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Nutritional Requirements and Diet Choices of the Pygmy Rabbit (*Brachylagus idahoensis*): A Sagebrush Specialist

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Abstract Sagebrush (*Artemisia tridentata*) comprises up to 99% of the winter and 50% of the summer diets of pygmy rabbits (*Brachylagus idahoensis*). Few animals specialize on such plants as sagebrush, which contain high levels of plant chemicals that can be toxic. We investigated the nutritional requirements of pygmy rabbits and their ability and propensity to consume sagebrush alone and as part of a mixed diet. We compared diet choices of pygmy rabbits with that of a generalist forager, the eastern cottontail (*Sylvilagus floridanus*). Pygmy rabbits had a moderately low nitrogen requirement (306.5 mg N/$kg^{0.75}$/d), but a relatively high energy requirement, needing 750.8 kJ digestible energy/$kg^{0.75}$/d to maintain their body mass while residing in small cages. They digested fiber in pelleted diets similarly to other small hindgut fermenters, but both cottontails and pygmy rabbits digested the fiber in sagebrush better than expected based on its indigestible acid detergent lignin content. Pygmy rabbits consumed more sagebrush than cottontails, regardless of the amount and nutritional quality of supplemental pellets provided. When consuming sagebrush alone, they ate barely enough to meet their energy requirements, whereas cottontails ate only enough sagebrush to meet 67% of theirs. Both rabbit species ate more sagebrush as the quality and quantity of supplemental pellets declined, and more greenhouse-grown sagebrush than sagebrush grown outside. Urine was more acidic when consuming sagebrush than when consuming pellets, indicating detoxification by the liver. Pygmy rabbits do not require sagebrush to survive, but seem to tolerate terpenes and other plant chemicals in sagebrush better than cottontails do.

Keywords Cottontail rabbit · Pygmy rabbit · Sagebrush · Specialist · Terpenes

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Introduction

Low levels of nutrients and high concentrations of plant secondary metabolites (PSMs) often make it difficult for herbivores to meet their daily nutritional requirements. Therefore, most herbivores have adapted a generalist strategy to foraging, eating many different types of plants to meet their nutritional requirements and reduce toxic effects of PSMs (Freeland, 1991; Dearing et al., 2000; Marsh et al., 2003; Sorensen and Dearing, 2003). Out of more than 1000 mammalian herbivores known, only about 1% are considered dietary specialists, obtaining most or all of their nutritional requirements from one plant source (Freeland 1991; Dearing et al., 2000). Specialist herbivores have likely coevolved with their forage plant, developing detoxification pathways specific to its PSMs (McArthur et al., 1991; Marsh et al., 2003; Sorensen and Dearing, 2003; Sorensen et al., 2004).

Monoterpenes are bitter-tasting volatile oils, many of which are responsible for the characteristic aromatic odors of plants such as sagebrush, *Eucalyptus* spp., and conifers (Harborne, 1991). They act as feeding deterrents (Sinclair et al., 1988; Gershenzon and Croteau, 1991; Meyer and Karasov, 1991), and at higher concentrations can cause acidosis (Cork and Foley, 1991), destroy or inhibit growth of rumen flora (Nagy et al., 1964; Burritt et al., 2000), irritate mucous membranes, cause neurotoxicity, diuresis and nephritis (Dearing et al., 2000), and require animals to spend additional energy in detoxification (Sorensen et al., 2005). In some instances, terpenes themselves may not be toxic, but as volatile compounds act as an easy-to-detect cue for more potent PSMs they are associated with, such as formylated phloroglucinol compounds (FPCs), lactones, and phenolics (Radwan et al., 1982; Bray et al., 1991; Lawler et al., 1999).

Although most animals avoid eating large amounts of plants that contain toxic PSMs, some animals specialize on them. Koalas (*Phascolarctos cinereus*) and the greater glider (*Petauroides volans*) feed almost exclusively on *Eucalyptus* spp., known to have high levels of terpenes, tannins, and FPCs (Lawler et al., 1998; Moore and Foley, 2000; Marsh et al., 2003). Other herbivores also rely on terpene-containing plants, but to a lesser extent. For example, the terpene-containing juniper (*Juniperus monosperma*) comprises 80–95% of the diet of the Stephen's woodrat (*Neotoma stephensi*; Dial, 1988). Likewise, pygmy rabbits (*Brachylagus idahoensis*) are restricted to deep-soil sagebrush-steppe habitat, consuming up to 99% of their diet in big sagebrush (*Artemisia tridentata*) in winter and up to 50% in summer (Green and Flinders, 1980b; Thines et al., 2004), despite high concentrations of terpenoids (6–23%), and monoterpenes (1–4%) in particular (White et al., 1982b; Meyer and Karasov, 1991). Although little is known about PSMs other than terpenes and sesquiterpenes in sagebrush, 10 coumarins (Shafizadeh and Melnikoff, 1970; Brown et al., 1975) and 13 flavonoids (Brown et al., 1975; Thines, 2006) have been identified.

Several wildlife species, such as mule deer (*Odocoileus hemionus*; Cluff et al., 1982), pronghorns (*Antilocapra americana*; Ngugi et al., 1992), and black-tailed jackrabbits (*Lepus californicus*; Uresk, 1978), consume sagebrush as a portion of their seasonal diet, but no other known mammal depends on sagebrush as extensively for food as does the pygmy rabbit. Consuming moderate amounts of sagebrush (15–50% of the diet) causes digestive upset, reduced rumen motility, rumen lesions, rumenitis, and even death in mice, mule deer, sheep, and cattle (Johnson et al., 1976; White et al., 1982b; Harborne, 1991). Therefore, sagebrush is generally considered toxic to mammals, especially livestock (Burritt et al., 2000).

Although sagebrush dominates 1.1 million km^2 of the western United States and Canada (Meyer and Karasov, 1991), pygmy rabbits only occupy patchily distributed deep soil habitats dominated by tall, dense big sagebrush (Green and Flinders, 1980a). However,

much of this small (400–500 g), burrowing rabbit's habitat within the Great Basin of the western United States has been degraded, fragmented, and converted to other uses such as agriculture for over a century (McAllister, 1995; Federal Register, 2003). An evolutionarily distinct population of pygmy rabbits in the Columbia Basin of Washington has been listed as endangered by the U.S. Fish and Wildlife Service (USFWS; Federal Register, 2003), and USFWS has received petitions for listing the species range-wide (Federal Register, 2005). However, little is known about the extent to which pygmy rabbits depend on sagebrush for food, how they cope with a diet high in terpenes, and their requirements for energy and nitrogen (N).

In this study, we examined the nutrient requirements of pygmy rabbits and their propensity and ability to select and consume sagebrush. As a comparison, we also examined nutritional requirements and sagebrush consumption by eastern cottontail rabbits (*S. floridanus*), a larger leporid that is considered a dietary generalist, but is also known to occasionally inhabit the sagebrush-steppe region with pygmy rabbits (McAllister, 1995). We expected pygmy rabbits to eat more sagebrush relative to their size than cottontails when sagebrush was offered alone and as part of a mixed diet. We expected them to voluntarily eat enough sagebrush to meet their energy requirements and maintain body mass, and to obtain more digestible nutrients from sagebrush, but less from nonsagebrush foods, than cottontails. Finally, we expected both pygmy rabbits and cottontails to eat more sagebrush as the quality and quantity of other diet items declined.

Methods and Materials

To examine energy and protein requirements and fiber tolerances of pygmy rabbits, we conducted digestion trials with 2–5 adult pygmy rabbits (451 ± 15 g) from populations found in Idaho. All had been born in captivity as part of a captive breeding program at the E.H. Steffen Center at Washington State University (WSU). As a comparison, we also used 3–4 adult eastern cottontails (1209 ± 70 g), captured on the WSU campus. Standard husbandry practices and the experimental protocol were approved by the WSU Institutional Animal Use and Care Committee (#3097). When not used in experiments, animals were housed on soil in large outdoor pens or in stainless steel rabbit cages indoors, and fed a maintenance diet consisting of one or more completely balanced pelleted rabbit diets (e.g., LabDiet® 5326 Hi Fiber Rabbit, PMI Nutrition International, Brentwood, MO, USA; Bunny Basics/T®, Oxbow Pet Products, Murdock, NE, USA). They were also fed a mix of fresh forbs and sagebrush grown in our greenhouse without pesticides, and only supplemented with N for fertilizer. Tap water, trace mineral block, and the pelleted rations were provided *ad libitum*.

In January–March 2003–2005, we conducted total-collection, single-diet digestion trials using a series of seven pelleted diets and sagebrush grown in the greenhouse and outside (Table 1). Cottontails participated in three of the pelleted trials and the greenhouse sagebrush trial. The diets ranged in fiber [24–55% neutral detergent fiber (NDF)] and N content (0.5–4.5%) (Table 1). During the trials, animals were housed individually in stainless steel digestion crates with Dri-Dek® flooring (Kendall Products, Naples, FL, USA) and plastic nest boxes in a covered barn. Electric heaters maintained the air temperature outside the crates at an average daily temperature that ranged from 0 to 5°C. The digestion trials lasted from 3 d (sagebrush) to 5 d (pellets) following a 10-d pretrial during which the animals were gradually introduced to the trial food by increasing its contribution to the diet, and acclimated to stainless steel digestion crates. At the end of each trial,

Table 1 Composition of pelleted rations and sagebrush (*Artemisia tridentata*) fed to captive pygmy rabbits (*Brachylagus idahoensis*) and eastern cottontails (*Sylvilagus floridanus*)

Diets	Date	Nitrogen content (% DM)	NDF (% DM)	ADL (% DM)	Gross energy content (kJ/g)
Pellet 1: WSU custom high-quality diet	3/4/05	3.1	28.5	2.6	17.8
Pellet 2: WSU custom low-quality diet	2/17/05	2.3	55.0	8.3	16.8
Pellet 3: WSU custom deer diet	2/22/03	2.8	32.3	4.5	18.2
Pellet 4: Purina® Advanced Nutrition Breeders Formula	1/15/04	3.4	41.5	6.7	18.3
Pellet 5: Mazuri® Moose Diet	1/30/03	3.2	37.7	6.0	19.3
Pellet 6: Lab Diet® 5326 Hi Fiber Rabbit	1/14/03	2.5	49.3	6.5	18.7
Pellet 7: WSU custom low-protein diet	3/14/03	0.5	24.2	1.4	18.3
Greenhouse sagebrush	2/8/05	4.5	41.7	10.3	18.8
Outside sagebrush	3/17/05	2.5	32.1	8.9	20.2

NDF: neutral detergent fiber; ADL: acid detergent lignin; WSU: Washington State University.

animals were moved back to their original pens and weaned back onto their maintenance diets during a 10-d post trial. Animals were weighed before, at least once during, and after each digestion trial. Food and water were offered *ad lib*, and a mineral block was provided. If an animal lost more than 20% of its pretrial body mass, it was removed from the trial.

During the sagebrush trials, fresh sagebrush was offered in the morning and augmented twice during the day as needed. To ensure that sagebrush diets were homogenous, sagebrush was grown in a dedicated greenhouse or food plot outside. Greenhouse sagebrush was grown from seed in individual containers, and watered daily. Greenhouse seedlings used in trials were 2–3 mo old and about 20 cm tall. Sagebrush grown outside in food plots were 3-yr-old shrubs that had originated from greenhouse seedlings. Only current annual growth was collected, the woody component of sagebrush was minimized, no flowers or inflorescences were included, and all leaves were consistent in size and age. Sagebrush was harvested each day immediately before feeding from several flats in the greenhouse or at least six shrubs outside. Orts were collected, weighed, and corrected for dry matter (DM), then subtracted from the forage given the previous day to determine daily DM intake (DMI) of each animal.

Feces fell to mesh screens placed below each digestion crate, urine was funneled into bottles containing ≈5 ml of HCl to maintain a pH < 7 and reduce loss of N as ammonia. Each day, the pH of urine (before it reached the HCl) was measured with a pH meter or pH paper between 0700 and 0800 hr. The remainder of the urine was collected daily and stored at −20°C. Samples of the food, feces, and orts were dried daily at 100°C for 24 hr to determine DM content. Additional samples of food and feces were dried at 60°C for 3 d and ground to pass a 1-mm screen and pooled at the end of the 3- to 5-d trial for later nutritional analysis. Samples of sagebrush intended for terpene analyses were harvested from at least six plants between 1000 and 1130 hr to avoid diurnal and interplant variation in terpene concentration (Nicholas, 1973) and stored at −40°C. We made composites of samples intended for tannin analysis from at least six plants, froze them at 20°C, and freeze-dried them before grinding.

We measured the gross energy content of food, feces, and urine by using bomb calorimetry (Table 1). Fiber composition of food and feces was determined from sequential detergent analysis (Goering and Van Soest, 1970) with filter bags, sodium sulfite, and alpha

amylase (Ankom Fiber Analyzer $^{200/220®}$; Ankom Technology, Fairport, NY, USA) to determine NDF, acid detergent fiber (ADF), and acid detergent lignin (ADL; Table 1). Kjeldahl analysis and a carbon–nitrogen analyzer (TruSpec®; LECO Corporation, St. Joseph, MI, USA) was used to determine N content of feces, food, and urine. Crude protein (CP) content (%) was estimated as 6.25 times the N content (Robbins, 1993). The capacity of condensed and hydrolyzable tannins to bind proteins in food was determined by the bovine serum albumin (BSA) method (Martin and Martin, 1982).

To extract volatile terpenes from whole plant tissue of greenhouse and outside sagebrush, we used direct simultaneous steam distillation pentane extraction of the frozen (−20°C) plant material with a Likens–Nickerson apparatus (J&W Scientific, Folsum, CA, USA). In this process, we combined plant material with 100 ml deionized water, boiled it for 20 min, and collected distillate in 25 ml of pentane with a standard added to the pentane. After drying and weighing the distilled plant material, we diluted the distillate with pentane (200 µl of sample with 800 µl of pentane) and quantified the components by comparing detector response with a standard of known quantity using capillary gas chromatography (GC) on a Hewlett-Packard 5890 Series II GC equipped with a flame ionization detector (at 230°C), 7673 A autosampler, and a ZB5 column (30 m × 0.25 mm i.d.; Phenomenex, Torrance, CA, USA) using hydrogen (13 psi head pressure) as the carrier gas. Cool on column injection (40°C) was used with a temperature program starting at 40°C, ramped at 10°C/min to 250°C, and then programmed at 40°C/min to 300°C. Data were analyzed by using Chrom Perfect Spirit v. 5.5.2 (Justice Laboratory Software, Denville, NJ, USA). We identified components of the distillate by comparing their mass spectra with spectra from the NIST02 Mass Spectral Library with Windows Search Program v.2.0 (National Institute of Standards and Technology, Gaithersburg, MD, USA). GC-mass spectrophotometry (GC-MS) was performed on a Hewlett-Packard 5840A-5985B MSD system (at 70 eV) using a ZB5 column (30 m × 0.25 mm i.d.; Phenomenex, Torrace, CA, USA) with the same temperature program as described for GC analysis.

Metabolic fecal nitrogen (MFN, g N/100 g feed) for pygmy rabbits was estimated as the negative *y*-intercept of the line of regression of digestible N (g N/100 g feed) against dietary N (%) for all foods. True N (%) was determined by the slope of this regression line. Similarly, the *y*-intercept of the regression line of urinary N (mg N/kg$^{0.75}$/d) against dietary N intake (mg N/kg$^{0.75}$/d) was used to estimate the endogenous urinary N (EUN, mg N/ kg$^{0.75}$/d). The *x*-intercept of the regression line for N balance (N ingested − N excreted, mg N/kg$^{0.75}$/d) against dietary N intake (mg N/kg$^{0.75}$/d) estimated maintenance N requirements (the amount of N an animals must consume to counteract the minimum constant losses from feces and urine) of pygmy rabbits and cottontails. Minimum dietary protein requirements were derived from the equation [(EUN + MFN (DMI) − 6.25)/DMI/0.74] (Robbins, 1993). Because none of the diets had enough tannins to precipitate proteins in BSA analysis, all diets were included, but only animals gaining mass or losing <1% of their pretrial body mass per d of the trial were included in these analyses. We compared digestible N, urinary N, and N balance among pygmy rabbits and cottontails with analysis of covariance.

Maintenance energy requirements for both pygmy and cottontail rabbits in small cages were estimated from the *x*-intercept of the regression of average daily change in body mass as a function of digestible energy intake (DEI; kJ/kg$^{0.75}$/d), where DEI was the product of DMI (g/kg$^{0.75}$/d), gross energy content of the food (kJ/g), and apparent energy digestibility (AED, %) for each diet. The slope of this line represented body mass change per unit of additional energy intake. We compared intake and digestibility among foods between pygmy rabbits and cottontails by using a two-way analysis of variance with interactions, with Tukey's multiple comparison test at $\alpha = 0.05$.

To compare intake of sagebrush by pygmy rabbits and cottontails as a function of the amount and nutritional quality of supplemental foods, we ran a series of single- and double-choice experiments. In these experiments, we measured the rabbits' voluntary consumption of sagebrush grown in the greenhouse and outside as we varied the amount of high-quality (pellet 1: high N, low fiber) or low-quality (pellet 2: low N, high fiber; Table 1) pellets offered. We used two to three pygmy rabbits and three cottontails for these experiments. Our first experiments determined the amount of sagebrush and high- or low-quality pellets consumed when each was offered *ad lib*. In subsequent experiments, we offered rabbits pellets at 50% and 25% of their *ad lib* intake of pellets and measured sagebrush consumption. We also measured sagebrush consumption when no supplementary pellets were offered, and consumption of greenhouse and outside sagebrush when offered together with 15% of their *ad lib* consumption high-quality pellets. Because of its limited availability, we only conducted experiments with outside sagebrush with 50% *ad lib* intake of both high- and low-quality pellets for both rabbit species, and with greenhouse sage/15% pellets and no pellets for pygmy rabbits.

Food offered was weighed and corrected for DM, and orts were collected, weighed, and subtracted to determine the dry mass of pellets and sagebrush consumed. For trials with both sagebrush types, we separated the food offered and refused into stems and leaves to account for any possible differences in preference for plant parts. We compared DMI and DEI of greenhouse sagebrush consumed by pygmy rabbits and cottontails, and the relationship between urine pH and sagebrush consumption, by using analysis of covariance, with species and pellet quality as class variables and dry mass of pellets consumed as a covariate. Additionally, we compared voluntary intake of greenhouse and outside sagebrush by pygmy rabbits and cottontails when fed 50% of their *ad lib* intake of low- and high-quality pellets by using analysis of variance, and paired *t*-tests.

Results

Energy and Protein Requirements

Pygmy rabbits required 750.8 kJ DE/kg$^{0.75}$/d and cottontails required 549.2 kJ DE/kg$^{0.75}$/d to maintain their body mass when residing in small cages between 0°C and 5°C (Fig. 1). Pygmy rabbits had an MFN of 7.5 g N/kg feed (Fig. 2), and an EUN of 22.9 mg/kg$^{0.75}$/d (Fig. 3). True N digestibility was 88%. They required at least 306.4 mg N/kg$^{0.75}$/d for

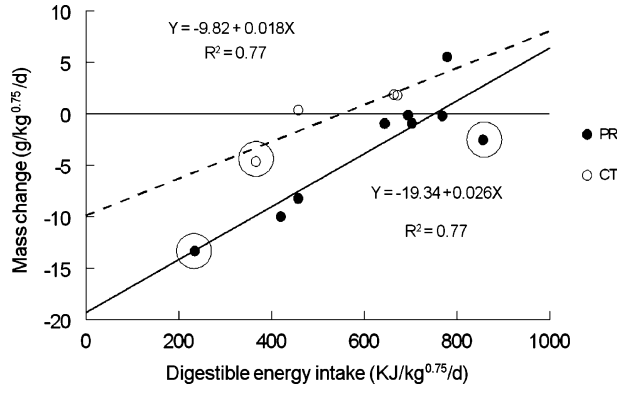

Fig. 1 Body mass gain as a function of digestible energy intake of captive pygmy rabbits (PR, solid line) and cottontail rabbits (CT, dashed line) when consuming pelleted rations and sagebrush (*Artemisia tridentata*, circled points). Daily digestible energy requirements are found at the intersection of the regression lines with the *x*-axis

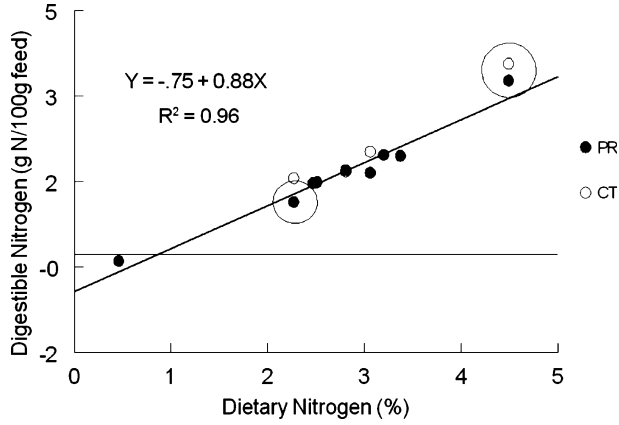

Fig. 2 Mean apparent digestible nitrogen of captive pygmy rabbits (PR) and cottontail rabbits (CT) as a function of dietary nitrogen for pelleted rations and sagebrush (*A. tridentata*, circled points). Metabolic fecal nitrogen is the negative *y*-intercept

maintenance (Fig. 4). Cottontails had a similar digestible N ($F = 1.13$, $P = 0.32$), N balance ($F = 0.80$, $P = 0.39$), and urinary N ($F = 0.02$, $P = 0.90$) relative to dietary N or N intake to pygmy rabbits (Figs. 2, 3, and 4). Based on their EUN and MFN, when pygmy rabbits eat >9 g DM/d, they require a minimum of 7% CP in their diet.

Digestibility of Foods

Pygmy rabbits digested DM, apparent energy (AE), neutral detergent solubles (NDS) and NDF of the high-quality diet (pellet 1), and NDS and NDF of the low-quality diet (pellet 2), to a lesser extent than the larger cottontails (all $P < 0.05$, Table 2). However, the two rabbit species digested high-quality pellets and greenhouse sagebrush equally (all $P > 0.05$, Table 2). Both pygmy and cottontail rabbits digested DM, AE, and NDF to a greater extent when eating high-quality pellets and greenhouse sagebrush than when eating low-quality pellets (all $P < 0.05$, Table 2). Pygmy rabbits achieved a lower DM digestibility (DMD) and AED on outside sagebrush than on high-quality pellets and greenhouse sage, but greater than that on low-quality pellets ($P < 0.05$, Table 2). Both pygmy and cottontail rabbits digested NDF as a function of the proportion of indigestible of the cell wall (i.e., ADL) similarly to other small hindgut fermenters, and to a lesser extent than ruminants and large

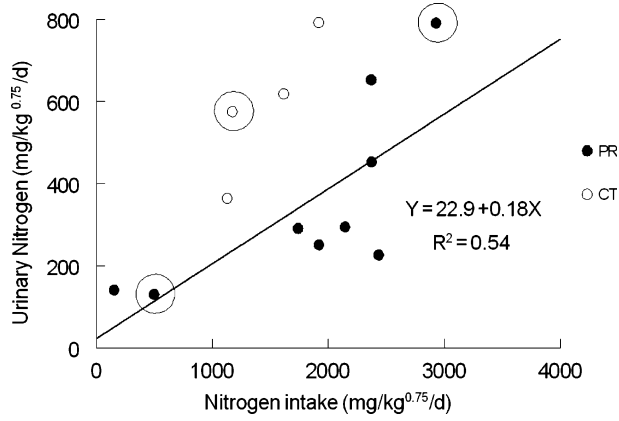

Fig. 3 Mean urinary nitrogen as a function of nitrogen intake of captive pygmy rabbits (PR) and cottontail rabbits (CT) consuming pelleted rations and sagebrush (*A. tridentata*, circled points). Endogenous urinary nitrogen is the *y*-intercept of the regression line

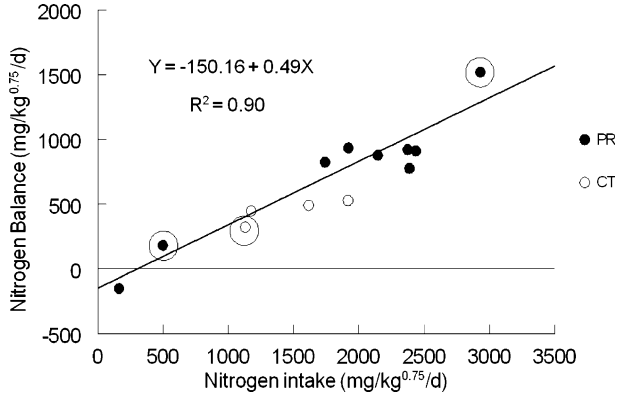

Fig. 4 Mean nitrogen balance as a function of total nitrogen intake of captive pygmy rabbits (PR) and cottontail rabbits (CT) consuming pelleted rations and sagebrush (*A. tridentata*, circled points). Minimum nitrogen requirements are found at the *x*-intercept

hindgut fermenters (Fig. 5). However, they digested NDF of greenhouse sagebrush better than expected from its ADL content, and better than the other diets (all $P < 0.05$; Table 2, Fig. 5).

A greater proportion of energy consumed was excreted in the urine of both pygmy rabbits ($\overline{X} = 9.5 \pm 2.4\%$) and cottontails ($\overline{X} = 14.1 \pm 2.7\%$) when eating sagebrush than when eating high-quality or low-quality pellets ($\overline{X}_{\text{pygmy rabbits}} = 0.7 \pm 0.2\%$, $\overline{X}_{\text{cottontails}} = 2.8 \pm 0.2\%$, $F = 9.87$, $P < 0.001$). Pygmy rabbits and cottontails excreted in their urine the same proportion of energy consumed when eating greenhouse sagebrush ($F = 0.53$, $P = 0.52$). In contrast, the proportion of ingested energy excreted in the feces when consuming sagebrush was lower than the amount excreted when eating low-quality pellets (all $P < 0.05$), and lower than (i.e., pygmy rabbits eating greenhouse sagebrush, $P < 0.05$) or equivalent to (i.e., pygmy rabbits eating outside sagebrush and cottontails eating greenhouse sagebrush, both $P > 0.05$) the energy excreted when eating high-quality pellets. Pygmy rabbits and cottontails excreted in feces the same proportion of ingested energy when eating greenhouse sagebrush ($F = 4.12$, $P = 0.14$).

Terpene Content of Sagebrush

Using GC and GC-MS, we identified six major peaks (mostly monoterpenes) present in the greenhouse and outside sagebrush (Table 3). Greenhouse sagebrush had over twice the total concentration (% DM) of terpenes of the outside sage, and among the major peaks, had more than twice the amount of artemiseole, 1,8-cineole/eucalyptol, and methyl santolinate, and 10 times more santolina epoxide than did the outside sagebrush. However, greenhouse sagebrush had only half the camphor of outside sagebrush (Table 3). Camphor made up nearly 50% of the major distilled volatile oils of the outside, but only 12% of the greenhouse, sagebrush.

Voluntary intake of sagebrush and pellets

When only pellets were offered, pygmy rabbits ate the same dry mass and digestible energy (DE) relative to their metabolic body mass ($kg^{0.75}$) as did cottontails (both $P > 0.05$, Table 2). Both pygmy ate more mass of low- than high-quality pellets ($F = 24.4$, $P = 0.001$), but consumed equivalent DE ($F = 0.19$, $F = 0.67$, Table 2).

Table 2 Mean body mass change, digestibility, and intake of pelleted diets and sagebrush (*Artemisia tridentata*) fed captive pygmy rabbits (PR, *Brachylagus idahoensis*) and eastern cottontails (CT, *Sylvilagus floridanus*)

Diet	Body mass change (%/d)		Dry matter digestibility (%)		Apparent energy digestibility (%)		Neutral detergent soluble digestibility (%)		Neutral detergent fiber digestibility (%)		Dry matter intake ($g/kg^{0.75}/d$)		Digestible energy intake ($kJ/kg^{0.75}/d$)	
	PR	CT	PR	CT	PR	CT	PR	CT	PR	CT	PR	CT	PR	CT
Pellet 1: WSU high quality	0.0	0.2	60.3^{*bc}	69.7^{ab}	61.5^{*bc}	71.4^{ab}	72.2^{*b}	83.8^{a}	17.7^{*b}	34.3^{b}	70.1^{ab}	52.8^{b}	769^{abc}	672^{a}
Pellet 2: WSU low quality	0.7	0.2	40.2^{f}	44.1^{c}	43.3^{ef}	46.7^{c}	70.0^{*b}	75.1^{b}	15.7^{*b}	18.7^{c}	107.3^{a}	84.4^{a}	779^{ab}	664^{a}
Pellet 3: WSU deer	−0.1	0.0	56.7^{cd}	62.5^{b}	60.2^{cd}	65.9^{b}	75.5^{b}	80.0^{ab}	17.1^{b}	25.6^{bc}	68.4^{*ab}	40.3^{bc}	703^{*abc}	458^{b}
Pellet 4: Purina® Breeders	−0.1	–	50.0^{de}	–	51.3^{de}	–	73.2^{b}	–	17.3^{b}	–	70.3^{ab}	–	644^{abc}	–
Pellet 5: Mazuri® Moose	−1.0	–	43.7^{ef}	–	46.7^{de}	–	71.3^{b}	–	-1.8^{c}	–	54.4^{bc}	–	458^{bcd}	–
Pellet 6: Lab Diet® Rabbit	0.0	–	39.5^{f}	–	42.6^{f}	–	64.2^{c}	–	14.1^{b}	–	94.5^{a}	–	695^{abc}	–
Pellet 7: WSU low protein	−3.6	–	69.6^{a}	–	72.2^{a}	–	87.7^{a}	–	13.1^{b}	–	33.6^{bc}	–	429^{cd}	–
Greenhouse sagebrush	−0.3	−0.4	68.3^{ab}	73.7^{a}	70.3^{ab}	74.6^{a}	77.4^{b}	82.4^{a}	55.6^{a}	61.4^{a}	65.3^{*abc}	26.2^{c}	857^{*a}	366^{b}
Outside sagebrush	−1.6	–	54.8^{cd}	–	57.2^{cd}	–	77.8^{b}	–	6.2^{b}	–	20.2^{c}	–	234^{d}	–

An asterisk denotes significant difference between PR and CT on each diet, and different letters denote significant differences among diets within a column ($\alpha = 0.05$).

WSU: Washington State University.

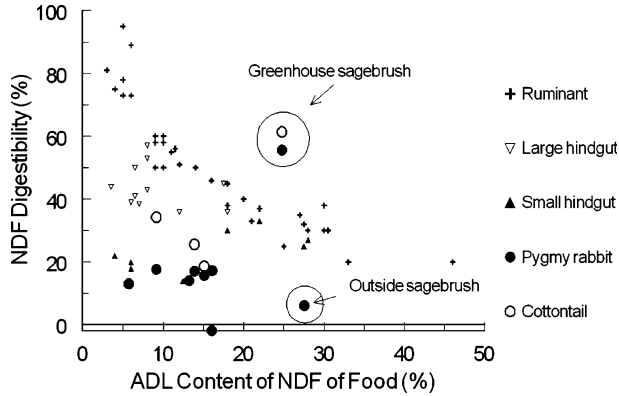

Fig. 5 Mean digestibility of neutral detergent fiber (NDF) of pygmy rabbits and cottontail rabbits as a function of the proportion of acid detergent lignin (ADL) in the cell wall of pelleted rations and sagebrush (*A. tridentata*, circled points) they consumed. Data for ruminants, large hindgut fermenters, and small hindgut fermenters are provided for comparison (Robbins, 1993)

Regardless of the amount of high- and low-quality pellets offered, pygmy rabbits voluntarily consumed more greenhouse sagebrush than cottontails relative to their metabolic body mass ($F = 5.72$, $P < 0.001$, Table 4). For example, pygmy rabbits ate five times more greenhouse sagebrush relative to their size than cottontails did when given *ad lib* access to both high-quality pellets and sagebrush (Table 4). However, sagebrush comprised only 8.1 ± 2.5% DM consumed by pygmy rabbits when offered with *ad lib* high-quality pellets, and 14.8 ± 2.5% when offered with *ad lib* low-quality pellets. Cottontails ate sagebrush as 0.7 ± 0.2% of their diet when eating high-quality pellets and 1.8 ± 0.8% when eating low-quality pellets. Both pygmy rabbits and cottontails ate more greenhouse sagebrush relative to their metabolic body mass when offered low-quality than high-quality pellets at 50% and 25% *ad lib* ($F = 37.85$, $P < 0.001$). For example, pygmy rabbits doubled their intake of sagebrush when offered 25% *ad lib* of low-quality pellets than when offered 25% *ad lib* of high-quality pellets (Table 4). Both rabbit species ate more greenhouse sagebrush as the amount of either type of pellet offered declined ($F = 30.22$, $P < 0.001$, Table 4).

When offered together with 50% *ad lib* intake of both high- and low-quality pellets, pygmy rabbits and cottontails ate a similar amount of greenhouse and outside sagebrush ($t < 6.48$, $P > 0.08$, Table 4). However, when no supplementary pellets were offered, pygmy

Table 3 Retention time on gas chromatograph-mass spectrophotometer (GC-MS), gas chromatograph (GC), % of dry plant tissue, and % of six major peak area of distilled oil of six terpenoid compounds found in sagebrush (*Artemisia tridentata*)

Compound name	Retention time on GC-MS (min)	Retention time on GC (min)	Outside sagebrush		Greenhouse sagebrush	
			% Dry mass	% Major peak area	% Dry mass	% Major peak area
Artemiseole	6.49	4.71	0.36	19	0.75	16
1,8-Cineole/eucalyptol	7.48	5.56	0.26	14	0.71	15
E-β-Santolina epoxide	7.63	5.90	0.07	4	1.02	21
Z-β-Santolina epoxide	7.72	5.99	0.12	7	1.05	24
Methyl santolinate	8.96	7.26	0.20	11	0.52	11
Camphor	9.32	7.36	0.91	45	0.54	12
Total major peaks	–	–	1.92	100	4.59	100
Total minor peaks	–	–	1.44	–	4.11	–
Total all peaks	–	–	3.36	–	8.70	–

Table 4 Dry mass ($\overline{X} \pm$ SE) of pellets, greenhouse sagebrush, or outside sagebrush (*Artemisia tridentata*) consumed by pygmy rabbits (PR, *Brachylagus idahoensis*) and cottontails (CT, *Sylvilagus floridanus*) when offered 4 different amounts of high- and low-quality pellets

Pellet type	Amount pellets offered	Amount sagebrush consumed (g/kg$^{0.75}$/d)									
		No sage		Greenhouse sagebrush (*ad lib*)				Outside sagebrush (*ad lib*)			
		PR	CT	PR		CT		PR		CT	
		Pellets	Pellets	Pellets	Sage	Pellets	Sage	Pellets	Sage	Pellets	Sage
No pellets	None	–	–	–	62.3 ± 6.8	–	26.2 ± 0.4	–	20.0 ± 5.8	–	–
High-quality pellet 1	*Ad lib*	69.5 ± 9.0	52.8 ± 1.0	61.1 ± 8.9	5.3 ± 1.9	44.3 ± 1.4	0.3 ± 0.1	–	–	–	–
	50% *ad lib*	–	–	29.5 ± 0.5	13.3 ± 1.3	21.5 ± 1.1	1.6 ± 0.4	30.1 ± 0.8	26.4 ± 0.7	21.1 ± 0.9	8.3 ± 2.1.
	25% *ad lib*	–	–	15.3 ± 0.4	30.4 ± 1.2	11.1 ± 0.7	4.0 ± 0.4	–	–	–	–
Low-quality pellet 2	*Ad lib*	106.0 ± 12.3	84.4 ± 6.1	67.4 ± 9.2	11.5 ± 0.8	71.3 ± 2.6	1.3 ± 0.5	–	–	–	–
	50% *ad lib*	–	–	32.6 ± 1.0	25.8 ± 0.8	35.5 ± 1.8	3.9 ± 1.2	38.9 ± 1.7	33.9 ± 8.3	36.8 ± 1.8	4.5 ± 2.2
	25% *ad lib*	–	–	18.1 ± 0.2	52.2 ± 64	18.0 ± 1.0	16.4 ± 1.5	–	–	–	–

rabbits ate three times more dry mass of greenhouse sagebrush than outside sagebrush (t = 5.01, P = 0.02, Table 4).

When consuming all mixed diets of pellets and sagebrush, pygmy rabbits acquired about twice the DEI of cottontails relative to their metabolic body mass (all $F < 12.77$, all $P < 0.02$; Fig. 6). By voluntarily eating more greenhouse sagebrush, both pygmy rabbits and cottontails achieved a higher total DEI when eating mixed diets (at 50% and 25% *ad lib*) including low-quality pellets than those that included high-quality pellets (both $F > 10.49$, both $P < 0.02$). As the DEI of pellets decreased, so did the total DEI of cottontails (F = 25.77, $P < 0.001$) when offered greenhouse sagebrush *ad lib*. However, pygmy rabbits ate enough greenhouse sagebrush to maintain their total DEI as their DEI of pellets varied from 100% to 0% of their diet (F = 4.26, P = 0.11; Fig. 6). However, when pellet intake was restricted to 25–50% *ad lib*, pygmy rabbits voluntarily ate enough sagebrush to meet between 67% and 110% of their requirements, and met only 30% of their requirements when eating outside sagebrush alone. In contrast, cottontails only met their daily DE requirements when given *ad lib* access to low- and high-quality pellets alone and with *ad lib* access to sagebrush (Fig 6). Cottontails met only 67% of their daily energy requirements on greenhouse sagebrush alone, and when pellets were restricted to 25–50% *ad lib*, they only ate enough sagebrush to meet 35–67% of their DE requirements. However, mass gain during the mixing trails was not consistently related to pellet type, pellet amount, sagebrush type, or rabbit species (F = 2.05, P = 0.11).

When offered *ad lib* access to both greenhouse and outside sagebrush together with 15% of their *ad lib* intake of high-quality pellets, pygmy rabbits ate five times more leaves of greenhouse ($\overline{X} = 50.82 \pm 3.2$g) than outside sagebrush ($\overline{X} = 10.35 \pm 1.4$g, t = 6.73, P = 0.003). Pygmy rabbits ate the same proportion of leaves and stems of greenhouse and outside sagebrush (t = 1.52, P = 0.20).

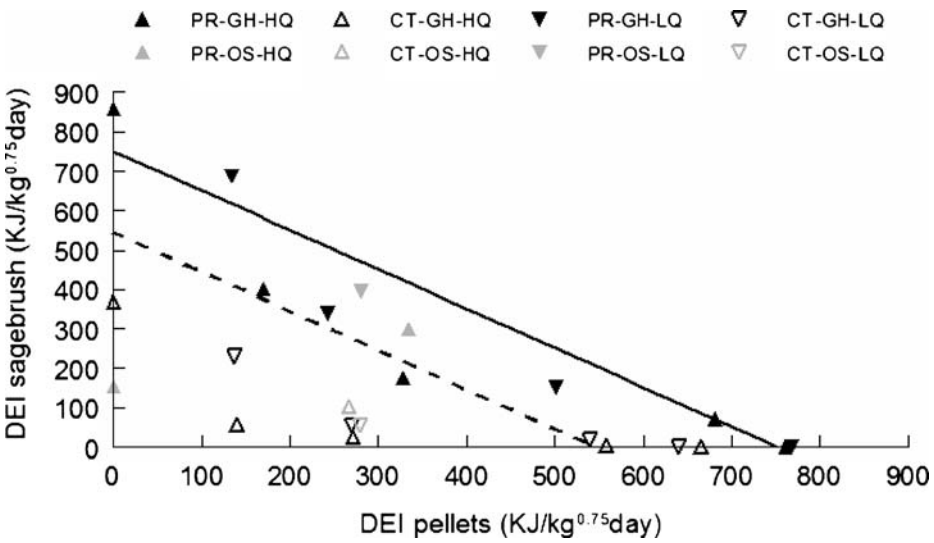

Fig. 6 The amount of digestible energy (DEI) of greenhouse (GH) and outside (OS) sagebrush (*Artemisia tridentata*) voluntarily consumed by pygmy rabbits (PR) and cottontails (CT) each day when offered different amounts of a high-quality (HQ) or low-quality (LQ) grain-alfalfa pelleted. The diagonal lines indicate the amount of additional energy in sagebrush pygmy rabbits (solid line) and cottontail rabbits (dashed line) would have to consume to meet their daily energy requirements and maintain body mass when eating pellets

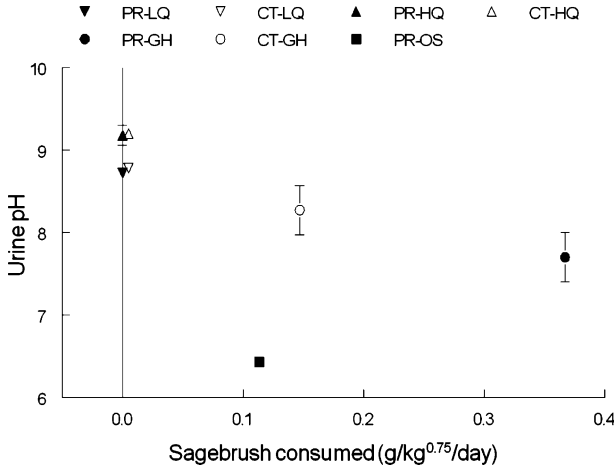

Fig. 7 pH of urine when in relation to the dry mass of greenhouse (GH) and outside (OS) sagebrush (*Artemisia tridentata*) eaten by captive pygmy rabbits (PR) and cottontail rabbits (PR) when offered *ad libitum*, and when consuming diets of high-quality (HQ) or low-quality (LQ) pelleted grain-alfalfa offered *ad libitum*

pH of Urine

When consuming high-quality pellets, low-quality pellets, and greenhouse sagebrush alone *ad lib*, pygmy rabbits and cottontails had the same urine pH ($F = 2.66$, $P = 0.13$), even when including DMI of sagebrush as a covariate ($F = 0.06$, $P = 0.53$; Fig. 6). Both had a higher urine pH when eating high-quality pellets than low-quality pellets, and a lower urine pH when eating only greenhouse sagebrush than either pellet ($F = 27.38$, $P < 0.001$; Fig. 7). Despite eating about half the total mass of outside sagebrush, the urine pH of pygmy rabbits was lower when eating outside sagebrush than when eating greenhouse sagebrush ($t = 5.0$, $P = 0.01$; Fig. 7).

Discussion

Pygmy rabbits had relatively high energy and low protein requirements. Daily energy expenditure (i.e., basal metabolic rate + activity + thermoregulation + heat increment, kJ/d) tends to scale as 591.6 kJ/kg$^{0.75}$ for eutherian mammals (Robbins, 1993). When living in small cages, the daily energy requirement of our 1.2-kg cottontails (549.2 kJ/kg$^{0.75}$/d) was consistent with this scaling, and consistent with Rose, (1973), who found energy requirements of eastern cottontails to range from 531–607 kJ DE/kg$^{0.75}$/d when ambient temperature ranged from 10°C to 20°C. Likewise, snowshoe hares (*L. americanus*, 1.5 kg) needed only 510 kJ/kg$^{0.75}$ (Holter et al., 1974) to maintain their body mass. In contrast, pygmy rabbits (0.45 kg) required DE of 750.8 kJ/kg$^{0.75}$/d to maintain their mass in small cages, 27% more than predicted from the scaling relationship for eutherian mammals, and 36% higher per unit metabolic mass than eastern cottontails. Similarly, when measuring oxygen consumption of pygmy rabbits under different thermal conditions, Katzner et al. (1997b) found that pygmy rabbits had a higher resting metabolic rate than other eutherian mammals relative to metabolic body mass. McNab, (1988) showed that small herbivores such as lagomorphs tend to have higher basal metabolic rates relative to their size than other mammals, and that energy requirements for small folivores scale with body mass$^{0.545}$. The lower critical temperature for pygmy rabbits is 15–20°C (Katzner et al., 1997b); thus they were always expending 18% more energy than they would at thermal neutrality during our

feeding trials, which were conducted at 0–5°C. Because of their larger size, the thermal neutral zone of cottontails is predicted to be two times wider than that of pygmy rabbits (Katzner et al., 1997b); thus cottontails may not have had to expend as much energy to keep warm in our trials.

Not only do pygmy rabbits have a higher energy requirement relative to body mass than similar mammals, their behavior and habitat demand that they acquire additional energy from their food, especially in winter. They do not hibernate, and they live in cold, exposed sagebrush-steppe rangelands in the northwestern United States. Additional activity and cold temperatures increases the energy expenditure of cottontails by 30–60% (Rose, 1973) and pygmy rabbits by up to 60% (Katzner et al., 1997b). Sagebrush, despite its terpene concentration, has a high DE content, especially relative to grass, one of the few other foods available in the winter. For example, NDF content of two common grass species in pygmy rabbit habitat in the Columbia Basin of Washington ranged from 67% in summer to 78% in winter, whereas sagebrush in the same area contained only 36–47% NDF (Thines et al., 2004). However, the detoxification of terpenes and other PSMs in sagebrush likely increases energy demands further, as evidenced by the high energy content of urine while eating sagebrush. Sorensen et al., (2005) found that woodrats ate more, increased energy intake, and reduced their basal metabolic rate and activity when eating high-terpene juniper diets. Therefore, many herbivores that specialize on plants with high levels of PSMs have developed energy conservation methods, such as low reproduction (e.g., woodrats, *Neotoma* spp.; Meyer and Karasov, 1991) and low metabolic rates, requiring them to eat less (e.g., arboreal marsupials, Cork and Foley, 1991). On the other hand, the small body size of pygmy rabbits is advantageous in eliminating PSMs from the body. The steady-state plasma concentration of toxins scales with body mass$^{0.39}$; thus, small animals maintain lower concentrations of toxins in their bodies than large animals and can ingest more relative to their size (Freeland, 1991). Therefore, the size of pygmy rabbits, the smallest leporid in North America, may be an adaptation to excreting toxins, thus allowing them to consume large amounts of sagebrush.

Although pygmy rabbits have a relatively high energy requirement, they seem to have a lower N requirement (306.5 mg N/kg$^{0.75}$/d) than many other mammals. Their N balance falls below the mean for eutherian mammals (582 ± 235 mg/kg$^{0.75}$/d; Robbins, 1993), such as blacktailed jackrabbits (950 mg/kg$^{0.75}$/d; Nagy et al., 1976). However, herbivorous marsupials that specialize on terpene and tannin-containing foods, such as ringtail possums (290 mg/kg$^{0.75}$/d) and koalas (275 mg/kg$^{0.75}$/d), have similar N requirements to pygmy rabbits (Robbins, 1993).

Pygmy rabbits have an MFN of 7.5 g N/kg feed, which is within the normal range for mammals (1–9 g N/kg feed; Robbins, 1993). This MFN is also within the range found in other lagomorphs such as blacktailed jackrabbits (4.6 g N/kg feed; Nagy et al., 1976), domestic rabbits (*Oryctolagus cuniculus*, 8.0 g N/kg feed; Slade and Robinson, 1970), and snowshoe hares (9.1 N/kg feed; Holter et al., 1974). EUN was 22.9. mg/kg$^{0.75}$/d, lower than that of the average nonruminant eutheran mammal (160 ± 22 mg N/kg$^{0.75}$/d), such as blacktailed jackrabbits (128 mg/kg$^{0.75}$/d; Nagy et al., 1976). However, the lowest excretion of urinary N we measured on pygmy rabbits eating low N diets was 97 mg/kg$^{0.75}$/d (Fig. 3). Thus, the standard method for estimating EUN may have underestimated EUN for pygmy rabbits.

When eating more than 9 g DM/d, pygmy rabbits require a diet of at least 7% CP, which is similar to the minimum dietary protein requirements of nonruminant eutherians and browsing ruminants (Robbins, 1993). The National Research Council, (1977) recommends that domestic rabbits be fed a diet with a CP content (6.25 N) of 13–18% for maintenance,

and a dietary level of 16% CP is considered adequate for growth and reproduction. Sagebrush in the greenhouse (18%), outside (15.6%), and in pygmy rabbit habitat in the Columbia Basin (9.5–11.4%; Thines et al., 2004) had at least twice the CP content of grasses (3.6–5%) available in native habitat (Thines et al., 2004.), and is adequate to meet the requirements for domestic and pygmy rabbits.

The smaller pygmy rabbits generally digested foods less efficiently than the larger cottontails, large hindgut fermenters, and ruminants (Fig. 5). Because smaller herbivores have a higher metabolic rate relative to their fermentation capacity (i.e., size of ceca), they have a faster throughput of ingesta and less efficient digestion of plant fiber (Demment and Van Soest, 1985). Fiber digestibility of pygmy rabbits and cottontails in our study was generally consistent with that of European rabbits (*O. cuniculus*; Kuijper et al., 2004) and snowshoe hares (Holter et al., 1974).

Despite terpenes in sagebrush, both pygmy rabbits and cottontails digested the NDS of sagebrush and pelleted diets equally, and pygmy rabbits digested the NDS of greenhouse and outside sagebrush similarly (Table 2). Furthermore, both digested the NDF of greenhouse sagebrush better than the pelleted diets without terpenes or other PSMs, even relative to the indigestible portion of the cell wall (Table 2, Fig. 5). Outside sagebrush was poorly digested, yet had a lower content of terpenes overall. Because sagebrush did not precipitate BSA, the biological activity of any condensed tannins in sagebrush was likely too low to affect digestibility. These findings suggest that terpenes and other PSMs in sagebrush do not reduce fiber or soluble digestion in either rabbit species, and that pygmy rabbits, which "specialize" on sagebrush, do not seem to have developed a mechanism for digesting this high-terpene food better than the generalist cottontail. Specific monoterpenes are known to be toxic to rumen microorganisms *in vitro* at 0.7–1.3 µl/ml and reduce *in vitro* and *in situ* digestibility (Schwartz et al., 1980; Welch and Pederson, 1981). However, nearly 80% of terpene concentration of sagebrush is lost by the time it reaches the stomach of pygmy rabbits (White et al., 1982b) and the rumen of mule deer (Cluff et al., 1982), likely through evaporative losses during chewing and eructation. Terpenes may also be absorbed into the bloodstream from the digestive system before they can influence digestibility of forages. Therefore, concentrations of monoterpenes in the digestive system of pygmy rabbits feeding on sagebrush are likely not high enough to reduce digestibility (White et al., 1982b; Gershenzon and Croteau, 1991).

On the other hand, urine pH of both cottontails and pygmy rabbits declined and urinary energy increased when sagebrush was added to the diet, indicating that consuming sagebrush incurs a metabolic cost to rabbits. However, the specialist pygmy rabbit did not have a consistently higher urine pH or lower urinary or fecal energy than the generalist cottontail, suggesting that pygmy rabbits may not have an increased capacity to avoid or metabolize terpenes or other PSMs. They had a lower urine pH when consuming solely outside sagebrush, which had a lower overall monoterpene content but a higher content of camphor, which is known to deter feeding by lagomorphs eating conifers (Rodgers et al., 1993). Outside sagebrush may also have had higher levels of unmeasured PSMs.

Both pygmy rabbits and cottontails were able to meet their DE requirements by increasing their DM intake of pellets as the nutritional quality of pellets declined. To do this, pygmy rabbits consumed more relative to their body mass than cottontails did, increasing DM intake from 9.6% of body mass on high-quality pellets to 14.0% on low-quality pellets, whereas cottontails increased from 5.3% to 8.2% of their body mass when shifting from high- to low-quality pellets (Table 2). When the amount of either type of pellet was restricted, both chose to eat more sagebrush, but cottontails did not consume

enough additional sagebrush to meet their energy requirements (Fig. 6). Regardless of the amount and quality of pellets offered, pygmy rabbits ate more sagebrush overall, and in relation to energy requirements, than did cottontails. However, when allowed free access to pellets containing a DE content similar to sagebrush, they selected pellets over sagebrush, which comprised only 8% of their diet. Under the same conditions, sagebrush comprised only 0.7% of the cottontails' diets. When offered low-quality pellets containing only half the protein and 60% of the DE content of sagebrush, pygmy rabbits increased their use of sagebrush to 15%, and cottontails to 1.8%, but sagebrush still did not make up a substantial portion of the diet of either rabbit. Likewise, Stephen's woodrats, a terpene specialist, consumed twice as much juniper (27% of diet) as did whitethroat woodrats (*N. albigula)*, a generalist (10%), when given *ad lib* access to a control diet with a low N content (1.25%) and high ADF (23%) similar to juniper (Dearing et al., 2000).

Like our captive rabbits, wild pygmy rabbits in native habitats expand their use of sagebrush from 11–50% in early summer, when alternative forages are more available and of higher nutritional quality, to 72–98% in the winter, when grasses and forbs are rare and less nutritious (Green and Flinders, 1980b; Thines et al., 2004). In contrast, domestic lambs (generalist foragers) actually ate more (Villalba and Provenza, 2005) or the same amount (Burritt et al., 2000) of a terpene-containing food as the energy and protein content of an alternative food increased. Therefore, the presence of high-quality foods in the environment may either decrease or increase diet breadth used by generalist and specialist herbivores.

Despite the lower fiber content of the outside sagebrush, pygmy rabbits chose to eat less of it when offered alone and when offered with 50% *ad lib* high- and low-quality pellets than of greenhouse sagebrush. In addition, pygmy rabbits ate five times more greenhouse sagebrush than outside sagebrush when they were simultaneously offered. Pygmy rabbits may have preferred greenhouse sagebrush because it contained nearly twice the protein content and was more digestible, despite its higher fiber content (Tables 1 and 2). Although the greenhouse sagebrush had higher monoterpene levels overall than outside sagebrush, and had higher levels of all specific terpene compounds identified except camphor, the specific effects of these terpenes and other PSMs on preference and digestion in pygmy rabbits is unknown. White et al., (1982a) found no relationship between total monoterpenoid content and dietary preference of sagebrush by pygmy rabbits, but pygmy rabbits did prefer plants from individual populations, and preference was negatively correlated with the monoterpene α-thujone (not found in our sagebrush samples). However, urine pH was lower when our pygmy rabbits ate solely outside sagebrush than solely greenhouse sagebrush, suggesting that the terpenoid compounds or other PSMs in the outside sagebrush required more detoxification, and thus may have been a less desirable food choice. Similarly, snowshoe hares preferred mature white spruce (*Picea glauca*) over juvenile white spruce that contained four times more camphor, and camphor added to pellets deterred feeding by captive hares (Sinclair et al., 1988).

The terpenoid content of both types of sagebrush we fed our pygmy rabbits likely differed to some degree from sagebrush consumed in their native habitat. Monoterpene concentration in sagebrush varies greatly with climate, soils, age, season, genetics, and plant part (Welch and McArthur, 1981; Gershenzon and Croteau, 1991; Zhang and States, 1991). Total terpene content measured in wild sagebrush varies from 0.37% to 3.7% DM, and the composition of terpenoids, such as α-thujone, camphor, α-pinene, 1,8-cineole, β-thujone, and terpineol, differs among populations, individuals, and plant parts (Welch and McArthur, 1981; Cluff et al., 1982; White et al., 1982a). For example, camphor content of oils distilled from sagebrush varies from 0% to 70.3% dry mass (Welch and McArthur, 1981). The reproductive structures and new growth, which we fed to pygmy rabbits in our

experiments, generally have higher levels of monoterpenoids than older, structural components (Gershenzon and Croteau, 1991). Our greenhouse sagebrush was fertilized with N, whereas the outside sagebrush was not, which might explain, in part, the higher N content and the different terpenoid composition (Gershenzon and Croteau, 1991). Similarly, when white spruce was grown in a nursery, it had about 30% less camphor than wild white spruce seedlings (Rodgers et al., 1993). Snowshoe hares selectively ate more nursery-grown white spruce than naturally regenerated spruce seedlings, and in most cases avoided the naturally regenerated spruce all together. Plants growing in low light and poor soil are expected to invest in carbon-based defenses, whereas those grown in fertile soils and high light (e.g., greenhouses) are more likely to invest in N-based defenses (Rodgers et al., 1993).

Because pygmy rabbits sacrificed protein and DE in their diet when they selected low-quality pellets over sagebrush in our experiments, and because wild pygmy rabbits decreased their use of sagebrush when other nutritious forages were available in the spring (Thines et al., 2004), pygmy rabbits do not seem to be constrained by the type of obligatory relationship with sagebrush that animals like koalas have with *Eucalyptus* spp. (Marsh et al., 2003). Sagebrush clearly exacts a cost to its specialist forager. However, because pygmy rabbits ate more sagebrush than cottontails and were able to meet their DE requirements while feeding exclusively on sagebrush, they have likely adapted mechanisms to keep the costs of ingesting terpenes relatively low. Adaptations for dealing with PSMs include avoiding ingesting them, reducing their absorption from the gut, and detoxifying them in the liver (McArthur et al., 1991).

Nonspecialist and specialist foragers alike often avoid PSMs by carefully selecting individual plants and plant parts (Zhang and States, 1991; Lawler et al., 1998; Dearing et al., 2000; Marsh et al., 2003). For example, small herbivores such as mountain hares (*L. timidus*), black-tailed jackrabbits, and woodrats often discard young leaves and eat the stems of plants, which have lower concentrations of PSMs but also have lower N and DE content (Gershenzon and Croteau, 1991; Meyer and Karasov, 1991; Palo et al., 1992). Pygmy rabbits ate leaves and stems in proportion to availability when eating greenhouse and outside sagebrush, but when given the opportunity during pretrials, we noticed that cottontails tended to eat stems and discard leaves of sagebrush.

Specialist herbivores may have more transporter proteins or transporter proteins more specific to a particular PSM, which move PSMs out of cells and into the intestinal lumen, thus reducing absorption of PSMs into the circulation (Sorensen et al., 2004). For example, the specialist Stephen's woodrat had lower blood levels of α-pinene, a terpene in juniper, and excreted 40% more in the feces than did the generalist whitethroat woodrat (Sorensen and Dearing, 2003; Sorensen et al., 2004). If absorbed, PSMs can be eliminated from the bloodstream through enzymatic reactions in the liver that add a functional group or conjugate the PSM to change its solubility (McArthur et al., 1991; Dearing et al., 2005). However, even specialist herbivores that encounter PSMs in their normal diet have a threshold, albeit higher than the generalists, to the amount of PSMs that can be processed and will not voluntarily ingest terpenes above a crucial daily amount (Boyle et al., 1999; Marsh et al., 2003; Boyle and McLean, 2004). This threshold is caused, in part, by the finite tolerance of the kidney for excreting acidic metabolites produced during detoxification (Freeland and Janzen, 1974; Foley, 1992; McLean et al., 1993; Foley et al., 1995). Therefore, urine pH is a general indicator of overall detoxification processes. Dearing et al., (2000) found that urine pH declined from about 8.7 when a generalist and specialist woodrat consumed a nonterpene control diet, to about 7.7 on a juniper diet. The specialist, which had a slightly higher pH when consuming juniper, may have produced metabolites that were less acidic, or were able to buffer the acidic metabolites better than the generalist. In our study,

urine of both pygmy rabbits and cottontails declined to a similar degree from a nonterpene pelleted diet to a sagebrush diet (Fig. 7). However, specialist pygmy rabbits did not maintain a higher urine pH on greenhouse sagebrush than generalist cottontails did.

In summary, pygmy rabbits have a relatively high metabolism and moderately low N requirement, and can acquire high levels of DE from sagebrush that dominates their natural habitat. However, their low voluntary intake of sagebrush in the presence of low-quality non-terpene foods and their increased excretion of acids and energy in the urine when consuming sagebrush suggests that consuming sagebrush exacts a cost even for this "specialist". However, because pygmy rabbits voluntarily consumed more sagebrush than cottontails regardless of the quantity and quality of supplementary foods, these small rabbits likely detoxify the monoterpenes and other PSMs better than the generalist rabbit species that overlap their range. Any "obligate-like" relationship between pygmy rabbits and their deep soil sagebrush habitat (Green and Flinders, 1980b) likely occurs not only because pygmy rabbits are better competitors for sagebrush as forage, but also because this habitat provides cover suitable for thermoregulation and hiding (Katzner and Parker, 1997a; Katzner et al., 1997b), and reproductive habitat suitable for digging natal burrows (Rachlow et al., 2005). Future studies examining behavioral and physiological responses to individual monoterpenes and other PSMs in sagebrush and detoxification pathways will enhance our understanding of the tolerances and requirements of this unique rabbit.

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