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Woodrat (*Neotoma*) herbivores maintain nitrogen balance on a low-nitrogen, high-phenolic forage, *Juniperus monosperma*

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Abstract The acquisition of adequate quantities of nitrogen is a challenge for herbivorous vertebrates because many plants are in low nitrogen and contain secondary metabolites that reduce nitrogen digestibility. To investigate whether herbivores maintain nitrogen balance on plant diets low in nitrogen and high in secondary compounds, we studied the effect of juniper (*Juniperus monosperma*) ingestion on the nitrogen balance of two species of herbivorous woodrats (*Neotoma stephensi* and *N. albigula*). These woodrat species feed on the foliage of juniper: *N. stephensi* is a juniper specialist, whereas *N. albigula* is a generalist that incorporates some juniper in its diet. Based on the nitrogen contents of the natural diets of these woodrats, we predicted that the generalist would be in negative nitrogen balance on a juniper diet whereas the specialist would not be affected. We found that both species of woodrat had low-nitrogen requirements (334.2 mg N/kg$^{0.75}$/day) and that a diet of 50% juniper did not result in negative nitrogen balance for either species. However, excretion patterns of nitrogen were altered; on the 50% juniper diet, fecal nitrogen losses increased ~38% and urinary nitrogen losses were half that of the control diet. The results suggest that absorption and detoxification of juniper secondary compounds may be more important for restricting juniper intake by the generalist than nitrogen imbalance.

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Introduction

Maintaining nitrogen balance is a challenge for herbivorous vertebrates. Plants contain low levels of nitrogen compared to most other food sources (Robbins 1993). Furthermore, many plants also produce phenolic compounds such as tannins that may effectively reduce the amount of nitrogen that is absorbed. Many studies have documented that plant phenolics complex nitrogen in vitro and also reduce both in vitro and in vivo digestibilities of nitrogen (Dearing 1997; Felicetti et al. 2000; Hanley et al. 1992; Holechek et al. 1990; Iason and Palo 1991; Jansman et al. 1995; Lindroth and Batzli 1984; Lindroth et al. 1986; Robbins et al. 1991; Robbins et al. 1987; Shipley and Felicetti 2002). The depression in nitrogen digestibility caused by some plant secondary compounds implies that herbivores may not be able to achieve nitrogen balance on plant diets. A few studies have examined the effect of natural forages rich in phenolics on the nitrogen budget of herbivores (Chilcott and Hume 1984; Cork 1986; Felicetti et al. 2000; Foley 1992; Foley and Hume 1987; Holechek et al. 1990; Shipley and Felicetti 2002). The results of these studies were equivocal: some herbivores (ringtail possum, koala, brushtail possum, greater glider; Chilcott and Hume 1984; Cork 1986; Foley 1992; Foley and Hume 1987) achieved positive nitrogen balance on low-nitrogen, high-phenolic diets, whereas other herbivores did not (goats, porcupines, duikers; Felicetti et al. 2000; Holechek et al. 1990; Shipley and Felicetti 2002).

To further investigate whether herbivores can maintain nitrogen balance on plant diets both low in nitrogen and high in secondary compounds, we studied the effect of juniper (*Juniperus monosperma*) ingestion on the nitrogen balance of two species of herbivorous woodrats

(*Neotoma stephensi* and *N. albigula*). These woodrat species occur in sympatry and feed on the foliage of juniper. *Neotoma stephensi* is a juniper specialist that feeds almost exclusively on juniper year round (Dial 1988; Vaughn 1982). In contrast, *N. albigula* is a generalist that feeds on a variety of plant species with no single species comprising more than 35% of the diet (Dial 1988). Juniper foliage is the second most abundant component in the diet of *N. albigula* and comprises 17–33% dry mass. The nitrogen content of juniper foliage, at 1% N or less, is below that estimated for nitrogen balance in nonruminant herbivores (~1.28% N; Karasov 1982). Furthermore, juniper contains high concentrations of terpenes and phenolics, the latter being potential reducers of nitrogen digestibility (Hagerman and Butler 1991; Harborne 1991).

We had two objectives in this study. The primary objective was to determine whether woodrats maintain nitrogen balance on a juniper diet low in nitrogen and high in secondary compounds. Previous research demonstrated that both the woodrat species maintained nitrogen equilibrium on low-nitrogen diets lacking secondary compounds (Dearing et al. 2000). A secondary objective was to examine the role of dietary specialization on nitrogen balance. We hypothesized that *N. stephensi*, because of its ability to specialize on juniper, would be in positive nitrogen balance on a diet containing 50% juniper foliage. We predicted that the generalist, *N. albigula*, would not be able to maintain nitrogen balance on a diet containing 50% juniper foliage because it typically does not consume that much juniper in nature and many of the plants in its diet contain more nitrogen than juniper (Dial 1984; Dial 1988).

Materials and methods

Capture and husbandry

Both species of woodrats were trapped near Wupatki National Park, 45 km NE of Flagstaff, AZ (35° 30′N 111°27′ W) and transported to the University of Utah Animal Facility. Animals were housed individually in cages (48×27×20 cm) with bedding and cotton batting. Animals were placed on a 12–12-h light-dark cycle for at least 6 months prior to the experiment and were kept on this cycle throughout. The 6-month acclimation was necessary, in part, to accommodate required quarantine procedures for ensuring that the animals were not infected with hantavirus. While prolonged captivity can possibly alter the physiology of animals, previous work on *N. albigula* and *N. stephensi* showed that substantial physiological differences in digestive physiology relative to juniper consumption persisted between these species even after 6 months in captivity (e.g., Sorensen et al. 2005). All animals were fed Harland Teklad ground rabbit chow (formula 2120) and water *ad lib*, prior to experimentation. Woodrats had continuous access to a running wheel (Nalgene Wheel Assembly 640–0700) for at least 10 days prior to experimentation as well as during all feeding trials.

Nitrogen balance trials

Nitrogen balance was measured on two treatments, a control diet and a juniper diet (foliage of *J. monosperma*) in *N. stephensi* ($N = 9$) and *N. albigula* ($N = 9$). Woodrats were maintained on the control diet for 11 days. The control diet was a formula designed to simulate water, nutrient, energy and fiber content of juniper, but was free of plant secondary compounds (Table 1, Dearing et al. 2000; Sorensen et al. 2005). The control diet did not perfectly mimic the juniper diet (e.g., it was lower in lignin than juniper) but it was more similar to juniper than any standard chow available. Immediately, following control diet, woodrats were fed an acclimation diet containing a homogenous mix of 75% control and 25% ground juniper for 3 days. Immediately, following the acclimation period, woodrats were fed a diet containing 50% control and 50% juniper for 21 days (referred to as "juniper diet"). A 50% juniper diet was selected because this was the maximum concentration on which both species could be sustained without rapid and excessive loss of body mass (i.e., >3% loss within 3 days). For juniper diet treatments, juniper foliage was crushed on dry ice to produce plant fragments that were <1.0 mm in size and added to the control diet. It was necessary to crush juniper to eliminate selective foraging. Juniper was kept on dry ice during diet preparation

Table 1 Nutrient contents (dry matter basis) of the control and juniper diets

Dietary component	Control diet	50% Juniper diet
Dry mass (%)	47	47
Energy (kJ/g)	16.97	19.3
Nitrogen (%)	1.2	1.01
Fiber (ADF, %)	25.4	30
Lignin (%)	1.7	7.3
Nonstructural carbohydrates (%)	41.2	29.4
Sugar (%)	14.2	12.6
Starch (%)	27	16.8
Crude fat (%)	4.1	10
Total phenolics (mg/g)	3.4	12.7
Alpha-pinene (mg/g)	0.00	7.08

Nitrogen was quantified using a Carla Erba 1108 Elemental Analyzer (Milan, Italy). Fiber was determined from quantifying the acid detergent fiber (ADF, Goering and Van Soest 1970) of diet using an Ankom fiber analyzer 200/200 (Ankom, Fairport, NY, USA). Lignin, nonstructural carbohydrates, sugar, starch and crude fat were quantified by Dairy One Forage Analysis (Ithaca, New York). Total phenolics were extracted from diets in 85% methanol following procedures in Torti et al. (1995). Extracts were assayed for total phenolics using the Folin-Coicalteu method (Singleton and Rossi 1965) and a tannic acid standard (Sigma#1764 KCNT). Alpha-pinene was measured by gas chromatography as described in Sorensen et al. (2005)

to minimize volatilization of terpenes. All diets were made fresh daily and were provided in excess of intake requirements to maintain body mass. Juniper foliage for the diet was collected from randomly selected juniper trees at the woodrat trapping site and stored immediately on dry ice until arrival at the University of Utah where it was stored at −20°C. Food intake (dry mass) was measured daily.

During the nitrogen balance trials (11 days control, 3 days acclimation, 21 days juniper diet), woodrats were kept in "metabolic cages" that were identical to standard cages they had been housed in with one exception: the plastic floor was replaced with a stainless steel wire bottom (Nalgene# 676-2154) to permit the separation and collection of urine and feces. The metabolic cages were suspended over a screen of stainless steel that retained fecal pellets but allowed urine to pass through. Urine was captured below the stainless steel mesh on a plexiglass ramp that drained into a collection cup. A feeder (Nalgene# 650-0104) was attached to cages to prevent selective foraging and to facilitate collection of uneaten food. A small piece of cotton batting was provided for bedding. Nitrogen content of urine and feces were averaged during 3-day collection periods at the end of control (starting on day 9) and juniper diet (starting on day 19) treatments. Fecal and urinary collections were combined separately and stored at −20°C. Nitrogen content of diet and subsample of each 3-day-pooled urine and fecal sample was measured with a Hach's microdigester (Digesdahl; Loveland, CO, USA) using the Kjeldahl procedure. All samples were run in duplicate.

Body mass was measured at the beginning of each diet treatment and monitored every 5 days on the control diet and every 3 days on the juniper diet. Any animal losing more than 12% of body mass during the juniper diet treatment was removed from the experiment.

We calculated three values related to nitrogen economy. First, nitrogen balance was calculated as the difference between nitrogen intake and output during the collection periods. Second, we calculated apparent digestibility of nitrogen as:

((g nitrogen in−g nitrogen out)/g nitrogen in) ×100.

Lastly, the maintenance nitrogen requirement (MNR) was calculated by linearly regressing the means of nitrogen balance and nitrogen intake for each species and treatment (Shipley and Felicitti 2002). The MNR was estimated as the nitrogen intake that resulted in zero nitrogen balance as determined from the relationship between nitrogen intake and balance (Hume 1986).

Statistical analysis

The results of dry matter intake and nitrogen parameters are presented as a function of metabolic body mass ($M^{0.75}$) for direct comparison with previously published values. However, the use of such ratios in statistical analyses may result in erroneous results (Packard and Boardman 1988). To control for metabolic body mass without using ratios, we conducted ANCOVAs with metabolic body mass as the covariate. All ANCOVAs were repeated measures with woodrat species as the between subject factor and diet treatment (control, 50% juniper) as the within subject factor and an interaction term (species × treatment). Body mass on the different diet treatments was compared with a repeated measures ANOVA with woodrat species as the between subject factor and diet treatment (control, 50% juniper) as the within subject factor and an interaction term (species × treatment). When a significant main effect or interaction occurred, a Tukey's HSD was performed within the ANOVA or ANCOVA to further examine differences between means. We used a repeated measures ANCOVA to compare apparent digestibility of nitrogen with nitrogen intake as the covariate, woodrat species as the between subject factor and diet treatment (control, 50% juniper) as the within species factor and an interaction term (species × treatment).

Because complete data sets are required for repeated measures analysis, three woodrats (two *N. albigula* and one *N. stephensi*) were removed from the final analysis due to missing measurements (e.g., spillage of urine container). Thus, the sample sizes used in the analyses were eight *N. stephensi* and seven *N. albigula*.

Results

Body mass and intake

The juniper diet treatment affected both body mass and food intake of woodrats. There were significant changes in the body mass of both species after 3 weeks on the 50% juniper diet (ANCOVA Diet: $F_{1,\ 13}=4.6$, $P=0.05$; Species: $F_{1,\ 13}=0.3$ $P=0.6$, Interaction: $F_{1,\ 13}=61.5$, $P=0.0001$). The generalist lost 11.4% body mass whereas the specialist gained 6.9% after 3 weeks on the 50% juniper diet (Table 2). There was a significant diet effect and interaction effect on dry matter intake when differences in metabolic body mass were controlled (ANCOVA Diet: $F_{1,\ 12}=28.6$, $P=0.0002$; Species: $F_{1,\ 13}=0.2$, $P=0.66$; Interaction: $F_{1,\ 12}=19.8$, $P=0.0008$). The specialist increased intake of dry matter on the juniper diet by 35%, whereas the generalist has similar intakes on the control and juniper diet (Table 2).

Nitrogen intake

When controlling for metabolic body mass, there was no significant difference in nitrogen intake between species or diet treatments (ANCOVA species: $F_{1,\ 13}=0.35$ $P=0.57$; Diet: $F_{1,\ 12}=1.8$, $P=0.21$). However, there was a significant interaction term (Interaction: $F_{1,\ 12}=20.9$, $P=0.0006$). On the control diet, the generalist had a

Table 2 Mean body mass, dry matter intake (*DMI*), nitrogen intake (*NI*), fecal nitrogen (*FN*), urinary nitrogen (*UN*), nitrogen balance (*NB*), and apparent digestibility of nitrogen (*ADN*) for *Neotoma albigula* ($N=7$) and *N. stephensi* ($N=8$) on control and juniper diets

	Body mass(g)	DMI(g/day)	NImg/kg$^{0.75}$/d	FNmg/kg$^{0.75}$/d	UNmg/kg$^{0.75}$/d	NBmg/kg$^{0.75}$/d	ADN(%)
Control							
N. albigula	202.2 (8.2)	13.3 (0.50)	487.9 (23.1)	227.5 (14.0)	189.7 (20.0)	70.7 (28.0)	52.6 (1.9)
N. stephensi	189.6(6.0)	10.9 (0.44)	420.6 (23.9)	244.6 (24.9)	137.9 (24.1)	38.0 (29.1)	43.5 (3.7)
Juniper							
N. albigula	179.1 (8.2)	12.7 (0.68)	461.6 (19.6)	316.2 (12.8)	97.5 (6.6)	47.9 (17.9)	31.8 (2.0)
N. stephensi	202.8 (5.7)	14.6 (0.36)	485.8 (14.1)	341.5 (23.6)	82.9 (6.5)	61.4 (26.0)	28.7 (4.9)

higher nitrogen intake than the specialist, whereas on the juniper diet, the specialist had greater nitrogen intake than on the control diet (Table 2).

Excretion of nitrogen

The excretion of fecal nitrogen increased by 39% when animals were on the juniper diet (ANCOVA, Diet: $F_{1,12}=20.2$, $P=0.0007$; Table 2). There was no significant effect of species (ANCOVA Species: $F_{1,13}=1.3$, $P=0.27$) or interaction (ANCOVA Interaction: $F_{1,12}=0.17$, $P=0.69$). Excretion of nitrogen in the urine decreased by 50% when woodrats consumed the juniper diet (ANCOVA Diet: $F_{1,12}=18.5$, $P=0.001$). There was no significant effect of species (ANCOVA Species: $F_{1,13}=3.4$, $P=0.09$) or an interaction (Interaction: $F_{1,12}=0.87$, $P=0.37$).

Nitrogen balance

There was no effect of juniper on nitrogen balance (ANCOVA Diet: $F_{1,12}=0.04$, $P=0.83$) and there was no difference in nitrogen balance between woodrat species (ANCOVA Species: $F_{1,13}=0.0003$, $P=0.99$). The interaction term was not significant (ANCOVA Diet: $F_{1,12}=2.6$, $P=0.13$). Woodrats were in positive nitrogen balance on both the control and juniper diets (Table 2).

Apparent digestibility of nitrogen

There was a significant effect of diet treatment on ADN even after controlling for differences in nitrogen intake (ANCOVA Diet: $F_{1,12}=18.1$, $P=0.001$). Woodrats on the juniper diets had ADNs 40–52% lower than on the control diet (Table 2). There was no significant difference between species (ANCOVA Species: $F_{1,13}=2.5$, $P=0.14$) and no significant interaction (ANCOVA Interaction: $F_{1,12}=0.5$, $P=0.48$).

Maintenance nitrogen requirement

The means of nitrogen intake and balance for each species on each diet were marginally correlated but the coefficient of determination was high ($P=0.06$, $N=4$, $R^2=0.88$). Maintenance nitrogen requirement was estimated to be 334.2 mg N/ kg$^{.75}$ /day ($y=-146.3+0.433x$) for both generalists and specialists.

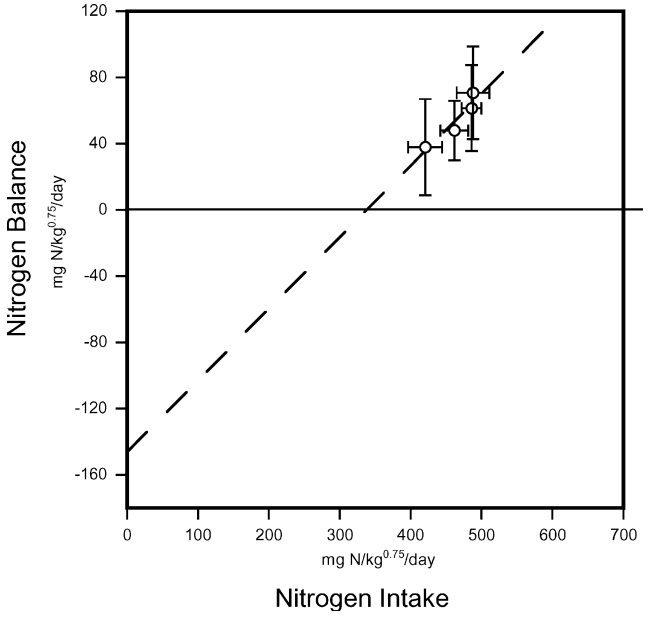

Fig. 1 Estimation of the maintenance nitrogen requirement (MNR) from the relationship of nitrogen balance and nitrogen intake in specialist and generalist woodrats (Hume 1986). The MNR was estimated to be 334.2 mg N/kg$^{0.75}$/day ($y=-146.3+0.433x$)

Discussion

We compared the nitrogen budgets of woodrat herbivores consuming a low-nitrogen diet lacking secondary compounds to that of a low-nitrogen juniper diet high in secondary compounds. Ingestion of the juniper diet significantly decreased the apparent digestibility of nitrogen and altered nitrogen excretion patterns, resulting in a significant increase in fecal nitrogen and a significant decrease (40–50%) in urinary nitrogen. Despite these significant changes in nitrogen excretion, woodrats remained in nitrogen balance in the sense that nitrogen intake exceeded excretory outputs. Below, we discuss the ramifications of our findings.

Nitrogen requirements

Woodrats had a low maintenance requirement for nitrogen (334.2 mg N/kg$^{.75}$ /day), which likely plays a role in their ability to successfully utilize low-nitrogen plants like juniper. The MNR for woodrats is considerably less than the average nitrogen requirement for eutharians (582 mg N/kg$^{0.75}$/day ± 235, Robbins 1993) but is similar to that of marsupials (356 mg N/kg$^{0.75}$/day) as well as some other rodents such as porcupines (346 mg N/kg$^{0.75}$/day, Felicetti et al. 2000; Fournier and Thomas 1997; Robbins 1993). Many of these animals regularly consume foods low in nitrogen (Fournier and Thomas 1997).

What factors are responsible for the low MNR in woodrats? The MNR of animals is primarily determined by losses of nitrogen in the urine (''endogenous urinary nitrogen'') and feces (''metabolic fecal nitrogen'') under basal conditions (Robbins 1993). Endogenous urinary nitrogen (EUN) and metabolic fecal nitrogen (MFN) are estimated using regression equations whose parameters are determined from the results of nitrogen balance studies on animals fed diets that range in nitrogen content (typically 1–5% N). In this study, we did not estimate the EUN and MFN because the woodrats were not fed the appropriate range of dietary nitrogen. However, below, we compare the pattern of nitrogen excretion in woodrats to that of other animals to address the potential mechanism for the low MNR of woodrats.

Fournier and Thomas (1997) argue that metabolic fecal nitrogen represents a greater avenue of nitrogen loss for animals on low-nitrogen diets. For example, porcupines (*Erethizon dorsatum*) have a low MNR that is the result of exceptionally low excretion of nitrogen in the feces (< 29% of the rodent average), despite the fact that their urinary nitrogen excretion is the highest reported for eutharians (Fournier and Thomas 1997). However, porcupines cannot maintain nitrogen balance on diets containing phenolics even at sufficient nitrogen intakes because nitrogen losses in the feces increase tremendously while losses of urinary nitrogen do not change. In contrast, Cork (1986) proposed that herbivores consuming low-nitrogen, high-phenolic diets maintain nitrogen balance diets through low urinary outputs that compensate for high fecal nitrogen losses. The koala (*Phascolarctos cinereus*) and brushtail possum (*Trichosurus vulpecula*) exhibit this ratio of nitrogen excretion on low-nitrogen, high-phenolic diets (Cork 1986; Foley and Hume 1987). We predict that woodrats will also have a low EUN given the pattern seen in other studies of herbivores in positive nitrogen balance while consuming phenolic-rich forages. The reduction in losses of urinary nitrogen of woodrats consuming the juniper diet compared to the control support this prediction.

Phenolics and nitrogen metabolism

Phenolics in general and tannins in particular have repeatedly been demonstrated to lower the apparent nitrogen digestibility of diets. An implication of such studies was that decreased nitrogen digestibility results in negative nitrogen balance. Indeed, some studies have reported negative nitrogen balances for herbivores on tannin-rich diets (Felicetti et al. 2000; Holechek et al. 1990; Shipley and Felicetti 2002). In contrast, in this study, we found that although nitrogen digestibilities decreased on the juniper diet (high-phenolic, low-nitrogen), woodrats remained in positive nitrogen balance. Moreover, there was no significant effect of juniper diet on nitrogen balance.

A plausible interpretation of our results is that the nitrogen balance of woodrats is not significantly impacted by phenolic-rich forage; however, it is possible that the reduction in nitrogen digestibility observed in this study could be biologically significant under natural circumstances. For example, animals have much greater nitrogen demands during growth, reproduction and during periods of increased metabolism (Karasov 1982). A reduction in nitrogen digestibility may therefore compromise these important activities. In addition, many animals utilize nitrogen-based conjugates in the detoxification of plant secondary compounds (Klaassen 1996). If phenolics decrease nitrogen availability, the use of nitrogen-containing conjugates for detoxification may be constrained and ultimately limit total food intake. The complex interactions between nitrogen availability and nitrogen-dependent activities such as growth, reproduction, metabolism and detoxification underscore the need for further experimentation in understanding how decreased nitrogen digestibilities affect herbivores.

Although there was no effect of juniper diet on nitrogen balance, there was a significant effect on nitrogen excretion patterns. On the juniper diet, excretion of nitrogen increased significantly in the feces and decreased significantly in the urine compared to nitrogen excretion patterns on the control diet. These patterns of nitrogen excretion have been documented in other mammals consuming phenolic-rich forages (Cork 1986; Foley and Hume 1987). The increase in fecal nitrogen of animals on phenolic-rich diets appears to originate from a variety of endogenous sources including gut mucosa, digestive enzymes and salivary proteins that bind tannins (Foley et al. 1999; Jansman et al. 1995; Mehansho et al. 1987; Skopec et al. 2004). The relative contributions from these endogenous sources are currently controversial; however, they are thought to comprise the majority of the nitrogen present in the feces of animals consuming a phenolic-rich diet.

Specialist versus generalist

Contrary to our predictions, the juniper diet did not differentially affect the nitrogen balances of the specialist and generalist. In general, theory predicts that the generalist would have been more impacted than the specialist (Futuyma and Moreno 1988). Data from another pair of sympatric woodrats where one is a

specialist (*N. fuscipes*) on oak (*Quercus californicus*) leaves and the other (*N. lepida*) is a generalist, supported this prediction (Atsatt and Ingram 1983). In a laboratory feeding trial, *N. fuscipes* consumed two fold more oak leaves than *N. lepida*, yet the fecal nitrogen content of *N. fuscipes* was only half that of *N. lepida*. Nitrogen content of the food and urine were not measured in their study, but given the differences in food intake and fecal nitrogen, it seemed likely that the generalist was in negative nitrogen balance.

We found it particularly surprising that the generalist in this study was able to maintain nitrogen balance on the juniper diet. In nature, the generalist feeds on plants species with higher nitrogen contents compared to juniper (Dial 1984). Furthermore, the generalist was in negative energy balance as indicated by mass loss during the juniper treatment (see Sorensen et al. 2005 for a complete discussion of energy balance). That the generalist could be in positive nitrogen balance under these conditions underscores the ability of woodrats to cope with low-nitrogen diets.

It is plausible that both specialist and generalist woodrats in our study produce tannin-binding salivary proteins (TBPs) that mitigate the negative effects of dietary phenolics (Mehansho et al. 1987; Mehansho et al. 1983). The increase in fecal nitrogen of woodrats on the juniper diet is a common result for herbivores that produce TBPs on phenolic-rich diets (Dearing 1997; Robbins et al. 1991; Skopec et al. 2004). Additionally, the nitrogen digestibilities of another species of woodrat on other forage diets appear unaltered by the presence of dietary tannins. The apparent digestibility and nitrogen balance of *N. lepida* is unaffected by high concentrations of the phenolic resin from creosote (Karasov 1989; Meyer and Karasov 1989). Thus, the possibility exists that the specialist and generalist are equally good at extracting adequate nitrogen from juniper and that the absorption and processing of toxins (phenolics or others such as terpenes) are more important in governing the ingestion of juniper for the generalist (Dearing et al. 2000; Dearing et al. 1999; Sorensen et al. 2005).

A potential criticism of our nitrogen balance calculations is that they are underestimations because we did not control for losses of ammonia in the urine either through cold temperature or the addition of acid to the urine collection vials. We were unable to find any data in the literature describing the magnitude of the error created by ammonia loss using these collection procedures. Moreover, we suggest that there was likely little ammonia lost from the urine samples due to the pH of the urine. The urine pH of woodrats on diets similar to that fed in this study was neutral or acidic (Dearing et al. 2000). At acidic pHs, ammonia is present in its nonvolatile ammonium. It is only at alkaline pHs (>9.2) that a significant amount of ammonium is converted into ammonia (Foley 1995, 1992). This relationship between low pH and low ammonia is precisely why many researchers add glacial acetic acid to urine vials during nitrogen studies (e.g., Fournier and Thomas 1997). Our assertion that ammonia loss from urine was minimal is supported by data from another nitrogen balance study where urine of woodrats was collected on ice to minimize loss of ammonia (Dearing et al. 2000) The nitrogen balance estimates of Dearing et al. (2000) were in the same range as those presented here.

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