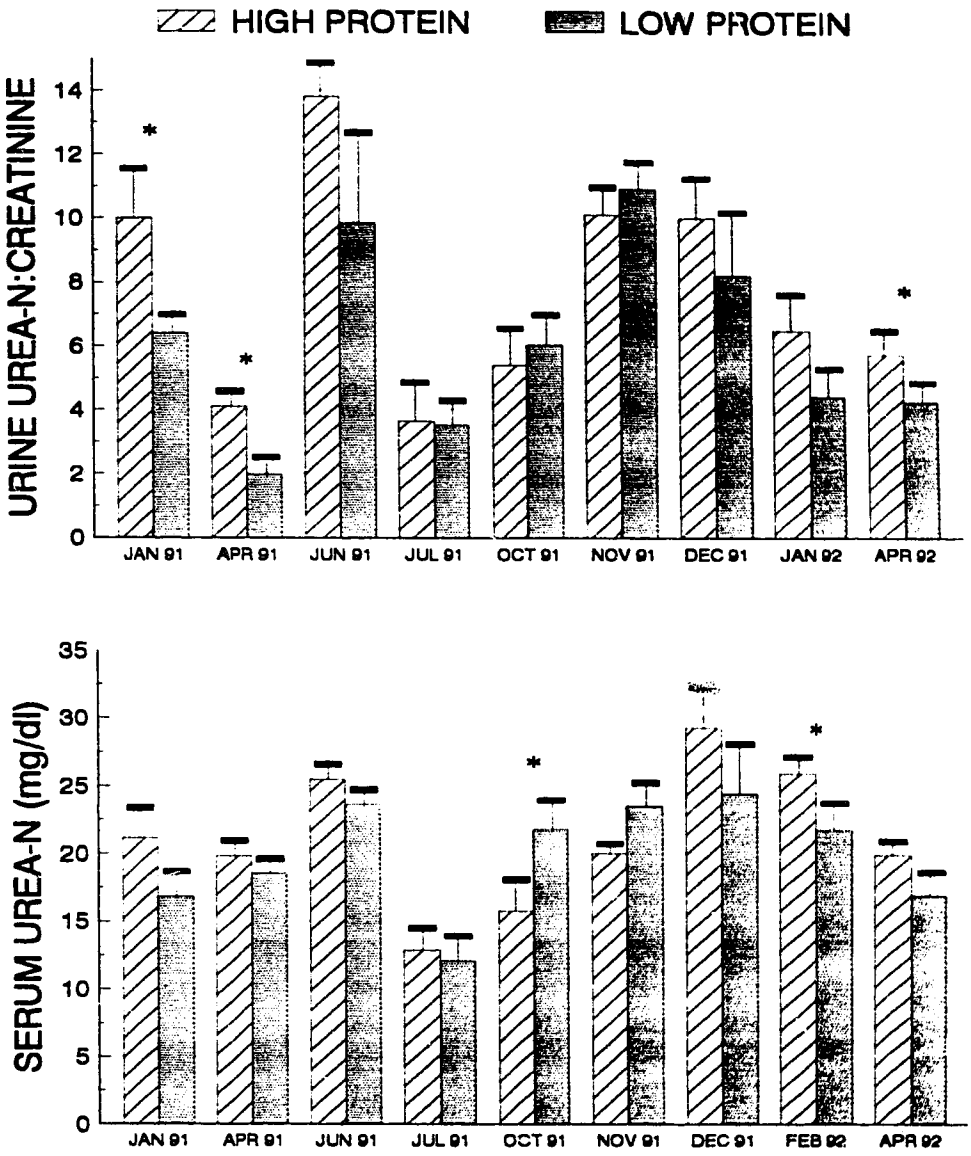

Figure 3-1: Monthly urine urea-N:creatinine (mean±SE) and and serum urea-N concentrations (mean±SE) in adult female wapiti on high and low protein diets. Animals were fed the experimental diets from Jan 91 to Apr 91 and from Nov 91 to Apr 92. Significant differences between groups are indicated by * ($P<0.05$).

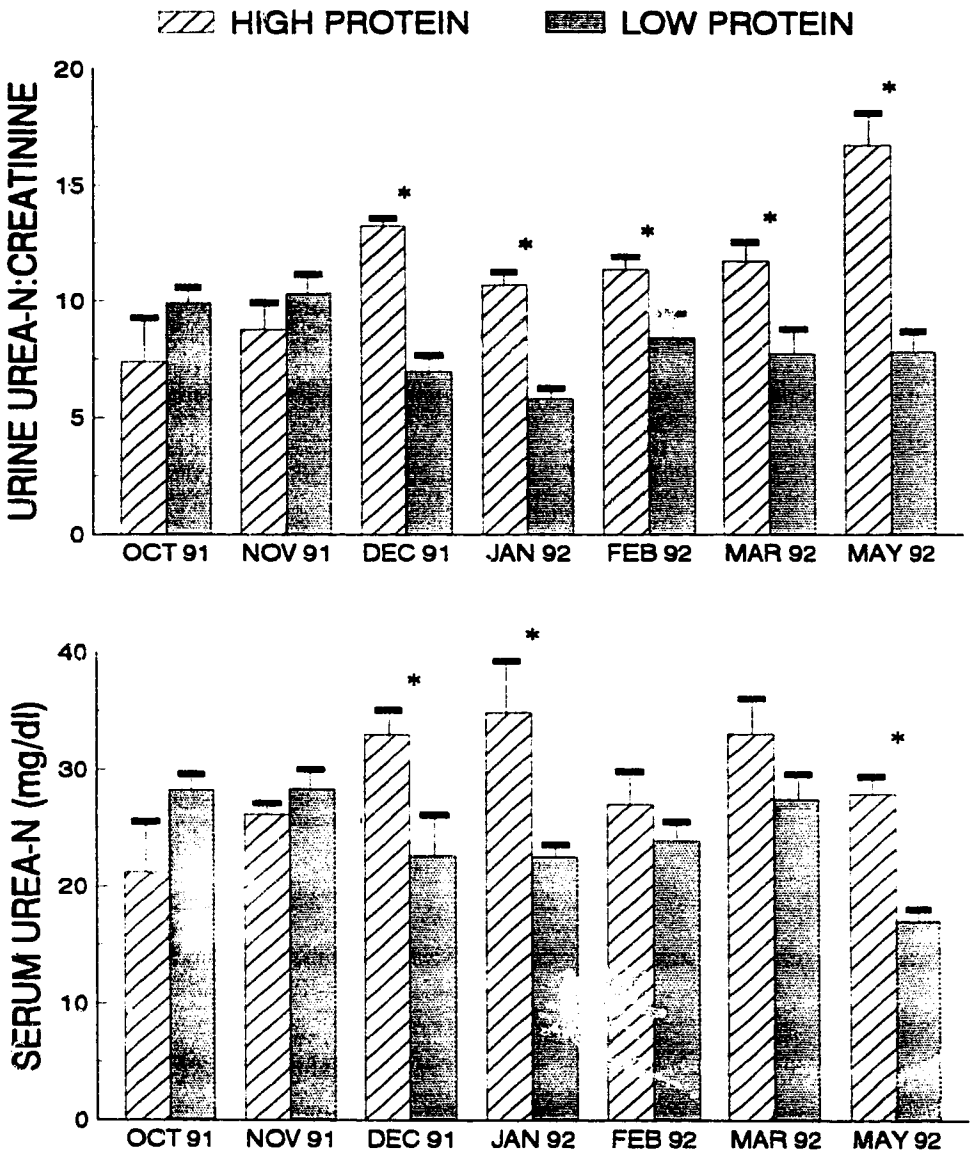

Figure 3-2: Monthly urine urea-N:creatinine (mean±SE) and serum urea-N concentrations (mean±SE) in adult female reindeer fed high and low protein diets. Animals were on the experimental diets from Dec 91 to May 92. Significant differences between groups are marked by * ($P<0.05$).

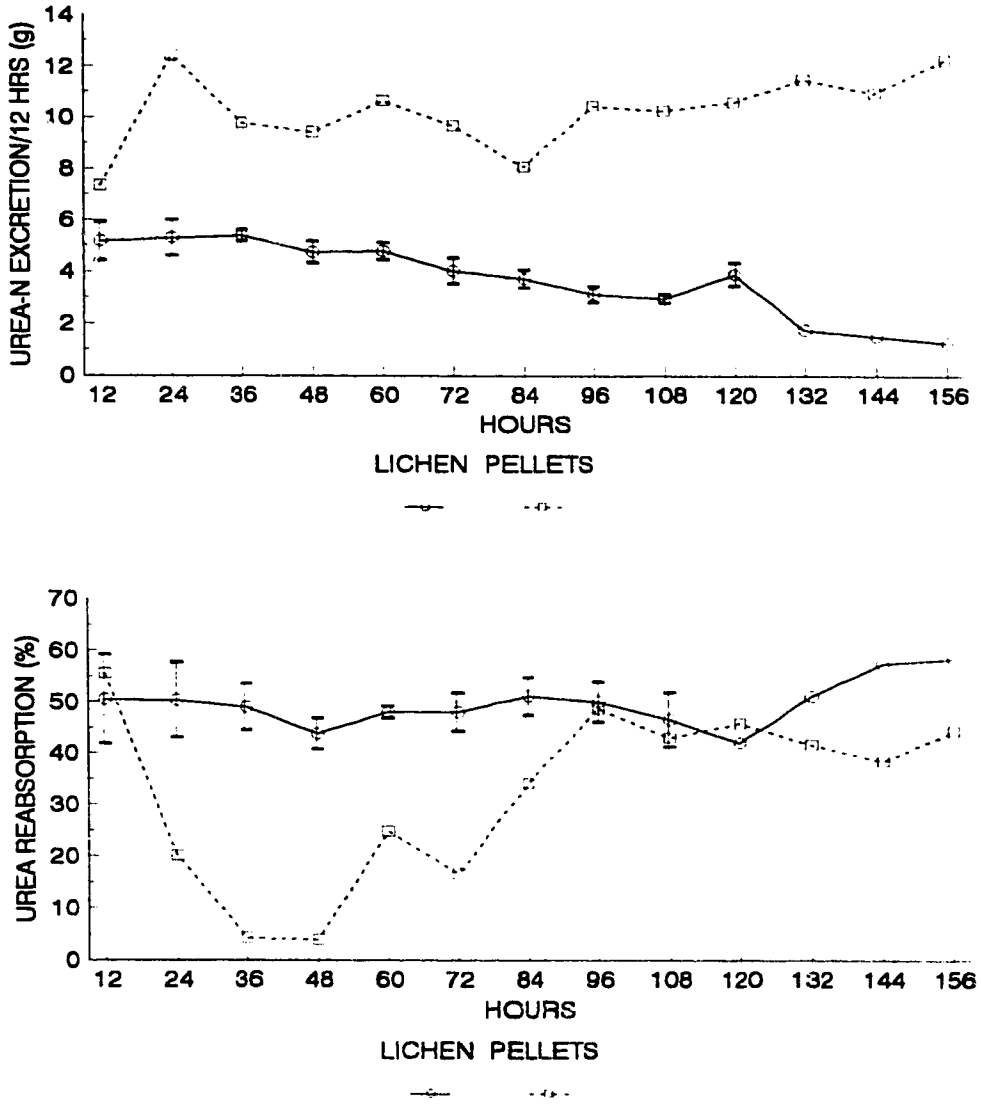

Figure 3-3: Urinary urea-N excretion (mean±SE) and reabsorption in the kidney (mean±SE) in adult female reindeer fed lichen (n=4) or high protein pellets (n=1).

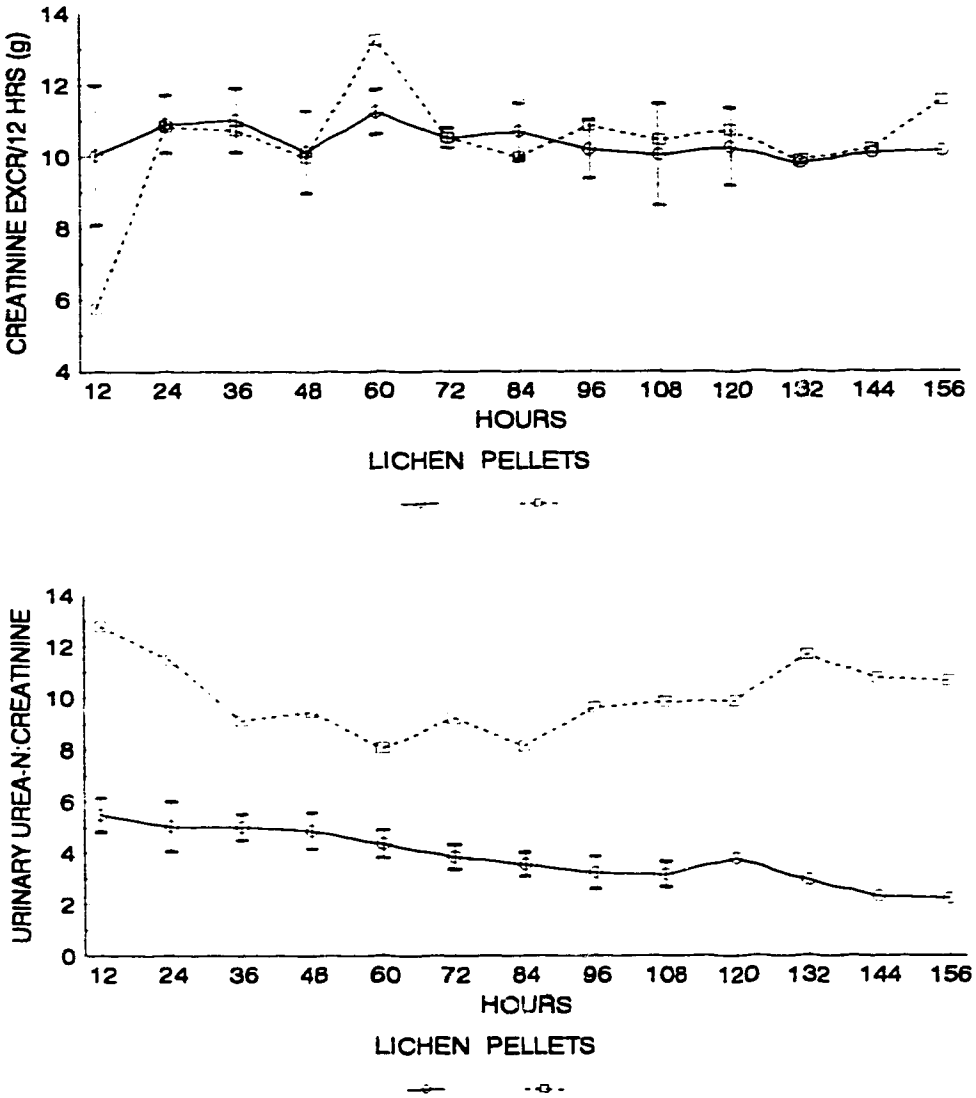

Figure 3-4: Urinary creatinine excretion (mean±SE) and urinary urea-N:creatinine ratios (mean±SE) in adult female reindeer fed lichen (n=4) or high protein pellets (n=1).

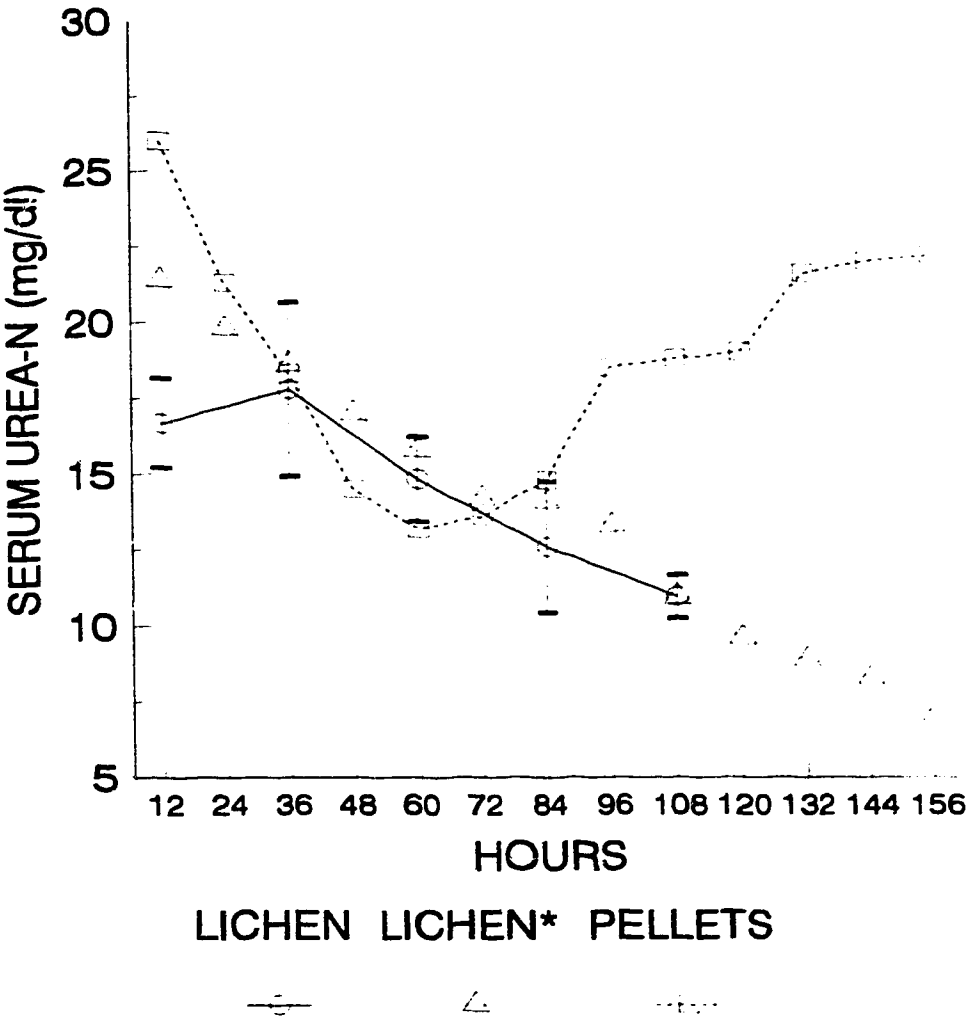

Figure 3-5: Serum urea-N concentration (mean±SE) in adult female reindeer fed lichen (n=4) and high protein (n=1). (* = sampled on a 12 hour interval).

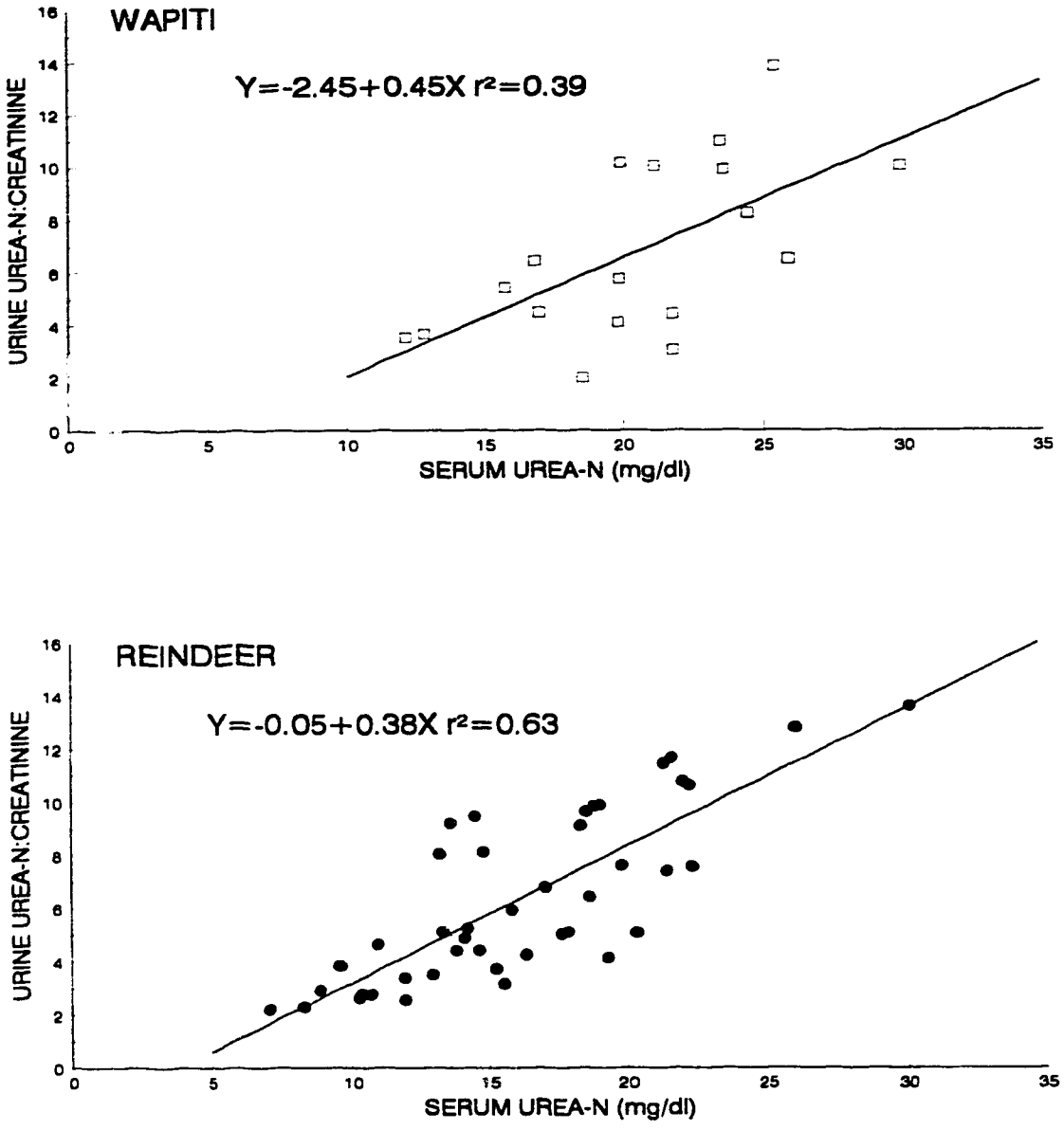

Figure 3-6: Serum urea-N concentration versus urine urea-N:creatinine in adult female wapiti and reindeer. LOW and HIGH groups were combined for the analysis.

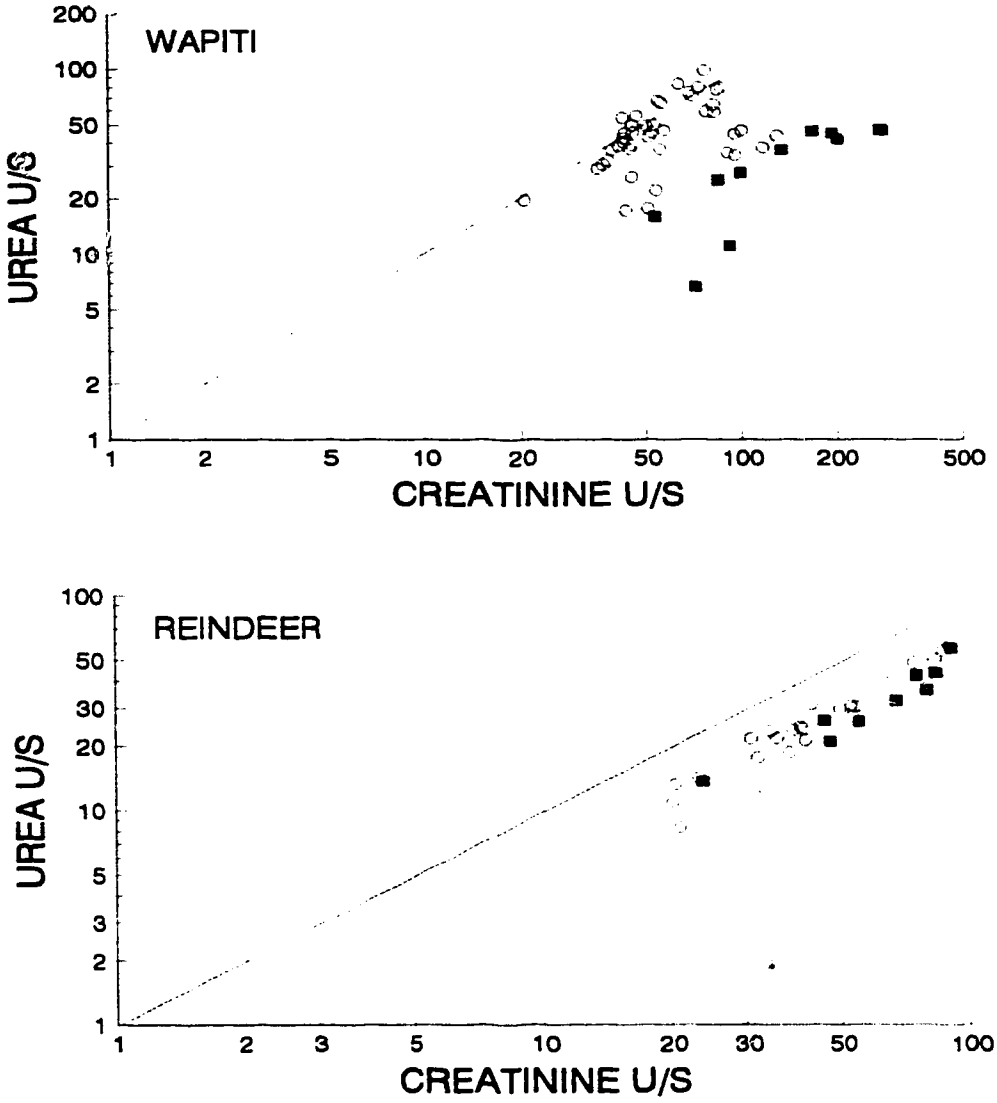

Figure 3-7: Urea U/S ratios vs creatinine U/S ratios in adult female wapiti and reindeer fed high (○) and low (■) protein diets and a reindeer calf fed lichen (·) (Hove and Jacobsen 1975).

Chapter 4

Urinary excretion of N^{τ}-methylhistidine in wapiti and reindeer: an invalid indicator of protein turnover

Introduction

Northern ruminants typically face low dietary quality and intake over winter (Morgantini and Hudson 1989, Nieminen and Heiskari 1989, McEwan and Whitehead 1970). In response to lower protein intake ruminants reduce nitrogen losses by decreasing urea-N excretion and by recycling urea-N to rumen microbes (Robbins *et al.* 1974, Wales *et al.* 1975). If these processes are not sufficient to make up for the low nitrogen intake then animals will utilize endogenous proteins, primarily striated muscle, as a source of amino acids. An increase in net protein catabolism over winter in northern ungulates has been suggested, based on comparisons of muscle weights from animals harvested from the same population in fall and spring (Adamczewski *et al.* 1987, Ouellet 1992) but direct evidence has not been obtained. It would also be advantageous for animals with restricted nitrogen intake to reduce muscle protein turnover as the process requires a net energy expenditure. Unfortunately, it has not been possible to study protein turnover in free ranging animals.

In this study urinary excretion of N^{τ}-methylhistidine (N^{τ}-MH) was evaluated as an indicator of myofibrillar protein degradation in wapiti (*Cervus elaphus*) and reindeer (*Rangifer tarandus*). It was decided to determine if urinary N^{τ}-MH:creatinine ratios (UN^{τ}-MHC) in urine may, in some species, provide a superior indication of protein catabolism than urinary urea-N:Creatinine ratios (UUC). UUC have been investigated as a potential indicator of physiological processes in free ranging ungulates and have proved useful in assessing nutritional status (DelGiudice and Seal 1988, DelGiudice *et al.* 1989, 1991a, 1991b, Cool 1992). However, UUC increase only when animals are experiencing severe under-nutrition, or alternately, when the diet provides high levels of nitrogen (Parker *et al.* 1993). Ruminants reduce urea-N excretion by increasing urea-N reabsorbtion in the

kidney and by more efficient recycling of urea-N to rumen microbes (Robbins *et al.* 1974, Wales *et al.* 1975, Cool 1992, this study). These processes must therefore be overwhelmed before UUC increase. In addition, urea is produced whenever high levels of amino acids are released into the blood whether they originate in the diet or endogenously. As N^{τ}-MH is a product of a single process, the post-translational methylation of specific histidine residues in the actin of all muscle fibres and in the myosin of white muscle fibres (Lukanski *et al.* 1981), and has been shown to be quantitatively excreted in some species, UN^{τ}-MHC promised to be more sensitive than UUC to changes in muscle protein degradation.

There are several practical advantages to having a sensitive indicator. Information on muscle protein degradation would enable wildlife managers to determine the status of free ranging animals in winter (ie. if they are catabolizing endogenous protein as a source of nitrogen) before the animals are in very poor condition. It would reveal if animals which appeared to be in poor condition were improving or declining in condition. This would provide advanced warning of the reproductive and calf survival effects of declining condition. This information would also provide information on whether or not northern ruminants are able to reduce protein turnover in response to winter conditions.

Unfortunately, because N^{τ}-MH excretion varies between species, its use as an index of protein degradation needs to be validated for each species before it can be used. Harris and Milne (1981a) listed a number of assumptions which need to be confirmed before N^{τ}-MH excretion can be used. These included; 1) there is no N^{τ}-MH in the diet, 2) N^{τ}-MH released from protein breakdown is rapidly eliminated from the body, and 3) N^{τ}-MH is not metabolized.

N^{τ}-MH excretion has been validated in several species. Harris and Milne (1981a) concluded that the excretion of N^{τ}-MH in urine is a valid index of muscle protein breakdown in cattle. Tomas *et al.* (1984) used N^{τ}-MH excretion in the urine as an index of myofibrillar protein breakdown in rats. In other species the results were not as promising. N^{τ}-MH does not appear to be a valid index of muscle protein breakdown in sheep, pigs, or goats as they only slowly excrete intravenously injected labelled N^{τ}-MH

in urine (Harris and Milne 1980, 1981b, Brown *et al.* 1987). The slow excretion in these species has been attributed to a large expandable balenine pool (N^{τ}-MH-beta alanine).

The objectives of this study were to determine if N^{τ}-MH was quantitatively excreted by wapiti and reindeer, to investigate factors influencing N^{τ}-MH excretion and to determine if N^{τ}-MH is metabolized in these animals. Physiological indicators need to be evaluated in a situation where complicating factors such as stress and diet can be controlled. These experiments were therefore conducted on captive-reared wapiti and reindeer.

Materials and methods

The experimental procedures in this study met the requirements of the Canadian Council on Animal Care and were approved by the Animal Care Committee, Department of Animal Science, University of Alberta (wapiti) and by the Animal Care Committee, Department of Biology, University of Alaska, Fairbanks (reindeer).

Daily excretion of N^{τ}-methylhistidine by wapiti and reindeer

Daily excretion of N^{τ}-MH was determined for six adult female wapiti aged 4-6 years at the University of Alberta's Ministik Wildlife Research Station (MINISTIK) near Edmonton and six adult female reindeer aged 5-8 years at the Large Animal Research Station (LARS) at the University of Alaska, Fairbanks.

The trial for wapiti was conducted in January 1991. All animals were fed a pelleted diet with 8.5% crude protein *ad libitum* (see Chapter 2 for ingredients). No animal products were in the mixture. Animals were placed in 2.4 x 1.2 m metabolic crates at ambient temperature (-7 to -5°C) for 24 hours. The crates were equipped with vinyl coated grated floors which allowed urine to be separated from feces and collected in underlying trays. The daily volume of urine produced was recorded and a sample was collected for later analysis. Serum was recovered from blood samples taken from the jugular vein immediately after the animals were removed from the crates. Serum and urine samples were stored at -20C until analyzed.

Trials for reindeer were conducted in November 1991 and January 1992. In November six reindeer were fed a pelleted diet containing 18.8% crude protein *ad libitum* (see Chapter 2 for ingredients). No animal products were in the mixture and no N^{τ}-MH was detected in the pellets. After the November trial, three of the animals were selected at random and were switched to a pelleted diet containing 8.5% crude protein. This diet was similar to that provided to the wapiti and contained no animal products. (see Chapter 2 for ingredients). These animals remained on this diet through the January trial.

During the trials, animals were placed in 0.6 x 1.2 m metabolic crates equipped with vinyl coated grated floors and plastic fecal separation screens which allowed urine and feces to be separated with little cross contamination. Three 24 hour collections were made per animal to help compensate for daily fluctuations. As the crates did not allow the animals to turn around or lie down, collections were separated by two days to minimize stress and to allow the animals to exercise. Between collections the animals were held in individual 4 x 5 m pens. The daily volume of urine produced was recorded, urine was thoroughly mixed and a subsample was collected for later analysis. Blood samples were taken via venapuncture after each of the three urine collections when the animals were removed from the crates and serum was separated and collected. Serum and urine samples were stored at -20°C until analyzed.

Isotope recovery

Adult female reindeer from the Large Animal Research Station at the University of Alaska Fairbanks were used to determine the quantitative recovery of N^{τ}-[$^{14}CH_3$]MH in urine. Trials were conducted in January 1992 (3 animals) and May 1992 (2 animals). Animals were housed indoors in horse stantions. The ambient temperature remained between 10 and 15°C.

Animals were offered a pelleted reindeer diet containing 18.8% crude protein *ad libitum* (same ration as November daily excretion trials). Four of the animals refused the pellets while in the metabolic crates even though they consumed them when out of the metabolic crates. Mid-way through the trial these four animals were supplemented with lichen. In January, animals were provided snow *ad libitum* while in May they had free

access to water. Body weights were obtained at the beginning and end of each trial. 7 days prior to the trial, a catheter was surgically implanted into one jugular of each animal. Animals were anaesthetized using a combination of Xylazine and Ketamine. At the start of the trial (time 0) animals were injected with approximately 200μCi of N^{τ}-[$^{14}CH_3$]MH (14.4mCi/mmol, radiochemical purity >95%, Amersham, Buckinghamshire England) in a solution of 0.9% saline and a concentration of 5.5056 μCi/ml. The precise amount of solution injected was determined by weighing the syringe before and after injection. The injection was made into the jugular which did not contain the catheter. After injection, animals were placed in 2.4 x 1.2 m metabolic crates equipped with vinyl coated expanded metal floors, a fine screen under the grating to collect fecal material and a urine collection tray to funnel urine into a collection bucket. Blood samples were taken via venous catheter just prior to injection and at 12 hour intervals thereafter. The blood samples were centrifuged and serum was collected. Urine volume was determined every 12 hours, the urine was thoroughly mixed, a 50 ml aliquot was collected and preserved by freezing. Serum and urine samples were stored at -20°C until analyzed.

Chemical analyses

Urine and serum were analyzed for N^{τ}-MH content using an HPLC procedure (Dalla Libera 1991). Samples were deproteinized with 0.200 ml of 3.0M $HClO_4$ and centrifuged at 3000 rpm for 15 minutes. Deproteinized samples were analyzed using a Varian Model 5500 Liquid Chromatograph with a Varian 2070 spectrofluorometer detector and a Varian 9090 auto analyzer (Varian Canada, Calgary AB). Separations were done on a 15 m x 4.6 mm 3 micron reverse phase column (Supelco Inc., Bellefonte PA).

Radioactivity in serum and urine was determined via liquid scintillation. Approximately 0.100-0.200 ml of urine or serum was placed in 14 ml of Ecolite(+) liquid scintillation fluid (ICN Biomedicals Inc. Irvine CA) and counted in a Tricarb 1600CA Liquid Scintillation Counter (Packard Instrument Co. Downers Grove IL). Cumulative excretion of N^{τ}-[$^{14}CH_3$]MH was determined based on urine volume and specific radioactivity of urine.

Urine flow rate (UFR) was calculated from the total urine production while animals were in the metabolic crates. Glomerular filtration rate (GFR) and tubular secretion of N^{τ}-MH was determined using the renal clearance of creatinine (Kaplan and Pesce 1989). Creatinine concentrations (µmol/ml) were determined using a colorimetric method based on the Jaffé reaction (Sigma Diagnostics, St. Louis MO). The proportion of N^{τ}-MH filtered at the glomerulus which was excreted was determined using the N^{τ}-MH/creatinine clearance ratio ((urine N^{τ}-MH[nmol/ml]/serum N^{τ}-MH[nmol/ml])/(urine creatinine[µmol/ml]/serum creatinine[µmol/ml])).

To determine if radioactivity in urine was associated with N^{τ}-MH, injectate and urine samples were run through ion-exchange columns as described by Haverberg *et al.* (1974). Fifteen fractions containing two column volumes each were collected, an aliquot was taken for measurement of radioactivity, and radioactivity was counted in a Tricarb 1600CA Liquid Scintillation Counter (Packard Instrument Co. Downers Grove IL).

Statistical analyses

Statistical analyses were conducted using procedures from SAS (SAS 1988). The relationships between weight, GFR and UFR, and N^{τ}-MH excretion was analyzed using least squares regression (PROC REG). Differences between trials and groups were evaluated using least squares analysis of variance (PROC GLM). Differences between animals were assessed using and Tukey's studentized range test.

Results

Daily urinary excretion of N^{τ}-MH

Daily urinary excretion of N^{τ}-MH by six adult female wapiti maintained on a low protein diet (8.5% CP) averaged 35.5±7.5 µmol/day (mean±SE) (Table 4-1). Corrected for weight, excretion averaged 0.13±.02 µmol/kg/day. Serum concentrations of free N^{τ}-MH for the same six animals averaged 147±8 nmol/ml.

Excretion of N^{τ}-MH (µmol/day) by six reindeer in November was weakly correlated with body weight (r^2=0.56) (Figure 4-1). Based on the regression equations,

typical daily excretion for a 100 kg animal in November was estimated to be 3.47 μmol/kg/day.

In November, there were no significant differences either in daily N^{τ}-MH excretion or daily excretion of N^{τ}-MH by body weight between the two reindeer treatment groups ($P>0.5$). By January, daily excretion and daily excretion by body weight in reindeer fed a low protein diet had declined significantly ($P<.001$) and were significantly lower than in animals fed a high protein diet ($P<.001$) (Table 4-1). In November there was no significant difference in serum concentration between the two reindeer treatment groups. By January, serum concentrations increased significantly ($P<.001$) in the low protein group.

Low daily excretion of N^{τ}-MH was also recorded for reindeer which refused a pelleted ration and were fed lichen during the isotope recover trials (RD 007, WINKLE and RD176 in January and WINKLE in May) (Table 4-1). RD 212, which consumed an average of 0.75 kg of high protein ration per day, had a very high N^{τ}-MH excretion rate. Serum N^{τ}-MH concentrations were the highest in the animal with the lowest N^{τ}-MH excretion (WINKLE, May 1992).

Renal handling of N^{τ}-MH

There were no significant differences in UN^{τ}-MHC between trials or dietary groups in the excretion trials (Table 4-2). GFR and UFR were significantly lower ($P<0.05$) in the low protein group in January and were significantly higher ($P<0.05$) in the fed animal (RD 212) than in voluntarily fasted animals in the isotope recovery trials.

A strong linear correlation ($r^2=0.75$) was observed between the rate of N^{τ}-MH excretion and UFR in all animals, with the rate of N^{τ}-MH excretion increasing with increased UFR (Figure 4-2). There was no correlation between N^{τ}-MH excretion and GFR ($r^2=0.15$) (Figure 4-2). Mean N^{τ}-MH/creatinine clearance ratios were significantly different between animals fed high and low protein diets (HIGH 0.129±0.008 SE n=26, LOW 0.078±0.006 SE n=9 $P<0.001$).

Quantitative excretion of N^{τ}-[$^{14}CH_3$]MH

Cumulative recovery of injected N^{τ}-[$^{14}CH_3$]MH averaged 60.6%±1.9 (mean±SE n=3) after 115 hours during the January isotope recovery trial, while in May, 56.9% of the label was recovered from one animal and 72.4% was recovered from another after 156 hours (Figure 4-3). In both trials, between 15 and 20 percent of the label was recovered in the first six or seven hours following injection.

Metabolism of N^{τ}-MH

By determining the cumulative excretion of N^{τ}-[$^{14}CH_3$]MH, it is possible to determine the amount of N^{τ}-[$^{14}CH_3$]MH remaining in the body and the proportion of this amount cleared during the following 12 hour period. Figure 4-4 indicates that in all animals the proportion of the remaining dose cleared declines over time.

A comparison of the relationship between the radioactivity (^{14}C) remaining in serum and the amount of injected radioactivity (^{14}C) not accounted for by the urine provides insights into the location of the remaining N^{τ}-[$^{14}CH_3$]MH. Assuming that blood volume remains constant (5 l was used as a standard) over the trial a decreasing proportion of the label remaining is accounted for by serum (Figure 4-5).

The presence of a N^{τ}-MH metabolite was indicated by differences between urine and the injectate in recovery of radioactivity from ion exchange fractions (Figure 4-6). HPLC analysis of volumes 11 and 12 from the urine samples indicated the radioactivity peak was not associated with free N^{τ}-MH. Hydrolysis of urine samples (6M HCl at 110° for 2 hours) did not eliminate the radioactivity peak.

Discussion

Daily urinary excretion of N^{τ}-MH

In wapiti, urinary excretion of N^{τ}-MH is very low relative to serum levels of free N^{τ}-MH. N^{τ}-MH excretion is also very low relative to cattle on a live weight basis. This indicated that there was a large body pool of free N^{τ}-MH and only minute amounts were

being excreted in the urine, effectively invalidating the use of N^{τ}-MH to monitor muscle protein degradation in wapiti.

Excretion of N^{τ}-MH by adult female reindeer was comparable on a live weight basis to that recorded for male cattle by Harris and Milne (1981a) (4.04 μmol/kg/day for a 100kg animal). The authors suggest that female cattle would excrete slightly less. Daily excretion of N^{τ}-MH by adult female reindeer (3.0 to 3.5 μmol/kg/day for a 100kg animal) suggests that a large proportion of the free N^{τ}-MH was being excreted daily and invited further investigation.

Harris and Milne (1981a) observed a strong linear correlation between N^{τ}-MH excretion and live body weight in male cattle ranging in weight from 50-500 kg. Although the range of weights analyzed in this study was small (83-122 kg), N^{τ}-MH and body weight were also linearly correlated in adult female reindeer.

These comparisons of excretion data with cattle suggested that N^{τ}-MH may be rapidly excreted in reindeer, as observed in cattle. The observation that the reindeer on the low protein diet reduced N^{τ}-MH excretion also supported the suggestion that N^{τ}-MH was being quantitatively excreted as the percent striated muscle mass (%SMM), calculated from creatinine excretion (see Chapter 2 for calculations), was lower in these animals. As the major source of N^{τ}-MH is striated muscle, it would be expected that N^{τ}-MH excretion would be lower in this reindeer group. These reindeer also had a lower nitrogen balance than the reindeer fed a high protein diet. Haverburg *et al.* (1975) attributed decreases in N^{τ}-MH excretion in rats fed a low protein diet to metabolic adaptation to conserve muscle loss due to breakdown. This may also have been occuring in reindeer.

However, serum concentrations of free N^{τ}-MH in reindeer were much higher than recorded in cattle (Harris and Milne 1981a). Concentrations were more comparable with those of adult sheep (Harris and Milne 1980, 1987) and adult pigs (Harris and Milne 1981b). In addition, animals on a low protein diet had significantly higher serum levels of N^{τ}-MH than animals on a high protein diet. This suggested that free N^{τ}-MH is being retained in the blood and that there were changes in renal handling of N^{τ}-MH.

Renal handling of N^{τ}-MH

To conserve energy and water, reindeer decrease water flux in winter (Cameron and Luick 1972). This may have implications in the excretion of N^{τ}-MH. Brown *et al.* (1987) suggested that water flux may affect N^{τ}-MH excretion as female goats with restricted water intake had lower N^{τ}-MH excretion rates than males with water *ad libitum*. Observations from this study support this suggestion.

In the May isotope recovery experiment, the animal under voluntary water restriction and with low UFR had lower and more consistent rates of N^{τ}-MH excretion than the animal with high water intake. Differences in UFR during the excretion trials also mirrored N^{τ}-MH excretion with animals in the low protein group in January having significantly lower N^{τ}-MH excretion and UFR. The strong correlation observed between UFR and N^{τ}-MH excretion, along with the lack of correlation with GFR, indicates that N^{τ}-MH excretion is affected by the volume of urine produced even if there is no change in the amount of plasma filtered.

Hoffer (1990) also observed that N^{τ}-MH excretion declined in fasting humans despite no change in GFR and concluded there was a change in renal handling of N^{τ}-MH. Hoffer (1990) suggested that renal handling of N^{τ}-MH is related to the free amino acid concentration in blood (ie, if free amino acid concentrations decline then the rate of N^{τ}-MH excretion declines in parallel due to increased amino acid reabsorption in the kidney). This did not appear to occur in this experiment as animals with low excretion rates had elevated serum N^{τ}-MH concentrations. The observed decrease in the proportion of filtered N^{τ}-MH reabsorbed by reindeer on low protein diets with decreased urine flows indicates that these animals had increased tubular reabsorption of N^{τ}-MH. This supports Hoffer's (1990) suggestion that there were changes in renal handling of N^{τ}-MH but it would appear that this is related to urine flow rather than just to amino acid concentration.

The observations that N^{τ}-MH excretion is affected by water flux is an important factor in evaluating the use of N^{τ}-MH excretion as a indicator of protein catabolism in any species. It is of particular importance in reindeer where seasonal changes in water flux have been documented. These observations also cast doubt on whether N^{τ}-MH was being quantitatively excreted and was reflecting protein turnover. The definitive method

to determine quantitative excretion is through the use of labelled N^{τ}-MH (Harris and Milne 1980, 1981a, 1981b).

Recovery of injected N^{τ}-[$^{14}CH_3$]MH

Urinary excretion of N^{τ}-MH has been validated as an indicator of muscle protein breakdown in only a few species based on the quantitative excretion of N^{τ}-[$^{14}CH_3$]MH. In cattle, over 90% of injected N^{τ}-[$^{14}CH_3$]MH was recovered in urine within 7 days (Harris and Milne 1981a). N^{τ}-MH excretion was also found to be quantitative in man (Long *et al.* 1975), New Zealand White Rabbit (Harris *et al.* 1977), and rats (Young *et al.* 1972). In rats, much of the N^{τ}-MH in urine is in an acetylated form however this does not interfere with the quantitative excretion (Sainz *et al.* 1984). Quail (*Coturnix coturnix japonica*) and domestic fowl excreted over 90% of injected N^{τ}-[$^{14}CH_3$]MH over seven days (Saunderson and Leslie 1983).

N^{τ}-MH excretion has failed to be a reliable indicator of protein turnover in several species. Recovery of N^{τ}-[$^{14}CH_3$]MH injected into sheep became asymptotic at less than 50% after 7 days (Harris and Milne 1980) while pigs excreted less than 25% of the injected dose over the same period (Harris and Milne 1981b). In a study on male and female dairy goats, Brown *et al.* (1987) reported the cumulative percentage of recovered radioactivity only reached 40% - 50% after 7 days. In male Saanen goats, Nishizawa *et al.* (1989) obtained nearly 100% recovery after 7 days suggesting that the technique was valid. However, a similar study with female Saanen goats only obtained 55.4% recovery after six days (Nishizawa *et al.* 1992). Domestic turkeys were similar in that they only excrete 49% of injected N^{τ}-[$^{14}CH_3$]MH during seven days (Saunderson and Leslie 1983).

The recovery of injected radioactivity in adult female reindeer was substantially lower than that observed in species where urinary excretion of N^{τ}-MH has been validated. Recovery was similar to that observed in goats (Brown *et al.* 1987, Nishizawa *et al.* 1992) and sheep (Harris and Milne 1980). The difference in recovery is mainly a function of excretion during the first 24 hours. Cattle excreted 50-75% of the label in the first day

whereas sheep, goats, and reindeer only excreted 10-40%. This suggests that reindeer contain a large pool of free or reversibly bound N^{τ}-MH into which the N^{τ}-[$^{14}CH_3$]MH rapidly mixed.

Evidence of N^{τ}-MH metabolism

Metabolism of N^{τ}-MH has also been indicated in some studies. Schwartz *et al.* (1973) identified a pathway for biosynthesis of N^{τ}-methylhistamine (N^{τ}-MHA) via the decarboxylation of N^{τ}-MH in rats. Labelled 1-methylimidazole-4-acetic acid, a metabolite of N^{τ}-MHA, was detected in the urine of mice after injection with N^{τ}-[$^{14}CH_3$]MH (Murray *et al.* 1985). Brown *et al.* (1987) speculated that the N^{τ}-[$^{14}CH_3$]MH had undergone decarboxylation to N^{τ}-MHA in goats. Nishizawa et al. (1992) suggested that labelled metabolites recorded in female goat urine were N-acetyl-N^{τ}-MH and 1-methylimidazole-4-acetic acid.

In this study, the declining proportion of the ^{14}C remaining in the body which was excreted in the urine and the declining proportion of the remaining radioactivity which is accounted for by serum volume suggests that N^{τ}-[$^{14}CH_3$]MH was constantly taken up by other tissues. In addition, the occurrence of a second peak in the ion exchange fractions suggests that a metabolite of N^{τ}-[$^{14}CH_3$]MH may exist in reindeer urine. This metabolite was not identified nor was the proportion of urinary radioactivity attributable to the metabolite determined. However, the observation that the peak was not removed by acid hydrolysis suggests it was not N-acetyl-N^{τ}-MH nor a peptide of N^{τ}-MH.

In goat urine, Nishizawa *et al.* (1989) found about 20% of the activity in two peaks other than N^{τ}-MH. One peak was subsequently identified as N-acetyl-N^{τ}-MH as the activity was transferred to the N^{τ}-MH peak after hydrolysis (Nishizawa *et al.* 1992). The second peak was tentatively identified as 1-methylimidazole-4-acetic acid, a metabolite of N^{τ}-MHA.

Several authors have postulated why there should be such a difference in the excretion of N^{τ}-MH between species and, in some cases, between age and sex classes of the same species. Harris and Milne (1980) suggested that those species with the lowest concentration of the peptide balenine (N^{τ}-MH-beta alanine) in muscle showed the fastest

clearances of N^{τ}-[$^{14}CH_3$]MH. Balenine, likely synthesized in muscle along with carnosine and anserine, was at first thought to only be found in those animals which retained N^{τ}-[$^{14}CH_3$]MH but has now been shown to occur as a normal constituent of muscle in all animals tested. Wapiti have very high balenine levels (Harris and Milne 1987, Plowman and Close 1988) which fits with the low daily excretion of N^{τ}-MH. Reindeer have not been analyzed for balenine, however, based the present results and Harris and Milne's (1980) suggestion, reindeer should have high balenine concentrations.

Conclusions

This study indicates that urinary excretion of N^{τ}-MH does not provide a reliable measure of protein degradation in wapiti or reindeer. In wapiti only minute amounts of N^{τ}-MH are excreted despite a high concentration of free N^{τ}-MH in serum. As found in their close relatives, red deer, wapiti would also likely have a high concentration of balenine. In reindeer, N^{τ}-MH is not quantitatively excreted in urine despite excretion levels comparable, on a live weight basis, with cattle.

An assessment of the renal handling of N^{τ}-MH in reindeer demonstrated that water flux influences N^{τ}-MH excretion. Nutrition also affects N^{τ}-MH excretion, likely due to changes in water flux related to nutritional status. Changes in water flux and renal handling of N^{τ}-MH need to be considered in studies using N^{τ}-MH excretion as an indicator of protein degradation.

As in several other species, reindeer appear to metabolize N^{τ}-MH as N^{τ}-[$^{14}CH_3$]MH is removed from the serum faster than can be explained by excretion in urine. The metabolites of N^{τ}-[$^{14}CH_3$]MH are not known but some of the metabolic products are released into urine. Further study is needed to identify the products and pathways of N^{τ}-MH in reindeer.

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Table 4-1. Urinary excretion and serum concentrations of N$^{\tau}$-methylhistidine in adult female wapiti and reindeer.

Group[1]	Daily Excretion (µmol/day)	Daily Excretion by Wt. (µmol/kg/day)	Serum Concentration (nmol/ml)
WAPITI - January 1991			
LOW n=6	35.5±7.5	0.13±.02	147±8
REINDEER - November 1991			
HIGH n=3	336.4±41.4[a]	3.25±0.39[a]	20.1±3.8[a]
LOW n=3	384.3±50.8[a]	3.70±0.18[a]	18.6±1.0[a]
REINDEER - January 1992			
HIGH n=3	304.9±30.2[a]	2.95±0.13[a]	19.9±5.2[a]
LOW n=3	199.5±15.3[b]	2.06±0.07[b]	32.8±2.8[b]
Animal No.[2]			
REINDEER - January 1992			
RD 007 n=5[3]	146.0±6.7[a]	1.50±0.07[a]	30.1±0.6[a] n=9
WINKLE n=5[3]	195.5±23.1[a]	1.70±0.21[a]	38.5±2.3[a] n=10
RD 176 n=5[3]	242.3±23.2[a]	2.22±0.21[a]	46.2±1.3[a] n=7
REINDEER - May 1992			
WINKLE n=7[3]	113.7±13.2[a]	1.03±0.12[a]	97.4±3.6[b] n=13
RD 212 n=7	336.6±17.2[b]	4.18±0.21[b]	37.8±2.2[a] n=13

[1] n refers to number of animals sampled in the group.
[2] n refers to number samples from each animal.
[3] animals refused the pelleted ration so were supplemented with lichen.
Note: Means with different letters in the same column are significantly different (P<0.01)
Data are expressed as mean±SE.

Table 4-2. Urinary N^{τ}-methyhistidine:creatinine (UN^{τ}-MHC), glomerular filtration rates (GFR) and urinary flow rates (UFR) in adult female reindeer.

Group[1]	UN^{τ}-MHC (x1000)	GFR (ml/min)	UFR (ml/min)
REINDEER - November 1991			
HIGH n=3	17.28±2.66[a]	70.30±6.07[a]	1.48±0.37[a]
LOW n=3	15.31±0.58[a]	77.44±8.86[a]	1.76±0.03[a]
REINDEER - January 1992			
HIGH n=3	17.24±1.62[a]	59.19±0.40[a]	1.17±0.29[a]
LOW n=3	15.03±1.52[a]	34.17±4.08[b]	0.61±0.08[b]
Animal No.[2]			
REINDEER - January 1992			
RD 007 n=10[3]	7.50±0.27[a]	76.76±1.29[a]	0.57±.08[a]
WINKLE n=10[3]	9.75±0.79[a]	79.22±1.74[a]	0.32±0.04[a]
RD 176 n=10[3]	11.84±0.80[ab]	80.84±2.79[a]	0.40±0.04[a]
REINDEER - May 1992			
WINKLE n=14[3]	7.75±0.35[a]	83.50±6.43[a]	0.28±0.04[a]
RD 212 n=14	17.30±0.84[b]	118.41±6.18[b]	2.32±0.22[b]

[1] n refers to number of animals sampled in the group.
[2] n refers to number samples from each animal.
[3] Animals refused the pelleted ration so were supplemented with lichen.
Note: Means with different letters in the same column are significantly different ($P<0.05$)
Data are expressed as mean±SE.

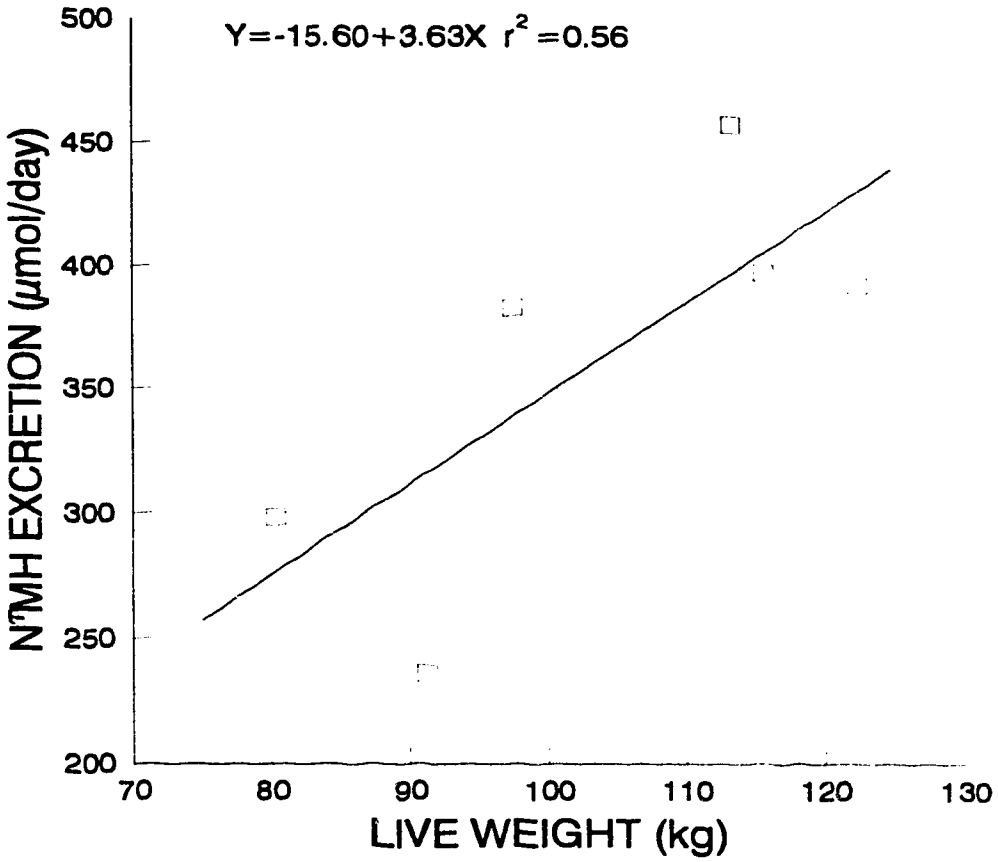
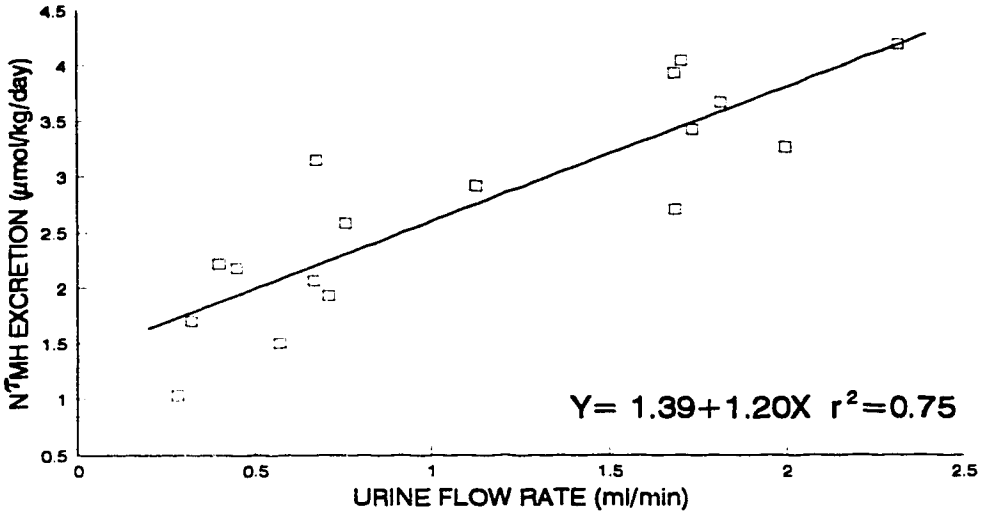


Figure 4-1: Relationship between daily excretion of N^{τ}-methylhistidine and live weight in adult female reindeer fed a high protein diet (18.5% CP).



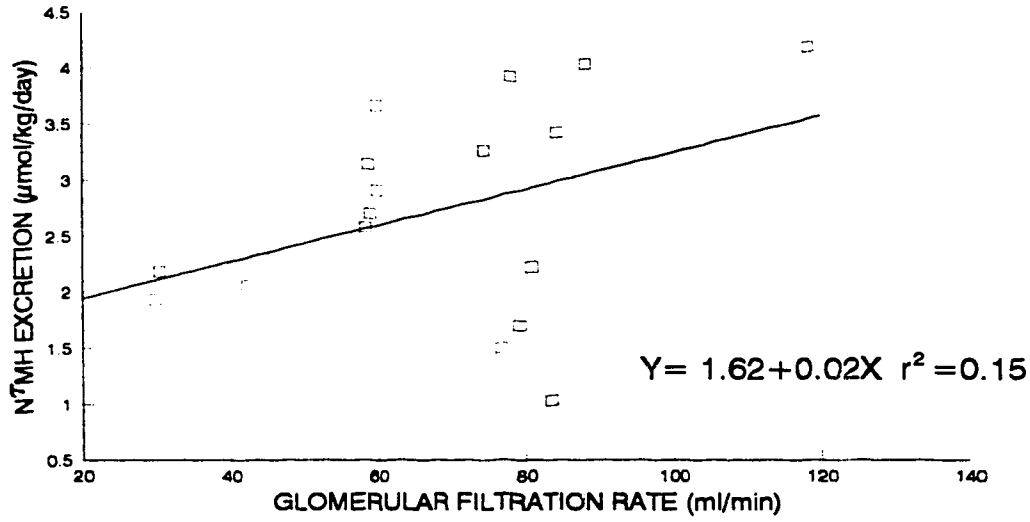


Figure 4-2: Relationship between rate of urinary excretion of N^{τ}-methylhistidine and urine flow rate and glomerular glomerular filtration rates in adult female reindeer.

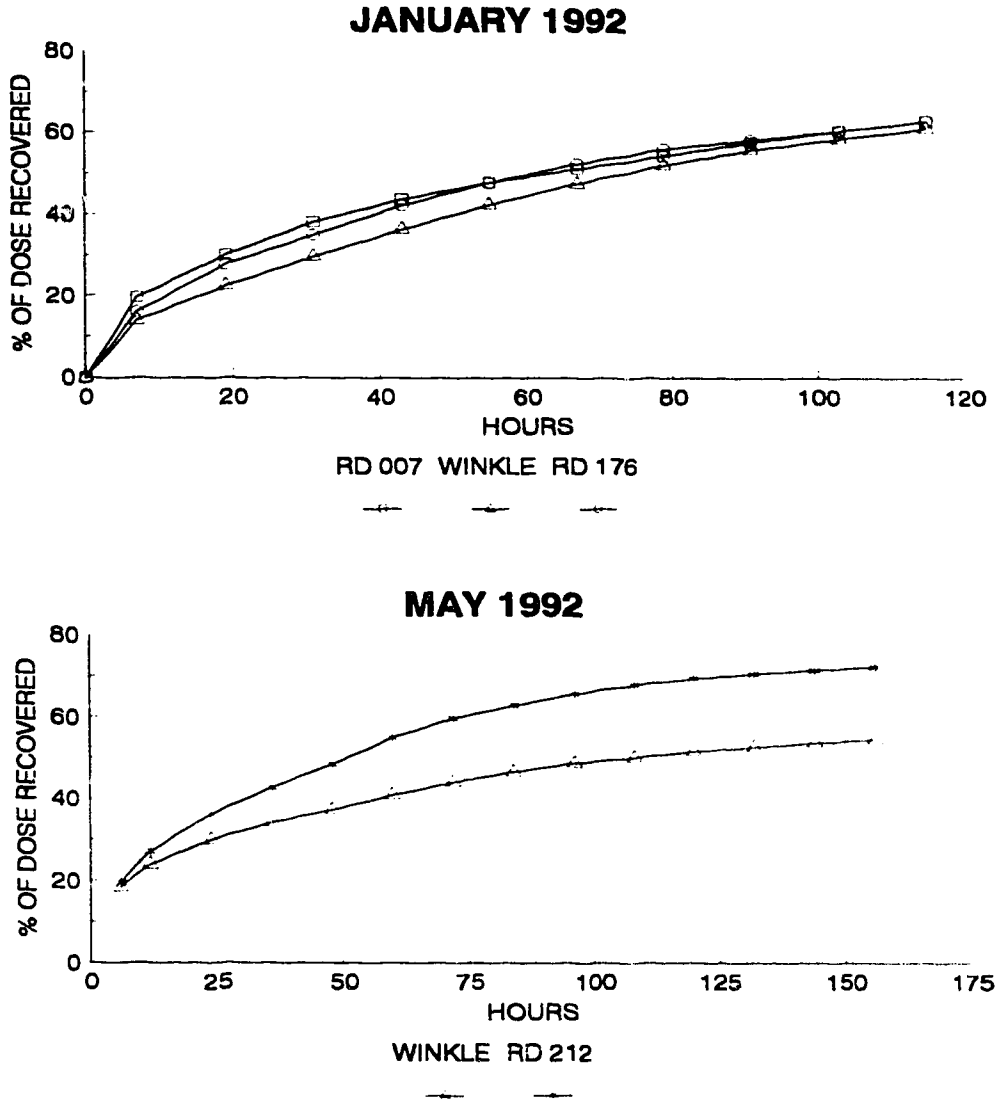


Figure 4-3: Cumulative excretion of radioactivity in urine from individual adult female reindeer injected with ^{14}C labelled N^{τ}-methylhistidine.

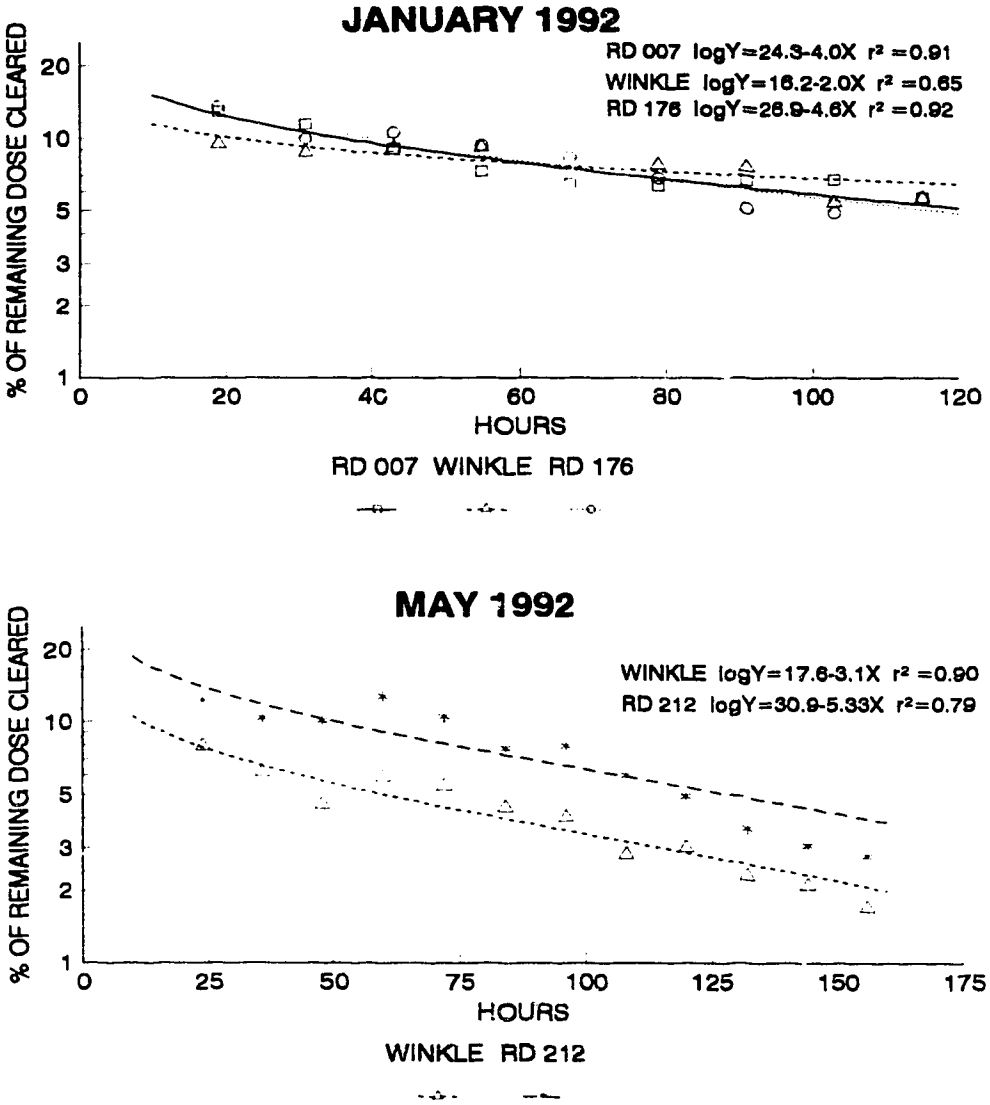


Figure 4-4: Proportion of radioactivity remaining in the body of individual adult female reindeer injected with ^{14}C labelled N^{τ}-methylhistidine cleared during the following 24 hours.

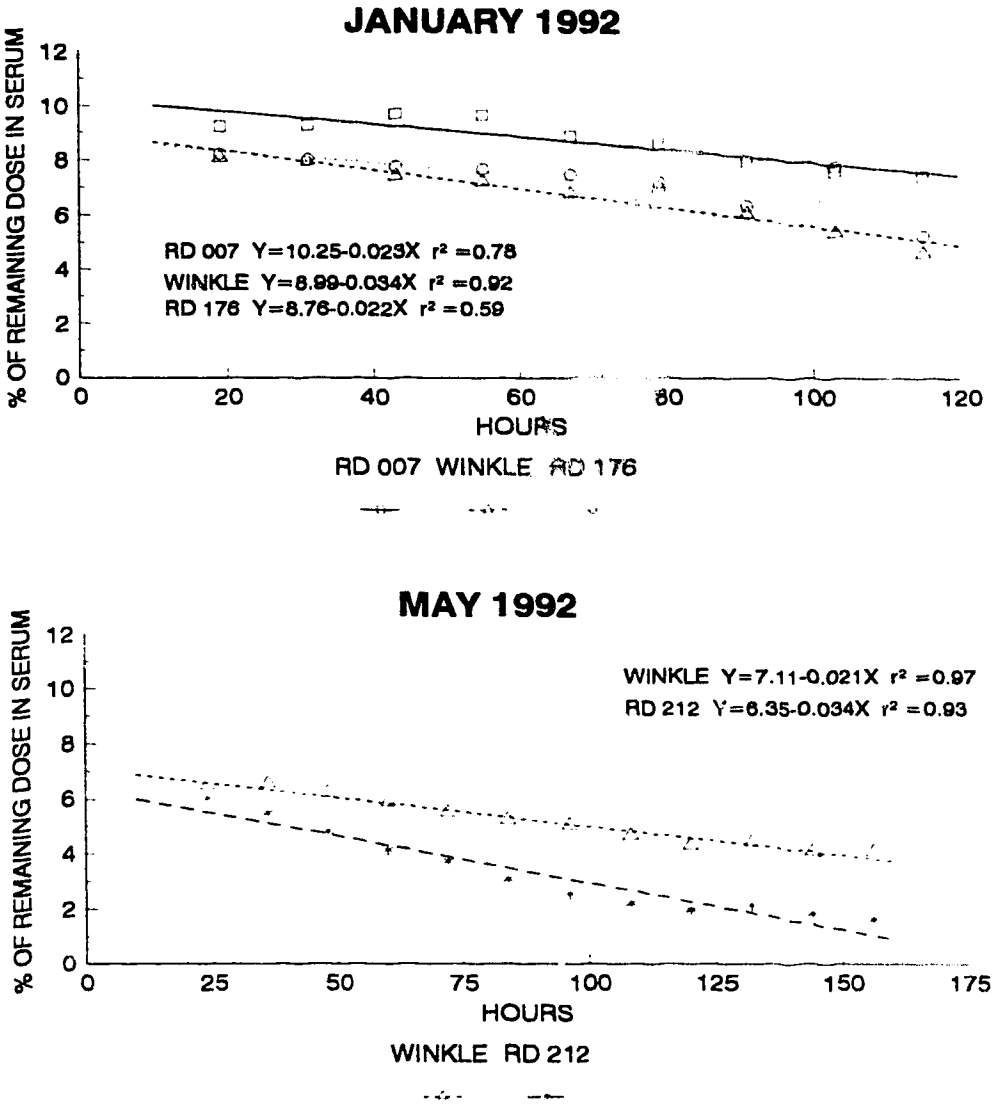


Figure 4-5: Proportion of radioactivity remaining in the body of individual adult female reindeer injected with ^{14}C labelled N^{τ}-methylhistidine accounted for by activity in serum assuming a constant (5 l) volume of serum.

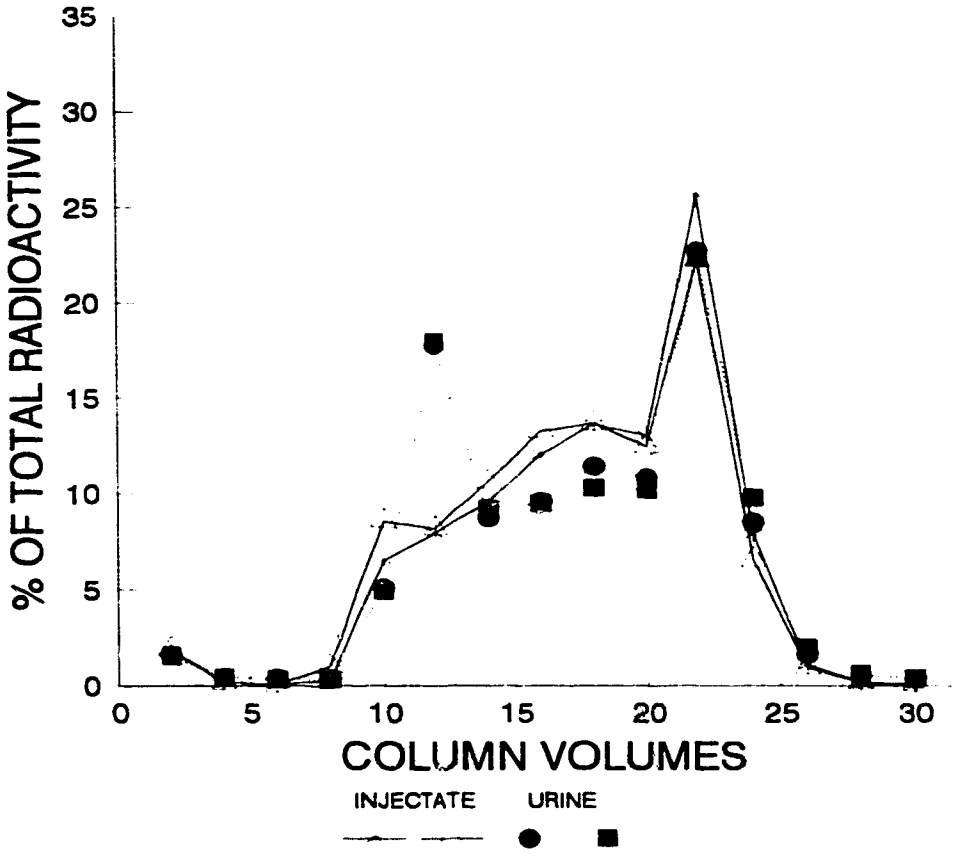


Figure 4-6: Proportion of total radioactivity in ion exchange fractions from urine collected from reindeer 12 hours after injection with ^{14}C labelled N^{τ}-methylhistidine and and from the injectate (in duplicate). 1.0M Pyridine was added to the column after 20 column volumes were displaced with 0.2M Pyridine.

Chapter 5

Seasonal changes in body composition of female caribou as they relate to fall set points

Introduction

Seasonal changes in body composition in *Rangifer tarandus* spp. have been studied in a number of populations. On arctic islands, the amplitude of the annual cycle is large with protein and fat reserves accumulated over summer and catabolized over winter (Thomas *et al.* 1976, Reimers *et al.* 1982, Adamczewski *et al.* 1987a). Similar cycles were observed in the introduced reindeer (*R.t. tarandus*) on South Georgia (Leader-Williams and Ricketts 1983). The cycle in mainland caribou (*R.t. groenlandicus*, *R.t. caribou* and *R.t. granti*) populations has been much less pronounced and much more variable (Dauphiné 1976, Parker 1981, Huot 1989, Boertje 1990, Allaye-Chan 1991).

These changes in body composition, and differences in cycles have generally been assumed to reflect forage quality and availability (Klien 1970, Reimers and Ringberg 1983, Adamczewski *et al.* 1987a, Parker 1981, Messier *et al.* 1988, Huot 1989) and have therefore been used to assess caribou summer and winter ranges. However, physical condition of ungulates is also influenced by other environmental and physiological factors including appetite, metabolic rate, seasonal changes in nutrient partitioning, disease, reproductive status, energy expenditures (eg. for migration), insect harassment, and stress.

There is evidence in the literature that these factors interact to result in set points in body composition and weight in ungulates. Set point being a target body composition which animals, through physiological processes, preferentially seek and maintain. In wapiti (*Cervus elaphus*) and mule deer (*Odocoilius hemionus*), seasonal weight change is largely dependent upon fall set points (Hudson *et al.* 1985, Renecker and Samuel 1991). Weight gain from spring to fall has been shown to be related to spring weight (Suttie *et al.* 1983, Watkins *et al.* 1991) in red deer and wapiti. The mechanism for this is increased feed intake (Wairimu *et al.* 1992). In reindeer, compensatory weight changes

occured in response to hormone induced weight changes (Ryg 1983). Very low interannual variation in fall body composition and weight within barren-ground caribou herds reported by Dauphiné (1976), Allaye-Chan (1991), and Ouellet (1992) also supports fall set points. Ouellet (1992) suggested that food availability in winter somehow influences body composition in the following fall in barren-ground caribou thus contending that fall body composition is not just related to the quality and availability of forage over summer.

The objective of this study was to determine if there is evidence for a fall set point in the Bathurst caribou herd as was suggested for Southampton Island and Coats Island by Ouellet (1992) and Adamczewski *et al.* (1987a),respectively. A further objective was to compare seasonal changes in body composition in Bathurst and Southampton Island caribou with the literature to determine if fall body composition depended on previous over winter changes in body composition or level of fatness at the nadir of condition, as suggested by Berg and Butterfield (1976) and if possible to identify a mechanism regulating set points.

Study areas

Two barren-ground caribou populations were contrasted (Figure 5-1). The island population occupies Southampton Island, a large island (43,000)km^2) located at the north end of Hudson Bay. The mainland herd occupies a large range (250,000 km^2) extending from Great Slave Lake to the arctic coast. This herd, called the Bathurst herd in reference to the proximity of its calving area to Bathurst Inlet, is one of the five major barren-ground caribou herds found on the mainland NWT. The history, environment and demographics of these herds has been described elsewhere (Gates *et al.* 1986, Heard 1989, Ouellet 1992), however a brief summary is provided here.

In 1991, an estimated 13,700±1,600 SE one year old and older barren-ground caribou occupied Southampton Island (Ouellet 1992). This population is the result of an introduction of 48 barren-ground caribou from Coats Island in 1967. The origin of caribou on Coats Island is unknown but they have been on the island since early in the

century (Gates *et al.* 1986). The increase in the number of caribou on Southampton Island since the introduction is similar to previous estimates of maximum growth for reindeer and caribou populations (Ouellet 1992). Predictably, recruitment has been high with the 69 calves:100 cows observed the spring of 1987 being typical (Ouellet 1992). The Bathurst caribou herd numbered approximately 320,000 animals one year old and older in 1990 (NWT Dept. Ren. Res. files). This population has also had good recruitment with over 30 calves: 100 cows since 1985.

The range of the Bathurst caribou herd encompasses a wide variety of habitat types. Their annual movements take them from dense boreal forests to lichen dominated tundra habitats. Detailed descriptions of the geography and plant life of these two biomes are presented in Kelsall (1968), Rowe (1972), and Thompson *et al.* (1978). In contrast, Southampton Island caribou are restricted to the tundra on the island year round.

Despite the differences in ranges, the diets of the two populations are similar. The winter diet of both populations consists mostly of terrestrial lichen supplemented with sedges (*Carex* sp.) and horsetails (*Equisetum* sp.) in early winter, and birch (*Betula* sp.) and willow (*Salix* sp.) in late winter (Scotter 1965, Scotter 1967, Miller 1976, Thomas and Hervieux 1986, Gates *et al.* 1986). The summer diets include a wide range of plants, however willow, sedges, *Dryas integrifolia, Hedysarium* sp. and *Pedicularis* sp. are among those most often mentioned in the literature (Thomas *et al.* 1984, Ouellet 1992). The green portions of these plants are high in crude protein (10-25%) and crude fibre (25-50%) providing an abundance of nitrogen and energy (Nieminen and Heiskari 1989, Ouellet 1992).

The migratory nature of the Bathurst herd contrasts greatly with the relatively sedentary nature of the Southampton Island population. The Bathurst herd moves annually between winter ranges in the boreal forest and summer ranges on the tundra (Heard 1989). Surveys of caribou distribution on Southampton Island suggest that they remain in essentially the same areas throughout the year (Ouellet 1992).

Another significant contrast between the herds is the level of predation. The Bathurst herd is subject to predation by both wolves and grizzly bears, while there are no

predators on Southampton Island. There may also be a difference in the level of harassment by biting and parasitic insects. Although biting insects, such as black flies and mosquitos, and parasitic insects, such as warbles and nose bots, are found on both ranges, Southampton Island caribou may be able to find greater relief from the insects than can Bathurst caribou. Situated in Hudson Bay, Southampton Island does not experience the warm summer temperatures common on the Bathurst range. The proximity to the ocean also results in almost constant winds and the rugged uplands on eastern Southampton Island allow caribou to take advantage of even light winds.

Methods

Between March 1990 and March 1992, 55 male and 94 female caribou were collected from the Bathurst caribou herd and 39 male and 99 female caribou were collected from Southampton Island (Table 5-1). The collections on Southampton Island were conducted in the spring (May 1990 and 1991) and in early winter after the rut, (November 1990 and 1991) within 40km of Coral Harbour. Ten collections on the Bathurst herd were spaced between March 1990 and March 1992. The collections were combined into five seasons; (Late winter = February-April, Spring = May-June, Summer = July-August, Fall = Sept-October, Early winter = November-January).

Sampling protocol

Animals were shot by native hunters who were instructed on the number of males, females and calves to harvest. Within these categories, selection of animals was random. The animals were processed at a central location where fresh weights and carcass measurements were taken and indicator bones and muscles were collected and frozen. Physical parameters recorded and methodology used are summarized in Table 5-2.

Indicator muscle, bone, and fat measurements were used to estimate the weight of muscle, bone and fat in each carcass using the following equations determined for caribou by Adamczewski *et al.* (1987b):

ln(**muscle wt** [kg])= -2.791 + 1.071 * ln(gastrocnemius wt [g])

fat wt (kg)= -0.246 + depth of back fat (cm) + 26.401 * Riney-trimmed kidney fat [g]

ln(**bone wt** [kg])= -4.878 + 1.237 * ln(femur wt [g])

To adjust for differences in frame size between animals of different ages, calculated muscle and fat weights were standardized to bone weights to give muscle to bone ratios (Muscle:Bone) and fat to bone ratios (Fat:Bone).

Statistical analyses

Statistical analyses were all conducted using SAS (SAS Inc. 1988). Seasonal differences were analyzed using least squares analysis of variance (PROC GLM). Comparisons between seasonal means were based on Tukey's studentized range test (HSD). Location differences in morphometric measurements were analyzed using t-tests (PROC TTEST). The frequency of age classes in the samples from the two locations was analyzed using the likelihood ratio chi-square (PROC FREQ).

Results and discussion

Body size

There was a significant difference in the frequency of the non-calf age classes sampled ($P<0.05$), with more older females and males harvested from the Bathurst herd (Table 5-1). The difference in frequency of age classes harvested reflects the expected composition of the two herds. The Southampton Island herd is rapidly increasing (Ouellet 1992) and would be expected to have a higher proportion of young animals relative to the Bathurst herd which is stable.

Body lengths of adult female (>30 months) Bathurst caribou and Southampton Island caribou were similar, however, jaw length, metatarsus length, femur length and femur weight were significantly larger in the sample of Bathurst caribou than their Southampton Island counterparts (Table 5-3). No differences were detected in males.

These animals were considered to be at full adult size as no significant changes in body or bone lengths were detected after 30 months in Southampton Island females (Ouellet 1992). The reason for size differences in females between herds is demonstrated by plotting jaw length, metatarsus length and femur length against age in months (Figure 5-2). Growth of the metatarsus and femur continued longer in Bathurst caribou than in Southampton Island caribou. This is likely related to the fact that 100% of the Southampton Island yearlings sampled in May were pregnant while none of yearlings and only 40% (4 of 10) of two year olds were pregnant in the Bathurst herd. The demands of gestation and lactation in the pregnant yearlings prevented them from continuing to grow over their third summer.

Evidence for set points in body composition

By using Muscle:Bone and Fat:Bone as indices of muscle and fat mass respectively, body composition could be compared between herds and with other herds without consideration of age class or differences in frame size. Both the Bathurst and Southampton Island herds showed considerable consistency between years in early and late winter Muscle:Bone and Fat:Bone. Variation between animals from the same herd during the same time period was slight. There was also very little difference in Fat:Bone between early and late winter (Table 5-4).

Fat:Bone data for females (>11 months) for early and late winter were combined and plotted along with Fat:Bone estimated from data on female caribou from George River (Huot 1989), Porcupine (Allaye-Chan 1991), and Coats Island (Adamczeski *et al.* 1987a) caribou and reindeer from Svalbard (Riemers *et al.* 1982) (Figure 5-3). The George River and Porcupine caribou herds demonstrated the same stability in Fat:Bone between early and late winter, as Bathurst and Southampton Island caribou, with slight increases in fat deposits. A similarly slight increase in fat deposits was also recorded by Thomas and Kiliaan (1986) on the Beverly herd (data not shown). Data from Kaminuriak caribou also fit this pattern with only slight declines in backfat and kidney fat through the winter (Dauphiné 1976). Data from these authors could not be used to estimate Fat:Bone.

Although there was seasonal stability within the mainland and Southampton Island herds, there were large differences between the herds. Fat:Bone were significantly higher on Southampton Island than in Bathurst caribou in both early winter (1.12±0.07 SE n= 31 vs 0.35±0.05 SE n=14 P<0.0001) and late winter (1.15±0.06 SE n=50 vs 0.30±0.05 SE n=60 P<0.0001). Fat levels in the George River herd approximated those of the Bathurst herd while Porcupine caribou were comparible to Southampton Island caribou.

In contrast to the stability in fat reserves of the mainland and Southampton Island herds, Svalbard reindeer and Coats Island caribou have very high fat reserves in early winter and these are essentially depleted by late winter (Figure 5-3).

Changes in muscle mass have not been as well studied as changes in fat reserves, however, a comparison between Southampton Island, Bathurst and Coats Island caribou indicate that Muscle:Bone undergo the same cycle as was observed for fat (Figure 5-4) with Coats Island caribou losing muscle mass over winter (Adamczewski *et al.* 1987a). Riemers *et al.* (1982) also recorded considerable loss of protein over winter in Svalbard reindeer.

In early winter there was no difference in Muscle:Bone between Southampton Island and Bathurst caribou (7.02±0.14 SE n=29 vs 7.32±0.22 SE P>0.10). In spring Muscle:Bone had declined significantly in Southampton Island caribou (P<0.05) and was significantly lower than Bathurst caribou (6.87±0.08 SE vs 7.37±0.12 SE P<0.001).

These data can be used to argue for the existence of fall and spring set points in *Rangifer*. The low annual variation in fall fat and protein reserves between animals in a population suggests that animals have a target body composition they strive for. The observation that fat reserves in the fall are lower than the physiological maximums (as demonstrated by Svalbard and Coats Island animals) in the mainland and Southampton Island herds also suggests a set point. An obvious question which arises from these data is that if a fall set point is responsible for fall body composition why would the Bathurst and George River herds have a fall set point with fat reserves so low that pregnancy rates are reduced. In the Bathurst herd no yearlings (0/8) and only 40% (4/10) two year olds were pregnant. In the George River Herd 11% (1/9) of yearlings and 70% of two year

olds were pregnant (Messier *et al.* 1988). This constrasts with Southampton Island where 100% of the yearlings and two year olds were pregnant.

The comparison of early winter and late winter body composition does not tell the whole story on the mainland caribou ranges. Huot (1989) predicted that there would be a significant loss of muscle mass and fat reserves from spring to summer in the George River herd as caribou moved to the calving grounds and were faced with the demands of migration, late gestation and early lactation combined with senescent vegetation and insect harassment. This loss was documented on the Bathurst range with caribou in summer (July) having significantly lower Muscle:Bone and Fat:Bone than other seasons (Table 5-4). A complete lack of backfat and essentially no kidney fat resulted in the calculated dissectible fat being less than zero. Caribou in the Porcupine herd also catabolized reserves at least into June (Allaye-Chan 1991).

Repletion of protein and fat reserves in the Bathurst herd took place in August with Muscle:Bone and Fat:Bone returning to late winter values by early September. Allaye-Chan (1991) and Ferguson and Gauthier (1992) also observed that most fat and protein deposition occurred before September and that only small amounts of fat were deposited between September and November. This raises the question why Bathurst caribou did not deposit reserves to the same extent as Coats Island and Porcupine caribou ie. was the difference due to set point mechanisms or environmental constraints?

A comparison of 18 month old (yearling) caribou in the fall from the Bathurst and Southampton herds provides some insights. Growth of yearlings in the two herds was similar as there were no significant differences between herds in bone length or bone weight nor was there any significant difference in Muscle:Bone (Table 5-5). In contrast, Fat:Bone and depth of backfat were significantly higher in Southampton Island yearlings. As fall fat reserves may be a determinant of fecundity in caribou (Dauphiné 1976, Thomas 1982, Crête *et al.* 1993, Cameron *et al.* 1993) it seems logical that yearling females would strive to at least reach a body composition which would allow them to breed, as is observed on Southampton Island (Ouellet 1992) and in captive caribou (Crête *et al.* 1993). Thus, the observation that no yearling caribou from the Bathurst herd were able to attain sufficient fat reserves to breed could be interpreted as suggesting that

summer nutrition was insufficient. However, consideration should also be given to the adaptive significance of delaying reproduction.

It is possible that forage quality is limiting on the Bathurst summer range, however, there is no evidence of deterioration of the Bathurst summer range as suggested by Couturier *et al.* (1990) for the George River herd, and the Bathurst summer range is much larger than that of the George River herd. Insect harassment has been linked to summer loss of fat reserves in the mainland herds (Kelsall 1968) and to lower weight gain in reindeer calves (Helle and Tarvainen 1984). Insects may prevent Bathurst caribou from depositing fat reserves during the summer when forages are most nutritious. If insect harassment continues late into the summer then it may not be possible for caribou to deposit body reserves prior to plant senscence and winter snows. If insect harassment is limiting the ability of yearlings to deposit fat it will also likely affect the ability of other animals, and especially lactating females, to deposit fat. Thus there is good reason to believe that environmental factors, rather than set point mechanisms prevented fat deposition in Bathurst caribou.

This doesn't help explain the situation on Southampton Island where Ouellet (1992) demonstrated that the differences in fall body composition between Coats Island and Southampton Island are not due to summer forage nor genetics, and harassment by biting insects is not a consideration. Nor does it argue that mainland herds don't have a set point but rather it suggests that Bathurst caribou did not attain their fall set point.

Set point mechanisms

The existence of set points requires a biological mechanism for regulation of body composition (Mrosovsky and Powley 1977). Adamczewski *et al.* (1987a) concluded that Coats Island caribou were at their set point in October and November, but did not suggest how the set point might be set or regulated. Ouellet (1992) suggested that food availability in winter somehow influences body composition in the following fall. A possible mechanism for this involves what Boertje (1990) referred to as "appetite drive". Appetite being the culmination of neurological and gastrointestinal factors influencing feed intake (Bailey and McLaughlin 1987). Simply stated, animals which have had to

catabolize significant body reserves over winter will respond by eating more over summer thereby depositing more reserves.

Changes in "appetite drive" may explain differences in body composition observed between caribou on lichen and non-lichen winter ranges. Caribou with access to lichen on the winter range can meet energy intake requirements (Boertje 1990) and minimize nitrogen loss (Hove and Jacobsen 1975). These animals would be expected to have a lower "appetite drive" during summer therefore deposit lower levels of reserves. This scenario fits with observations of the Southampton Island and Porcupine caribou herds which maintain or deposit fat reserves and usually maintain protein reserves over winter (Allaye-Chan 1991, Ouellet 1992). Caribou from these herds do not deposit the large amounts of fat observed on Coats Island despite, on Southampton Island at least, the lack of insect harassment and excellent summer ranges. A similar dampening of body condition cycles with *ad libitum* feeding in wapiti (Hudson *et al.* 1985) also supports the interaction between loss of body reserves and subsequent deposition of reserves. In captive caribou, Crête *et al.* (1993) observed that the very high daily forage consumption recorded during the first summer after capture was not repeated in the following summers and that consumption of pelleted feeds in particular was lower. Unfortunately, no information is available on how this related to body composition.

In contrast, caribou and reindeer without access to lichen winter ranges cannot maintain energy and protein reserves over winter (Riemers *et al.* 1982, Leader-Williams and Ricketts 1983, Adamczewski *et al.* 1987a). If this depletion of reserves gives these animals enhanced "appetite drive", when quality vegetation becomes available it would result in increased intake and in rapid and greater deposition of fat and protein reserves. This scenario is consistent with the early and rapid repletion in Coats Island caribou (Adamczewski *et al.* 1987a) and Svalbard reindeer (Reimers *et al.* 1982, Reimers and Ringberg 1983) and the large difference (50%) in the weight of the digestive tract between summer and spring in Svalbard reindeer (Reimers and Ringberg 1983). It is also consistent with studies on reindeer by Ryg (1983) which demonstrated that compensatory weight gains were inversely proportional to induced weight loss and that this resulted

from increased dry-matter intake. Wairimu *et al.* (1992) and Watkins *et al.* (1991) demonstrated similar compensatory weight gains in wapiti and also attributed this to higher forage intakes. Crête *et al.* (1993) found that caribou captured from the George River herd in the spring rapidly increased food consumption when offered a pelleted feed.

The effect of loss of body reserves is likely to be most pronounced in the first year of life when most of the summer nutrient intake is partitioned to bone and muscle (Ringberg *et al.* 1981). This may also be the most important time for establishing set points. On Coats Island, calves lost essentially all fat reserves over winter, including femur marrow fat (Adamczewski *et al.* 1987a), while Southampton Island and Bathurst calves still had fat reserves in the spring.

As suggested by the Bathurst data, there may also be environmental constraints which prevent animals from reaching set point. If insect harassment is limiting feeding during the summer, a year with low insect number would see Bathurst caribou deposit considerably more fat and possibly breed as yearlings. The appetite drive model suggests, however, they still wouldn't deposit as much as Coats Island caribou as they do not experience a similar loss of reserves over winter.

Conclusions

Results from this study, and those from others, suggest that fall set points exist in caribou but vary between herds. It also appears that deposition of fall reserves depends on loss of reserves over winter, not necessarily spring condition. This "floating" set point implies that, other than a physiological maximum, the set point is not an intrinsic mechanism but can be modulated by environment. The effect of loss of body reserves on "appetite drive" appears to be one way that the set point can be established without animals having to "anticipate" their needs over the following winter.

The possibility of caribou having a floating set point in body composition which is mediated by nutritional experience should be considered when physical condition is used to assess the demographics of caribou herds. Body reserves in the fall need to be interpreted with consideration of the requirements for body reserves over winter.

Further investigation of fall set points and the mechanisms involved is needed. The strongest evidence of fall set points would be provided by monitoring gut fill and forage intake of caribou during late summer and fall. A reduction in forage intake once a particular body composition is attained would be strong evidence for a set point. A question of particular interest, and importance to population dynamics is whether or not yearling caribou from the Bathurst herd are unable to deposit because of nutritional constraints or if their set point for fatness is lower than that which permits them to breed.

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Table 5-1: Location, month, sex and age class of caribou collected between March 1990 and March 1992.

Month	Sex	Southampton I.			Bathurst Herd		
		Calf	Ylg	Adult	Calf	Ylg	Adult
Mar90	♂	-	-	-	-	-	1
	♀	-	-	-	1	3	10
May90	♂	-	4	7	-	-	-
	♀	2	6	21	-	-	-
Nov90	♂	5	6	0	-	-	-
	♀	7	1	16	-	-	-
Feb91	♂	-	-	-	-	1	1
	♀	-	-	-	-	2	1
Mar91	♂	-	-	-	2	5	3
	♀	-	-	-	-	5	9
May91	♂	-	4	11	-	-	-
	♀	2	9	14	2	-	8
Jun91	♂	-	-	-	-	2	7
	♀	-	-	-	-	-	1
Jul91	♂	-	-	-	-	3	7
	♀	-	-	-	1	4	4
Sep91	♂	-	-	-	1	2	9
	♀	-	-	-	-	-	8
Nov91	♂	-	2	-	-	-	-
	♀	-	6	17	-	-	-
Dec91	♂	-	-	-	-	2	3
	♀	-	-	-	-	1	4
Jan92	♂	-	-	-	-	1	-
	♀	-	-	-	-	5	5
Feb92	♂	-	-	-	1	1	1
	♀	-	-	-	-	-	12
Mar92	♂	-	-	-	-	1	1
	♀	-	-	-	-	-	8
Totals	♂	5	16	18	4	18	33
	♀	11	22	68	4	20	70

Table 5-2: Parameters considered and methodology in caribou collections from the Bathurst and Southampton Island herds.

Sex

Body Weight: Total body weight (±0.5 kg) without antlers.

Body Length: Total body length (±0.5 cm) measured along the body contour from the last caudal vertebra to the intersection between the fur and the black part of the upper lip.

Depth of Backfat: Maximum depth (±1.0 mm) of backfat measured along an incision 45° forward from the base of the tail.

Estimated Age: Estimated age based on visual inspection of tooth wear and eruption (Miller 1974) and annuli counts of the first incisor (Matson's Laboratory).

Riney-trimmed Kidney and Kidney Fat Weight: Weight of kidney (±1.0 g) and Riney fat (±1.0 g) (Riney 1955).

Kidney Fat Index: Weight of Riney fat/Weight of kidney *100.

Jaw Length: Mandible length (±1.0 mm) measured from end of bone where incisors erupt to "heel" of mandible (Langvatin 1977).

Femur Length: Total femur length (±0.1 mm) (Langvatin 1977).

Femur Weight: Total femur weight (±0.1 g) with tendons and ligiments removed.

Metatarsus Length: Total Metatarsus length (±0.1 mm) (Langvatin 1977).

Femur Marrow Fat: Percent (±0.5%) fat content based on the dry-weight method corrected for mineral content (1.0444*(final wt/initial wt)-0.065) Neiland (1970).

Gastrocnemius Weight: Weight of gastrocnemius (±1.0 g) from left side trimmed of tendons and the flexor digitorum.

Table 5-3: Comparision of body size measurements from adult caribou (>30 months) from the Bathurst herd and Southampton Island.

	Southampton Island		Bathurst Herd
Females:			
Body Length (cm)	174.90±0.91 n=58	n.s.	176.21±0.96 n=78
Jaw Length (mm)	256.58±1.17 n=53	*	262.75±0.94 n=81
Metatarsus Length (cm)	25.54±0.09 n=58	*	26.72±0.09 n=85
Femur Length (cm)	26.33±0.09 n=58	*	27.21±0.19 n=85
Femur Weight (gm)	292.53±3.08 n=52	*	309.58±2.90 n=85
Males:			
Body Length (cm)	196.68±2.75 n=18	n.s.	192.60±1.60 n=48
Jaw Length (mm)	290.76±3.52 n=17	n.s.	288.11±1.79 n=55
Metatarsus Length (cm)	27.01±0.22 n=17	n.s.	28.24±0.14 n=53
Femur Length (cm)	29.08±0.22 n=18	n.s.	29.01±0.34 n=48
Femur Weight (gm)	419.81±11.27 n=18	n.s.	403.05±5.81 n=47

Note: Values are expressed as Mean±SE. n.s. - values in the same row are not significantly different.
* - Values in the same row are significantly different (P<0.05).

Table 5-4: Estimated Muscle:Bone and Fat:Bone ratios for male and female caribou (> 11 months) harvested from the Bathurst and Southampton Island caribou herds.

	Muscle:Bone	Fat:Bone
Bathurst Herd - Males		
Late Winter 90	7.87±0.50 n=2 ab	0.14±0.01 n=2 b
Late Winter 91	7.12±0.21 n=8 ab	0.14±0.01 n=8 b
Spring 91	6.49±0.34 n=9 ab	0.18±0.04 n=9 b
Summer 91	5.74±0.30 n=10 b	0.03±0.05 n=8 b
Fall 91	6.25 n=1 ab	0.71±0.18 n=2 a
Early Winter 91	7.02±0.17 n=6 ab	0.20±0.05 n=6 b
Late Winter 92	8.20 n=1 a	0.28 n=1 b
Bathurst Herd - Females		
Late Winter 90	7.78±0.23 n=12 a	0.81±0.12 n=12 a
Late Winter 91	7.33±0.32 n=14 a	0.34±0.60 n=15 bc
Spring 91	6.93±0.25 n=9 a	0.08±0.02 n=9 cd
Summer 91	5.59±0.19 n=8 b	-0.06±0.01 n=8 d
Fall 91	7.29±0.31 n=8 a	0.55±0.11 n=8 ab
Early Winter 91	7.33±0.22 n=14 a	0.35±0.06 n=14 ab
Late Winter 92	7.51±0.26 n=15 a	0.50±0.10 n=15 ab
Southampton Island - Males		
Spring 90	6.30±0.14 n=10 a	0.70±0.11 n=11 a
Early Winter 90	6.83±0.36 n=4 a	0.42±0.13 n=4 a
Spring 91	6.69±0.16 n=15 a	0.73±0.10 n=15 a
Early Winter 91	6.62±0.20 n=2 a	0.67±0.19 n=2 a
Southampton Island - Females		
Spring 90	6.83±0.11 n=27 b	1.07±0.06 n=27 a
Early Winter 90	7.70±0.28 n=7 a	1.11±0.06 n=8 a
Spring 91	6.93±0.11 n=23 b	1.24±0.11 n=23 a
Early Winter 91	6.81±0.14 n=22 b	1.12±0.09 n=23 a

Note: Values are expressed as mean±SE. Means in columns within a group with different letters are significantly different (P<0.05).

Table 5-5: Comparison of bone, muscle and fat measurements from yearling female caribou (18 months) from the Bathurst and Southampton Island caribou herds.

	Southampton Island		Bathurst Herd
Muscle:Bone	6.51±0.20 n=5	n.s.	6.92±0.36 n=6
Fat:Bone	1.28±0.14 n=6	***	0.34±0.08 n=6
Jaw Length (mm)	242.5±7.1 n=7	n.s.	233.5±2.6 n=7
Metatarsus Length (cm)	25.16±0.55 n=7	n.s.	25.87±0.39 n=7
Femur Length (cm)	25.74±0.22 n=6	n.s.	26.49±0.30 n=6
Femur Weight (gm)	289.1±11.3 n=6	n.s.	299.7±10.4 n=6
Depth of Backfat (cm)	2.72±0.34 n=7	*	0.21±0.14 n=7

Note: Values are expressed as mean±SE. n.s. - values in the same row are not significantly different.
* - Values in the same row are significantly different ($P<0.05$).
** - Values in the same row are significantly different ($P<0.01$).
*** - Values in the same row are significantly different ($P<0.001$).

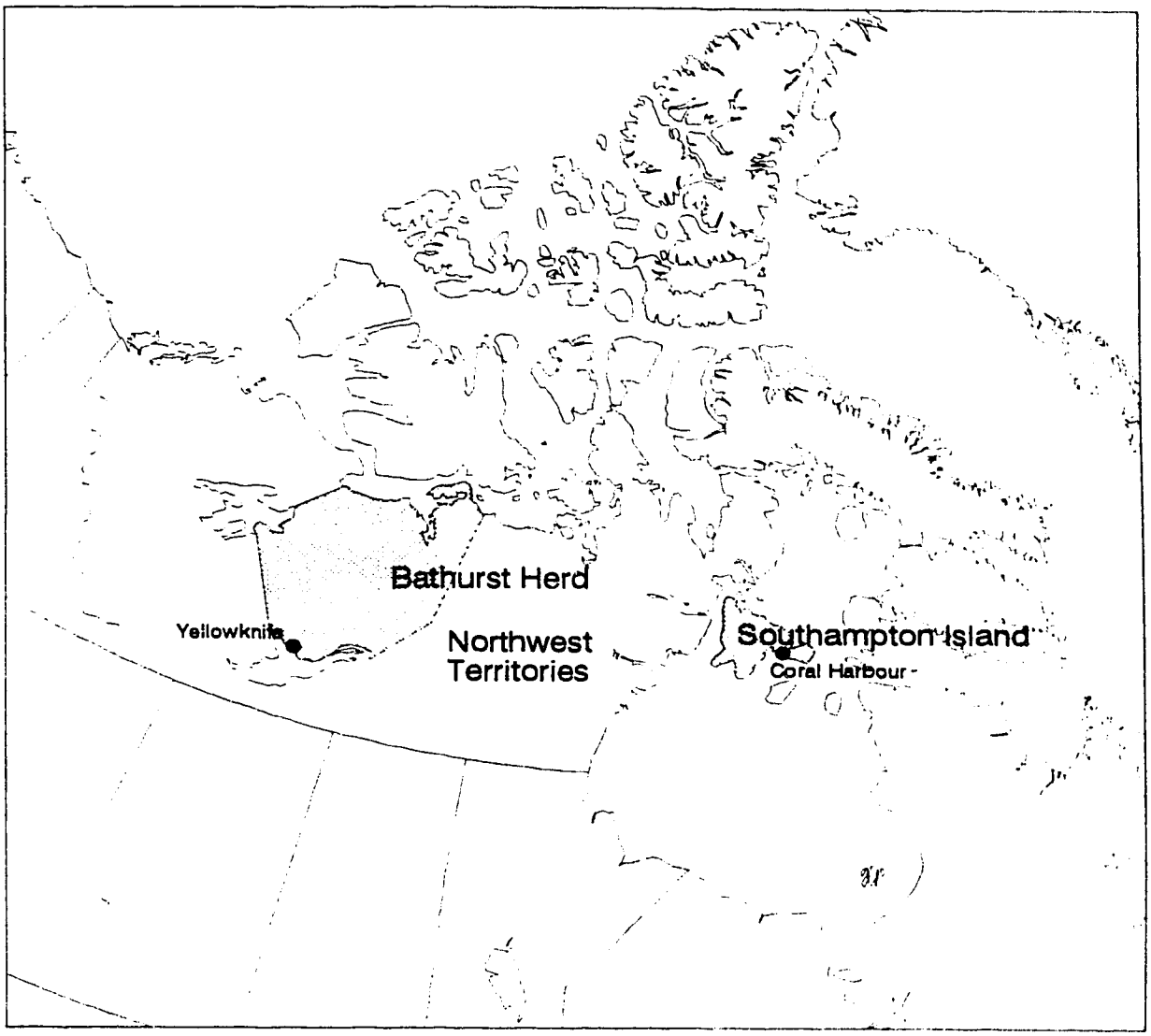

Figure 5-1: Ranges of the Bathurst and Southampton Island caribou herds. Collections were conducted 50-100 km northeast of Yellowknife and 20-40 km northeast of Coral Harbour.

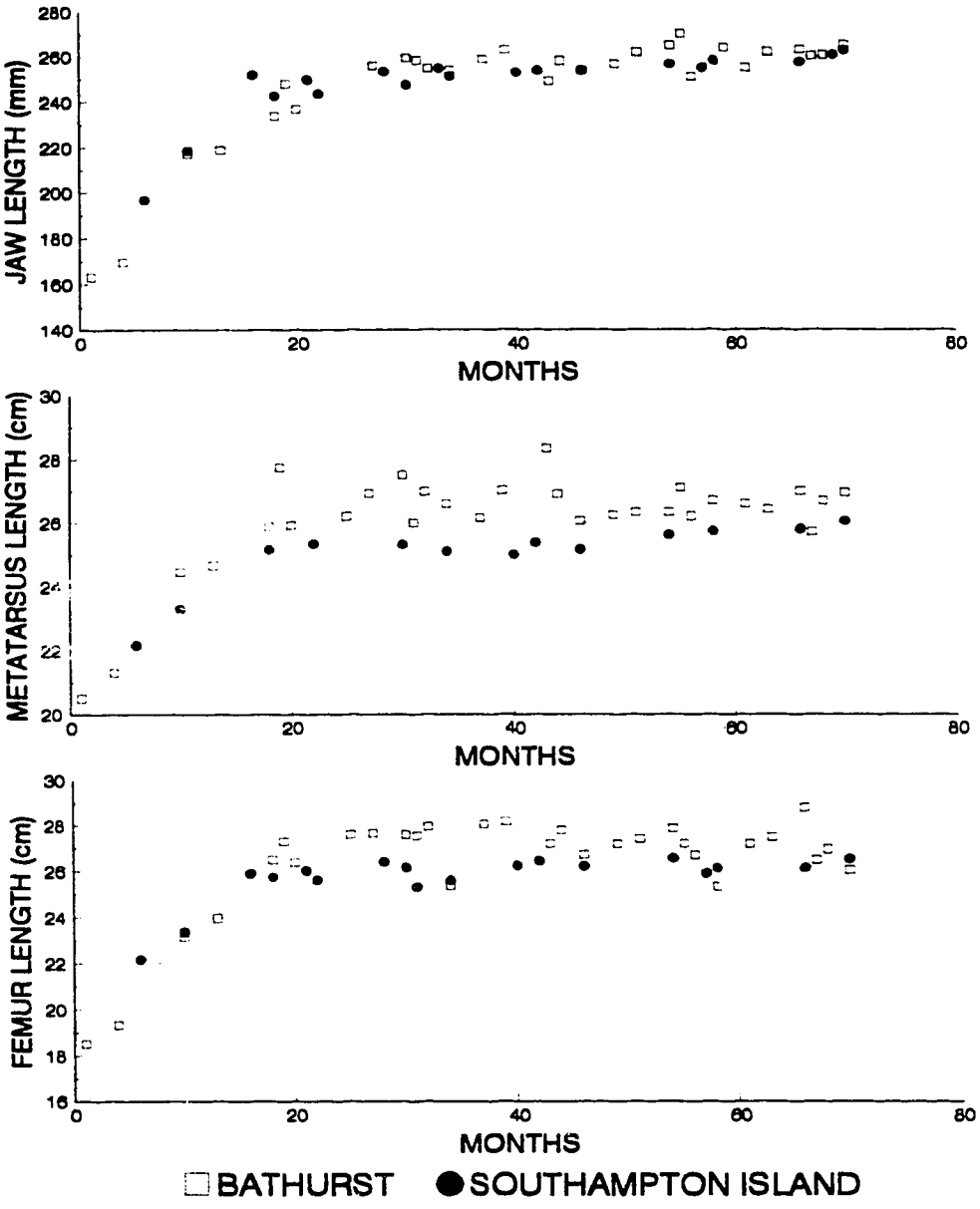

Figure 5-2: Jaw length, metatarsus length and femur length in female caribou aged 1 to 72 months from the Bathurst and Southampton Island caribou herds.

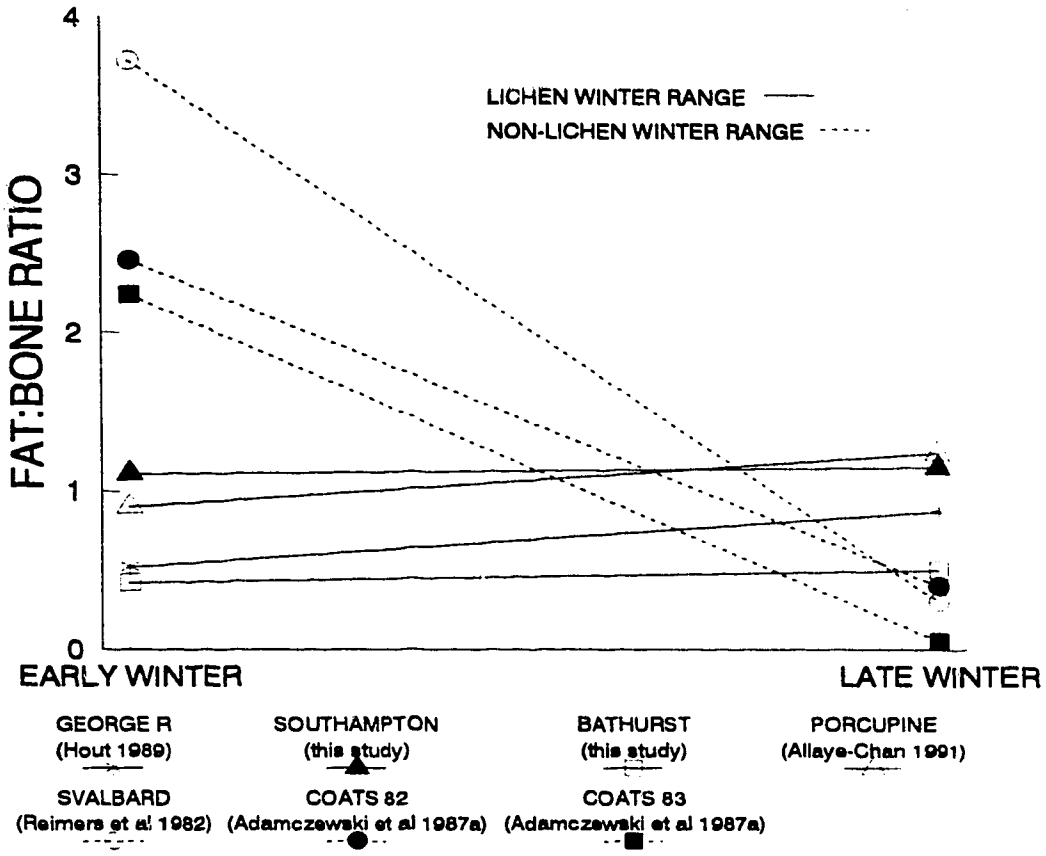

Figure 5-3: Over winter changes in Fat:Bone ratios in adult female reindeer and caribou on lichen and non-lichen winter ranges.

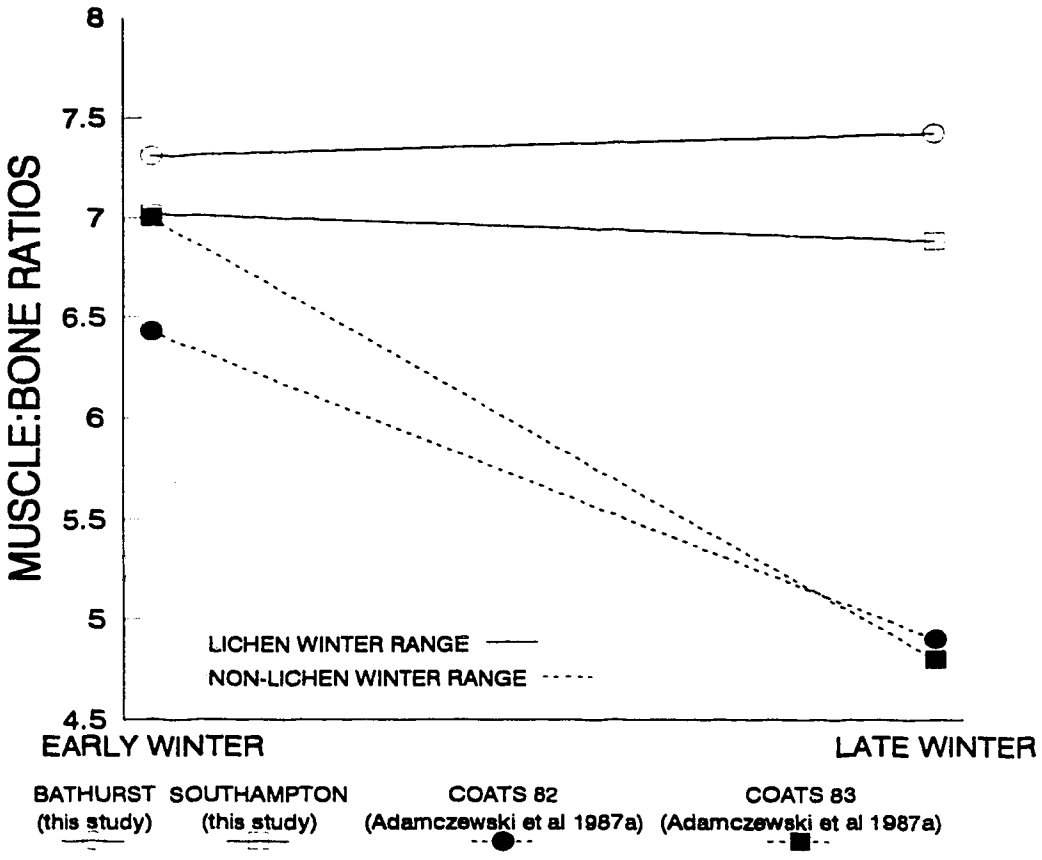

Figure 5-4: Over winter changes in Muscle:Bone ratios in adult female reindeer and caribou on lichen and non-lichen winter ranges.

Chapter 6

Urinary urea nitrogen excretion and urea nitrogen to creatinine ratios in relation to body composition of caribou

Introduction

Monitoring body composition has been relied on heavily in assessments of the demography of caribou populations (Messier *et al.* 1988, Couturier *et al.* 1990). Physical condition, as reflected in body composition, has been shown to affect productivity and recruitment through effects on fecundity and neonatal calf survival (Dauphiné 1976, Thomas 1982, Reimers 1983, White 1983, Crête *et al.* 1993). Historically, indices of body composition have been taken from direct carcass measurements. Body measurements, tissue weights, and standard fat measurements were identified as useful indicators of physical condition (Adamczewski *et al.* 1987b, Huot 1988, Allaye-Chan 1991). All of these indices required either killing or at least handling the animals.

Recently, several studies have focused on the use of urinary urea nitrogen:creatinine (UUC) to indirectly assess body composition (DelGiudice and Seal 1988, DelGiudice *et al.* 1989, 1991a, 1991b, Cool 1992). Collection and analysis of urine from snow allows for sample collection without stress due to capture or harvesting animals. This is of particular importance in the assessment of low density or endangered populations. Elevated UUC have been documented in undernourished white-tailed deer (*Odocoileus virginianus*) (DelGiudice *et al.* 1987, DelGiudice and Seal 1988), moose (*Alces alces*), and wapiti (*Cervus elaphus*) (DelGiudice *et al.* 1991a, DelGiudice *et al.* 1991b, Cool 1992). The management use of UUC was demonstrated by DelGiudice and Seal (1988) when they were able to classify deer into three categories; early undernutrition, prolonged-reversible undernutrition and prolonged-irreversible undernutrition.

The use of UUC depends upon the increased excretion of urea nitrogen (urea-N) in response to accelerated catabolism of endogenous proteins. Catabolism of endogenous

fat and protein reserves is typical of over wintering northern ungulates. When energy intake is limited, as in most cases of undernutrition, fat reserves are mobilized first with catabolism of endogenous protein increasing as fat reserves become depleted (Torbit *et al.* 1985, DelGiudice *et al.* 1987). In some situations, particularly with reindeer and caribou (*Rangifer tarandus*) consuming lichen diets, nitrogen intake can be limiting (Steen 1968, Neiminen and Heiskari 1989). Steen (1968) suggested that reindeer on a lichen diet must catabolize endogenous protein to provide a supply of amino acids for protein synthesis and nitrogen for rumen microbes, even when energy intake allows them to accumulate fat.

Although UUC have been used to indirectly assess body composition, only one study has been conducted to determine if changes in UUC reflect body composition. In black-tailed deer (*O. hemionus sitkensis*) UUC did not consistently reflect individual animal body composition (Parker *et al.* 1993). The objective of this study was to determine if urine and serum nutritional indices reflected body composition in caribou in the spring. Specifically, two urinary indices, UUC and urinary N^{τ}-methylhistidine (N^{τ}-MH) to creatinine ratios (UN^{τ}-MHC), and two serum indices, serum urea-N concentrations (SUN) and serum N^{τ}-MH concentrations (SN^{τ}-MH), were assessed by comparing them with proportions of fat and muscle in harvested caribou. UN^{τ}-MHC and SN^{τ}-MH were included in the analysis as N^{τ}-MH excretion had been shown to be an indicator of endogenous myofibrillar protein degradation in some species (Harris and Milne 1981, Tomas *et al.* 1984, Long *et al.* 1988). SUN was also evaluated as DelGiudice and Seal (1988) found SUN was elevated in malnourished deer.

Methods

Sampling protocol

During late winter and spring in 1990, 1991 and 1992 (February through May), 55 adult, 13 yearling and 6 calf caribou were collected from the Bathurst caribou herd and 48 adult, 23 yearling and 4 calf caribou were collected from Southampton Island.

Animals were shot by native hunters who were instructed on the sex of animals to harvest but to otherwise take animals at random. Immediately after death, a blood sample was taken by slicing through the carotid artery in the lower neck. A urine sample was collected either directly as it drained from the animal or from the snow. The animals were then taken to a central location where fresh weights and carcass measurements were taken and indicator bones and muscles were collected and frozen. Blood samples were centrifuged upon returning to camp and serum was retained. Samples were frozen at -10°C (urine and serum at -20°C) until analyzed in the laboratory. Physical parameters considered and methodology used are summarized in Table 6-1.

Indicator muscle, bone, and fat measurements were used to estimate the weight of muscle, bone and fat in each carcass using the following equations determined for caribou by Adamczewski *et al.* (1987b):

ln(**muscle wt** [kg])= -2.791 + 1.071 * ln(gastrocnemius wt [g])

fat wt (kg)= -0.246 + depth of back fat (cm) + 26.401 * Riney-trimmed kidney fat [g]

ln(**bone wt** [kg])= -4.878 + 1.237 * ln(femur wt [g])

To adjust for differences in frame size between animals of different ages, estimated muscle and fat weights were standardized to bone weights to give muscle to bone ratios (Muscle:Bone) and fat to bone ratios (Fat:Bone).

Chemical analyses

Creatinine concentrations (mg/dl and µmol/ml) in serum and urine were determined using a colorimetric method based on the Jaffé reaction (Sigma Diagnostics, St. Louis MO). Urinary and serum concentrations of urea nitrogen (urea-N) (mg/dl) were determined using a colorimetric urea assay kit based on the diacetyl monoxime reaction (Sigma Diagnostics, St. Louis MO). Urine and serum samples were analyzed for N^{τ}-Methylhistidine (N^{τ}-MH) (µmol/ml) using an HPLC procedure (Dalla Libera 1991). Samples were deproteinized with 0.200 ml of 3.0M $HClO_4$ and centrifuged. Samples were analyzed using a Varian Model 5500 Liquid Chromatograph with a Varian 2070

spectrofluorometer detector and a Varian 9090 auto analyzer (Varian Canada, Calgary AB). Separations were done on a 15 cm x 4.6 mm 3 micron reverse phase column (Supelco Inc., Bellefonte PA).

Urinary creatinine excretion is strongly correlated with muscle weight and is highly consistent within species (Vestergaard and Leverett 1958, Kertz *et al.* 1970, Chetal *et al.* 1975, Forbes and Bruining 1976). Therefore, creatinine coefficients from reindeer were used to estimate urea nitrogen excretion from caribou based on the ratio of urea to creatinine in urine and body weight using the formula:

TTLUREA = ((CREATCO * WT)/UCREAT*UUREA)/1000

where TTLUREA= total daily urea-N excretion (g), CREATCO = creatinine coefficient (16.16 mg/kg/day) determined from lean adult female reindeer (unpublished data), WT = weight (kg). UCREAT = urine creatinine concentration (mg/dl) and UUREA = urine urea-N concentration (mg/dl).

Statistical analyses

Differences between variable means for sex and location were analysed using least squares analysis of variance (PROC GLM) (SAS 1988).

Results and Discussion

Herd comparisons

Thomas (1982) and Nieminen and Laitinen (1986) classified the physical condition of caribou and reindeer based on bone marrow and kidney fat. Thomas (1982) classified caribou with a kidney fat index (KFI) less than 30 or femur marrow fat (FMF) less than 50% as being in poor condition. Nieminen and Laitinen (1986) used a KFI of less than 25 or FMF less than 20% as their basis for classifying reindeer in poor condition.

Even using the less stringent numbers proposed by Thomas (1982), none of the animals harvested on Southampton Island could be classified as being in poor condition. In contrast, 39% (34 of 87) of the Bathurst caribou harvested had a KFI less than 30 and 6% (4 of 70) had a FMF less than 50%. These differences in fat reserves are reflected

in the large differences in FATBONE ratios observed for both males and females (Figure 6-1).

Herd differences in fatness were reflected in UUC for female caribou with Bathurst females excreting significantly more urea-N than Southampton Island females. The lack of a similar difference for males is likely due to the small sample size as urine collection from harvested males was much more difficult. Although the differences were not significant, SUN showed a trend towards being lower in Bathurst caribou.

The higher average UUC in caribou from the Bathurst herd suggests that some of the animals were either catabolizing protein or eating a higher protein forage (DelGiudice and Seal 1988). The first suggestion was supported by the fact that the area of the collection was still 100% snow covered so no new vegetation was available to have resulted in a higher UUC. However, DelGiudice and Seal (1988) also observed elevated SUN in undernourished animals whereas this was not observed in the Bathurst caribou.

UN^{τ}-MHC and SN^{τ}-MH were similar for Bathurst and Southampton Island females and Bathurst males (Figure 6-1). The high UN^{τ}-MHC observed for Southampton Island males appears to be a result of higher serum N^{τ}-MH concentrations. Six of the males on Southampton Island had serum N^{τ}-MH concentrations in excess of 100 nmol/ml. This is much higher than observed in reindeer and is in the range observed for wapiti (Chapter 4). The reason for the higher serum N^{τ}-MH concentrations in Southampton Island males is not apparent. Increased excretion of N^{τ}-MH has been associated with starvation and growth in rats and cattle (Nishizawa *et al.* 1977, Wassner *et al.* 1977, Jones *et al.* 1990). Fat levels would suggest that the animals were not starving but growth cannot be ruled out. Ouellet (1992) observed that males on Southampton Island grew through the winter resulting in a large sexual dimorphism.

Biochemical indicators of body composition

DelGiudice and Seal (1988) classified animals with UUC below 4.0 as in early undernutrition, UUC between 4 and 23 were thought to indicate prolonged-reversible undernutrition and UUC over 23 were indicative of prolonged-irreversible undernutrition.

All the UUC values from the harvested caribou were below 1 suggesting that no animals were undernourished.

However, plotting UUC values from Bathurst caribou in the spring versus KFI and FMF suggests that caribou with UUC greater than 0.25 had depleted body reserves (Figure 6-2). All of the caribou with UUC greater than 0.25 had KFI less than 40 and all but 2 would have been classified as in poor condition using Thomas' (1982) criteria of KFI less than 30. These animals also demonstrated some loss of FMF with those animals with significant loss of FMF having the highest UUC.

It should be noted that although all caribou with UUC greater than 0.25 had depleted fat reserves, not all caribou with low KFI and low FMF had high UUC. A similar pattern was observed in Sitka black-tailed deer (*O. hemionus sitkensis*) where UUC was compared with fat reserves determined using tritiated water (Parker *et al.* 1993). The reason for this is because KFI and FMF depend on past nutrition while UUC reflects current nutrition. It is possible that caribou with low fat reserves could still be obtaining sufficient energy in the diet. This would result in low UUC even though they are in poor physical condition.

The only other study which compares UUC to fat levels was limited to analysis of winter killed wapiti (DelGiudice *et al.* 1991a). These animals had UUC in excess of 70 and FMF <10% suggesting severe and prolonged undernutrition. None of the Bathurst caribou had reached this state and their undernutrition was likely reversible.

DelGiudice and Seal (1988) also suggested that SUN could be used to classify the phases of undernutrition with SUN <20 mg/dl indicating early undernutrition, SUN from 20 to 40 mg/dl indicating prolonged-reversible undernutrition, and SUN over 40 mg/dl indicating prolonged-irreversible undernutrition. In this study, SUN did not correspond as well as UUC to fat levels although the only animal with elevated SUN also had high UUC and low KFI (Figure 6-2).

UN^{τ}-MHC showed no relationship with either KFI or FMF (Figure 6-2) supporting the conclusions from Chapter 4 that N^{τ}-MH excretion would not be a good index of protein catabolism in caribou. It remains possible that in later stages of malnutrition excretion of N^{τ}-MH would increase, as has been observed in starving rats (Wassner *et al.*

1977), however, once animals are severely malnourished monitoring N^{τ}-MH would have no advantages over UUC or visual classification of condition, and would be more expensive.

Nitrogen conservation in over wintering caribou

Caribou are typically able to maintain most, if not all, their muscle mass over the winter (Huot 1989, Ouellet 1992) while consuming a mainly lichen diet with very low (<3%) crude protein (Scotter 1965, Scotter 1967, Thomas and Hervieux 1986). To do this, urinary nitrogen loss must be minimal. Urinary excretion of urea-N is typically the most significant source of urinary nitrogen loss (Dukes 1947). The level of urinary nitrogen loss can therefore be assessed by looking at urinary urea-N excretion.

The mean estimated daily urea-N excretion for adult caribou from both study areas in late winter/spring was 0.11±0.01 SE g (n=76 range=0.011-0.510). Adult female caribou on the Bathurst range with UUC less than 0.25 excreted, on average, slightly more urea-N at 0.14±0.02 g/day while caribou with UUC greater than 0.25 excreted an average of 0.38±0.08 g/day. Urea-N excretion is best evaluated based on dietary nitrogen intake. The late winter diet of Bathurst caribou is primarily terrestrial lichen (Thomas and Hervieux 1986) with a nitrogen content of approximately 4 g/kg (Scotter 1965). With an apparent digestibility of 75% (Thomas and Kroeger 1980) these lichen would provide approximately 3 g N/kg. Therefore, the urinary loss of urea-N observed in animals with UUC greater than 0.25 could be made up by ingesting 125 g of lichen.

The low excretion of urea-N in wild caribou, even in those with elevated UUC, is evident when contrasted with adult female reindeer on a low protein (7.9%) pelleted diet, which excreted an average of 7.9 g urea-N/day (n=3), and reindeer on a high protein diet (18.8%) which excreted an average of 26.6 g urea-N/day (Chapter 2). Cattle on a 12% CP diet excreted over 28 g urea-N per day but were able to reduce this to less than 2.5 g urea-N/day when fed a 4% CP diet and deprived of water (Livingston *et al.* 1962). Captive caribou fed a simulated winter diet excreted an average of 18.0 g urea-N/day (Wales *et al.* 1975).

Hove and Jacobsen (1975) found reindeer held on a lichen diet reabsorbed an average of 93% of urea filtered at the glomerulus. Calculating from their figures indicated that these animals were excreting an average of 0.091 g urea-N/day. This is within the range observed in wild caribou so it is likely that the caribou from the Bathurst and Southampton Island herds were also reabsorbing over 90% of urea filtered.

The only other wild ruminant for which comparable abilities to reduce nitrogen loss has been documented is the camel. Schmidt-Nielsen *et al.* (1957) reported that a camel grazed in the sandy desert for 4 weeks then maintained on a low N diet of dates and hay for an additional 17 days with no access to water reduced urea N excretion to less than 0.3 g/day.

Conclusions

The results of this study indicate that UUC can be used to monitor the nutritional status of free ranging caribou on lichen winter ranges and that analysis of urine in snow can be used to conduct physiological assessments of individual caribou and caribou populations, as has been demonstrated in deer (DelGiudice *et al.* 1989) and wapiti (DelGiudice *et al.* 1991c). The observation that UUC did not increase above 0.25 until fat reserves were being depleted (KFI<40) suggests that this value could be used to distinguish caribou in prolonged undernutrition in late winter/spring. Further data are needed from caribou in very poor condition to determine the UUC values for animals with severe prolonged undernutrition. None of the animals collected during this study would have been expected to die of malnutrition. It would be expected that UUC would continue to increase as the severity and period of undernutrition increased.

Serum urea-N concentration was a poor indicator of physical condition. It is possible that, at the levels of protein catabolism occurring in animals with low fat reserves, animals were able to recycle much of the urea-N produced into the gut, thus serum levels did not increase. The fact that urea-N excretion was still low relative to dietary nitrogen sources supports this.

Serum N^{τ}-MH and UN^{τ}-MHC did not provide any information on physical condition supporting the conclusions of Chapter 4, that N^{τ}-MH excretion is not a valid index of protein turnover in *Rangifer*.

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Table 6-1: Parameters considered and methodology in caribou collections from the Bathurst and Southampton Island herds.

Sex

Body Weight: Total body weight (±0.5 kg) without antlers.

Body Length: Total body length (±0.5 cm) measured along the body contour from the last caudal vertebra to the intersection between the fur and the black part of the upper lip.

Depth of Backfat: Maximum depth (±1.0 mm) of backfat measured along an incision 45° forward from the base of the tail.

Estimated Age: Estimated age based on visual inspection of tooth wear and eruption (Miller 1974) and annuli counts of the first incisor (Matson's Laboratory).

Riney-trimmed Kidney and Kidney Fat Weight: Weight of kidney (±1.0 g) and Riney fat (±1.0 g) (Riney 1955).

Kidney Fat Index: Weight of Riney fat/Weight of kidney *100.

Jaw Length: Mandible length (±1.0 mm) measured from end of bone where incisors erupt to "heel" of mandible (Langvatin 1977).

Femur Length: Total femur length (±0.1 mm) (Langvatin 1977).

Femur Weight: Total femur weight (±0.1 g) with tendons and ligiments removed.

Metatarsus Length: Total metatarsus length (±0.1 mm) (Langvatin 1977).

Femur Marrow Fat: Percent (±0.5%) fat content based on the dry-weight method corrected for mineral content (1.0444*(final wt/initial wt)-0.065) Neiland (1970).

Gastrocnemius Weight: Weight of gastrocnemius (±1.0 g) from left side trimmed of tendons and the flexor digitorum.

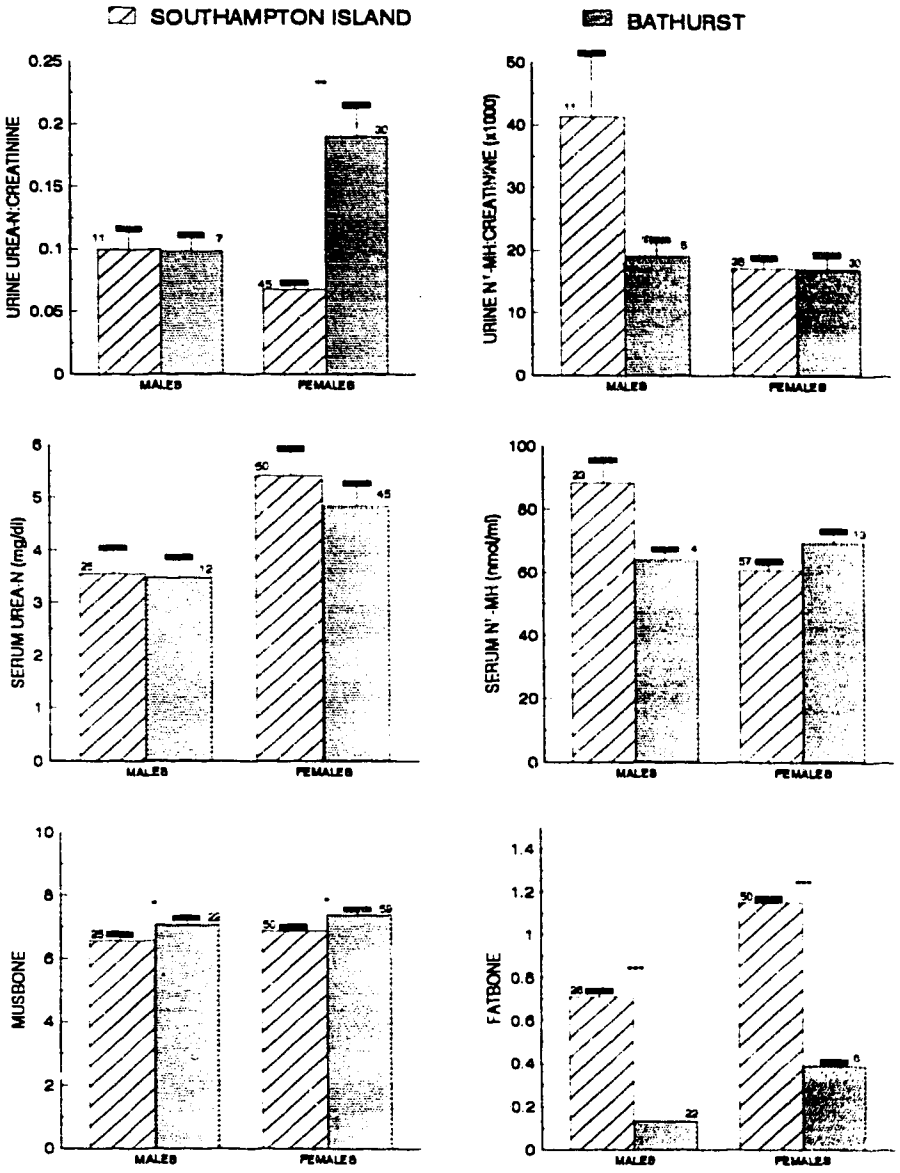

Figure 6-1: Herd and sex differences in urine urea-N:creatinine, urine N^{τ}-MH:creatinine, serum urea-N, serum N^{τ}-MH, muscle:bone (MUSBONE), and fat:bone (FATBONE) in late winter (mean±SE). (*P<0.05, **P<0.001, *** P<0.0001)

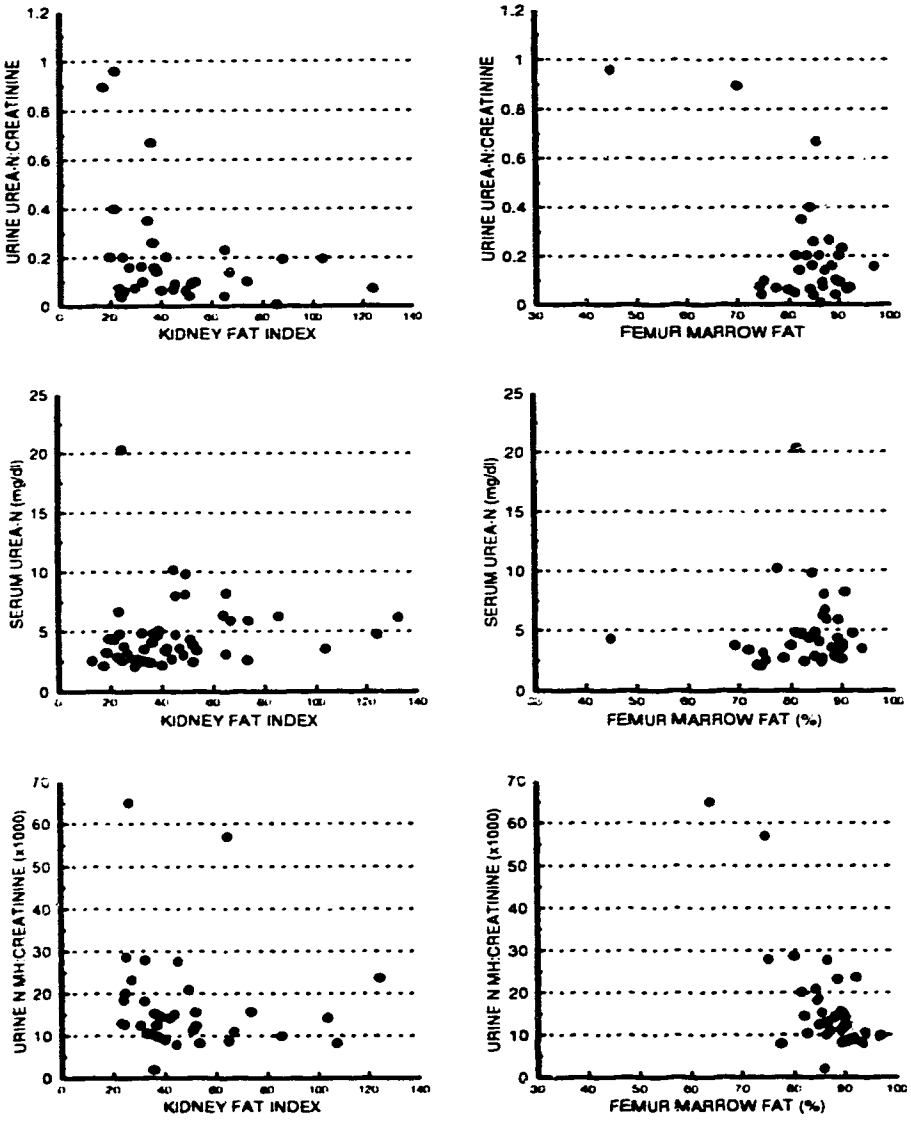

Figure 6-2: Urine urea-N:creatinine, serum urea-N, and urine N -MH:creatinine versus kidney fat index and percent femur marrow fat in adult female Bathurst caribou in late winter/spring.

Chapter 7
General discussion

Physiological adaptations in northern ungulates enable them to survive and prosper despite large annual cycles in forage quality and availability. Flexibility to adapt to changing conditions is important for species which live in these unpredictable environs. Thus it can be expected that northern ungulates will demonstrate a diversity of compensatory mechanisms to nutritional cycles.

In this study, reindeer (*Rangifer tarandus tarandus*) and wapiti (*Cervus elaphus*) demonstrated different approaches to compensate for low dietary nitrogen. Indirect evidence suggests that wapiti on the low protein diet had higher gut fill and feed intake relative to wapiti on a high quality diet. This strategy, which would result in a longer retention time and more complete digestion of the less nutritious winter diet, has also been observed in Roe deer (*Capreolus capreolus*) (Holand 1992) and lean white-tailed deer (*Odocoileus virginianus*) fawns (Verme and Ozoga 1980).

In contrast, reindeer reduced food intake slightly when fed the low protein diet and weight changes suggested they did not change gut fill significantly. Reduced food intake in winter has been observed in many of the cervids including barren-ground caribou (*R.t. groenlandicus*), white-tailed deer, moose (*Alces alces*) and wapiti on good quality winter diets (McEwan and Whitehead 1970, Verme and Ozoga 1980, Suttie *et al.* 1983, Renecker and Hudson 1990). In caribou, this reduction in intake has been tied to lower metabolic requirements (Boertje 1990).

Neither of these strategies enabled animals to maintain a positive nitrogen balance and both species catabolized endogenous proteins. The strategy used by wapiti appeared to allow them to maintain larger fat reserves through the winter than the strategy used by reindeer. This use of body reserves was not necessarily detrimental as it did not affect reproductive success.

The use of endogenous proteins did not result in an increased loss of nitrogen in the urine. Both species reduced urea-N excretion in response to the low protein diet. In

wapiti this was accomplished by changes in renal function and a reduction in urine volume. Changes in renal function resulted in a smaller proportion of urea-N filtered being excreted or alternately a larger portion of urea-N filtered being reabsorbed. Changes in renal function were also reported by Mould and Robbins (1981) who attributed the curvilinear relationship between plasma urea-N and urea-N excretion to changes in the filtering capacity of the kidney.

In reindeer, the proportion of urea-N filtered which was excreted remained the same, but reduced urine flow aided in reducing urea-N excretion. In reindeer fed lichen, reduced urea-N excretion was also aided by a reduction of serum urea-N concentrations. This suggests that reindeer on lichen can remove urea-N from the blood efficiently, presumably into the rumen.

Wales *et al.* (1975) concluded that caribou have the potential to recycle considerable amounts of urea-N in the winter. This is supported by the large population of ureolytic bacteria observed in Svalbard reindeer in winter (Orpin and Mathiesen 1990). Nitrogen recycling could therefore be of significance in maintaining nitrogen balance in reindeer.

Although the reindeer in this study did not change reabsorption at the kidney level, data from Hove and Jacobsen (1975) and from harvested caribou (Chapter 6) indicate that with very low serum urea-N (ie. ≤5.0 mg/dl) they can virtually eliminate urea-N excretion.

It was not possible to investigate protein turnover in this study as urinary excretion of N^{τ}-MH was invalidated as an index of protein turnover. This puts wapiti, reindeer and presumably caribou in the same class as sheep, goats, and pigs (Harris and Milne 1980, Harris and Milne 1981a, Brown *et al.* 1987). The effect that urine flow had on N^{τ}-MH excretion, on renal reabsorption of N^{τ}-MH, and on the recovery of labelled N^{τ}-MH needs to be investigated further. These observations suggest that N^{τ}-MH may not be excreted quantitatively in other species if they have restricted water flux. In cattle, validation of N^{τ}-MH was conducted using cattle with free access to water (Harris and Milne 1981b), perhaps excretion would not have been quantitative if water flux was reduced.

That caribou would reduce protein turnover in winter seems intuitive and has been suggested by tracer studies (Blanchard and Luick 1980). Methods for monitoring protein turnover in free ranging animals, particularly over winter, would be useful in assessing physiological status. An increase in turnover or net catabolism of endogenous proteins in winter would indicate nutritional stress. An indicator not influenced by recycling other metabolic processes would be highly sensitive and could provide data in earlier phases of undernutrition. It would also provide data on the nitrogen balance of animals, not just the energy balance.

Comparisons of fall body composition of caribou and reindeer on lichen and non-lichen winter ranges support the existence of fall set points in body composition, and particularly in fall fat reserves. These set points may be tied to previous nutritional experience through mechanisms similar to those observed in compensatory gain studies, namely increased feed intake and possibly increased selection for high quality feeds (Ryg 1983, Watkins *et al.* 1991, Wairimu *et al.* 1992). Evidence for this occurring in wild caribou is still circumstantial but it warrants further study.

That caribou do not become as fat as physiologically possible needs to be considered in using fall condition to assess summer nutrition. Certainly very poor condition in the fall would suggest poor summer nutrition, as caribou would at least be expected to strive for fat reserves which would permit ovulation (Crête *et al.* 1993). However, once beyond this point the relationship between summer nutrition and body composition is not as clear.

As has been proposed for other wild cervids (DelGiudice *et al.* 1987, 1989, 1991b), the use of urinary urea-N:creatinine (UUC) as an indicator of physiological status and as an indirect indicator of body composition may also be applicable to caribou. UUC values of 0.25 (mg:mg) could be interpreted to mean that fat reserves are partially depleted, that dietary sources of energy are insufficient, and therefore endogenous proteins are being catabolized. This cutoff is much lower than that suggested by DelGiudice and Seal (1988) for white-tailed deer, however, urea-N excretion by well nourished caribou on lichen diets is also much lower than observed for deer. The use of serum urea-N

concentrations, urinary N^{τ}-MH:creatinine, and serum N^{τ}-MH concentrations as indicators of physiological status were not supported.

Calculation of total urea-N excretion in free ranging caribou demonstrated that, even when UUC increased in caribou due to accelerated endogenous protein catabolism, the actual quantity of urea-N lost in urine was minimal. This reflects a reduction in water flux and efficient recycling of urea-N into the rumen which would reduce serum urea-N concentrations and allow for increased reabsorption of urea-N in the kidney. As demonstrated for other ungulates (e.g. DelGiudice *et al.* 1991a), severe undernutrition and starvation could only result in increased UUC as recycling becomes less efficient without sufficient energy intake thereby increasing serum urea-N concentrations.

Murphy and King (1985) pointed out that an animal's nutritional status can only be properly evaluated in the context of its environment. This study demonstrates that wapiti, reindeer and caribou have a wide repertoire of adaptations which allows them to minimize the impact of low winter forage quality and availability. This study also raises several questions about these adaptations. Both reindeer and wapiti changed feed intake when placed on the low protein diet but it is not clear under what conditions animals will attempt to make up for dietary quality by increasing intake versus reducing intake and metabolic requirements. The observations that reindeer did not increase tubular reabsorption of urea-N despite being on a nitrogen deficient diet, yet caribou on a lichen diet in the wild have very high reabsorption raises the questions: under what conditions does reindeer kidney function change? And what is the role of lichen in nitrogen conservation? The failure of N^{τ}-MH excretion to be a valid index of myofibrillar protein turnover in wapiti and reindeer requires a return to the search for such an index.

Further investigations of set points in wild caribou populations are recommended. In particular, body composition and gut fill should be monitored during late summer and fall to determine if caribou reduce intake once a certain body composition is attained. The effect of previous winter's nutrition on fall body composition also needs further investigation as does the impact of insect harassment on fall body composition. Further data are also needed to determine what UUC would be indicative of severe undernutrition in caribou.

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