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CREOSOTE BUSH (*Larrea tridentata)* RESIN INCREASES WATER DEMANDS AND REDUCES ENERGY AVAILABILITY IN DESERT WOODRATS (*Neotoma lepida*)

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Abstract—Although many plant secondary compounds are known to have serious consequences for herbivores, the costs of processing them are generally unknown. Two potential costs of ingestion and detoxification of secondary compounds are elevation of the minimum drinking water requirement and excretion of energetically expensive metabolites (i.e., glucuronides) in the urine. To address these impacts, we studied the costs of ingestion of resin from creosote bush (*Larrea tridentata*) on desert woodrats (*Neotoma lepida*). The following hypotheses were tested: ingestion of creosote resin by woodrats (1) increases minimum water requirement and (2) reduces energy available by increasing fecal and urinary energy losses. We tested the first hypothesis, by measuring the minimum water requirement of woodrats fed a control diet with and without creosote resin. Drinking water was given in decreasing amounts until woodrats could no longer maintain constant body mass. In two separate experiments, the minimum drinking water requirement of woodrats fed resin was higher than that of controls by 18–30% (about 1–1.7 ml/d). We tested several potential mechanisms of increased water loss associated with the increase in water requirement. The rate of fecal water loss was higher in woodrats consuming resin. Neither urinary water nor evaporative water loss was affected by ingestion of resin. Hypothesis 2 was tested by measuring energy fluxes of woodrats consuming control vs. resin-treated diets. Woodrats on a resin diet had higher urinary energy losses and, thus, metabolized a lower proportion of the dietary energy than did woodrats on control diet. Fecal energy excretion was not affected by resin. The excretion of glucuronic acid represented almost half of the energy lost as a consequence of

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resin ingestion. The increased water requirement and energy losses of woodrats consuming a diet with resin could have notable ecological consequences.

Key Words—Woodrats, creosote resin, secondary compounds, herbivores, water requirement, osmolarity, urine, metabolizable energy, glucuronic acid, detoxification.

INTRODUCTION

The potential consequences of ingestion of plant secondary metabolites (PSMs) are numerous. Herbivores consuming diets with PSMs may exhibit decreased digestibility, alteration of the central nervous system, and decreased reproduction among others (Freeland and Janzen, 1974; Haukioja, 1980; Meyer and Karasov, 1989; Belovsky and Schmitz, 1991; Bryant et al., 1992; Silverstein, et al., 1996). However, the effect of PSM ingestion on water balance of herbivores has been largely ignored (Dearing et al., 2001). Previous studies (Mangione et al., 2000; Dearing et al., 2001, 2002) implied that ingestion of PSMs increases water flux in desert herbivores. For example, desert woodrats increased water intake by 50–76% with increasing levels of dietary toxin (Mangione et al., 2000). If this increase in water consumption is obligatory, it doubles the normal minimum requirement of preformed water for desert woodrats consuming dietary toxins (Karasov, 1989). The consequences of an increase in water consumption could have significant implications for diet selection by herbivores whose only source of preformed water is that in plants.

Besides affecting water balance, PSMs may impact energy availability. There are three ways that PSMs may reduce energy availability. First, they can dilute the available energy in food, as they typically cannot be used as a source of energy (Jakubas et al., 1993b). Second, they may retard the extraction of available food energy. Decreased energy extraction can occur if PSMs inhibit digestive enzymes or bind to food components such that they cannot be digested or absorbed, as may occur for tannin–protein complexes (Glick and Joselyn, 1970a,b; Lindroth et al., 1984; Robbins et al., 1987a,b, 1991). Lastly, they can cause postingestive energy loss through increased energy metabolism during detoxification (Iason and Murray, 1996), excretion of conjugation molecules (Dash, 1988; Remington, 1990; Guglielmo et al., 1996), or excretion of endogenous materials such as salivary proteins (Dietz et al., 1994; McArthur et al., 1995). These effects are measurable as reductions in the digestibility or metabolizability of energy. Regardless of the exact mechanism, reductions in dietary energy availability can compromise the energy balance of herbivores ingesting PSMs.

In this study, we examined how ingestion of plant toxins affects energy availability and water requirements of a herbivorous rodent. We focused on woodrats (*Neotoma lepida*) from the Mojave desert because they feed on creosote (*Larrea tridentata*), renowned for its toxic secondary chemistry (Cameron and Rainey,

1972; Karasov, 1989). Leaves of creosote contain between 10 and 25% phenolic resin (by dry mass), 40% of which is NDGA (nordihydroguaiaretic acid), a well-documented toxin to mammals (Grice et al., 1968; Goodman et al., 1970; Mabry, et al., 1977; Sheikh et al., 1997). The remainder of the resin is a complex mixture of partially O-methylated flavones and flavonols (Rhoades, 1977). Water content of creosote in the Mojave desert ranges from about 0.5 to 1.0 ml/g dry mass, depending on season, with water content being highest in spring following winter rains and lowest in fall just prior to winter rains (Nagy et al., 1976; Schmidt-Nielsen, 1979; Karasov, 1989).

We tested the hypothesis that ingestion of PSMs increases the minimum water requirement of herbivores. We predicted that herbivores eating a diet with PSMs would require more drinking water to maintain body mass than those consuming the same diet without toxins. We investigated the following potential causes of increased water loss as a result of dietary PSMs: i) increase in osmotic load due to an increase in either food intake or organic acid load as a result of detoxification and excretion of PSM metabolites in urine; ii) a diuretic effect of the resin resulting in higher urine water content; iii) an increase in fecal water content; or iv) an increase in evaporative water loss rate as might occur if metabolic rate increased. Components of water influx and efflux were determined in desert woodrats drinking the minimum water necessary for body mass maintenance in the presence and absence of creosote resin in the diet.

We also tested whether creosote resin decreases energy digestibility and metabolizable energy. In previous studies, Meyer and Karasov (1989) demonstrated that the phenolic resin from creosote leaves had no effect on either dry matter digestibility or nitrogen digestibility. Although this result suggests that creosote resin does not function as a digestibility reducer as proposed by Rhoades (1977), energy digestibility was not measured. Also, the resin might increase energy losses postabsorption, due to detoxification of PSMs. Woodrats significantly increase the amount of detoxification products (e.g., glucuronides and sulfates) in the urine after the ingestion of creosote resin (Mangione et al., 2001). Losses of glucose, glycine, and sulfate moieties used in conjugation could reduce the energy available to woodrats. We predicted that metabolizable energy would be lower in animals fed resin-treated diets than in control diets. We compared the energy fluxes of desert woodrats on diets with and without creosote resin.

METHODS AND MATERIALS

Field Site and Sample Collection. Desert woodrats were trapped at Beaver Dam, Grand Co., UT (37°06′N, 113°58′W). The vegetation at Beaver Dam was primarily composed of creosote bush, black brush (*Coleogyne ramosissima*), Joshua

tree (*Yucca brevifolia*), desert almond (*Prunus fasciculata*), and, less commonly, cholla (*Opuntia spp.*).

Woodrats were captured between April 10 and 12, 1996, using Tomahawk and Sherman live traps baited with peanut butter and oats (see Mangione et al., 2000, for details). All woodrats were transported to the animal facility at the University of Utah and kept in quarantine closets for 2–5 m while they were determined to be free of Sin Nombre hantavirus (Dearing et al., 1998) and then were transported to the Department of Wildlife Ecology, University of Wisconsin, Madison, WI. Ten kilograms (wet mass) of creosote leaves were collected on April 11, 1996, at Beaver Dam from a stand of creosote near the trapping areas. A mixture of young and mature foliage was clipped from stems not bigger than 0.3 cm in diam. The foliage was placed onto dry ice and kept at -20°C until the resin was extracted.

Animal Housing and Diet Preparation. All feeding trials were conducted at the University of Wisconsin – Madison. The experimental protocols were approved by the Research Animal Resources Center (RARC), University of Wisconsin – Madison. Woodrats were housed in metal cages ($47 \times 30 \times 21$ cm) with screened bottoms. Animals were provided with cotton bedding and a ceramic bowl in which to nest. The room was kept at 21°C and 65% relative humidity on a 12:12 L/D cycle. When not involved in experiments, woodrats were provided water *ad libitum*, high-fiber rabbit chow *ad libitum* (Harlan Teklad 8630, Wisconsin), and occasionally apples.

Resin from creosote leaves was extracted by soaking the leaves and stems in diethyl ether (1 part wet leaves: 5 parts solvent) for 45 min. The ether solution was filtered through Whatman N$^{\circ}$4 filter paper, poured into beakers in a water bath at 40°C, and the ether removed by boiling for 2–4 hr until the filtrate reached a dense and viscous consistency. The resin was stored at -25°C for up to 7 m prior to use.

The resin diet was prepared by adding 3.7 g of resin to 25 ml of 95% ethanol per 100 g of ground rabbit chow. Results from other experiments indicated that this concentration was near the maximum tolerable amount that adult woodrats would ingest without losing $>10\%$ body mass (Mangione et al., 2000). The resin/ethanol solution and the chow were thoroughly mixed, and dried overnight (room temperature) until the ethanol was evaporated (confirmed gravimetrically). Control and resin-treated diets were pelleted and stored in the freezer until used. Because both heat and water can alter the properties of phenolics in diets (Price et al., 1980) and alter diet palatability (Dietz et al., 1994; Lindroth et al., 1984), we blanketed the pellet machine with a plastic bag filled with crushed ice to minimize heating during pelleting. The amount of water used to make the pellets (both control and resin-treated diets) was minimized by adding 7–10% (volume solution/mass of food) of a 60% ethanol solution. The final pellets were dried overnight to constant mass at room temperature and then stored in the freezer until use.

EXPERIMENTAL DESIGN

Water Requirement. Two experiments were performed to determine whether resin consumption resulted in an increase in minimum water requirement and to ensure that the urine collection method used in the first experiment yielded reliable results.

Experiment 1. Minimum water requirement of woodrats ($N = 6$) was determined for each of the following sequential treatments; resin-free diet, resin-containing diet, and resin-free diet (hereafter called control #1, resin, and control #2, respectively). Woodrats were given water and food *ad libitum* for 1 m between treatments. To determine the minimum water requirement, the water ration (measured daily to ± 0.1 g; density of water = 1 ml/g) was reduced gradually over a 3-wk period until the woodrat could not stabilize its body mass at a lower water ration. Specifically, at the beginning of each trial, water was offered *ad libitum*, and on the 4th day it was reduced to 60% of *ad libitum*. When a reduction in body mass (measured daily to ± 0.1 g) was detected, the ration offered the previous day was maintained until body mass stabilized for at least 2 d. At this point, a new reduction (20%) of water was imposed. If a woodrat lost body mass (2% or more) for more than 4 d, the ration was increased slightly (<0.5 ml/d) and body mass was checked again daily. When body mass stabilized for at least 2 d, the ration was reduced again to test if the woodrat would lose body mass and to ensure that the ration was, in fact, the minimum water required to maintain body mass. Considering the sensitivity of our measures of water and animal mass (± 0.1 g), we think that our precision in measuring minimum water requirement is <0.5 g/d.

A detailed example showing body mass fluctuations with water offered over time is given in Figure 1. In this example, on day 4, available water was decreased from 12 to 6.5 ml. This resulted in a rapid decline in body mass of approximately 5.5% from initial. Therefore, on d 5, available water was increased to 7 ml to reduce the continual weight loss. By d 9, body mass was reasonably stable so the available water was decreased to $\sim$5.4 ml/d. This decrease caused a continual decline in body mass, necessitating two increases of 0.5 ml/d on ds 12 and 14 to stabilize body mass. During these periods, increments or reductions in water of only 0.1 ml/d were enough to cause parallel changes in body mass. Thus, the minimum water requirement for this animal was determined to be 5.8 ml/d.

Once at minimum water, components of water influx and efflux were measured for 7 d (see bracket on Figure 1). During this period, food, water intake, and body mass were measured daily. Woodrats were restricted to a portion ($16 \times 19 \times 20$ cm) of their cage that permitted the separate collection of urine and feces. Pilot experiments showed that there were no differences in food intake when woodrats were in either section of the cage. Urine, uncontaminated by food

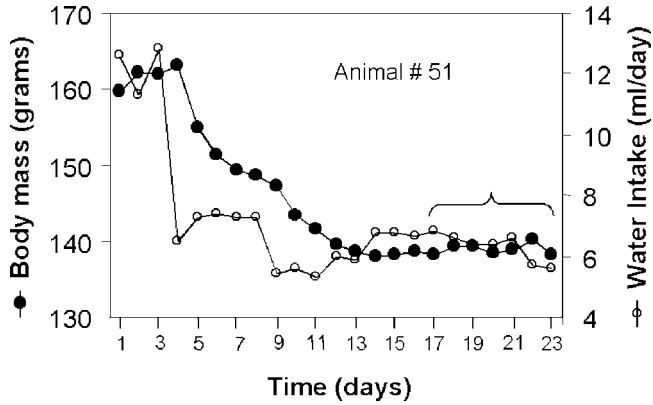

FIG. 1. A representative example of the relationship between available water and body mass (from experiment 1). Bracket shows the period for which water intake, food intake, body mass, and fecal output were measured daily for calculations.

and feces, was separately collected in plastic vials on ice to minimize bacterial growth and evaporation. All feces and urine were collected and dried. Feces were dried in the oven at 65°C to constant mass. Urine samples used for energy content quantitation were lyophilized. For calculation of total urine volume excreted, the small amounts of dry urine that adhered to the funnels and the bottom of the cage were collected with a hot distilled water rinse and added to the rest of the liquid urine collected. Fecal and urinary water losses were calculated as the product of the dry mass excreted multiplied by the respective water content measured in freshly collected samples. To determine the fecal water content, fresh feces were collected periodically during the night and day throughout experiment 1. Metabolizable energy of dry mass ingested was calculated from the results shown below for metabolizable energy coefficients. Evaporative water loss was calculated at the end of the experiment by measuring rates of body mass change during the last 12 hr of a 24-hr fast. In a fasting mammal whose respiratory quotient is approximately 0.7, mass change due to respiratory loss of CO_2 is approximately balanced by a greater retention of O_2, which is incorporated into metabolic water. Thus, any mass decrease is due largely to evaporation or urinary and fecal loss. Urine or feces were collected at 3-hr intervals in a tared container with mineral oil. This period gives enough time to obtain samples, but not enough to allow feces to disintegrate in the oil.

Experiment 2. A month and a half after completion of experiment 1, a second experiment was performed to corroborate the findings on water requirements. This experiment used a crossover design to block more effectively for any potential effect of time, and to test for possible overlooked urinary losses in the collection

system used in experiment 1. Initially, 6 woodrats were randomly divided into 2 groups, control and resin. Minimum water required was determined for each group following the procedure described in experiment 1. Woodrats were allowed to recover for approximately a month before being switched to the other treatment. At the end of each trial, food intake rate, body mass, and urine output were measured daily for 4 d. To minimize possible evaporation or losses of water from the system, urine was collected under mineral oil into a tray located 1 cm beneath the cage. Urine volume was measured in a graduated cylinder. Feces were collected in nylon mesh located 1 cm beneath the steel mesh of the cage floor. Even though an effort was made to collect fresh feces to determine the water flux by this route (as in experiment 1), the samples were incomplete. At the end of the first run in experiment 2, one of the woodrats was euthanized because it abruptly lost ~37% of body mass. Thus, the final sample size was 5 animals.

In both experiments, water intake was measured by weighing water bottles and correcting for both evaporation and spillage to the nearest 0.1 g. Spillage, ~0.3 ml/d, was determined by measuring the volume of water that dripped daily into a container with mineral oil. Dry matter intake was measured as the difference between what was given and what remained each day after the samples were dried at 50°C for 3 d. Water intake from food was calculated as the product of food intake and the water content of the pellets. Content of water in urine was measured for each animal in experiments 1 and 2 by drying an aliquot of urine to constant mass. Urine osmotic pressure was measured with a Wescor 510B Vapor Pressure Osmometer. When urine concentration exceeded the capacity of the osmometer, samples were diluted 1:5 with distilled water and remeasured. This may lead to 10–25% overestimation of the concentration because the ionization of salts increases with dilution (Karasov, 1989; Sweeney and Beuchat, 1993). In fact, we found an average overestimation of 24% for both controls and treatments when we compared 1:5 vs. 1:2 dilutions of urine (data not shown). However, these overestimates should not greatly confound the comparisons made within our study because dilutions were applied without bias to the two treatment groups.

Energy Availability. Energy losses in feces and urine were measured by bomb calorimetry using urine and fecal samples from experiment 1. Data on body mass, food and resin intake, fecal production, and urine production were measured for days 5, 6, and 7.

Energy Content of Feces and Urine. Urine and fecal samples from days 5, 6, and 7 of each of the treatments in experiment 1 were frozen at −25°C until analysis. Because the animals were in steady state, one sample per animal (days 5, 6, and 7 pooled) of feces and of urine was analyzed in duplicate. Feces were ground in a Wiley mill (1-mm screen). Before urine samples were pooled, they were lyophilized to constant mass, then, the same amount of dried urine from each day was weighed. The 3 samples were mixed and ground in a mortar with liquid nitrogen. Pooled samples of urine were used to make the pellets for

bomb calorimetry. The pellets were lyophilized again because the urine absorbed water after being ground and pelleted. Lyophilized urine pellets were stored in a vacuum-sealed desiccator until used. Fecal pellets were redried at 50°C, 24 hr before they were bombed. Energy content of urine and feces were measured using a Phillipson Microbomb Calorimeter (Gentry Instruments) with benzoic acid as a standard.

Digestibility and metabolizability of food energy were calculated using the equations:

$$\text{ADEC} = (\text{Ge}_i Q_i - \text{Ge}_f Q_f)/\text{Ge}_i Q_i \quad \text{and}$$

$$\text{MEC} = (\text{Ge}_i Q_i - \text{Ge}_f Q_f - \text{Ge}_u Q_u)/\text{Ge}_i Q_i$$

where ADEC and MEC are apparent digestible and apparent metabolizable energy coefficients (unitless proportions of energy), respectively; Q_i, Q_f, and Q_u are dry matter intake, fecal output, and urine output in grams/d; Ge_i, Ge_f, and Ge_u are the energy contents of food, feces, and urine in kJ/g (Robbins, 1993). Both ADEC and MEC were expressed as percentage of energy intake in Figure 6a and b).

Data Analysis. Components of water influx and efflux from experiment 1 (drinking water, dry matter intake, water content of the urine, fecal output, water content of feces water efflux from feces, and evaporative water loss) were compared using a repeated measures ANOVA with treatment as the repeated measure. Differences between treatments were examined with *post hoc* contrasts. Drinking water, dry matter intake, water content of the urine, and water flux from urine during experiment 2 were compared between treatments using repeated measures ANOVA with one grouping factor (the sequence in which the diet was offered, either control–resin or resin–control). Because one of the animals in experiment 2 died during the first trial, sample size used in the analysis was 5.

The effect of treatments on ADEC and MEC was tested with repeated measures ANOVA (treatments = control 1, resin, and control 2 as the repeated measures). Proportions were arcsine transformed before statistical analysis. This analysis is the most appropriate one because the samples are not independent from each other.

Because there is controversy regarding the comparison of ratio-based assimilation efficiencies (Beaupre and Dunham, 1995), we also tested for an effect of treatment on fecal energy and urinary energy excretion, using the ANCOVA method of Beaupre and Dunham (1995). We regressed fecal energy excretion rate as the dependent variable with food energy intake as the covariate. Urinary energy excretion was regressed against digestible energy intake as the covariate. In both analyses of ANCOVA, the interaction terms were not included in the models because they were not significant, so we tested only for possible differences

between treatments. Both repeated measures ANOVA and ANCOVA were done using (SYSTAT version 5.03; Wilkinson, 1992). In all cases, values are expressed as mean ± one standard error (N = number of woodrats).

RESULTS

Water Requirement. There was no consistent effect of resin ingestion on body mass of woodrats. In experiment 1, the body mass of woodrats eating resin (118.9 ± 11.5 g) was higher than the body mass of woodrats during the second control period (113.5 ± 10.5 g), but not the first control period [118.8 ± 11.7 g; $F(2, 10) = 5.21$, $P = 0.02$]. In experiment 2, there was no difference in body mass between woodrats consuming resin (114.7 ± 7.0 g) or control diet [Control: 116.5 ± 8.3 g; $F(1, 4) = 1.04$, $P = 0.36$].

The effect of resin on minimum water requirement was consistent across both experiments. The minimum requirement for drinking water was higher in woodrats fed resin than in controls, by 18% in experiment 1 [Control 1: 5.5 ± 0.25 ml/d; Resin: 6.5 ± 0.8 and 5.5 ± 0.33 ml/d; $F(2, 10) = 3.81$, $P = 0.059$], and by 30% in experiment 2 [Control: 5.6 ± 0.2 ml/d and Resin: 7.3 ± 0.5 ml/d; $F(1, 3) = 19.22$, $P = 0.022$]. There were no significant differences in food intake between control and resin treatments in either experiment [experiment 1: $F(2, 10) = 1.94$, $P = 0.193$, and experiment 2: $F(1, 3) = 1.47$, $P = 0.31$; Figure 2].

Fecal water loss was higher in woodrats eating resin diets [$F(2, 10) = 5.67$, $P = 0.023$], apparently because of the combination of small increases in both fecal water content [$F(2, 10) = 2.24$, $P = 0.15$] and fecal dry matter flux [$F(2, 10) = 2.86$, $P = 0.104$; Figure 3].

Urine water fluxes were not affected by resin in either experiment [experiment 1: $F(2, 10) = 2.25$, $P = 0.15$, and experiment 2: $F(1, 3) = 0.35$, $P = 0.59$]. Urine water content was not elevated in resin-fed woodrats in either experiment [experiment 1: $F(2, 10) = 3.09$, $P = 0.09$, and experiment 2: $F(1, 3) = 0.021$, $P = 0.89$; Figure 4]. Urine osmotic pressure was lower for woodrats fed the resin-treated diet. In experiment 1, woodrats ingesting resin had lower osmotic pressure than one of the two control groups [$F(2, 10) = 14.9$, $P = 0.001$]. Woodrats fed resin also had lower urine osmolarity than woodrats fed the control diet in experiment 2 [$F(1, 3) = 966.36$, $P < 0.001$; Figure 4]. In experiment 1, evaporative water loss varied among trials but there was no consistent effect of resin [control 1: 2.8 ± 0.2 g/d, resin: 3.6 ± 0.3 g/d, control 2: 4.3 ± 0.3 g/d; $F(2, 10) = 5.88$, $P = 0.02$].

Energy Availability. Fecal energy excreted per day was not different between treatments [$F(2, 14) = 0.27$, $P = 0.76$] but increased with food energy intake [$F(1, 14) = 142.5$, $P < 0.001$; Figure 4a]. Urinary energy excretion was higher (~40%) for woodrats eating the resin-treated diet [$F(2, 14) = 8.7$, $P = 0.004$]

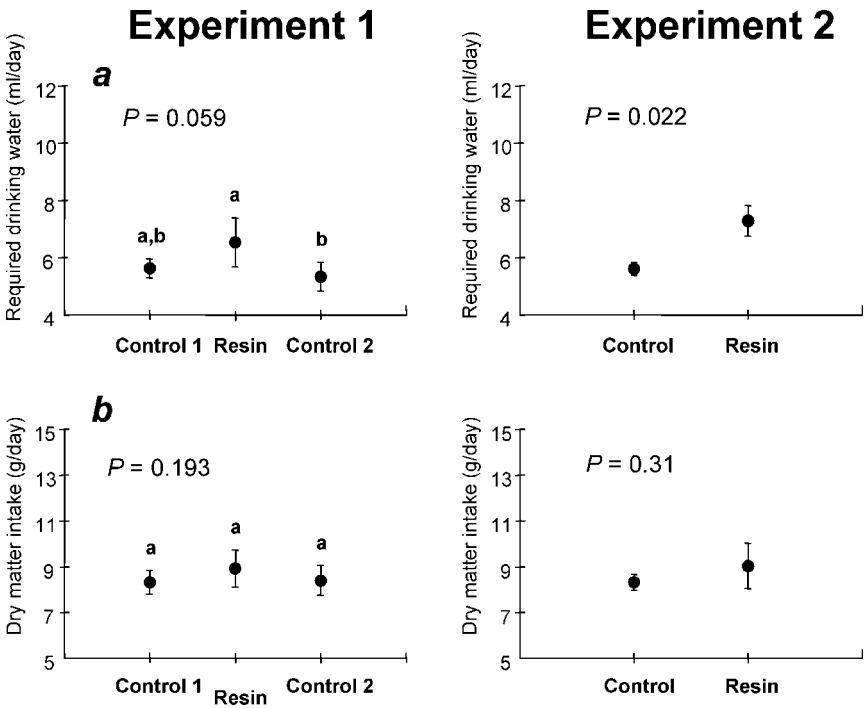

FIG. 2. Variation of required water (a) and dry matter (b) intake by woodrats consuming control and resin diets in experiments 1 and 2. Values are expressed as mean plus or minus one standard error. Means with the same lowercase letter indicate no significant difference. Differential contrasts were used to test for difference between continuous treatments in experiment 1 and special contrasts to test for differences between control 1 and control 2. For experiment 2, P values represent the overall significance for the repeated measures ANOVA with one grouping factor (diet sequence and either control-resin or resin-control).

and increased with digestible energy intake [$F(1, 14) = 5.52$, $P = 0.034$; Figure 4b].

The apparent digestible energy coefficient (ADEC) was not affected by the treatments [$F(2, 10) = 1.65$, $P = 0.24$; Figure 6a]. The metabolizable energy coefficient (MEC) was reduced by resin [$F(2, 10) = 5.21$, $P = 0.028$; Figure 6b]. MEC was lower in woodrats fed the resin-treated diet than in control 1 [$F(1, 5) = 10.17$, $P = 0.024$]. There was a similar trend between resin and control 2 [$F(1, 5) = 4.21$, $P = 0.095$; Figure 5b]. Urinary energy excretion as a percentage of apparent digestible energy was higher in woodrats fed resin than in control diets [$F(2, 10) = 20.26$, $P < 0.001$, control 1 vs. resin: $F(1, 5) = 23.15$, $P = 0.005$; control 2 vs. resin: $F(1, 5) = 23.89$, $P = 0.005$]. There were no

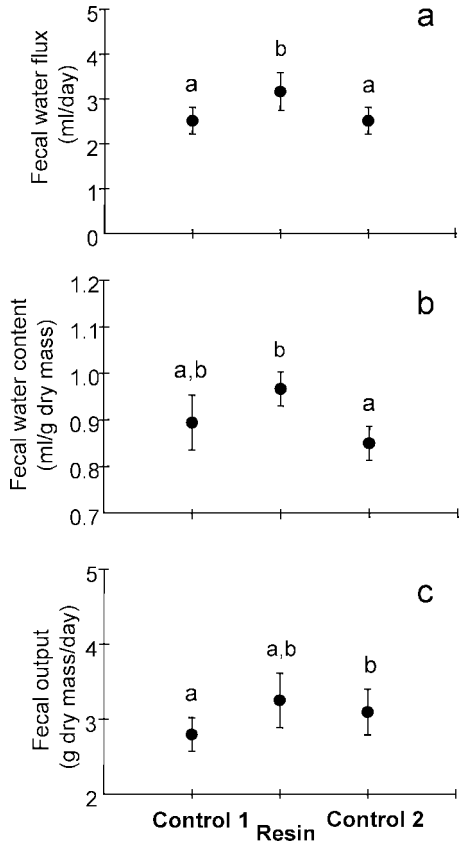

FIG. 3. Variation of fecal water flux (a), fecal water content (b), and fecal dry mass output (c) of desert woodrats consuming control versus resin diets for experiment 1. Values are expressed as mean plus or minus one standard error. Means with the same lowercase letter indicate no significant difference. Differential contrasts were used to test for difference between continuous treatments in experiment 1 and special contrasts to test for differences between control 1 and control 2.

differences between contro1 1 and control 2 with respect to urinary energy excretion [$F(1, 5) = 1.61$, $P = 0.25$; Figure 7].

DISCUSSION

The addition of creosote resin to the diet affected both the minimum water required and the energy available for woodrats. To our knowledge, this is

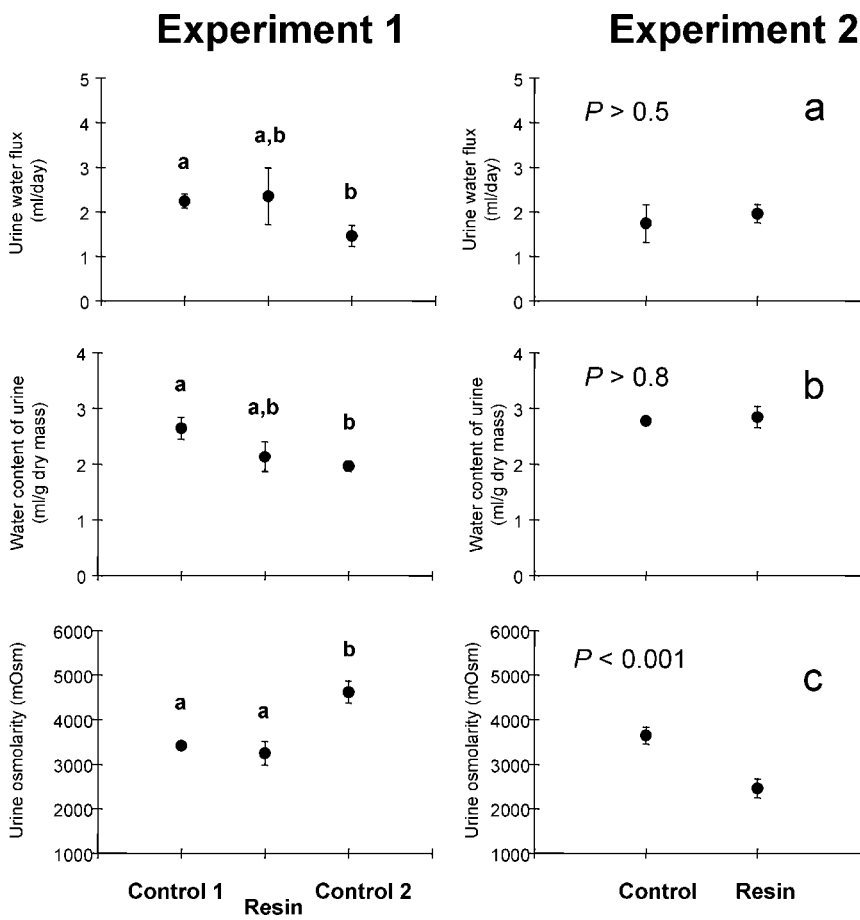

FIG. 4. Variation of urinary water flux (a), water content (b), and osmolarity of the urine (c) of desert woodrats fed control or resin diets for experiments 1 and 2. Values are expressed as mean plus or minus one standard error. Means with the same lowercase letter indicate no significant difference. Differential contrasts were used to test for difference between continuous treatments in experiment 1 and special contrasts to test for differences between control 1 and control 2. For experiment 2, P values represent the overall significance for the repeated measures ANOVA with one grouping factor (the sequence in which the diet was offered, either control-resin or resin-control).

the first experimental evidence on the effects of plant secondary metabolites on minimum water requirement of a herbivorous mammal. Our results support the hypothesis that the minimum water required for woodrats to maintain body mass increased with the ingestion of creosote resin. Also, we confirmed the prediction that

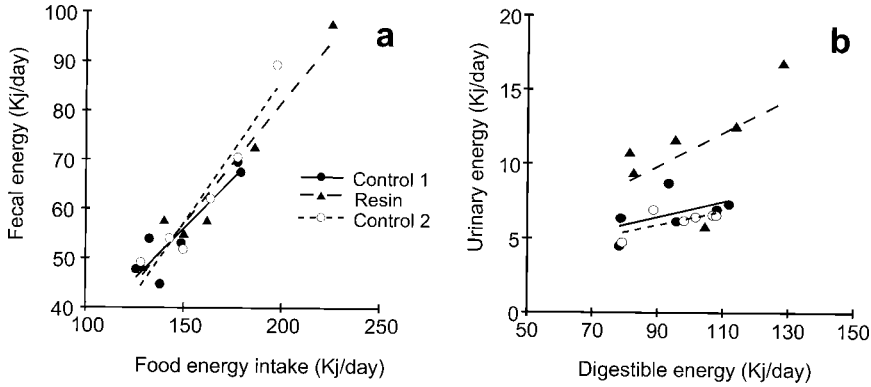

FIG. 5. Fecal energy output vs. food energy intake (a) and urinary energy excretion vs. digestible energy (b).

metabolizability of dietary energy was lower for woodrats fed resin diets compared to control diets.

Effect of Resin on Water Intake. The addition of resin to diet increased water intake and the minimum water requirement. The minimum water requirement for desert woodrats on control diets was 5.5 ml/d compared to 7 ml/d when resin was added (Figure 2; experiment 2). In comparison, *ad libitum* drinking rates are 12 ml/d for control diets vs. 15 ml/d for diets with 1–3% resin, and 25 ml/d for 5% resin diets (Mangione et al., 2000). Clearly, when woodrats are given free access to water, they drink in considerable excess of the minimum they need to match minimum water losses. When resin was added to the diet, the minimum requirement increased by 18–30% (1–1.7 ml/d above 5.5 ml/d). This increase (25%) is comparable to that observed for *ad libitum* intake of water by woodrats fed a 3% resin diet. In addition, ingestion of resin reduces estimates of metabolic water by reducing the amount of food available to be metabolized.

Effect of Resin on Avenues of Water Loss. On control diets, woodrats lost similar amounts of water (~2.5 ml/d) through each of the three possible avenues (urine, fecal, and evaporative). Resin appeared to increase minimum water loss rate mainly through an effect on fecal water loss. Fecal water loss rate was 0.7 ml/d higher in woodrats eating resin than in controls, and accounts for 70% of the difference in required water between control and resin diets. The increase in fecal water loss in woodrats eating resin was due to increases in both fecal water content and fecal dry matter excreted.

An increase in fecal water content may occur if fewer osmolytes are absorbed from the intestinal lumen causing retention of water by osmosis or by increasing the secretory rate of water. A component of creosote resin, NDGA, inhibits

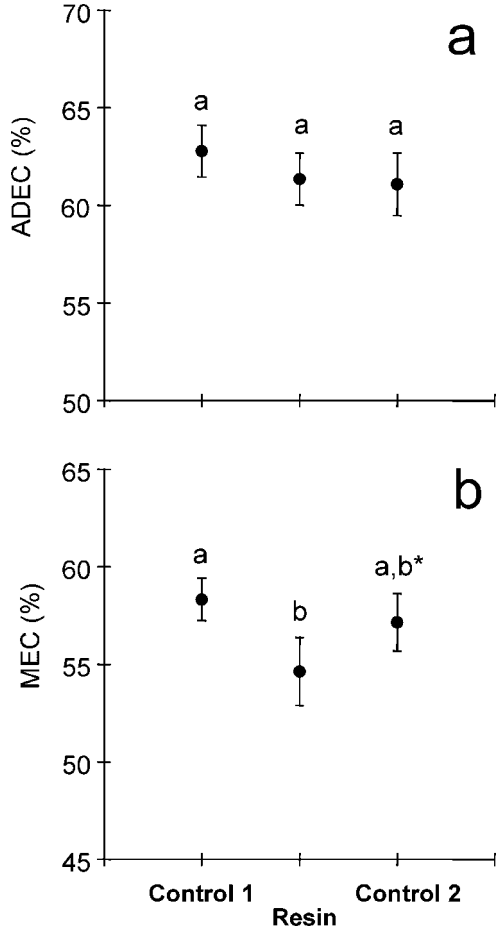

FIG. 6. Apparent digestible energy (a) and metabolizable energy coefficient (b) with treatments. Different letters indicate means are significantly different ($P < 0.05$). The asterisk indicates that the difference between resin and control 2 was significantly different ($P < 0.1$).

Na^+ K^+ ATPase of intestinal mucosa homogenates in rats (Kellett et al., 1993). The disruption of Na^+ transport may alter the mechanisms of solute-coupled water absorption in the large intestine. Thus, it is plausible that resin had a direct effect on osmolyte absorption. References regarding the effects of plant secondary compounds on Na^+ balance, retention, or excretion are abundant (Freeland et al., 1985; Navarro et al., 1994; Johnson et al., 1999; Dearing et al., 2001, and references

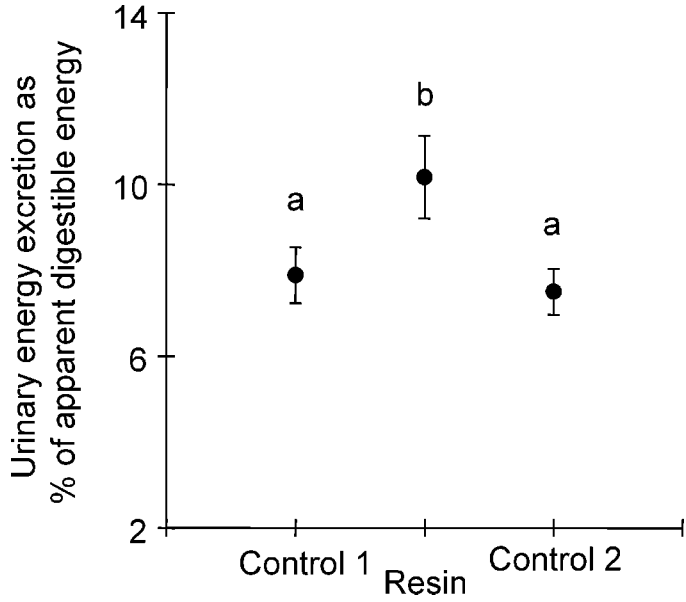

FIG. 7. Urinary energy excretion as a percentage of apparent digestible energy. Means with different letters are significantly different ($P < 0.05$).

therein). Most of these studies focused on the natruretic and diuretic effects of these compounds on animals, mainly laboratory rats (Galati et al., 1996). Dearing et al. (2001) also pointed out that the effect of secondary compounds on the water balance of wild herbivores has received little attention, but could be of tremendous importance to the fitness of the animal.

Increases in production of fecal dry matter could occur if resin ingestion reduced digestive efficiency (more feces produced per unit food consumed) or if larger quantities of resin-containing food were consumed. The first explanation seems unlikely, as Meyer and Karasov (1989) showed that resin had no significant effect on dry matter digestion, an observation we confirmed. The second explanation is not strongly supported by our data because food intake did not increase resin-treated diet (Figure 2).

Resin had relatively little effect on urinary or evaporative water loss. Although evaporative water loss differed significantly among treatments in experiment 1, it was not consistently higher in woodrats eating resin, but increased progressively with time. We do not know whether this reflects chronological changes in the woodrats themselves, which were all adults. It is possible that environmental conditions, e.g., relative humidity, which were thought to have been held relatively

constant, may have varied somewhat as the animal room was not an environmental chamber, but a standard animal facility.

The increase in minimum water requirement was not matched by a significant increase in urinary water loss. This was somewhat surprising, as NDGA has been reported to have a diuretic effect on humans (Timmermann, 1977). In addition to NDGA, many other PSMs have diuretic properties (Dearing et al., 2001). Moreover, 3% NDGA fed to lab rats produced cysts in their kidneys (Grice et al., 1968). This effect could decrease the kidney's capacity to minimize urinary water loss. However, neither the water content of the urine, nor the urinary water flux, was significantly affected by the resin (Figure 4). Curiously, urine osmotic pressure was lower in woodrats eating resin diets (Figure 4). Although these results imply a diuretic effect of resin, a simple comparison of osmolarity may be confounded by the different osmolytes excreted on the control vs. resin diets. Evidence of a substantial difference in osmolytes is indicated by the higher energy density and excretion of glucuronic acid metabolites in urine of animals consuming resin diets (Mangione et al., 2001). The results imply a change in urinary osmolytes; however, more research on the chemical composition of the osmolytes is necessary to explain these findings.

Ecological Implications of Increased Minimum Water Requirement. Even a small increase in the minimum water requirement of an animal, particularly a desert one, could result in myriad ecological consequences. The case documented by Karasov (1989) of woodrats feeding exclusively on creosote during the winter exemplifies the possible ramifications. In this case, the suitability of creosote as the only source of preformed water was marginal for desert woodrats, independent of the effect of creosote resin. The water content of the majority of individual creosote bushes measured by Karasov (1989) was not sufficient to meet the water requirements of woodrats on resin-free diets. The ingestion of creosote resin magnifies the problem: because the water requirement is greater when woodrats consume resin, even fewer creosote bushes than that estimated by Karasov (1989) will contain enough water to satisfy their water requirements. To meet water requirements, woodrats must be extremely selective in their choice of creosote bushes. Such dietary selectivity may necessitate that they travel longer distances between bushes with adequate water contents, thereby increasing the risk of predation. Furthermore, water requirements may be additionally enhanced via increases in evaporative water losses caused by increased movement associated with selective foraging. The difficulties associated with maintaining water balance while consuming creosote could be further exacerbated if resin concentration and water content were positively correlated. The water content of branches within a creosote bush varies significantly, with the top parts of the bush having greater water contents than the lower parts (Karasov, 1989). Resin content is higher in young leaves than in mature leaves within a bush (Meyer and Karasov, 1989). Thus, by

feeding on mature leaves on the upper portions of bushes, woodrats may maximize water intake and minimize resin intake.

Other mammals that feed on creosote may also be foraging selectively to optimize the water to resin ratio. According to Ernest (1994), jackrabbits (*Lepus californicus*) prefer to repeatedly forage from mature branches rather than current-year-growth branches, but water content was not measured in that study. Another example is *Ctenomys mendocinus*, a fossorial rodent from the Central Monte in Argentina, that forages on creosote bush (*Larrea cuneifolia*). There is no evidence of preference by *C. mendocinus* for water or resin content of *Larrea cuneifolia* branches (A. M. Mangione, 1990, 2002, personal observation). The interplay of resin and water on the water balance of desert herbivores and subsequent ecological consequences deserves further consideration.

Effect of Resin on Energy Availability. Our prediction that the metabolizability of dietary energy would be lower in animals fed resin diets compared to control diets was confirmed. Urinary energy loss was 40% higher in woodrats fed resin-treated diets compared to control diets, and the percentage of digestible energy excreted in woodrat urine was 24% higher on resin diets than control diets (Figure 5b). Woodrats had 4.5 and 6.3% lower MEC when fed resin-treated diets than control diets (Figure 6b). Resin had no effect on the percentage of ingested energy that was apparently digested (Figure 5a).

We have confirmed by two different methods (ratios and ANCOVA) that ingestion of creosote resin significantly increases urinary energy losses in woodrats fed resin-treated diets compared to control diets. How do these values compare to other values given in the literature, and is the magnitude of the energy drain biologically relevant? Jakubas et al. (1993a) and Guglielmo et al. (1996) suggested that herbivorous mammals have lower costs of detoxification via the glucuronic acid pathway than birds. According to these authors, the energy excreted in the form of glucuronic acid by herbivorous mammals represents 0.6–1.2% of the metabolizable energy intake (MEI = $\mathrm{Ge}_i Q_i$*MEC), and this percentage is considerably greater in birds eating natural forages (2–25 times). We estimated production of glucuronic acid in this study, from the previously established relationship between glucuronide excretion and maximum resin intake for Mojave woodrats (Mangione et al., 2001) [Glucuronide (mg/d) $= -0.41 + 0.457 *$ resin intake (mg/d)]. We incorporated the values of resin intake of this study (estimated from the feeding rate) into the equation above. The total energy associated with the excretion of glucuronic acid is the product of glucuronic acid excretion and its heat of combustion (13.5 kJ/g; Guglielmo et al., 1996). The energy lost as glucuronic acid was expressed as percentage of MEI. Our calculations reveal that energy in glucuronic acid was 1.9% of MEI, which is 1.6× greater than the highest values reported for mammals and similar to some of the values reported for birds (Jakubas et al., 1993a; Guglielmo et al., 1996). Total urinary energy excreted,

which includes glucuronic acid as well as other detoxification metabolites, was 12.4% of MEI in woodrats fed resin-treated diets compared to 7.3% for woodrats on control diets. This implies that almost half of the urinary energy lost as a consequence of creosote resin ingestion, may be the result of energy lost in the form of glucuronic acid, glycine, and sulfate conjugates, with glucuronic acid comprising 75% of the total moles of conjugates excreted in urine (Mangione et al., 2001).

The hypothesis that there is a lower cost of detoxification in mammals than in birds requires further evaluation. In this study, we fed woodrats naturally occurring allelochemicals (creosote resin) and the cost of detoxification as a percentage of MEI was similar to some of the values given for birds (Guglielmo et al., 1996). Moreover, the percentage of digestible energy excreted in the urine of woodrats fed resin-treated diet (10.2%) was similar to reported values for rodents and lagomorphs fed forage containing PSMs (green wheat and forbs; Robbins, 1993). The hypothesis that birds have greater detoxification costs can be properly tested only by feeding birds and mammals diets containing similar secondary metabolites.

In summary, plant secondary metabolites are thought to deter herbivores by toxicity and by reduction of digestibility of matter and/or nitrogen. This study quantified the two other costs: energy and water. Even if a herbivore has mechanisms to cope with the toxicity of PSMs, it may still confront the negative effects that PSMs exert on water and energy balance. These negative effects of PSMs should be factored into the numerous potential detrimental effects of secondary compounds on herbivore performance.

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