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PROTEIN CONSERVATION IN FEMALE CARIBOU (*RANGIFER TARANDUS*): EFFECTS OF DECREASING DIET QUALITY DURING WINTER

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Female caribou subsist primarily on lichens and some senescent browse during winter when demands for fetal growth add to costs of thermoregulation and mobility. Lichens, although potentially high in digestible energy, contain less protein than required for maintenance by most north-temperate ungulates. To understand the adaptations of caribou to the nutritional constraints of their primary food resource, we fed captive female caribou a sequence of 3 diets designed to resemble decreasing quality of forages during early, mid-, and late winter, respectively: high energy–high protein (HIGH), medium energy–low protein (MEDIUM), and low energy–low protein (LOW). In vitro digestibility of dry matter declined from 94% (HIGH) in November, to 66% (MEDIUM) in December and January, and to 53% (LOW) from February to April. Dietary protein averaged 19.8% in November and 4.3% from December to April. We used measures of body condition, stable isotopic signatures, and concentrations of nitrogen (N) metabolites to define protein dynamics in the animals. Subcutaneous rump fat declined between October and April from 2.3 cm ± 0.3 *SE* to <0.5 cm as intake of digestible energy declined from 44.0 ± 2.0 MJ/day to 16.3 ± 3.2 MJ/day. In erythrocytes, increasing enrichment of carbon (^{13}C) throughout winter suggested that caribou reused body lipids, and increases in ^{15}N during January and February indicated that they also recycled amino-N. Urinary N was primarily urea with an isotopic signature that tracked dietary ^{15}N through late winter. Plasma urea-N declined from 44.0 ± 2.6 mg/dl to 8.5 ± 1.2 mg/dl as nitrogen intake declined from 91.5 ± 5.3 g N/day to 14.1 ± 0.9 g N/day. Examination of these data suggests that caribou catabolized dietary C and N in preference to endogenous fat reserves and body protein. Female caribou appear to tolerate low intakes of protein and energy in winter by minimizing net loss of body protein and reapportioning body reserves to support fetal growth.

Key words: caribou, energy balance, lichens, nutritional ecology, protein balance, *Rangifer*, stable isotopes

The adaptability of ungulates to northern environments is strongly influenced by nutritional constraints that direct activity patterns and foraging strategies. Acquisition and conservation of energy and protein affect body composition, which in turn affects most aspects of survival and reproduction (Allaye-Chan 1991). For ungulates living in boreal and arctic regions, behavioral and physiological strategies are probably more responsive to the selective pressures of their harsh environment than is the case for species at temperate latitudes. Caribou (*Rangifer tarandus*) are the only ungulate species to have successfully colonized both arctic and boreal ecosystems, and they show both behavioral and physiological adaptations to the extreme seasonal declines in temperature and food resources of winter. Strategies for survival in *Rangifer* are well researched from the perspective of energy balance. Animals reduce energy requirements significantly by minimizing activity (e.g., Adamczewski et al. 1993) or migrating to more suitable winter ranges (e.g., Kelsall 1968) and by using physiological traits that allow thermoneutrality to −40°C (e.g., Cuyler and Øritsland 1993; Nilssen et al. 1984; Tyler and Blix 1990). The role of body fat as an energy reserve for survival and reproduction has been well documented (e.g., White 1992). Levels of energy intake directly affect changes in body weight and percentage body fat (Allaye-Chan 1991), which show a positive relationship with pregnancy rates in caribou (Cameron et al. 1991).

Our understanding of protein dynamics in *Rangifer*, by contrast, is relatively poor (McEwan and Whitehead 1970; Wales et al. 1975). Winter diets of caribou are dominated by lichens and some senescent browse that may contain low levels of protein (Johnson et al. 2000; Person et al. 1980; Säkkinen

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TABLE 1.—Dry matter digestibility (as determined by in vivo or nylon bag digestion[a]) and crude protein content of winter forages consumed by north-temperate ungulates in North America.

Species	Location	Forage	Digestibility (%)	Crude protein (%)	Source[b]
White-tailed deer	Michigan	Aspen browse	49	6.2	1
White-tailed deer	Michigan	Conifer	60	3.8	1
White-tailed deer	Michigan	Conifer	41	3.4	1
White-tailed deer	New Hampshire	Winter browse	39	7.2	2
White-tailed deer	New Hampshire	Conifer	48	8.2	2
Elk	Colorado	Winter leaves	38	5.5	3
Elk	Colorado	Browse stems	32.5	6.1	3
Elk	Colorado	Graminoids	44.5	4.2	3
Moose	Alaska	Winter browse	~36.5	~6.5	4
Moose	Alaska	Winter browse	~27.5	~6.5	5
Moose	Alberta, Canada	Winter browse	~48	~6.0	6
Moose	Michigan	Winter browse	~34.5	~6.5	7
Mule deer	Washington	*Alectoria* lichen	85	2.0	8
Reindeer	Alaska	Ground lichens	51	~2.5	9
Caribou	Washington	Arboreal lichen	82	~2.5	10

[a] Plant samples are suspended in nylon bags within the rumen, which allows for an "open-flow" system to determine digestion of specific forages (e.g., Person et al. 1980).
[b] 1, Ullrey et al. 1972; 2, Mautz et al. 1976; 3, Hobbs et al. 1981; 4, Risenhoover 1989; 5, Regelin et al. 1987; 6, Renecker and Hudson 1985; 7, Risenhoover 1987; 8, Robbins 1987; 9, Person et al. 1980; 10, Rominger 1995.

et al. 2001). Inadequate nitrogen (N) to maintain N balance may result in loss of muscle protein in adults and reduced growth in young animals (Robbins 1993; Swick and Benevenga 1977). Reproductive demands for N are particularly high when females must supply N for developing fetuses during winter, and for milk in early summer (Adamczewski et al. 1987; Parker et al. 1990). It has been shown that inadequate N intake can compromise the female and the fetus in utero or at term in several breeds of domestic cattle and sheep (e.g., Martin et al. 1997; Maynard et al. 1979). Maternal responses to N restriction may attenuate adverse effects on the offspring, especially in large mothers, but severe restriction will compromise the offspring. Low N intake reduces viability of white-tailed deer (*Odocoileus virginianus*) neonates (Sams et al. 1995) and could ultimately delay calving date and reduce calf survival in caribou (Cameron et al. 1993). Low-N diets such as lichens also may impair milk production for early calf growth and fail to maintain calves in their 1st winter (Bjarghov et al. 1976; Jacobsen et al. 1981). Studies on caribou have described deposition and mobilization of body protein and fat depots (e.g., Adamczewski et al. 1987), and directly correlated fetal development with maternal protein (Allaye-Chan 1991). However, the influence of protein intake on maternal protein has not been identified. Further, caribou may have adaptations that can minimize digestive and urinary N losses on low-N diets (Case 1994; Hove and Jacobsen 1975; Valtonen 1979; White et al. 1984), but the limits to N balance are unclear.

Crude protein content (defined as g N × 6.25) of north-temperate and arctic forage species declines to lowest levels during winter. The level of crude protein considered necessary to maintain minimum protein balance by wild adult ruminants ranges from 5 to 9% (Robbins 1993:175; average 7%—Van Soest 1994:289, 344). Minimal protein requirements have been reported in red deer (*Cervus elaphus*, 5%—Maloiy et al. 1970), white-tailed deer (4.1–5.8%—Asleson et al. 1996; Holter et al. 1979; Robbins et al. 1975), moose (*Alces alces*, 5.9%—Schwartz et al. 1987b); and elk (*Cervus elaphus nelsoni*, 5–7%—McCullough 1969). Winter diets for these species (Table 1) generally provide near-maintenance levels of protein, although protein balance may become negative if body protein is mobilized to contribute to energy demands. However, the winter diets of caribou are dominated by lichens, which, although relatively high in digestible energy, often contain only 2–3% crude protein (Person et al. 1980; Storeheier et al. 2002; Table 1). This protein level is substantially below what is required for maintenance and survival by other north-temperate ungulates.

For animals with adequate energy and protein intake, dietary carbon (C) and N are incorporated into the tissues when lipids and proteins are synthesized. Inadequate intake of energy results in a net loss of C when lipids and protein are catabolized. Inadequate intake of organic N results in the net loss of body protein and in reapportioning of those amino acids to support maintenance of critical tissues. These catabolic states of tissue mobilization also are associated with reuse of C and N within the body if intermediary metabolites such as amino acids and fatty acids are reincorporated or if catabolites such as urea are recycled back to tissues.

Stable isotopes allow assessments of C and N exchange between the body and the diet and between tissues within the body (see reviews in Gannes et al. 1997, 1998). Therefore, changes in isotopic signatures ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) should indicate the relative contributions of diet and metabolic processes such as N recycling or reutilization of lipid. The lighter isotopes of N and C (^{14}N, ^{12}C) typically participate in enzymatic reactions more than the heavier isotopes (^{15}N, ^{13}C). Consequently, N exchanges within tissues, and in the process of excretion, may increase the proportion of heavy N (^{15}N) retained in the body while increasing the proportion of light N (^{14}N) in products such as urinary urea.

The overall goal of this study was to increase our understanding of the adaptations of caribou to lichen ecosystems, and the influence of low-protein (but high-energy) lichens on body condition and allocation of endogenous fat and protein toward reproduction. We studied the responses of captive female caribou to a sequence of 3 diets designed to resemble decreasing quality of forages during early, mid-, and late winter, respectively: high energy–high protein (HIGH), medium energy–low protein (MEDIUM), and low energy–low protein (LOW). Our objectives were to examine dynamics of feed intake, body mass, and fat reserves; to use isotopic signatures as indicators of N and C sources; and to measure concentrations of N catabolites as indicators of protein conservation. The experimental design attempted to track the naturally occurring states of sufficient energy and protein content in the diet (e.g., autumn–early winter conditions), deficient dietary protein (e.g., midwinter lichen ranges in good condition), and deficient dietary protein and energy (e.g., late-winter low lichen availability and senescent

forages). Our 1st diet was designed to ensure that animals were not limited by either energy or protein. The consequences of the subsequent 2 dietary treatments may apply to nonmigratory caribou on small ranges that exhaust lichens as winter progresses, or to migratory animals that encounter areas of lower lichen availability and greater abundance of senescent sedges (e.g., Jorgenson et al. 2002).

Materials and Methods

Captive caribou on simulated winter diets.—Six adult female caribou were maintained at the Alaska Department of Fish and Game Moose Research Center near Soldotna, Alaska, in a 6-ha pasture. In late October 1999 through April 2000, animals were confined together in a single, ≈1-ha enclosure. The enclosure was denuded of most herbaceous vegetation and covered with snow for the duration of the experiments. Pelleted rations were provided in an adjacent covered pen, where animals had continuous access only to individual-specific Calan gates (American Calan, Inc., Northwood, New Hampshire). This controlled-access feeding system used a feed container, accessible only through a neck slot controlled by a 24-V electronically locking gate that was unlocked by an individual-specific ''key'' collar worn by the animal (Mazaika et al. 1988). Each caribou was trained to use its own gate feeder, and subsequently, animals were required to use the collar keys to access food. There was no competition or interaction among females for food. Three diets (high energy–high protein, medium energy–low protein, low energy–low protein), prepared by Alaska Garden and Pet (Anchorage, Alaska), were fed ad libitum sequentially over 2-month periods, commencing after winter pelage growth was complete in late October. Foods offered and rejected were weighed daily (±1 g) for all individuals to determine daily feed intake. All dietary treatments contained corn, beet pulp, and molasses, and small amounts of limestone flour and trace mineral salts to maintain a relatively consistent flavor and isotopic (^{15}N, ^{13}C) signature. Energy and protein contents of the diets were increased by inclusion of barley and soybean meal (HIGH) and progressively reduced (MEDIUM and LOW) by substitution of those concentrates with tapioca starch, crystaline cellulose, aspen sawdust, and rice hulls (LOW only).

Before confinement in the 1-ha enclosure, breeding activity of the caribou females was monitored in the larger pasture using a HeatWatch Estrus Detection System (DDx Inc., Denver, Colorado). This system involved a small radiofrequency transmitter and pressure sensor glued to the rump of the female. The transmitter sent information to a receiving computer base station each time the female was mounted. The estrus detection system and visual observations indicated that all animals bred between 8 and 15 October.

Female caribou in this study were not tamed, but were moved through a chute system for handling each month. All measurements and samples were taken between 0900 and 1200 h to minimize effects of time of day. Animals were weighed (±0.5 kg) on an electronic platform scale (Model 700, Tru-Test Limited, Aukland, New Zealand) to determine changes in live body mass. Blood was sampled by restraining the animals in a drop-floor squeeze chute immediately after the enclosed platform scale. Blood was collected by cephalic venipuncture in heparinized tubes, centrifuged at 300 × *g* to separate blood cells and plasma, and stored at −20°C for subsequent analyses. Blood sera from the late November sampling period was assayed for pregnancy-specific protein B to confirm pregnancy status (Stephenson et al. 1995). Animals were assessed for general changes in body condition by measuring subcutaneous fat at the rump with ultrasonography (Stephenson et al. 1998). The rump region was scanned by using an Aloka portable ultrasound device with a 5-Mhz, 8-cm linear array transducer (Model 500, Aloka, Inc., Wallingford, Connecticut) to determine subcutaneous fat thickness (±1 mm—Stephenson et al. 1998). Total body fat (y, %) for caribou was predicted from the equation $y = 5.76 + 2.27x$, where x is maximum subcutaneous fat thickness on the rump ($r^2 = 0.73$, $n = 9$—T. Stephenson, in litt.), as validated by total body grinding (e.g., Stephenson et al. 1998).

Every 2 weeks, caribou were allowed into another small pen adjacent to the 1-ha enclosure for collection of snow urine. This pen accumulated fresh snowfall over biweekly periods when animals were excluded. After urination events for each animal, samples of snow with urine in it were collected, thawed to <10°C, decanted into neoprene vials, and refrozen for analysis. Animals subsequently were returned to the 1-ha enclosure. We followed animal welfare protocols approved by the Alaska Department of Fish and Game, which meet guidelines recommended by the American Society of Mammalogists (Animal Care and Use Committee 1998).

Laboratory analyses.—Feed samples for each dietary treatment (composite samples obtained from multiple bags of each batch of food) were prepared for analysis by drying at 50°C and grinding through a 2-mm screen. All samples were analyzed in triplicate and averaged. Dry matter content was determined by drying to a constant mass at 100°C in a convection oven. Gross energy content (kJ/g) was determined by bomb calorimetry (Parr Instruments, Boleen, Illinois), total N by elemental analyzer (CNS2000, LECO Co., St. Joseph, Michigan), and neutral detergent fiber (NDF) by extraction with neutral detergent (Peltier et al. 2003). Digestibility was estimated by measures of in vitro dry matter digestibility (IVDMD) with rumen inocula from captive reindeer (e.g., Spaeth et al. 2002).

Plasma and urine samples were assayed for urea by the diacetylmonoxime method (Procedure 535, Sigma Chemical, St. Louis, Missouri; Marsh et al. 1965), and for creatinine by the alkaline picrate reaction (Procedure 555, Sigma Chemical; Heinegård and Tiderström 1973). Uric acid was analyzed by reaction with uricase and peroxidase (Procedure 686A; Sigma Chemical; Fossati et al. 1980) after dissolving urates in urine with an equal volume of 1% (weight:weight) Li_2CO_3 (Adeola and Rogler 1994).

Urea and ammonia N were isolated from plasma and urine by modifications of the methods of Barboza et al. (1997) and Nolan and Leng (1972). Samples were alkalized to volatilize ammonia, which was collected into boric acid. Urea was isolated by incubation with urease after ammonia had been removed. Distilled N was quantified by titration of the ammonia with hydrochloric acid. This method recovered 94 ± 8% and 68 ± 9% (mean ± *SD*) of N from ammonia and urea, respectively. Titrated solutions were overacidified with HCl and dried at 65°C to minimize subsequent losses of ammonia N. Dry ammonium borate was subsequently analyzed for isotopic enrichment of N.

Total N in urine was measured in dry material with an elemental analyzer. Oven drying recovered 99–83% (mean ± *SD*, 89 ± 2%) of N in liquid urine, whereas lyophilization only recovered 75–67% (71% ± 2%) of N, probably through loss of ammonia with sublimated water. Therefore, urine was acidified with boric acid and oven-dried at 65°C to minimize loss of ammonia. Plasma and blood were dried without acidification at 65°C. Isotope ratio mass spectrometry (IRMS; Europa Scientific 20-20 Continuous Flow IRMS, Europa Scientific, Chestershire, United Kingdom) was used to measure ^{15}N enrichment, which was expressed as δ (parts per thousand [ppt]) against air, and ^{13}C enrichment, which was expressed relative to the limestone standard (Peedee Belemnite; Wolfe 1992). We assumed that heparin concentrations in the vacutainers used to draw blood did not vary much among samples because they were of similar volumes, and also that the amount of N (and its contribution to isotopic analyses) from Na heparin was negligible compared with the blood.

TABLE 2.—Gross energy (kJ/g), protein (% N), and fiber (% neutral detergent fiber [NDF]) content and in vitro dry matter digestibility (% IVDMD) of high-energy–high-protein (HIGH), medium-energy–low-protein (MEDIUM), and low-energy–low-protein (LOW) diets consumed by captive female caribou during early, mid-, and late winter, respectively, 1999–2000. Data, presented as mean ± 1 *SD,* are averages of 2 batches of feed (only 1 batch was used for HIGH treatment).

	HIGH	MEDIUM	LOW
	26 Oct.–21 Dec.	22 Dec.–15 Feb.	16 Feb.–11 Apr.
kJ/g	17.54	17.38 ± 0.46	17.71 ± 0.16
% N	3.17	0.63 ± 0.34	0.73 ± 0.17
% NDF	19.41	35.32 ± 1.58	45.10 ± 2.45
% IVDMD	93.96	66.06 ± 5.62	53.25 ± 3.07

Statistical analyses.—Data for urine and urinary metabolites refer to snow urine samples. Estimates of daily feed intake were averaged for each of the 5-day periods preceding blood or urine samples. We used an α level of 0.05 for statistical significance in all analyses.

Because we resampled the same caribou in this study, we used a repeated-measures analysis of variance (ANOVA; SYSTAT 10.2, SYSTAT Software Inc., Point Richmond, California) to examine changes over time in body mass and subcutaneous fat, food intake, C and N signatures, fractions and ratios of urinary metabolites, and N metabolites in plasma. We tested for differences between pregnant and nonpregnant individuals because of the potentially profound effects of pregnancy on protein or energy assimilation even though the statistical power of this comparison was limited by our small sample size (Zar 1999). The repeated-measures analysis requires an observation for each individual in each sampling interval (monthly for mass, fat, and blood parameters and biweekly for urinary analyses). One individual was removed from all analyses because that animal developed a leg abscess that required oral medication and affected food intakes from February to April. Data were missing in some sampling intervals for the remaining 5 animals: urine samples were not collected in late November because of insufficient snow, feed intake was not available for several animals that were not habituated (despite training) to the feeding gates in late October, and a urine sample was not obtainable from 1 animal in April. In those cases, the incomplete sampling interval was omitted from the repeating series to conserve the sample size for ANOVA.

RESULTS

The 3 diets consumed by caribou resulted in increasing fiber content and a corresponding decrease in dry matter digestibility across the winter (Table 2). Consequently, estimated digestible energy content of the diet (% IVDMD × kJ/g) declined by almost 43% from early to late winter. The 2 low-protein dietary treatments in mid- and late winter were each produced in 2 batches and, therefore, were slightly more variable because of compositional changes within ingredients. Average dietary N content declined approximately 78% from early to mid- and late winter.

Three caribou in this study were diagnosed as pregnant by using the pregnancy-specific protein B assay. These adults (born in 1995 and 1996) had previously produced calves, beginning at 2–3 years of age, with average gestation lengths of 223 days. The 2 smallest adults born in 1997 and 1998 were not pregnant; they had not reproduced before and did not produce calves in the spring after this study (at age 2–3 years). Body mass was the only variable to differ significantly with pregnancy status. Pregnant and nonpregnant caribou were not different in food intake, body fat, isotopic signatures, or N metabolites. Therefore, we pooled data from all 5 adult females for final analyses and graphical presentations when pregnancy status was not a significant effect.

Food intake, body mass, depth of subcutaneous fat, and predicted total body fat of caribou all varied significantly throughout the winter (Fig. 1). Ad libitum dry matter intake by caribou declined from 2.7 kg/day in early winter to lowest levels of 1.87 kg/day on the low energy–low protein diet (Fig. 1A). Although bimonthly measures of food intake were most variable toward the end of winter, pregnancy status did not significantly interact with time; that is, food intakes were similar between groups at the end of winter. However, pregnant individuals weighed significantly more than the younger, nonpregnant animals in late October (mean ± *SE*, 118.3 ± 2.9 kg versus 94.8 ± 8.5 kg) as well as in late winter (121.3 ± 5.8 kg versus 89.9 ± 6.8 kg; Fig. 1B). Mass within each group at the end of the trials was not significantly different from the beginning, even though the 3 pregnant individuals gained a fetus during this time. Levels of body fat declined throughout the winter (Fig. 1C). Body fat peaked (rump fat thickness 2.3 ± 0.3 cm, 11.0% total body fat) in late November for 4 animals; the remaining smallest female reached maximum fatness in late October (0.81-cm rump fat thickness). Rump fat thickness in early April ranged from 0.1 to 0.53 cm, indicating 6.0–7.0% total body fat.

The estimated digestible energy intake of caribou declined even on the higher-energy diets in early and midwinter, and remained relatively stable at the lowest intakes on the low-energy diet in late winter (Fig. 2A). Peak fatness in late November coincided with the highest consumption of digestible energy at 44.0 MJ/day ± 2.0 *SE* (range: 40.8–50.5 MJ/day) based on the assumption that digestibility of gross energy was equivalent to IVDMD. Relative digestible energy intake was 407.5 ± 37.2 kJ kg^{-1} day^{-1} or 1,311.1 ± 96.2 kJ $kg^{-0.75}$ day^{-1}. The lowest energy consumption occurred in late March at 16.3 ± 3.2 MJ/day, which was equivalent to 205.3 ± 11.7 kJ kg^{-1} day^{-1} or 658.8 ± 30.7 kJ $kg^{-0.75}$ day^{-1}.

Patterns of C enrichment differed between blood and urine samples (Figs. 2B and 2C). The ^{13}C enrichment in red blood cells was lowest in early winter, and increased throughout the experiment (Fig. 2B), corresponding with declining energy intake and subcutaneous fat of animals (Figs. 1C and 2A). Enrichment of ^{13}C in snow urine varied across the winter, partially in response to general changes in dietary ^{13}C (Fig. 2C). The change to lower dietary ^{13}C enrichment in the low-protein diets also depleted urinary ^{13}C to the lowest enrichments in late January–February. However, urinary ^{13}C was significantly more enriched in late winter even though dietary signatures remained the same.

Daily N intake by caribou and enrichments of ^{15}N in blood and urine varied significantly over the winter (Fig. 3). Nitrogen intake declined from highest levels (91.5 g N/day ± 5.3 *SE*) in November throughout early and midwinter (Fig. 3A) in response to declines in both dry matter intake and dietary N content (Fig. 1A; Table 2). The lowest N consumption (6.1 ± 0.6 g N/day)

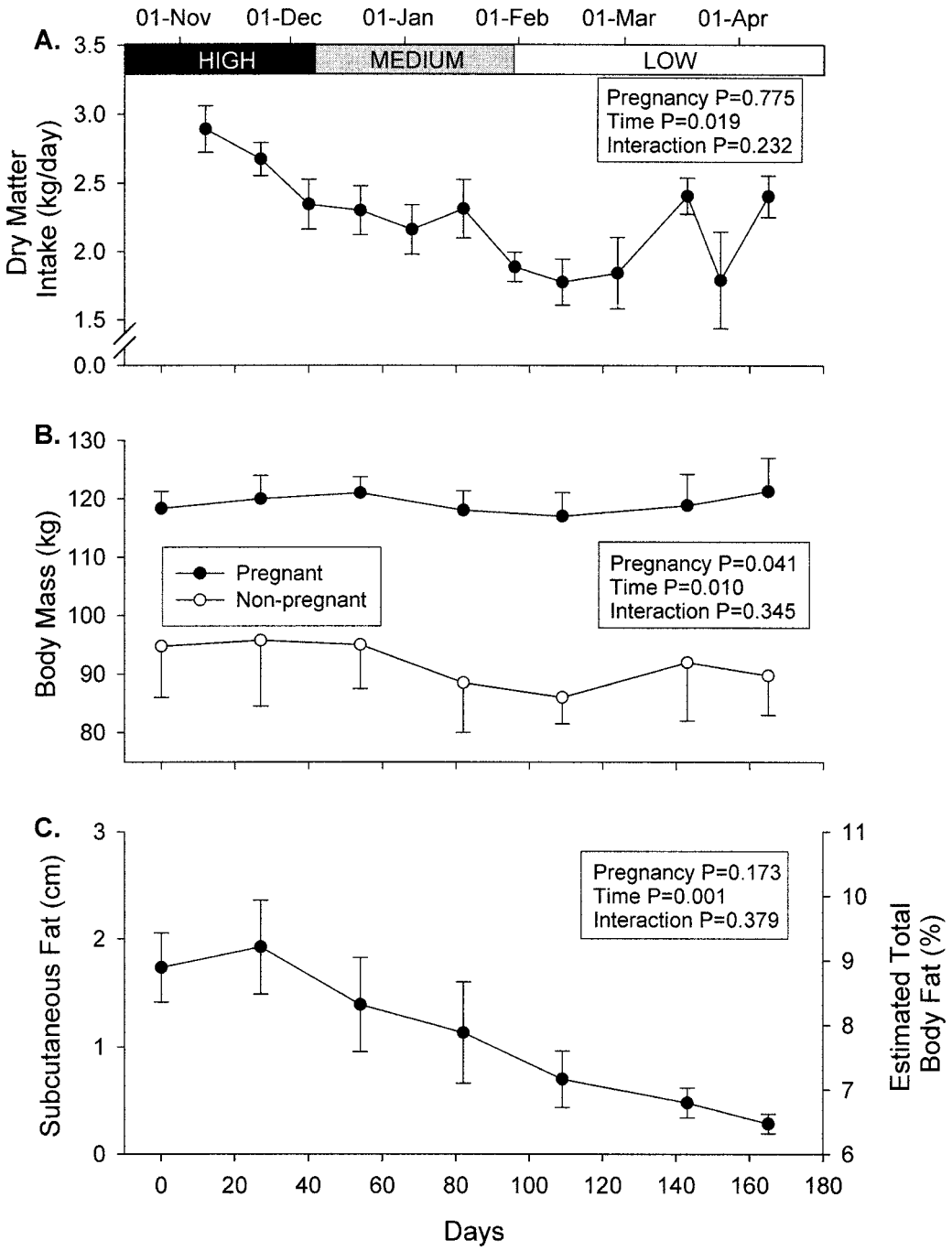

FIG. 1.—A) Dry matter intake, B) body mass, and C) subcutaneous fat measured at the rump and estimated total body fat of 5 captive caribou fed a sequence of high-energy–high-protein (HIGH), medium-energy–low-protein (MEDIUM), and low-energy–low-protein (LOW) diets during early, mid-, and late winter, respectively, between 26 October 1999 and 11 April 2000. Data on dry matter intake and subcutaneous fat showed no significant effects of pregnancy, and, therefore, were pooled for all 5 individuals. Data on body mass were pooled for 3 pregnant animals and for 2 nonpregnant individuals because of significant differences associated with pregnancy status. Data are shown as mean ± 1 *SE*.

coincided with the lowest food intakes (Figs. 1A and 3A), which were measured in February. A subsequent rise in food intake also increased N intake in early April to 14.1 ± 0.8 g N/day. The ^{15}N enrichment of red blood cells increased over time (Fig. 3B), even when dietary intake of N was low through mid- and late winter, indicating a slow use of endogenous amino-N for erythrocyte protein. The excretion of urinary ^{15}N directly reflected dietary enrichments (Fig. 3C), irrespective of the level of N intake. Similarly, the ^{15}N enrichment of urea-N within the urine samples also tracked dietary ^{15}N (Fig. 3D). Enrichment of residual nonamino-N (total urinary N minus ammonia and urea) was variable (1.1–4.0 ppt) throughout the winter and did not reflect dietary treatment.

Urea accounted for most of the N excreted by caribou during winter, but as a relative proportion of total N, it varied throughout the winter (Fig. 4A). Urinary urea tended to decline

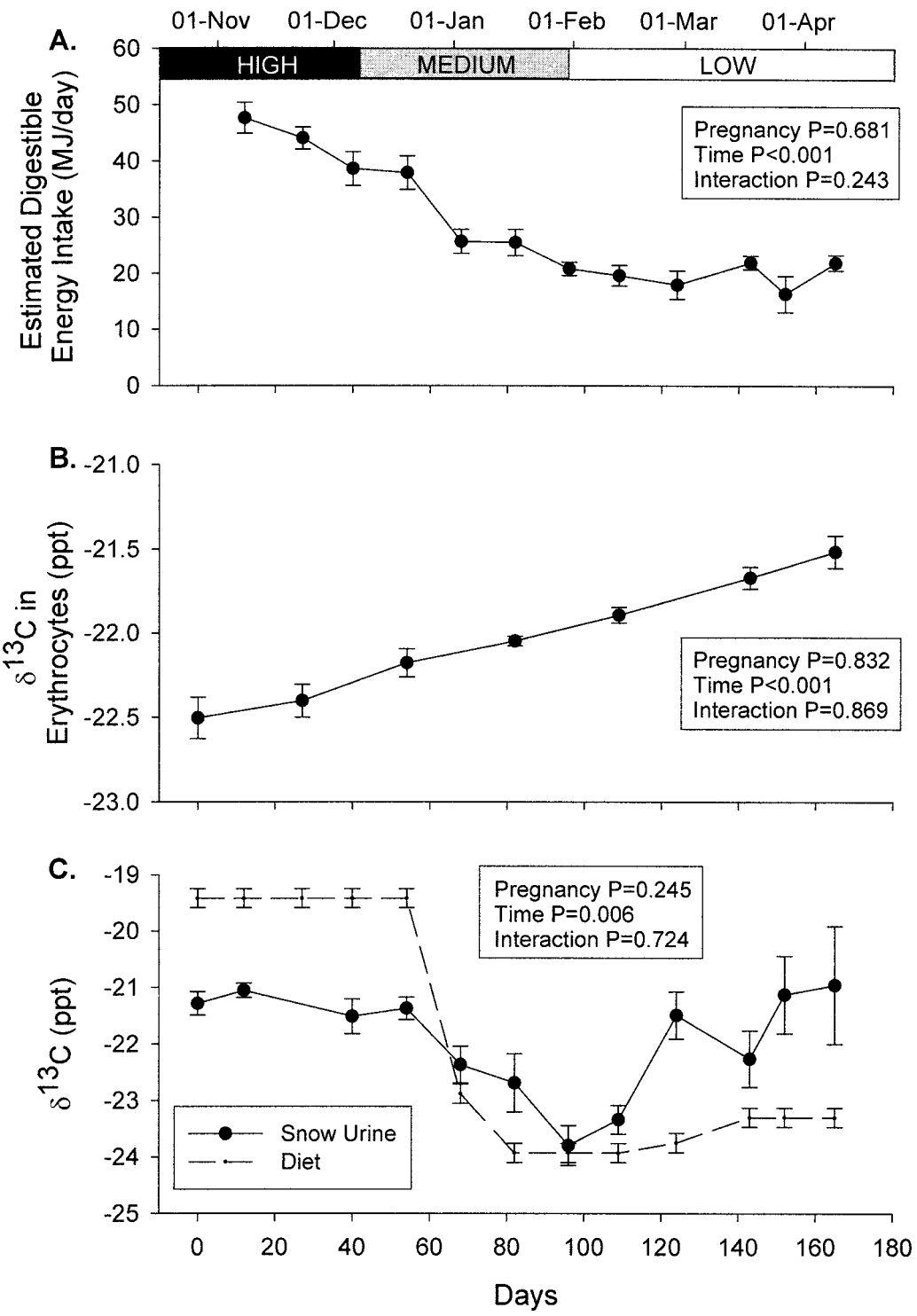

FIG. 2.—A) Energy intake, B) carbon (^{13}C) enrichment in erythrocytes, and C) urine collected in snow relative to diet for 5 captive caribou fed a sequence of high-energy–high-protein (HIGH), medium-energy–low-protein (MEDIUM), and low-energy–low-protein (LOW) diets during early, mid-, and late winter, respectively, between 26 October 1999 and 11 April 2000. Data showed no significant effects of pregnancy, and, therefore, were pooled for all 5 individuals. Data are shown as mean ± 1 *SE* except diet (mean ± 1 *SD*).

from the highest levels of 77% of total N measured at the start of the experimental period in early winter to lowest levels of 30% in midwinter as N intake declined (Fig. 3A). In contrast, the proportion of creatinine increased from 1.2% to 11.7% of total urinary N between early and midwinter (Fig. 4B). Increased intakes of N in late winter corresponded with a return to lower proportions of urinary N in creatinine and in highly variable levels of urea (Fig. 3A). The proportion of ammonia in urinary N was highest (2.4%) during high intake of N, reflecting deamination of excess dietary protein or regulation of

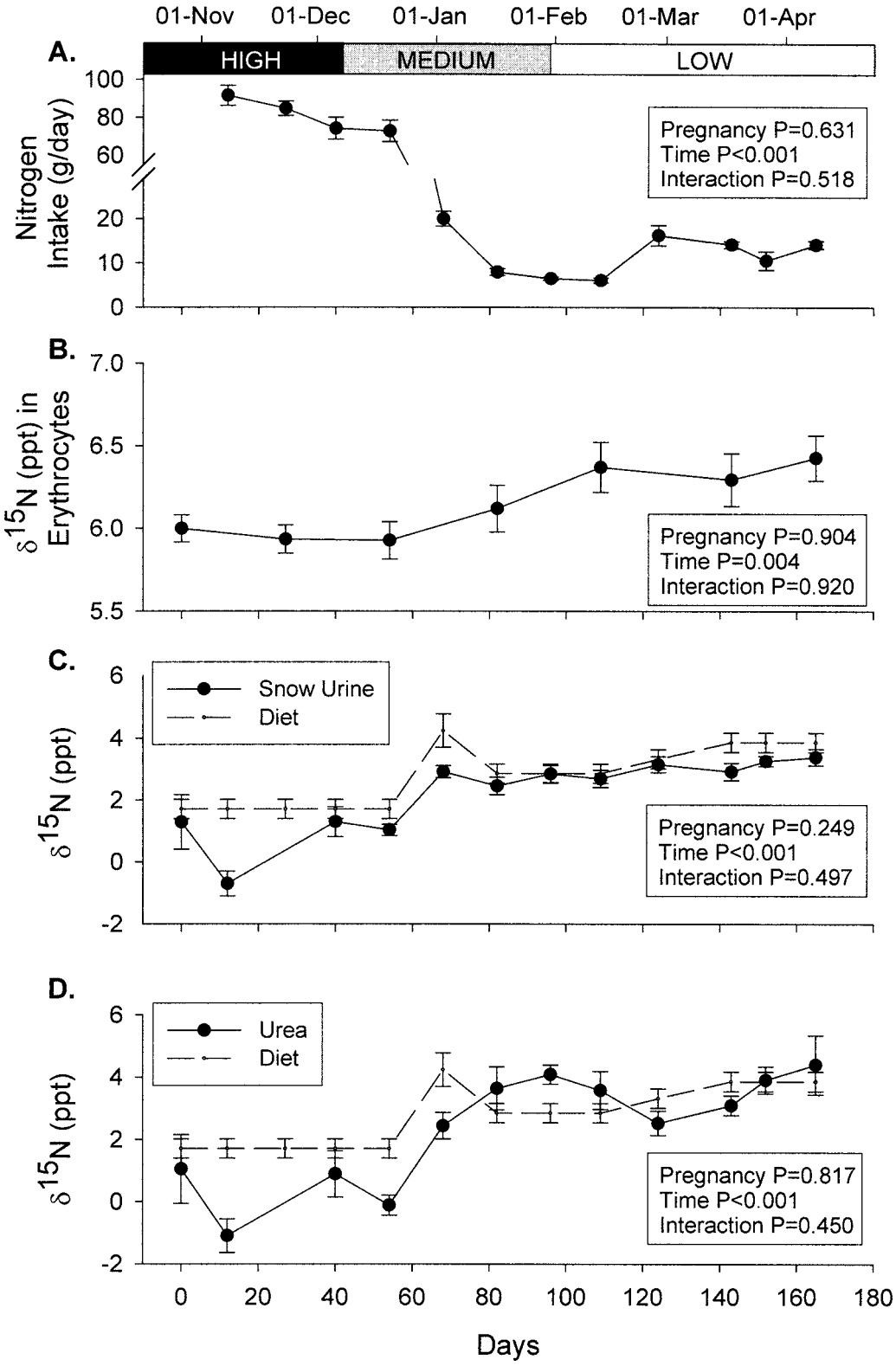

FIG. 3.—A) Nitrogen (N) intake, B) ^{15}N enrichment in erythrocytes, C) urine collected in snow relative to diet, and D) urea-N in snow urine relative to diet for 5 captive caribou fed a sequence of high-energy–high-protein (HIGH), medium-energy–low-protein (MEDIUM), and low-energy–low-protein (LOW) diets during early, mid-, and late winter, respectively, between 26 October 1999 and 11 April 2000. Data showed no significant effects of pregnancy, and, therefore, were pooled for all 5 individuals. Data are shown as mean ± 1 *SE* except diet (mean ± 1 *SD*).

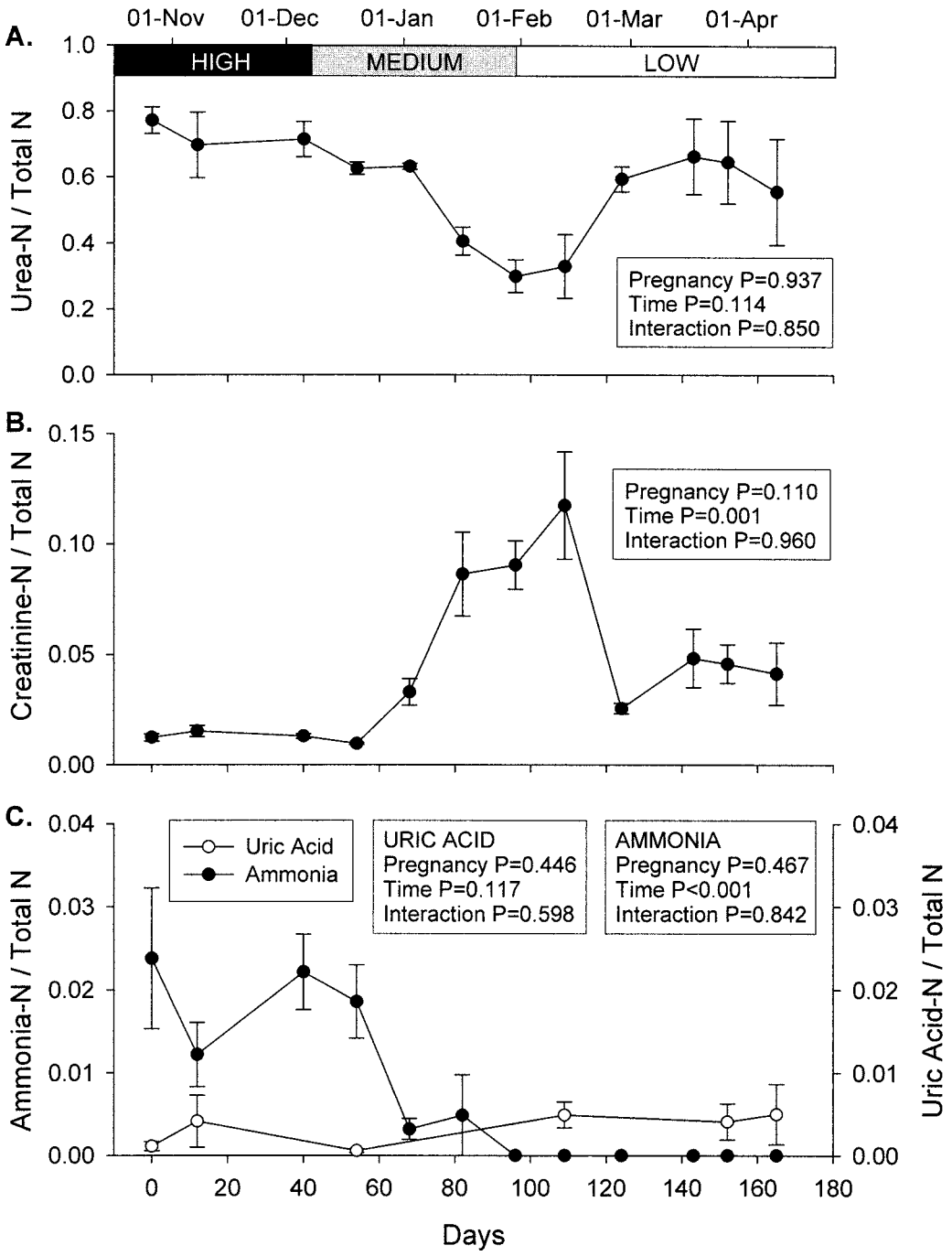

FIG. 4.—A) Ratios of urea nitrogen (N), B) creatinine N, and C) ammonia N and uric acid N to total N in urine collected in snow for 5 captive caribou fed a sequence of high-energy–high-protein (HIGH), medium-energy–low-protein (MEDIUM), and low-energy–low-protein (LOW) diets during early, mid-, and late winter, respectively, between 26 October 1999 and 11 April 2000. Data showed no significant effects of pregnancy, and, therefore, were pooled for all 5 individuals. Data are shown as mean ± 1 *SE*.

urinary pH (Fig. 4C). Soon after the change to low-protein diets, ammonia was no longer detectable in the urine samples. The proportion of uric acid was low (0.32% ± 0.08 *SE* of total urinary N) and did not change with time or diet (Fig. 4C).

Urea concentrations in plasma declined with decreasing N intake over winter (Fig. 5A) from 44.0 mg/dl ± 2.6 *SE* at the beginning of the experiment in late October, to 8.5 ± 1.2 mg/dl by late winter (April) after the switch to the low energy–low protein diet. Plasma creatinine peaked in midwinter (4.0–4.6 mg/dl; Fig. 5B), suggesting a release of creatinine from muscle and possibly a change in renal filtration when intakes of solutes and N were lowest.

Urea concentrations have typically been considered in relation to creatinine, facilitating comparisons that are not associated with water turnover and dilution (particularly for snow urine samples—e.g., DelGiudice et al. 1996). Changes in ratios

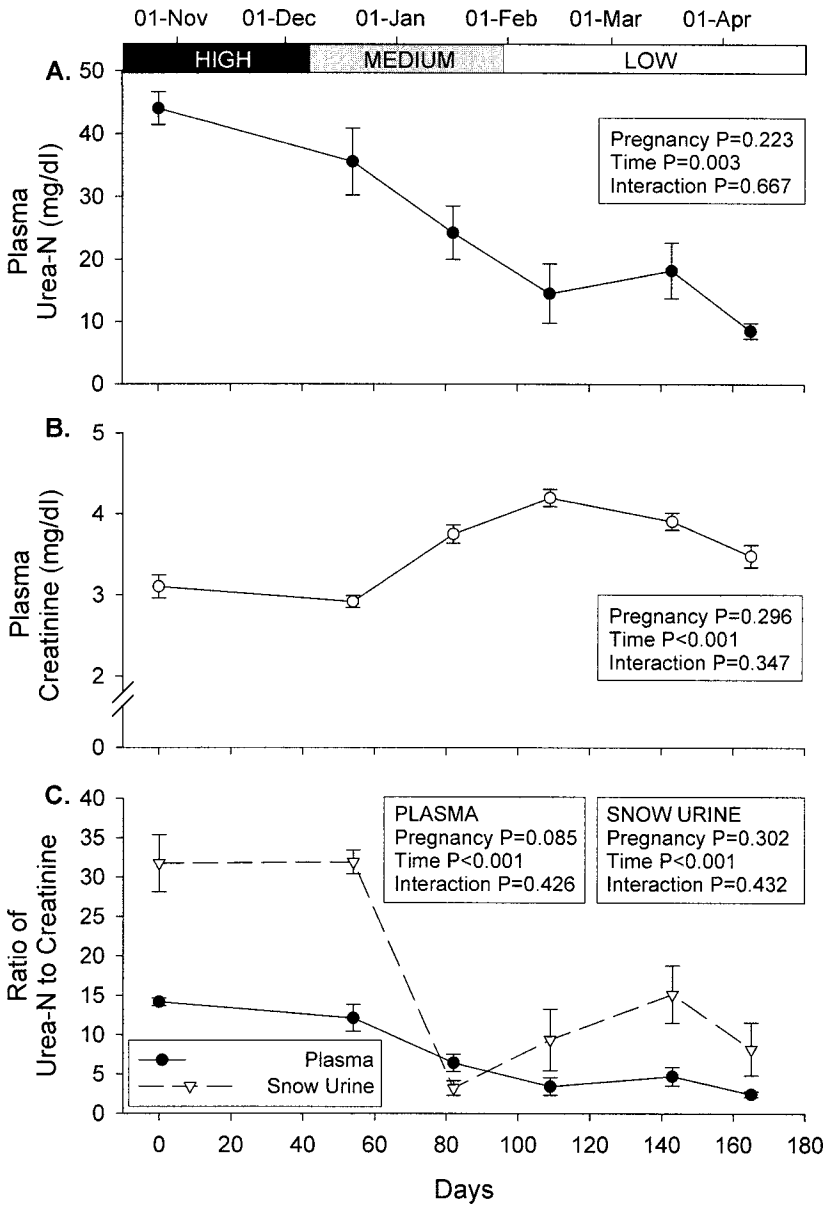

Fig. 5.—A) Plasma urea, B) plasma creatinine, and C) ratios of urea nitrogen (N) to creatinine in plasma and urine collected in snow for 5 captive caribou fed a sequence of high-energy–high-protein (HIGH), medium-energy–low-protein (MEDIUM), and low-energy–low-protein (LOW) diets during early, mid-, and late winter, respectively, between 26 October 1999 and 11 April 2000. Data showed no significant effects of pregnancy, and, therefore, were pooled for all 5 individuals. Data are shown as mean ± 1 *SE*.

of urea-N to creatinine (UN:C) varied significantly over the winter for both plasma and urine from caribou (Fig. 5C). The UN:C ratio in plasma declined throughout winter from 14.2 to 2.5, even though plasma creatinine increased at midwinter (Fig. 5B). UN:C ratios in urine were high (31.8) when N intake was highest. Lower urinary UN:C ratios in midwinter may reflect low urea levels as well as elevated excretion of creatinine (Fig. 4).

Discussion

Caribou were in negative energy balance through most of this study, as indicated by declines in body fat through winter. The average digestible energy intake of 660 kJ $kg^{-0.75}$ day^{-1} in late winter was just slightly lower than the digestible energy required for maintenance of body mass in pregnant white-tailed deer (670 kJ $kg^{-0.75}$ day^{-1}—Ullrey et al. 1969). Fasting metabolic rates (assumed to be basal metabolic rate [BMR]) in caribou range from 403 kJ $kg^{-0.75}$ day^{-1} (Fancy 1986) in nonreproductive females to estimated expenditures of 584 kJ $kg^{-0.75}$ day^{-1} in pregnant females during the last trimester of gestation (see Barboza and Bowyer 2001). The digestible energy intake observed in the animals in this study in late winter would support only the equivalent of 1.1 × BMR during pregnancy, which does not include additional costs of activity, and therefore coincides with the observed declines in body fat. Body mass did not change significantly over the course of this study. In pregnant animals, mass of the conceptus (fetus and amniotic fluids) would

have increased over time, but for both pregnant and nonpregnant animals, increasing (and variable) intake at the end of winter probably increased gut fill. Body fat stores (with no water) likely were replaced with lean tissues and fluids that contain a large fraction of water. Small increases in body mass also have been observed during hyperphagia in reindeer (Mesteig et al. 2000).

Even though food was provided ad libitum, it is likely that voluntary food intake during winter is under some endogenous control (Gedir and Hudson 2000; Schwartz et al. 1987a; Tyler et al. 1999). Consequently, loss of body fat may not depend on dietary quality during early and midwinter. In addition, as noted for moose and mule deer, individuals that have attained minimum body thresholds (Renecker and Samuel 1991; Schwartz et al. 1988) by autumn may be inclined to reduce intake and body condition over winter. Photoperiodic cues and depletion of body fat in late winter probably restored dietary responsiveness of caribou because food intake was highly variable during the last phase of this study (Fig. 1A) even though dietary energy content was unchanged. We did not have sufficient samples sizes to determine if body condition influenced intake at the end of winter, although there was some suggestion that animals in better condition consumed less digestible energy per day.

In early winter, caribou catabolized dietary C more than endogenous fat and protein because the urinary ^{13}C that was excreted was stable and the signature was depleted in relation to the high-energy diet (Fig. 2C). In late winter, however, C from body fat appears to have been used for energy. Increases in urinary ^{13}C during late winter were not due to dietary changes (Fig. 2C), but rather indicated catabolism of endogenous C. This is substantiated by the steady increases in ^{13}C enrichment of erythrocytes over winter (Fig. 2B); that enrichment most likely occurred because of recycling C from adipose tissue to erythrocyte membranes. The use of endogenous C is reflected in significant depletion of body fat (Fig. 1C) as well as the increased energy demands for growth of fetal tissue in pregnant individuals (Pekins et al. 1998).

Nitrogen intake in early winter was 2,135–2,519 mg N $kg^{-0.75}$ day^{-1}, considerably greater than the maintenance requirement of caribou estimated at 820 mg N $kg^{-0.75}$ day^{-1} in growing animals (McEwan and Whitehead 1970). Our observations of greater proportions of urea and ammonia in urinary N during early winter than in midwinter (Fig. 4) are consistent with an excess of dietary N. In midwinter, caribou probably conserved N when intake was only 186–238 mg N $kg^{-0.75}$ day^{-1}. At that time, low plasma urea (Fig. 5A) and urinary urea-N (Fig. 4A) suggest that urea excretion was minimized. Urea recycling could have increased N retention during midwinter when N intakes on the low-protein diet were lowest. The ability to recycle N normally lost during excretion has been purported to affect the margin of survival in free-ranging caribou (Case 1994; Wales et al. 1975), as well as in several other north-temperate ungulates (e.g., *Odocoileus virginianus*—Robbins et al. 1974; and *Cervus elaphus*—Mould and Robbins 1981). However, reducing the net oxidation and loss of amino-N from body protein may play a greater role during this period. We observed a gradual increase in N enrichment of erythrocytes in midwinter (Fig. 3B), presumably resulting from recycling of amino acids within the body. Most urinary N was probably derived from the diet that was similar in enrichment of ^{15}N (Fig. 3C). Dietary protein that is degraded to amino acids is apparently deaminated quickly, and the ammonia is returned to the pool of urea for excretion. Low enrichment or depeletion of urea-N compared with dietary N for most of the winter (Fig. 3D) suggests that recycling of amino-N via urea is small. However, urea recycling was indicated by enrichment of urea-N above dietary N at 80–100 days when intakes of dietary N (Fig. 3A) and the proportion of urinary N in urea (Fig. 4A) were lowest. Low concentrations of urea in plasma during late winter (Fig. 5A) suggest that the amount of N associated with this metabolite is small and thus recycling may only serve to minimize loss when dietary N intake declines below a minimum. These suggestions of N dynamics await direct confirmation from measures of N balance and urea kinetics in wintering *Rangifer*. However, examination of data from this study does suggest that caribou oxidize dietary N before endogenous pools of amino-N, which is consistent with the repartitioning of maternal C and N into fetal tissues by pregnant animals as winter progresses (Barboza and Bowyer 2001).

The use of endogenous fat and protein allows caribou to sustain fetal development while consuming diets that are low in digestible energy such as senescent browse or low in N such as lichens. Relationships between body mass and likelihood of parturition in caribou (Cameron et al. 1993; Chan-McLeod et al. 1999; Gerhart et al. 1996) are affected by both body lipids and protein. Body fat reserves must support the predictable demands of fetal growth as well as more variable increments for thermoregulation or mobility in deep snow (e.g., Cuyler and Øritsland 1993). Consequently, fat reserves at the start of winter can be influenced by energy demands from the previous winter (Parker et al. 1993; Renecker and Samuel 1991). Maternal energy supplies, from body fat that declines over the winter, may only become limiting to fetal development in the last stage of gestation. However, fetal development may be more sensitive to small changes in maternal body protein, which is likely the primary source for fetal protein accretion in late winter. Maternal N dynamics in midwinter may dictate the likelihood of continuing through the last trimester of gestation (72 d—McEwan and Whitehead 1972) when 80% of fetal mass is deposited (Barboza and Bowyer 2000; Oftedal 1985; Robbins and Robbins 1979). Thus, fetal tissues are probably most vulnerable to large energy demands on the mother during the end of gestation when body fat may be depleted and body protein may be used as an energy substrate. Furthermore, as dense energy-rich lipid stores are depleted, the rate of depletion of protein stores (muscle mass) likely will increase (Cook et al. 2001; Torbit et al. 1985). This relationship may be obscured in mass data of pregnant females because of the increasing tissue and fluids associated with a growing conceptus.

We attempted to conduct this experiment with gestating animals to define the mechanisms that allow caribou to subsist on low-protein diets when energy demands are high and protein demands are increasing during fetal development, but 2 individuals were not pregnant. Whether these animals experienced early embryonic mortality, as has been shown in caribou

with low body mass and low maternal fat content (Russell et al. 1998), is unknown. These individuals were the smallest and youngest. Small sample sizes in our study make it difficult to conclusively state that there are indeed no measurable differences (as we determined) in strategies for intake, allocation, and mobilization across body sizes or pregnancy states. We recommend additional research to compare strategies of gestating females with those of potentially still-growing nonpregnant females with fewer absolute body reserves. Nonetheless, to our knowledge, this study is unique in employing methods that combine concentrations and isotopic enrichments of blood and urinary metabolites with measures of intake and condition.

Our study tracks dietary constraints that are imposed on cervids in winter. Caribou rely heavily on lichens during winter pregnancy, which presents substantive nutritional challenges. Lichens differ from most other available winter forages because of high digestible energy and very low protein contents (Table 1). Whether the high energy content provides carbohydrates that sustain bacterial activity and facilitate endogenous recycling of N (Church 1975; Maynard et al. 1979) is unknown. The incorporation of N from urea into microbial protein potentially may be more efficient as a result of available energy in the diet (Wales et al. 1975), resulting in most effective utilization of protein. Strategies that spare the loss of body protein in caribou subsisting on low-protein diets become critically important for overwinter survival and successful reproduction. The apparent ability of wild caribou to continue using lichens through late winter and parturition requires further investigation of digestion, body composition, and N dynamics. Our results indicate that dietary N and endogenous N are handled differently by caribou during winter. Dietary N contributes to urinary N, whereas maternal protein from autumn is likely the primary source of fetal protein. Studies are needed to determine if the ability to recycle N helps meet the high N demands for fetal growth that coincide with low dietary availability of N on lichen ranges with limited alternative foods.

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Literature Cited

Adamczewski, J. Z., C. C. Gates, R. J. Hudson, and M. A. Price. 1987. Seasonal changes in body composition of mature female caribou and calves (*Rangifer tarandus groenlandicus*) on an arctic island with limited winter resources. Canadian Journal of Zoology 65:1149–1157.

Adamczewski, J. Z., R. J. Hudson, and C. C. Gates. 1993. Winter energy balance and activity of female caribou on Coats Island, Northwest Territories: the relative importance of foraging and body reserves. Canadian Journal of Zoology 71:1221–1229.

Adeola, O., and J. C. Rogler. 1994. Comparative extraction methods for spectrophotometric analysis of uric acid in avian excreta. Archives of Animal Nutrition 47:1–10.

Allaye-Chan, A. C. 1991. Physiological and ecological determinants of nutrient partitioning in caribou and reindeer. Ph.D. dissertation, University of Alaska, Fairbanks, Fairbanks.

Animal Care and Use Committee. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416–1431.

Asleson, M. A., E. C. Hellgren, and L. W. Varner. 1996. Nitrogen requirements for antler growth and maintenance in white-tailed deer. Journal of Wildlife Management 60:744–752.

Barboza, P. S., and R. T. Bowyer. 2000. Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. Journal of Mammalogy 81:473–489.

Barboza, P. S., and R. T. Bowyer. 2001. Seasonality of sexual segregation in dimorphic deer: extending the gastrocentric model. Alces 37:275–292.

Barboza, P. S., S. D. Farley, and C. T. Robbins. 1997. Whole-body urea cycling and protein turnover during hyperphagia and dormancy in growing bears (*Ursus americanus* and *U. arctos*). Canadian Journal of Zoology 75:2129–2136.

Bjarghov, R. S., P. Fjellheim, K. Hove, E. Jacobsen, S. Skjenneberg, and K. Try. 1976. Nutritional effects on serum enzymes and other blood constituents in reindeer calves (*Rangifer tarandus tarandus*). Comparative Biochemistry and Physiology, A. Comparative Physiology 55:187–193.

Cameron, R. D., W. T. Smith, and S. G. Fancy. 1991. Comparative body weights of pregnant/lactating and non-pregnant female caribou. Pp. 109–114 in Proceedings of the Fourth North American Reindeer and Caribou Symposium (C. E. Butler and S. P. Mahoney, eds.). Newfoundland Labrador Wildlife Division, St. Johns, Canada.

Cameron, R. D., W. T. Smith, S. G. Fancy, K. L. Gerhart, and R. G. White. 1993. Calving success of female caribou in relation to body weight. Canadian Journal of Zoology 71:480–486.

Case, R. L. 1994. Adaptations of northern ungulates to seasonal cycles in nitrogen intake. Ph.D. dissertation, University of Alberta, Edmonton, Canada.

Chan-McLeod, A. C. A., R. G. White, and D. E. Russell. 1999. Comparative body composition strategies of breeding and nonbreeding female caribou. Canadian Journal of Zoology 77: 1901–1907.

Church, D. C. 1975. Digestive physiology and nutrition of ruminants. 2nd ed. O&B Books, Corvallis, Oregon. Vol. 1.

Cook, R. C., J. G. Cook, D. L. Murray, P. Zager, B. K. Johnson, and M. W. Gratson. 2001. Development of predictive models of nutritional condition for Rocky Mountain elk. Journal of Wildlife Management 65:973–987.

Cuyler, L. C., and N. A. Øritsland. 1993. Metabolic strategies for winter survival by Svalbard reindeer. Canadian Journal of Zoology 71:1787–1792.

DelGiudice, G. D., M. A. Asleson, L. W. Varner, E. C. Hellgren, and M. R. Riggs. 1996. Creatinine ratios in random sampled and 24-hour urines of white-tailed deer. Journal of Wildlife Management 60:381–387.

Fancy, S. G. 1986. Daily energy budgets of caribou: a simulation approach. Ph.D. dissertation, University of Alaska, Fairbanks, Fairbanks.

FOSSATI, P., L. PRENCIPE, AND G. BERTI. 1980. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clinical Chemistry 26:227.

GANNES, L. Z., C. MARTINEZ DEL RIO, AND P. KOCH. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comparative Biochemistry and Physiology, A. Comparative Physiology 119:725–737.

GANNES, L. Z., D. M. O'BRIEN, AND C. MARTINEZ DEL RIO. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271–1276.

GEDIR, J. V., AND R. J. HUDSON. 2000. Seasonal intake determination in reproductive wapiti hinds (*Cervus elaphus canadensis*) using *n*-alkane markers. Canadian Journal of Animal Science 80:137–144.

GERHART, K. L., R. G. WHITE, R. D. CAMERON, AND D. E. RUSSELL. 1996. Body composition and nutrient reserves of arctic caribou. Canadian Journal of Zoology 74:136–146.

HEINEGÅRD, D., AND G. TIDERSTRÖM. 1973. Determination of serum creatinine by a direct colorimetric method. Clinica Chimica Acta 43:305–310.

HOBBS, N. T., D. L. BAKER, J. E. ELLIS, AND D. M. SWIFT. 1981. Composition and quality of elk winter diets in Colorado. Journal of Wildlife Management 45:156–171.

HOLTER, J. B., H. H. HAYES, AND S. H. SMITH. 1979. Protein requirements of yearling white-tailed deer. Journal of Wildlife Management 43:872–879.

HOVE, K., AND E. JACOBSEN. 1975. Renal excretion of urea in reindeer. Acta Veterinaria Scandinavica 16:513–519.

JACOBSEN, E., K. HOVE, R. S. BJARGHOV, AND S. SKJENNEBERG. 1981. Supplementary feeding of female reindeer on a lichen diet during the last part of pregnancy. Acta Agriculturae Scandinavica 31:81–86.

JOHNSON, C. J., K. L. PARKER, AND D. C. HEARD. 2000. Feeding site selection by woodland caribou in northcentral British Columbia. Rangifer Special Issue 12:159–172.

JORGENSON, J. C., M. S. UDEVITZ, AND N. A. FELIX. 2002. Forage quantity and quality. Pp. 46–53 in Arctic refuge coastal plain terrestrial wildlife research summaries (D. C. Douglas, P. E. Reynolds, and E. B. Rhode, eds.). United States Geological Survey, Biological Resources Division, Reston, Virginia.

KELSALL, J. P. 1968. The migratory barren-ground caribou of Canada. Department of Indian Affairs and Northern Development, Queen's Printer, Ottawa, Ontario, Canada.

MALOIY, G. M., R. N. B. KAY, AND E. D. GOODALL. 1970. Digestion and nitrogen metabolism in sheep and red deer given large or small amounts of water and protein. British Journal of Nutrition 24: 843–855.

MARSH, H., B. FINGERHUT, AND H. MILLER. 1965. Automated and manual direct methods for the determination of blood urea. Clinical Chemistry 11:624–627.

MARTIN, G. S., ET AL. 1997. Prepartum protein restriction does not alter norepinephrine-induced thermogenesis or brown adipose tissue function in newborn calves. Journal of Nutrition 127:1929–1937.

MAUTZ, W. W., H. SILVER, J. B. HOLTER, H. H. HAYES, AND W. E. URBAN. 1976. Digestibility and related nutritional data for seven northern deer browse species. Journal of Wildlife Management 40:630–638.

MAYNARD, L. A., J. K. LOOSLI, H. F. HINTZ, AND R. G. WARNER. 1979. Animal nutrition. 7th ed. McGraw-Hill, New York.

MAZAIKA, R., P. R. KRAUSMAN, AND F. M. WHITING. 1988. A gate system for feeding captive ungulates. Journal of Wildlife Management 52:613–615.

MCCULLOUGH, D. R. 1969. The Tule elk: its history, behavior and ecology. University of California Publications in Zoology 88:1–209.

MCEWAN, E. H., AND P. E. WHITEHEAD. 1970. Seasonal changes in the energy and nitrogen intake in reindeer and caribou. Canadian Journal of Zoology 48:905–913.

MCEWAN, E. H., AND P.E. WHITEHEAD. 1972. Reproduction in female reindeer and caribou. Canadian Journal of Zoology 50:43–46.

MESTEIG, K., N. J. C. TYLER, AND A. S. BLIX. 2000. Seasonal changes in heart rate and food intake in reindeer (*Rangifer tarandus tarandus*). Acta Physiologica Scandinavica 170:145–151.

MOULD, E. D., AND C. T. ROBBINS. 1981. Nitrogen metabolism in elk. Journal of Wildlife Management 45:323–334.

NILSSEN, K. J., J. A. SUNDSFJORD, AND A. S. BLIX. 1984. Regulation of metabolic rate in Svalbard and Norwegian reindeer. American Journal of Physiology 247:R837–R841.

NOLAN, J. V., AND R. A. LENG. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. British Journal of Nutrition 27: 177–194.

OFTEDAL, O. T. 1985. Pregnancy and lactation. Pp. 215–238 in Bioenergetics of wild herbivores (R. J. Hudson and R. G. White, eds.). CRC Press, Boca Raton, Florida.

PARKER, K. L., M. P. GILLINGHAM, T. A. HANLEY, AND C. T. ROBBINS. 1993. Seasonal patterns in body mass, body composition, and water transfer rates of free-ranging and captive black-tailed deer (*Odocoileus hemionus sitkensis*) in Alaska. Canadian Journal of Zoology 71:1397–1404.

PARKER, K. L., R. G. WHITE, M. P. GILLINGHAM, AND D. F. HOLLEMAN. 1990. Comparison of energy metabolism in relation to daily activity and milk consumption by caribou and muskox neonates. Canadian Journal of Zoology 68:106–114.

PEKINS, P. J., K. S. SMITH, AND W. W. MAUTZ. 1998. The energy cost of gestation in white-tailed deer. Canadian Journal of Zoology 76:1091–1097.

PELTIER, T. C., P. S. BARBOZA, AND J. E. BLAKE. 2003. Seasonal hyperphagia does not reduce digestive efficiency in an arctic grazer. Physiological and Biochemical Zoology 76:471–483.

PERSON, S. J., R. E. PEGAU, R. G. WHITE, AND J. R. LUICK. 1980. In vitro and nylon-bag digestibilities of reindeer and caribou forages. Journal of Wildlife Management 4:613–622.

REGELIN, W. L., C. C. SCHWARTZ, AND A. W. FRANZMANN. 1987. Effects of forest succession on nutritional dynamics of moose forage. Swedish Wildlife Research Supplement 1:247–264 (not seen, cited in Schwartz and Renecker 1997:445).

RENECKER, L. A., AND R. J. HUDSON. 1985. Estimation of dry matter intake of free-ranging moose. Journal of Wildlife Management 49:785–792.

RENECKER, L. A., AND W. M. SAMUEL. 1991. Growth and seasonal weight changes as they relate to spring and autumn set points in mule deer. Canadian Journal of Zoology 69:744–747.

RISENHOOVER, K. L. 1987. Winter foraging strategies of moose in subarctic and boreal forest habitats. Ph.D. dissertation, Michigan Technical University, Houghton.

RISENHOOVER, K. L. 1989. Composition and quality of moose winter diets in interior Alaska. Journal of Wildlife Management 53:568–576.

ROBBINS, C. T. 1987. Digestibility of an arboreal lichen by mule deer. Journal of Range Management 40:491–492.

ROBBINS, C. T. 1993. Wildlife feeding and nutrition. Academic Press, New York.

ROBBINS, C. T., R. L. PRIOR, A. N. MOEN, AND W. J. VISEK. 1974. Nitrogen metabolism of white-tailed deer. Journal of Animal Science 38:186–191.

ROBBINS, C. T., AND B. L. ROBBINS. 1979. Fetal and neonatal growth patterns and maternal reproductive effort in ungulates and subungulates. American Naturalist 114:101–116.

ROBBINS, C. T., P. J. VANSOEST, W. W. MAUTZ, AND A. N. MOEN. 1975. Feed analysis and digestion with reference to white-tailed deer. Journal of Wildlife Management 39:67–79.

ROMINGER, E. M. 1995. Late winter foraging ecology of woodland caribou. Ph.D. dissertation, Washington State University, Pullman.

RUSSELL, D. E., K. L. GERHART, R. G. WHITE, AND D. VAN DE WETERING. 1998. Detection of early pregnancy in caribou: evidence for embryonic mortality. Journal of Wildlife Management 62:1066–1075.

SÄKKINEN, H., ET AL. 2001. Plasma urea, creatinine, and urea : creatinine ratio in reindeer (*Rangifer tarandus tarandus*) and in Svalbard reindeer (*Rangifer tarandus platyrhynchus*) during defined feeding conditions and in the field. Physiological and Biochemical Zoology 74:907–916.

SAMS, M. G., R. L. LOCHMILLER, E. C. HELLGREN, M. E. PAYTON, AND L. W. VARNER. 1995. Physiological responses of neo-natal white-tailed deer reflective of maternal dietary protein intake. Canadian Journal of Zoology 73:1928–1936.

SCHWARTZ, C. C., M. E. HUBERT, AND A. W. FRANZMANN. 1988. Energy requirements of adult moose for winter maintenance. Journal of Wildlife Management 52:26–33.

SCHWARTZ, C. C., W. L. REGELIN, AND A. W. FRANZMANN. 1987a. Seasonal weight dynamics of moose. Swedish Wildlife Research Supplement 1:301–310.

SCHWARTZ, C. C., W. L. REGELIN, AND A. W. FRANZMANN. 1987b. Protein digestion in moose. Journal of Wildlife Management 51:352–357.

SCHWARTZ, C. C., AND L. A. RENECKER. 1997. Nutrition and energetics. Pp. 441–478 in Ecology and management of the North American moose (A. W. Franzmann and C. C. Schwartz, eds.). Smithsonian Institution Press, Washington, D.C.

SPAETH, D. F., R. T. BOWYER, T. R. STEPHENSON, P. S. BARBOZA, AND V. VAN BALLENBERGHE. 2002. Nutritional quality of willows for moose: effects of twig age and diameter. Alces 38:143–154.

STEPHENSON, T. R., K. J. HUNDERTMARK, C. G. SCHWARTZ, AND V. VAN BALLENBERGHE. 1998. Predicting body fat and body mass in moose with ultrasonography. Canadian Journal of Zoology 76: 717–722.

STEPHENSON, T. R., J. W. TESTA, G. P. ADAMS, R. G. SASSER, C. C. SCHWARTZ, AND K. J. HUNDERTMARK. 1995. Diagnosis of pregnancy and twinning in moose by ultrasonography and serum assay. Alces 31:167–172.

STOREHEIER, P. V., S. D. MATHIESEN, N. J. C. TYLER, AND M. A. OLSEN. 2002. Nutritive value of terricolous lichens for reindeer in winter. Lichenologist 34:247–257.

SWICK, R. W., AND N. J. BENEVENGA. 1977. Labile protein reserves and protein turnover. Journal of Dairy Science 60:505–515.

TORBIT, S. C., L. H. CARPENTER, D. M. SWIFT, AND A. W. ALLDREDGE. 1985. Differential loss of fat and protein by mule deer during winter. Journal of Wildlife Management 49:80–85.

TYLER, N. J. C., AND A. S. BLIX. 1990. Survival strategies in arctic ungulates. Rangifer Special Issue 3:211–230.

TYLER, N. J. C., P. FAUCHALD, O. JOHANSEN, AND H. R. CHRISTIANSEN. 1999. Seasonal inappetance and weight loss in female reindeer in winter. Ecological Bulletin 47:105–116.

ULLREY, D. E., W. G. YOUATT, H. E. JOHNSON, A. B. COWAN, R. L. COVERT, AND W. T. MAGEE. 1972. Digestibility and estimated metabolizability of aspen browse for white-tailed deer. Journal of Wildlife Management 36:885–891.

ULLREY, D. E., W. G. YOUATT, H. E. JOHNSON, L. D. FAY, B. L. SCHOEPKE, AND W. T. MAGEE. 1969. Digestible energy requirements for winter maintenance of Michigan white-tailed does. Journal of Wildlife Management 33:482–490.

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

VAN SOEST, P. J. 1994. Nutritional ecology of the ruminant. Cornell University Press, New York.

WALES, R. A., L. P. MILLIGAN, AND E. H. MCEWAN. 1975. Urea recycling in caribou, cattle and sheep. Pp. 297–307 in Proceedings of the First International Reindeer and Caribou Symposium, 9–11 August 1972 (J. R. Luick, P. C. Lent, D. R. Klein, and R. G. White, eds.). University of Alaska, Fairbanks.

WHITE, R. G. 1992. Nutrition in relation to season, lactation, and growth of north temperate deer. Pp. 407–417 in The biology of deer (R. D. Brown, ed.). Springer-Verlag, New York.

WHITE, R. G., E. JACOBSEN, AND H. STAALAND. 1984. Secretion and absoption of nutrients in the alimentary tract of reindeer fed lichens or concentrates during the winter. Canadian Journal of Zoology 62:2364–2376.

WOLFE, R. R. 1992. Radioactive and stable isotope tracers in biomedicine—principles and practice of kinetic analysis. Wiley-Liss, New York.

ZAR, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey.

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