

Urea Recycling in Muskrats (*Ondatra zibethicus*): A Potential Nitrogen-Conserving Tactic?

Kevin L. Campbell
Robert A. MacArthur*

Department of Zoology, University of Manitoba, Winnipeg,
Manitoba R3T 2N2, Canada

Accepted by G.K.S. 9/13/96

ABSTRACT

The rate of ^{14}C -urea hydrolysis was determined in 32 field-acclimatized muskrats maintained on natural diets during spring, summer, fall, and winter. We hypothesized that urea recycling occurs in muskrats during all seasons and that the conservation of tissue nitrogen via this mechanism is most prevalent in fall and winter, when forage protein levels are lowest. Muskrats exhibited higher rates of urea hydrolysis and a lower serum urea nitrogen-to-creatinine ratio in fall and winter than in spring and summer. Even after correcting for seasonal differences in blood urea pool size, the adjusted rate of urea hydrolysis was 67% higher in fall and winter than in spring and summer. There was no evidence that the maintenance nitrogen requirements of muskrats fed natural vegetation were affected by seasonal changes in the amino acid composition of the diet. We suggest that increased levels of urea recycling, coupled with adaptive mechanisms for reducing nitrogen excretion and possibly conserving carbon skeletons of essential amino acids, may allow muskrats to reduce their nitrogen requirements on fall and winter diets. Our finding that ^{14}C -urea hydrolysis occurred during all four sampling periods suggests that nitrogen derived from this source may also be critical to supporting large hindgut microbe populations that enable this rodent to exploit the appreciable fiber content of its aquatic plant diet throughout the year.

Introduction

At temperate and northern latitudes, the protein content of vegetation can fluctuate dramatically throughout the year. Dur-

ing periods when dietary protein is limiting, the digestibility and retention of nitrogen can be critical factors influencing growth, reproduction, and survival of free-ranging animals (Loeb et al. 1991). Consequently, considerable research has been done on seasonal changes in nitrogen metabolism of herbivores, including the role of urea recycling (Robbins et al. 1974; Mould and Robbins 1981). In animals demonstrating this phenomenon, urea is transferred from the bloodstream into the gastrointestinal tract, where it is hydrolyzed by bacterial urease into ammonia and carbon dioxide (Richards 1972; Nelson et al. 1975). Ammonia produced at this point can either be incorporated into bacterial protein or diffuse into the bloodstream. Upon reaching the liver, blood-borne ammonia joins the nitrogen pool available for the synthesis of nonessential amino acids (Richards 1972). In simple-stomached animals, the incorporation of nitrogen from inorganic compounds into amino acids is greater when the dietary intake of protein is inadequate to meet daily requirements (Richards 1972), a condition prevalent during hibernation or long-term fasts (Nelson et al. 1975; Harlow and Buskirk 1991).

To our knowledge, no previous study has addressed the potential benefits of urea recycling to nonhibernating, simple-stomached herbivores during periods of low protein availability. An excellent model for investigating this question is provided by the muskrat (*Ondatra zibethicus*). We previously reported (Campbell and MacArthur 1996) that muskrats consuming natural vegetation exhibit urinary nitrogen excretion rates that were nearly six times lower in fall and winter than in spring and summer. Consequently, the maintenance nitrogen requirement calculated for this rodent was more than 25% lower during fall and winter, when dietary protein levels of aquatic vegetation appeared to be lowest (Campbell and MacArthur 1996). The quality of dietary protein is known to influence the daily requirements for this nutrient in mammals (Robbins 1993). It is possible that, despite their low nitrogen content, the fall and winter diets of muskrats contain a higher proportion of essential amino acids than the diets in other seasons. Alternatively, muskrats may reduce nitrogen excretion in fall and winter by recycling urea. Throughout the year, muskrats consume forages containing high levels of neutral detergent fiber (43%–62%) and rely strongly on microbial fermentation to meet their daily energy requirements (Campbell and MacArthur 1994, 1996). However, in hindgut fermenters, an appreciable proportion of the daily protein intake is likely absorbed before it reaches the hindgut. Because urea

*To whom correspondence should be addressed. E-mail: macarthr@bldgduff.lan1.umanitoba.ca.

hydrolysis provides a nitrogen source for the synthesis of microbial proteins in the cecum (Chilcott and Hume 1984), this reaction may be essential for maintaining high microbial populations year-round, thus permitting muskrats to exploit the appreciable fiber content of their aquatic plant diets.

The principal objective of this study was to determine whether urea hydrolysis, a necessary step in urea recycling, occurs in muskrats and, if so, whether the intensity of this hydrolysis varies with seasonal shifts in the protein content of cattail (*Typha latifolia*), the dominant food source of muskrats in northern prairie marshes (Campbell and MacArthur 1994). We hypothesized that urea recycling occurs in muskrats throughout the year and that the conservation of tissue nitrogen via this mechanism is most prevalent during fall and winter, when maintenance nitrogen requirements and forage protein levels are lowest. In addition, we examined seasonal changes in the amino acid composition of the muskrat's natural forage to determine whether diet quality might also contribute to the low maintenance nitrogen requirement previously observed during fall and winter (Campbell and MacArthur 1996).

Material and Methods

Eight adult muskrats were livetrapped at Oak Hammock Marsh, Manitoba (50°06' N, 97°07' W), during each of four test periods: July 27–August 10, 1994 (three males, five females), October 11–20, 1994 (eight males), January 4–24, 1995 (five males, three females), and May 16–26, 1995 (six males, two females). Animals were housed individually at $14^{\circ} \pm 1^{\circ}\text{C}$ with a 12L : 12D photoperiod (MacArthur 1979) for 1–4 d prior to testing. Each muskrat was tested only once ($n = 32$ muskrats in total). In order to minimize disturbances to digestive physiology, including microbial function, muskrats were fed vegetation approximating their natural diet in each test period. Accordingly, during May and July, muskrats were fed fresh aquatic vegetation, consisting predominately of cattail shoots and leaves ad lib. Muskrats in the October trials were maintained exclusively on fresh cattail rhizomes ad lib. In January, muskrats were fed cattail rhizomes collected just prior to freeze-up and stored at 5°C (Campbell and MacArthur 1996). Muskrats were not fasted prior to metabolic testing.

To test for the occurrence and evaluate the intensity of urea hydrolysis, each muskrat was lightly anesthetized with halothane (M.T.C. Pharmaceuticals) and injected intraperitoneally with $5 \mu\text{Ci}$ of ^{14}C -urea ($8.92 \times 10^{-5} \text{ mmol L}^{-1}$) diluted in 0.5 mL sterile physiological saline (Harlow and Buskirk 1991). The dosage was measured to the nearest 0.1 mg by weighing the sample syringe on an analytical balance (Mettler model AJ100) before and after injection. The animal was then transferred to a darkened, 11.5-L glass box fitted with a heavy Plexiglas lid and a removable wire screen floor, where it remained for a period of 8 h. The metabolic chamber was in-

stalled in a controlled-temperature cabinet held at $22^{\circ} \pm 1^{\circ}\text{C}$, and dry, CO_2 -free air was pumped through the chamber at a rate of 2 L min^{-1} . Flow rate was monitored with a Matheson rotameter calibrated against a model 1057 Brooks Vol-U-Meter. A small fraction (5%) of the exhaust gas from the chamber was routed to a Beckman F-3 paramagnetic O_2 analyzer connected to a strip-chart recorder (SE-120, BBC Goerz Metrawatt). The remaining exhaust gas was passed sequentially through two organic CO_2 traps, each containing 250 mL of ethylene glycol monomethyl ether and ethanolamine in a 2 : 1 volume ratio (Jaffay and Alvarez 1961). As a precautionary measure, exhaust gas from the chamber was routed through a final CO_2 trap of soda lime before exiting to the atmosphere.

Upon completion of a trial, two 0.6-mL aliquots of fluid were removed from each organic CO_2 trap and placed into separate scintillation vials, each containing 15 mL of Beckman Ready Safe scintillation cocktail. Each vial was assessed for ^{14}C activity with a Beckman LS 6000TA liquid scintillation counter, and the values were summed to give total activity of expired ^{14}C . To correct for variation among animals in metabolic rate, the production of $^{14}\text{CO}_2$ was expressed as disintegrations per minute (dpm) of $^{14}\text{CO}_2$ expired per milliliter of O_2 consumed. The total O_2 consumed during the 8-h run was determined with a Jandel Digitizing Tablet and Sigma-Scan software to integrate the area beneath the O_2 tracing (Dyck and MacArthur 1993).

To test for seasonal changes in the ratio of serum urea to serum creatinine, blood samples were collected from a separate group of 59 muskrats livetrapped in Oak Hammock Marsh from May 1991 to April 1992. Serum urea and creatinine concentrations were determined with a Coulter discrete chemical analyzer (Manitoba Agriculture Veterinary Services, Winnipeg, Manitoba).

To document seasonal changes in the crude protein content of cattail in Oak Hammock Marsh, we periodically collected random plant samples in 1991. Cattail rhizome samples were also obtained from two separate food caches found inside winter feeding lodges in early December 1995. Plant samples were separated into stem, leaf, and rhizome components, oven-dried at 70°C , and ground through a 1-mm mesh screen in a Wiley mill. A portion of each ground sample was sent to a feed analysis laboratory (Department of Animal Science, University of Manitoba) for determination of crude protein content (Kjeldahl $\text{N} \times 6.25$).

To test for possible seasonal changes in protein quality of the diet, samples of the natural mixed forages fed to muskrats in a previous study (Campbell and MacArthur 1996) were evaluated for their amino acid composition. Details regarding diet assessment, feeding protocol, and the nutrient composition of mixed forages are presented in Campbell and MacArthur (1996). The spring diet tested consisted of 50% cattail shoot, 25% cattail rhizome, 20% bladderwort (*Utricularia vul-*

garis), and 5% sedge (*Carex atherodes*) shoot. The summer diet consisted of 70% cattail shoot, 10% cattail rhizome, and 5% each of sedge shoot, soft-stem bulrush (*Scirpus validus*) shoot, whitetop (*Scolocholoa festucacea*) shoot, and duckweed (*Lemna minor*). The fall diet consisted of cattail rhizomes (60%), cattail shoots (30%), and whitetop shoots (10%). The winter diet was composed entirely of cattail rhizomes. Following a procedure modified from Mills et al. (1989), a 100-mg portion of each forage sample was combined with 4 mL of 6 mol L⁻¹ HCl and 0.1 mL 2-octanol, evacuated for 1 min, then hydrolyzed at 110°C for 24 h. The hydrolysate was neutralized with 4.1 mL of 25% (wt/vol) NaOH and made up to 50 mL with a sodium citrate buffer (pH 2.2). The amino acid content of each sample was determined by ion exchange chromatography with ninhydrin detection on an LKB 4151 Alpha Plus amino acid analyzer.

Treatment effects were initially evaluated for differences due to season, sex, and sex × season with a two-way ANOVA. Since no sex-related differences were apparent ($P > 0.05$), data for both sexes were pooled in all subsequent analyses. Mean seasonal values for pooled data were compared with one-way ANOVA and Tukey's Studentized range test (SAS Institute 1990). Significance was set at the 5% level, and means are presented with 1 standard error (SE). We followed a university-approved animal welfare protocol (C91-50) while conducting this experiment. Upon completion of testing, muskrats were held for a minimum of 48 h before being released at their site of capture.

Results

The occurrence of ¹⁴C-urea hydrolysis was documented in all muskrats tested in this study, with peak levels recorded in October and January ($F_{3,28} = 23.58$, $P < 0.0001$; Fig. 1A). The percentage of injected ¹⁴C-urea that was hydrolyzed during the 8-h trial increased from an average of 6.7%–7.4% in May and July to 23.7%–28.7% in October and January ($P < 0.05$). The ¹⁴CO₂ production (dpm of ¹⁴CO₂ expired per milliliter of O₂ consumed) of fall- and winter-acclimatized muskrats was four times higher than values recorded from animals caught in spring and summer ($F_{3,28} = 16.13$, $P < 0.0001$; Fig. 1B).

The serum urea-to-creatinine ratio of muskrats demonstrated strong seasonal variation and was significantly lower from September to April than during May and July ($F_{5,53} = 11.36$, $P < 0.0001$; Fig. 2). Though measured in a different group of muskrats collected in a different year, serum urea-to-creatinine ratios (Fig. 2) appeared to vary inversely with the rate of ¹⁴C-urea hydrolysis (Fig. 1). It is important to note that muskrats sampled in both years exhibited similar seasonal patterns in body composition and that measurements of diet quality and vegetation structure were consistent within the two collection periods (K. L. Campbell, unpublished data).

The crude protein content of cattail shoots and leaves under-

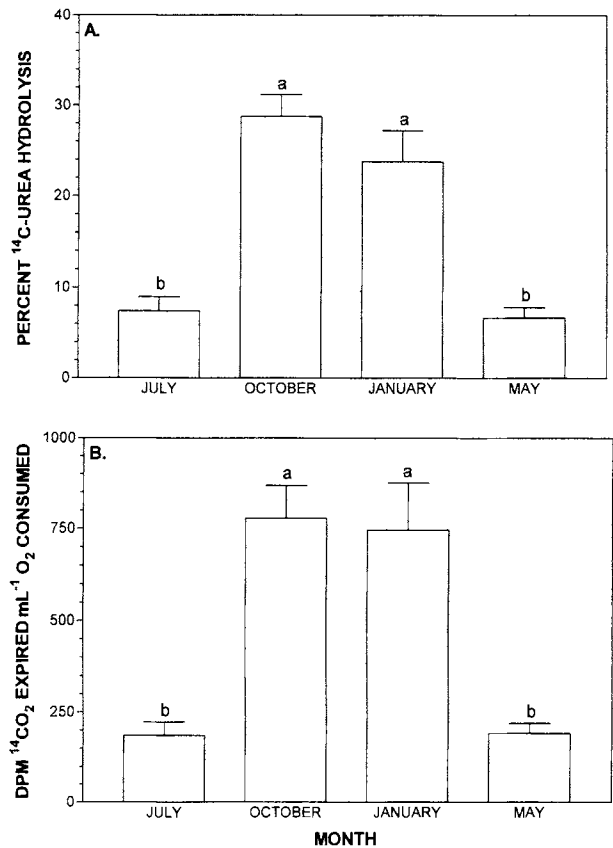


Figure 1. Seasonal changes in the proportion of urea hydrolyzed by 32 field-acclimatized muskrats ($n = 8$ per month). A, Percentage of injected ¹⁴C-urea collected as ¹⁴CO₂ in expired air. B, Disintegrations per minute (dpm) of ¹⁴CO₂ expired per milliliter of O₂ consumed. Vertical lines denote 1 SE. Means sharing the same letter are not significantly different ($P > 0.05$).

went dramatic seasonal changes, with shoot levels declining from 14.1% in June to only 2.2% by late September ($F_{2,15} = 55.91$, $P < 0.0001$; Fig. 3). Over this same period, the protein content of cattail rhizomes exhibited the opposite trend ($F_{5,22} = 3.89$, $P = 0.0112$; Fig. 3), with the highest recorded value (9.1%) observed in the rhizome samples recovered from two food caches in December. Nonetheless, this peak value for rhizomes is generally lower than the protein content of cattail shoots and leaves during the summer months (Fig. 3).

No discernable seasonal changes were apparent in the protein quality of the mixed diets approximating those consumed by free-ranging muskrats (Table 1). While the levels of individual amino acids varied seasonally, the total fraction of essential amino acids in each diet remained close to 42%.

Discussion

Our results strongly suggest that urea recycling occurs throughout the year in muskrats and that this nitrogen-conserving

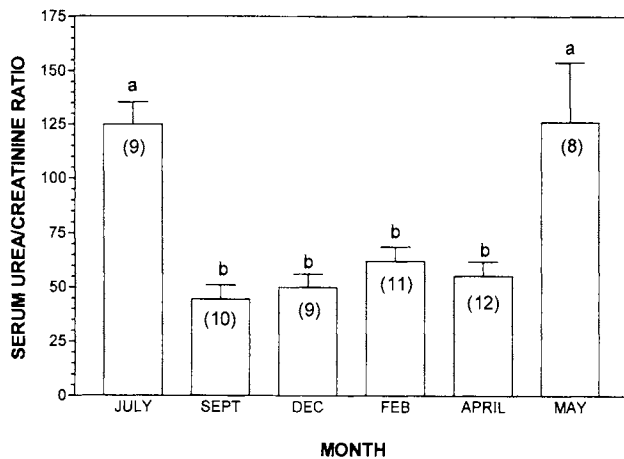


Figure 2. Seasonal changes in the ratio of serum urea nitrogen to serum creatinine in 59 field-acclimatized muskrats tested between May 1991 and April 1992. Vertical lines denote 1 SE. Means sharing the same letter are not significantly different ($P > 0.05$). Values in parentheses indicate number of muskrats sampled in each month.

tactic is most prevalent in fall and winter (Fig. 1). Animals on low-protein diets typically exhibit reduced plasma urea concentrations (Robbins 1993), and, in humans at least, the rate of urea hydrolysis is proportional to the concentration of urea in body fluids (Richards 1972). These observations have led to speculation (Harlow 1987) that protein-deficient diets are associated with a reduced rate of urea hydrolysis in nonruminant herbivores. However, muskrats demonstrated increased rates of urea hydrolysis in fall and winter (Fig. 1), when dietary protein (Table 1) and the serum urea-to-creatinine ratios (Fig. 2) were lowest. Low serum urea-to-creatinine values have been associated with the recycling of nitrogenous wastes and the conservation of lean body mass in polar bears (*Ursus maritimus*) (Ramsay et al. 1991). The low urea-to-creatinine values observed in muskrats from late fall to early spring are consistent with the relatively high level of urea hydrolysis observed during fall and winter and may be indicative of a reduced rate of total protein catabolism (Harlow and Buskirk 1991).

It occurred to us that this seasonal trend might be due, at least in part, to differences in ^{14}C -urea dilution arising from monthly variation in blood urea pool size. To address this possibility, we estimated the total blood urea content of muskrats in each season, using our serum urea concentrations and previous measurements of blood volume in field-acclimatized muskrats (MacArthur 1990). Serum urea values collected on dates closest to those of the urea hydrolysis trials were used in calculations. Our estimates of blood urea pool size followed a seasonal pattern similar to that of serum urea concentration, reaching minimal values in fall and winter (Fig. 4A). However, when the total blood urea pool was multiplied by the activity of expired $^{14}\text{CO}_2$ (dpm of $^{14}\text{CO}_2$ expired per milliliter of O_2

consumed) to compensate for variation in initial ^{14}C -urea dilution, a strong seasonal trend in the rate of urea hydrolysis was still apparent (Fig. 4B). The adjusted rate of urea hydrolysis was 67% greater in October–January than in May–July. Thus, the seasonal patterns reported herein cannot be attributed to monthly variation in blood urea pool size or metabolic rate and appear to reflect instead adaptive responses to temporal changes in diet quality. It is important to note that muskrats in this study were fed natural diets that varied seasonally in order to minimize disturbances in digestive function. Therefore, at this stage we cannot conclusively separate the effects of diet quality from other factors that might account for the observed seasonal trends in the rate of urea hydrolysis.

It is noteworthy that the digestive tract of muskrats undergoes pronounced seasonal changes, attaining maximal size in fall and winter (Virgl and Messier 1992; Campbell and MacArthur 1996). Animals with a larger gut capacity should theoretically possess higher levels of bacterial urease and thus should demonstrate higher rates of urea hydrolysis. Our results support this hypothesis, since seasonal changes in the adjusted rate of urea hydrolysis (Fig. 4) followed a trend similar to that of cecum size in muskrats (Campbell and MacArthur 1996).

Free-ranging muskrats rely on diets that undergo significant reductions in crude protein content between spring and fall (Table 1, Fig. 3). During this period, muskrats show a pronounced decline in nitrogen excretion (Campbell and MacArthur 1996). Two factors could contribute to this decline. One is the fact that the average energy content of the fall and winter diet (17.01 kJ g^{-1}) was, relative to the crude protein content (7.98%), higher than in spring and summer (16.87 kJ g^{-1} and

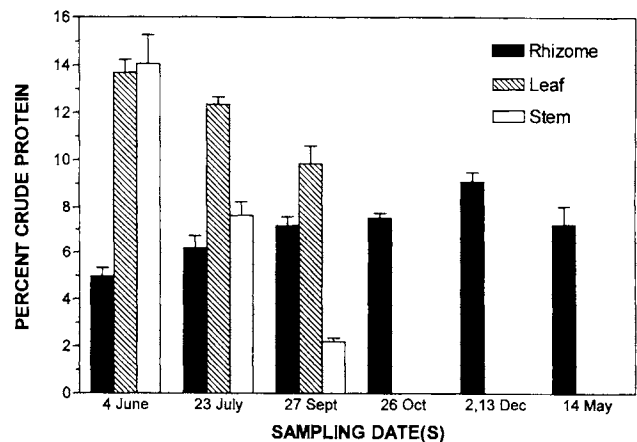


Figure 3. Seasonal changes in the crude protein content of the rhizomes, shoots, and leaves of cattail collected at Oak Hammock Marsh, Manitoba, from May to October 1991. The December value is based on samples collected from two muskrat food caches on December 2 and December 13, 1995. Each other monthly mean is based on plant samples collected from six randomly selected sites, except for October, when only two sites were sampled. Vertical lines denote 1 SE.

Table 1: Seasonal changes in the crude protein, amino acid, and ammonia content of natural mixed forages approximating those consumed by free-ranging muskrats

Component	May	July	September	December
% crude protein	11.3	9.0	7.7	8.3
% amino acid:				
Essential:				
Arginine	4.28	8.24	9.24	8.76
Histidine	2.37	2.20	2.73	2.50
Isoleucine	3.04	1.11	.15	2.45
Leucine	7.35	6.09	5.43	6.34
Threonine	4.38	5.58	4.35	4.42
Lysine	5.19	5.26	6.08	4.12
Methionine	2.88	2.53	1.37	2.66
Phenylalanine	5.41	4.60	4.48	4.67
Valine	6.56	6.73	8.76	6.92
Total	41.46	42.34	42.59	42.84
Nonessential:				
Aspartate	16.09	14.89	19.10	19.61
Glutamate	12.98	12.99	12.81	11.02
Serine	6.85	7.39	7.06	7.02
Proline	4.94	7.33	3.45	4.24
Glycine	5.78	4.84	5.26	5.17
Alanine	7.09	6.28	5.59	4.98
Tyrosine	1.96	1.14	.65	1.43
Total	55.69	54.86	53.92	53.47
% Ammonia	2.85	2.72	3.40	3.60

Note. In May, the diet consisted of 50% cattail (*Typha latifolia*) shoot, 25% cattail rhizome, 20% bladderwort (*Utricularia vulgaris*), and 5% sedge (*Carex atherodes*) shoot. In July, the diet consisted of 70% cattail shoot, 10% cattail rhizome, and 5% each of sedge shoot, soft-stem bulrush (*Scirpus validus*) shoot, whitetop (*Scolochloa festucacea*) shoot, and duckweed (*Lemna minor*). The September diet consisted of cattail rhizomes (60%), cattail shoots (30%), and whitetop shoots (10%). The December diet consisted exclusively of cattail rhizomes.

10.13% crude protein). This difference in the proportion of energy to protein in the diet may have resulted in more efficient use of dietary protein in fall and winter, and hence less nitrogen excretion in the urine (Robbins et al. 1974). Second, since reabsorption of urea by the kidneys is passive, and closely linked to the reabsorption of water, the amount of urea excreted increases with urine output (West 1985). Thus, the fall decline in nitrogen excretion could also reflect the marked seasonal changes in the water content of aquatic vegetation, and hence urine production, between spring–summer ($\bar{X} = 252 \text{ mL d}^{-1}$) and fall–winter ($\bar{X} = 39 \text{ mL d}^{-1}$; K. L. Campbell, unpublished data). Furthermore, urea resorption from the bladder is known to increase during periods of low urine output in several mammalian species (Nelson et al. 1975; Harlow 1987), and it is possible that during fall and winter muskrats also reduce urea excretion in this manner.

Ammonium ions derived from urea in the digestive tract

have two potential fates. They can be used by cecal and colonic bacteria for protein synthesis or transported to the liver, where they are incorporated into proteins or reconverted into urea. Nitrogen is thus free to circulate from the gut to the liver as ammonium ions and from the liver to the gut as urea, until its eventual loss in urine, feces, sweat, or sloughed cells (Robbins 1993). The observed increase in the rate of hydrolysis from May–July to October–January (Figs. 1 and 4B), combined with the decrease in urea excretion, suggests that ammonium ions have a greater potential to reenter this cycle in fall and winter than in spring and summer. Thus, with the assumption that adequate energy is available to the hindgut microflora, muskrats should be capable of substantially increasing the total amount of urea recycled during fall and winter. These are periods when forage diversity and dietary protein levels are lowest and when the thermoregulatory costs of aquatic foraging are greatest. A similar finding has been observed in ruminants,

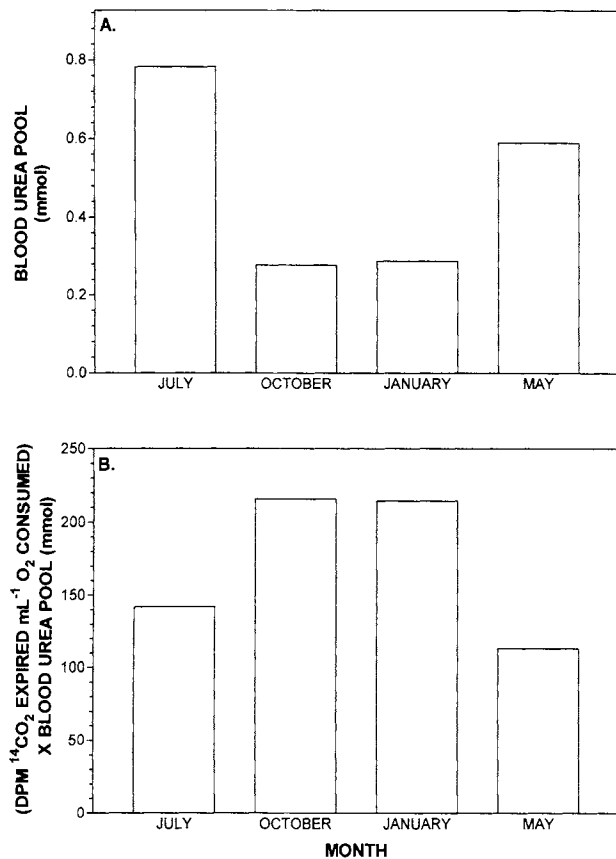


Figure 4. Seasonal changes in (A) the estimated blood urea pool size (mmol urea) and (B) the rate of ^{14}C -urea hydrolysis in muskrats, adjusted for initial ^{14}C -urea dilution (see text for details).

in which a greater proportion of the urea synthesized in the liver is recycled when dietary protein and plasma urea concentrations are lowest (Robbins et al. 1974; Mould and Robbins 1981). Although the nitrogen recovered by urea recycling in simple-stomached animals can be incorporated into at least three essential amino acids in the liver if their α -keto acid analogues are present, the extent of this conversion is believed to be nutritionally insignificant (Richards 1972).

Muskrats maintained exclusively on aquatic plant diets demonstrate a true protein digestibility of 96.5% (Campbell and MacArthur 1994). Thus, almost all ingested protein is absorbed prior to reaching the cecum. The proportion of recycled urea that is used by microbes in the gut of nonruminants ranges from 44% in domestic rabbits (Regoeczi et al. 1965) to 53%–59% in rock hyrax, *Procavia habessinica* (Hume et al. 1980). Our finding that ^{14}C -urea hydrolysis occurred during all four sampling periods suggests that nitrogen derived from this source may be critical to supporting large hindgut microbe populations that enable this rodent to exploit the appreciable fiber content of its aquatic plant diet (43%–62%; Campbell and MacArthur 1994, 1996) throughout the year. A similar

advantage has been suggested for the ringtail possum, *Pseudocheirus peregrinus*, which consumes high-fiber, low-nitrogen forage and relies strongly on microbial fermentation to meet its daily energy requirements (Chilcott and Hume 1984).

Ruminants and other foregut fermenters can absorb amino acids of bacterial origin directly from the small intestine (Robbins 1993). However, no active transport mechanism for amino acids has been demonstrated in the lower digestive tract of mammals (Chilcott and Hume 1984). Thus, in hindgut fermenters, bacterial proteins produced in the lower gut are presumably lost to the host, unless consumed and digested in the stomach and small intestine (Alexander 1993). Coprophagy is recognized as an important nitrogen-conserving tactic in animals that exhibit this behavior (Alexander 1993; Robbins 1993). Muskrats in this study were observed to practice coprophagy before metabolic trials in both summer and winter. However, no attempt was made to quantify seasonal changes in the frequency of this behavior.

Muskrats from our study area do not appear to lose lean tissue mass during fall and winter (K. L. Campbell and R. A. MacArthur, unpublished data). Furthermore, during these seasons, muskrats can meet their maintenance nitrogen requirements on diets containing only 10.7 mg nitrogen g^{-1} dry matter (assuming a dry matter intake of 75.7 $\text{g kg}^{-0.75} \text{d}^{-1}$; Campbell and MacArthur 1996). This value is close to the minimum nitrogen content of plant diets required for ruminants to remain in nitrogen balance (8 mg nitrogen g^{-1} dry matter; Robbins 1993) and is more than 18% below the minimum requirement predicted for ground squirrels, *Ammospermophilus leucurus* (Karasov 1982).

The question remains, then, how do muskrats meet their essential amino acid requirements in fall and winter on diets containing so little protein? One possibility is that muskrats actively select diets with superior protein quality during these particular seasons. Such selection would increase the diet's biological value (fraction of absorbed nitrogen retained in the body; Robbins 1993) and, in conjunction with urea recycling, could reduce the minimum nitrogen content of vegetation required to achieve nitrogen balance (Richards 1972). In a previous study (Campbell and MacArthur 1996), we observed that muskrats offered mixed natural diets have the ability to selectively increase the proportion of crude protein ingested over that presented in the diet. However, amino acid analyses of the mixed forage diets consumed by muskrats revealed little seasonal variation in the proportion of essential amino acids (Table 1), suggesting that muskrats do not increase their intake of essential amino acids by selective feeding.

Another potential mechanism whereby muskrats could reduce maintenance nitrogen requirements during periods when dietary protein is limiting involves differential regulation of essential and nonessential amino acid metabolism

(Lundberg et al. 1976). McFarlane and von Holt (1969) reported that rats fed a protein-deficient diet reduce catabolism of essential, but not nonessential, amino acids. It has been argued that increasing the rate of conversion of essential amino acids into liver proteins should facilitate the conservation of essential carbon skeletons, thereby decreasing their rate of oxidative metabolism and hence entry into the urea cycle (Lundberg et al. 1976).

It is well known that when dietary nitrogen sources are limiting, ruminants and macropod marsupials can increase the biological value of dietary nitrogen by recycling urea (Robbins et al. 1974; Mould and Robbins 1981). However, a similar response has not been documented in small nonruminant eutherians (Karasov 1982). Following the approach of Robbins et al. (1974) and assuming a true nitrogen digestibility of 96.5% and an endogenous urinary nitrogen loss of $0.041 \text{ g kg}^{-0.75} \text{ d}^{-1}$ (Campbell and MacArthur 1994), we estimated the mean (\pm SE) biological value of the aquatic plant diets consumed by muskrats. This value increased from $52.7\% \pm 7.2\%$ ($n = 16$) in spring and summer to $96.5\% \pm 1.9\%$ ($n = 15$) in fall and winter ($F_{1,29} = 34.75$, $P < 0.0001$). The gain in biological value cannot be attributed to seasonal changes in either dietary protein quality (Table 1) or daily nitrogen intake ($0.97 \text{ g kg}^{-0.75}$ in spring and summer; $0.95 \text{ g kg}^{-0.75}$ in fall and winter; Campbell and MacArthur 1996). This difference could reflect increased catabolism of dietary protein for energy in summer, with increased production and excretion of urea (Robbins et al. 1974). However, previous studies (Campbell and MacArthur 1996) indicate that muskrats can meet their daily maintenance energy requirements from nonprotein sources in all seasons. We suggest that the increased levels of urea recycling in fall and winter-acclimatized muskrats, together with adaptive mechanisms for reducing nitrogen excretion and possibly conserving carbon skeletons of essential amino acids, may permit muskrats to increase the biological value of aquatic vegetation. Such responses would enable these animals to lower both their daily maintenance nitrogen and essential amino acid requirements during those periods of the year when they are nutritionally challenged.

Acknowledgments

We would like to thank P. Mills, Department of Animal Science, University of Manitoba, for assistance in amino acid analyses. P. Charles, Department of Animal Science, University of Manitoba, performed the required protein analyses. We are grateful for the helpful suggestions and constructive criticisms provided by two anonymous reviewers. This study was funded by an operating grant to R.A.M. from the Natural Sciences and Engineering Research Council of Canada and by a University of Manitoba Fellowship to K.L.C.

Literature Cited

- Alexander R. McN. 1993. The energetics of coprophagy: a theoretical analysis. *J. Zool.* 230:629–637.
- Campbell K.L. and R.A. MacArthur. 1994. Digestibility and assimilation of natural forages by muskrats. *J. Wildl. Manage.* 58:633–641.
- . 1996. Seasonal changes in gut mass, forage digestibility and nutrient selection by wild muskrats (*Ondatra zibethicus*). *Physiol. Zool.* 69:1215–1231.
- Chilcott M.J. and I.D. Hume. 1984. Nitrogen and urea metabolism and nitrogen requirements of the common ringtail possum, *Pseudocheirus peregrinus*, fed *Eucalyptus andrewsii* foliage. *Aust. J. Zool.* 32:615–622.
- Dyck A.P. and R.A. MacArthur. 1993. Daily energy requirements of beaver (*Castor canadensis*) in a simulated winter microhabitat. *Can. J. Zool.* 71:2131–2135.
- Harlow H.J. 1987. Urea-hydrolysis in euthermic hibernators and non-hibernators during periods of food availability and deprivation. *J. Therm. Biol.* 12:149–154.
- Harlow H.J. and S.W. Buskirk. 1991. Comparative plasma and urine chemistry of fasting white-tailed prairie dogs (*Cynomys leucurus*) and American martens (*Martes americana*): representative fat- and lean-bodied animals. *Physiol. Zool.* 64:1262–1278.
- Hume I.D., K. Rübasmén, and W.v. Engelhardt. 1980. Nitrogen metabolism and urea kinetics in the rock hyrax (*Procapra habessinica*). *J. Comp. Physiol.* 138B:307–314.
- Jaffay H. and J. Alvarez. 1961. Liquid scintillation counting of carbon-14 use of ethanolamine-ethylene glycol monomethyl ether-toluene. *Anal. Chem.* 33:612–615.
- Karasov W.H. 1982. Energy assimilation, nitrogen requirement, and diet in free-living antelope ground squirrels *Amospermophilus leucurus*. *Physiol. Zool.* 55:378–392.
- Loeb S.C., R.G. Schwab, and M.W. Demment. 1991. Responses of pocket gophers (*Thomomys bottae*) to changes in diet quality. *Oecologia* 60:542–551.
- Lundberg D.A., R.A. Nelson, H.W. Wahner, and J.D. Jones. 1976. Protein metabolism in the black bear before and during hibernation. *Mayo Clin. Proc.* 51:716–722.
- MacArthur R.A. 1979. Dynamics of body cooling in acclimatized muskrats (*Ondatra zibethicus*). *J. Therm. Biol.* 4:273–276.
- . 1990. Seasonal changes in the oxygen storage capacity and aerobic dive limits of the muskrat (*Ondatra zibethicus*). *J. Comp. Physiol.* 160B:593–599.
- McFarlane I.G. and C. von Holt. 1969. Metabolism of amino acids in protein-calorie-deficient rats. *Biochem. J.* 111:557–563.
- Mills P.A., R.G. Rotter, and R.R. Marquardt. 1989. Modification of the glucosamine method for the quantification of fungal contamination. *Can. J. Anim. Sci.* 69:1105–1106.

- Mould E.D. and C.T. Robbins. 1981. Nitrogen metabolism in elk. *J. Wildl. Manage.* 45:323–334.
- Nelson R.A., J.D. Jones, H.W. Wahner, D.B. McGill, and C.F. Code. 1975. Nitrogen metabolism in bears: urea metabolism in summer starvation and in winter sleep and role of urinary bladder in water and nitrogen conservation. *Mayo Clin. Proc.* 50:141–146.
- Ramsay M.A., R.A. Nelson, and I. Stirling. 1991. Seasonal changes in the ratio of serum urea to creatinine in feeding and fasting polar bears. *Can. J. Zool.* 69:298–302.
- Regoeczi E., L. Irons, A. Koj, and A.S. McFarlane. 1965. Isotopic studies of urea metabolism in rabbits. *Biochem. J.* 95:521–535.
- Richards P. 1972. Nutritional potential of nitrogen recycling in man. *Am. J. Clin. Nutr.* 25:615–625.
- Robbins C.T. 1993. *Wildlife Feeding and Nutrition*. 2d ed. Academic Press, New York.
- Robbins C.T., R.L. Prior, A.N. Moen, and W.J. Visek. 1974. Nitrogen metabolism of white-tailed deer. *J. Anim. Sci.* 38:186–191.
- SAS Institute. 1990. *SAS/STAT User's Guide*. Version 6, 4th ed. Vol. 2. SAS Institute, Cary, N.C.
- Virgl J.A. and F. Messier. 1992. Seasonal variation in body composition and morphology of adult muskrats in central Saskatchewan, Canada. *J. Zool.* 228:461–477.
- West J.B. 1985. *Best and Taylor's Physiological Basis of Medical Practice*. 11th ed. Williams & Wilkens, Baltimore.