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Oxalate, calcium and ash intake and excretion balances in fat sand rats (*Psammomys obesus*) feeding on two different diets

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Abstract

Fat sand rats *Psammomys obesus* feed exclusively on plants of the family Chenopodiaceae, which contain high concentrations of chloride salts (NaCl, KCl) and oxalate salts. Ingestion of large quantities of oxalate is challenging for mammals because oxalate chelates Ca^{2+} cations, reducing Ca^{2+} availability. Oxalate is a metabolic end-point in mammalian metabolism, however it can be broken-down by intestinal bacteria. We predicted that in fat sand rats microbial breakdown of oxalate will be substantial due to the high dietary load. In addition, since a high concentration of soluble chloride salts increases the solubility of calcium oxalate in solution, we examined whether a change in the intake of chloride salts affects microbial oxalate breakdown and calcium excretion in fat sand rats. We measured oxalate, calcium and other inorganic matter (ash) intake and excretion in fat sand rats feeding on two different diets: saltbush (*Atriplex halimus*), their natural diet, and goose-foot (*Chenopodium album*), a non-native chenopod on which fat sand rats will readily feed and that has a similar oxalate content to saltbush but only 2/3 of the ash content. In animals feeding on both diets, 65–80% of the oxalate ingested did not appear in urine or feces. In animals consuming the more saline saltbush, significantly more oxalate was apparently degraded ($p < 0.001$), while significantly less oxalate was excreted in urine ($p < 0.01$) and in feces ($p < 0.05$). We propose, therefore, that fat sand rats rely on symbiotic bacteria to remove a large portion of the oxalates ingested with their diet, and that the high dietary salt intake may play a beneficial role in their oxalate and calcium metabolism.

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1. Introduction

Fat sand rats (*Psammomys obesus*) are diurnal gerbillid rodents widely distributed in the Sahara and Arabian deserts (Harrison and Bates, 1991). The species is strictly herbivorous, often feeding solely on plants of the family Chenopodiaceae. These plants are characterized by high concentrations of oxalate salts (Ellern et al., 1974; Noonan and Savage, 1999). The ingestion of large quantities of oxalate is challenging for most mammals, because oxalate

chelates Ca^{2+} , reducing Ca^{2+} availability in food and plasma (Concon, 1988). However, fat sand rats can consume a high-oxalate diet of chenopod plants with impunity and can thrive and reproduce on a diet of these plants alone (Daly and Daly, 1973; Degen et al., 1988; Kam and Degen, 1988) and sodium oxalate has been found in their urine (Korine et al., 2003).

Oxalate is an abundant metabolite in the plant kingdom and is produced by nearly all species (Franceschi and Loewus, 1995; Horner and Wagner, 1995). While in most plants it occurs as calcium oxalate crystals, some taxa, such as *Atriplex* sp. and *Halogeton* sp., contain large amounts of soluble oxalate, and consequentially their oxalate/calcium ratio exceeds 2:1 (Noonan and Savage, 1999). Organic acid synthesis in these plants may occur as a response to the

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accumulation of inorganic ions such as potassium and sodium, and oxalate production may be favored over the production of other organic acids (e.g., malate, citrate, etc.) since in terms of carbon it provides maximum charge balance capacity per carbon atom (Libert and Franceschi, 1980). Thus, an animal feeding on these plants is liable to ingest a surfeit of soluble oxalate. This excess oxalate not only reduces the calcium availability of the food itself (Massey et al., 1993; McConn and Nakata, 2004), but it may also diffuse into the blood and body tissue fluids with toxic consequences, such as corrosion of the buccal and the gastrointestinal tract epithelia, gastric hemorrhage, renal failure, hematuria and convulsions because ionized calcium becomes too low for normal muscle and nerve function (Concon, 1988; Noonan and Savage, 1999). Therefore, its diet presents the fat sand rat with two physiological challenges: (1) neutralization of the potentially harmful effects of the soluble oxalate, and (2) absorption of a sufficient amount of calcium for its biological needs.

Oxalate is a metabolic end-point in mammalian metabolism (Shirley and Schmidt-Nielsen, 1967; Khan, 1995; Noonan and Savage, 1999), however it can be degraded by anaerobic bacteria inhabiting the gastrointestinal tract. The bacterium species chiefly responsible for oxalate degradation in mammals is *Oxalobacter formigenes*, a gram-negative rod first described by Allison et al. (1985). This bacterium uses a two-step enzymatic pathway to break down oxalate to formate and carbon dioxide (Allison et al., 1985; Kuhner et al., 1996). The uptake of a proton in this process, which is coupled by an oxalate⁻² formate⁻¹ antiport system (Maloney et al., 1992), creates an inwardly directed proton motive force, driving ATP synthesis (Anantharam et al., 1989; Ruan et al., 1992), that accounts for all the energy requirements of this organism (Allison et al., 1985). The ability of other intestinal organisms to degrade oxalate has been less studied, but it was found that *Enterococcus faecalis* as well as some lactic acid bacteria can degrade oxalate (Hokama et al., 2000; Campieri et al., 2001; Weese et al., 2004), and their contribution to oxalate elimination in the gut may be significant. We expected, therefore, that bacterial degradation of oxalate would be the main means by which fat sand rats neutralize oxalate toxicity.

The first objective in this study was to quantify oxalate intake and excretion in fat sand rats eating either their natural diet of saltbush (*Atriplex halimus*), or goose-foot (*Chenopodium album*) (also commonly known as pigweed, lamb's-quarters, wild spinach, etc.), a plant that is not part of fat sand rats' natural diet, but that they readily eat. Goose-foot contains similar quantities of oxalate as saltbush, but smaller quantities of ash (noncombustible inorganic compounds), which in both plant species consist almost entirely of NaCl and KCl salts. The second objective was to measure calcium intake and excretion in order to determine the ratio of oxalate to calcium in both food and excreta, and the third objective was to estimate the magnitude and importance of bacterial degradation of oxalate in fat sand rats.

We predicted that bacterial oxalate degradation will be substantial in fat sand rats eating both diets, but since calcium oxalate becomes more soluble in a solution as the solution's ionic strength increases (Belliveau and Griffin, 2001), and hence more oxalate is available for bacterial degradation, we further predicted that when feeding on the more saline saltbush diet, bacterial degradation will be even more pronounced compared to the goose-foot diet. In addition, we examined the relationship between inorganic material (ash) intake and apparent oxalate degradation.

2. Materials and methods

2.1. Experimental animals

Seven individual fat sand rats were used in the experiments. They were captured with Havahart traps in October 2002 near Kibbutz Sede Boqer (30°52'N; 34°47'E), under permit number 17510 from the Israel Nature and National Parks Protection Authority. Animals were transferred to the laboratory at the Jacob Blaustein Institute for Desert Research at Midreshet Ben-Gurion, where they were kept in mesh cages (39 × 23.5 × 29 cm) at an ambient temperature of 27 °C and under a photoperiod regime of 12L:12D. Initially, the animals were fed solely on fresh leafy branches of saltbush, ad libitum. Animals were maintained for 8 months in captivity prior to experiments when their body mass was 20–30% higher than at the time of capture (225 ± 25 g vs. 175 ± 25 g).

2.2. Experimental food regimes

We used two different plants in our feeding trials: saltbush (*A. halimus*), and goose-foot (*C. album*). *A. halimus* is abundant in the area where animals were captured, and in our field observations at the site of capture, it was by far the most common food choice of the sand rats. In the wild, sand rats occasionally also fed on another chenopod, *Anabasis* sp., but when given both *A. halimus* and *Anabasis* sp. in captivity, they invariably preferred *A. halimus*.

C. album is a common garden weed that does not occur in arid habitats. We chose this plant for the experiment because it has a similar oxalate, but lower NaCl content, to *A. halimus*. Sand rats readily ate *C. album*, and maintained body mass when feeding on it. All *C. album* plants in our feeding trails were picked in the gardens of Kibbutz Sede Boqer.

Animals were not offered drinking water; thus water intake was from dietary preformed water.

2.3. Dry matter intake and excretion, and urine collection

To facilitate measurement of food intake and the collection of urine and feces, animals were placed in custom-built metabolic cages, allowing for the separation

of urine, food remains (orts) and feces, and were held there for 48 h. Every 24 h, each individual was provided with either 100 g of fresh *A. halimus* or 150 g of fresh *C. album*. The former was found by Degen et al. (1988) to supply all the daily dietary needs of a fat sand rat; the latter contains the same dry mass of plant material as 100 g of *A. halimus* and our animals maintained, or increased body mass when fed 150 g of *C. album*. After each 24 h period, the orts were carefully collected and weighed. Likewise, all feces were collected and weighed. All urine produced was captured in a funnel and collected in vials placed underneath the metabolic cages. Urine was stored at 0 °C and was assayed the day following sample collection. Twenty-four hour food intake was calculated by subtraction of orts mass from the initial food mass. Similar masses of fresh *A. halimus* and *C. album* as fed to the animals were kept beside the metabolic chambers and weighed before and after the experiment to allow correction for mass lost by evaporation. Orts and feces were oven dried at 60 °C for 72 h to constant mass before being measured for composition. Values for each animal for 24 h were taken as the average of the two 24 h periods each animal spent in a metabolic cage.

In order to minimize the effect of day in the feeding trials, we used a crossover experimental design where we randomly divided the seven individuals into two groups of four and three that fed on *A. halimus* and *C. Album*, respectively. Animals were allowed to habituate to their diet for at least 72 h before each feeding trial. After the initial feeding trial, we switched the diets of the two groups so that animals that initially fed on *A. halimus* fed on *C. album* and vice versa, and again we allowed a 72 h habituation period before the second feeding trial.

2.4. Oxalate, calcium, chloride, Na⁺, K⁺ and ash determination

The amount of oxalate in the food, feces and urine was determined with an oxalate oxidase assay (Sigma Kit#591). Feces and food samples were desiccated and then ground into a fine powder with a pestle and mortar. Fifty milligram aliquots of either ground plant or feces were heated at 60 °C for 3 h in a solution of 5 N HCl. Following this procedure, sub-samples of the solution were assayed for oxalate. Eight milliliter samples of urine were first acidified with 200 µL of glacial HCl to achieve dissociation of calcium and oxalate because the urine was basic (pH=9) following collection. Apparent oxalate degradation was determined by subtracting the amount of oxalate ingested in urine and feces from the amount of oxalate ingested, and expressed as percentage. We are aware that this method provides only an approximate estimate of bacterial degradation of oxalate and thus its interpretation should be made with caution. Though this measurement provides only circumstantial evidence of oxalate degradation by gut microflora, we add here that in a separate experiment we have cultured colonies of bacteria from fresh sand rat feces

that grow on anaerobic media with oxalate as the sole energy source (Niv Palgi, unpublished data).

To determine calcium content, plants, feces and urine were prepared as described for the oxalate assay. Ca²⁺ content was determined in dilutions of acidified samples with an atomic absorption spectrometer (Perkin-Elmer 1100 B). For determination of ash content, samples of food plants and feces were oven dried for 72 h at 60 °C, weighed, burnt at 580 °C for 5 h, and allowed to cool before weighing to 0±0.0001 g (Presica 40SM-200A electronic balance). Chloride was measured to ± 1 mmol/kg H₂O by titration (Corning 925 chloride analyzer). Sodium and potassium were measured to ± 1 mmol/kg H₂O with a flame photometer (Perkin-Elmer 1100 B). Five milligram ash samples of either plant material or feces were dissolved in 5 mL of double-distilled water for analysis. Urine samples (not acidified) were also diluted and analyzed. Osmotic concentration of urine was measured with a freezing point depression osmometer (Osmette II, Precision Systems Inc.). All analyses were done in triplicate. The average of each three triplicates was used as a single datum point.

2.5. Net water intake

Water content of food plants and feces was determined gravimetrically, by weighing samples before and after they were oven dried for 72 h at 60 °C. Net water intake was determined as water consumed in food minus water voided in feces and urine.

2.6. Statistical analysis

Paired *t*-tests were used to compare results between the two diets and we chose *p*<0.05 as the minimum acceptable level of significance. The Shapiro–Wilk test (Shapiro and Wilk, 1965) was done to establish that data distribution did not differ significantly from normality, and the O'Brien test (O'Brien, 1979) was used to establish that group variances were not significantly different.

2.7. Possible sources of non-dietary oxalate

Ascorbate can undergo spontaneous breakdown to oxalate in solutions with pH>7. Based on values from the literature for ascorbate content of saltbush and goose-foot (Streb et al., 1997), we estimated that fat sand rats feeding on these two plants might ingest up to 20 mg of ascorbate per day that would produce, if efficiency of this breakdown was 100%, 10 mg of oxalate. Endogenous production of oxalate in Wistar rats (*Rattus norvegicus*) of similar mass to fat sand rats (200–230 g) was less than 0.5 mg per day (Ferraz et al., 2002). Assuming that the rate of endogenous oxalate production in sand rats is the same, the combined inputs of oxalate from these non-dietary sources in fat sand rats are < 10% of the dietary oxalate intake, which is > 300 mg per day. As this is a conservative estimate, we

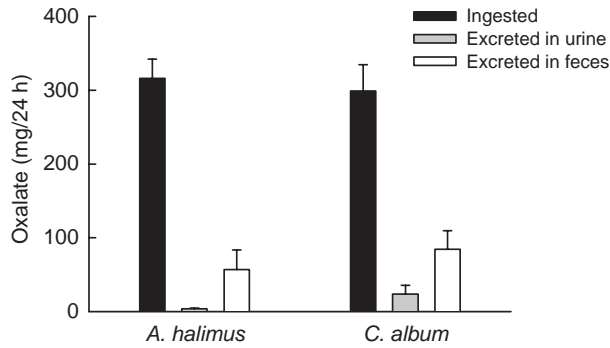


Fig. 1. Oxalate intake and excretion in fat sand rats feeding on two different diets. Bars represent quantities of oxalate in mg/24 h that fat sand rats ingested or excreted when feeding either on saltbush (*A. halimus*), or goose-foot (*C. album*). Fat sand rats feeding on saltbush excreted less oxalate in urine ($p < 0.01$) and feces ($p < 0.05$) than rats feeding on goose-foot, even though oxalate ingestion did not differ significantly ($p > 0.05$) between the two diets.

considered this input negligible for the purposes of this paper.

3. Results

Fat sand rats feeding on *A. halimus* and on *C. album* ingested similar quantities of oxalate, but excreted significantly less oxalate in urine (3.73 ± 0.8 mg/24 h vs. 24.2 ± 10.43 mg/24 h, $p < 0.01$) and feces (56.73 ± 27.3 mg/24 h vs. 84.6 ± 24.98 mg/24 h, $p < 0.05$) when feeding on *A. halimus* than on *C. album* (Fig. 1). Apparent oxalate degradation was significantly higher when eating *A. halimus* than when eating *C. album* ($81 \pm 8.5\%$ vs. $63.5 \pm 8.4\%$, $p < 0.001$) (Table 1). This occurred even though sand rats

Table 1

Data of food, water and electrolyte intake in excretion in fat sand rats feeding on two different diets

	<i>A. halimus</i>	<i>C. album</i>	Sample size
Body mass (g)	224 ± 26.9	230.9 ± 27.8**	N=7
Dry food intake (g/24 h)	18.79 ± 1.55	18.54 ± 2.20 NS	N=7
Dry faeces (g/24 h)	3.83 ± 1.13	4.94 ± 1.32*	N=7
Water intake (mL/24 h)	40.86 ± 3.36	65.75 ± 77.8***	N=7
Urine volume (mL/24 h)	9.85 ± 2.62	23.54 ± 3.68***	N=6
Water voided in feces (mL/24 h)	3.91 ± 1.12	3.55 ± 2.20 NS	N=7
Net water intake (mL/24 h)	27.95 ± 1.90	39.08 ± 3.35***	N=7
Urine osmotic concentration (mosm/kg H ₂ O)	4727 ± 198	3570 ± 635***	N=6
[Cl ⁻] in urine (mosm/kg H ₂ O)	1909 ± 88	1233 ± 229***	N=6
[Na ⁺] in urine (mosm/kg H ₂ O)	2124 ± 74	1615 ± 288**	N=6
[K ⁺] in urine (mosm/kg H ₂ O)	22.2 ± 4.3	16.6 ± 5.6*	N=6
Inorganic (ash) intake (g/24 h) ^a	6.76 ± 0.56	5.01 ± 0.59***	N=7
Ash in feces (g/24 h)	1.03 ± 0.30	0.98 ± 0.25 NS	N=7
% of apparent oxalate degradation	81 ± 8.5	63.5 ± 8.4***	N=7

NS=non-significant ($p > 0.05$) vs. *A. halimus*. Asterisks mark significant differences vs. *A. halimus*: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

^a Ash from food plants contained (in mol) 75% NaCl and 23% KCl (*A. halimus*), and 73% NaCl and 24% KCl (*C. album*).

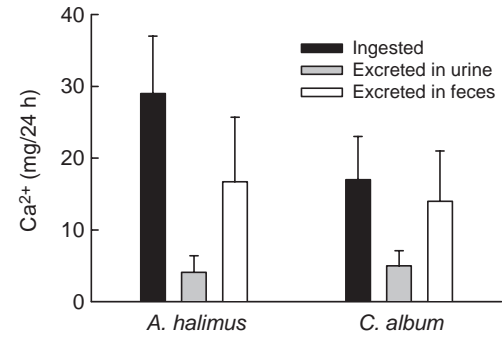


Fig. 2. Calcium intake and excretion in fat sand rats feeding on two different diets. Bars represent quantities of calcium in mg/24 h rats ingested or excreted when feeding either on saltbush (*A. halimus*), or goose-foot (*C. album*). Fat sand rats feeding on saltbush ingested more calcium ($p < 0.05$) than rats feeding on goose-foot. Calcium excretion in urine and faeces did not differ significantly ($p > 0.05$) between the two diets.

ingested more calcium (29.1 ± 7.9 mg/24 h vs. 17.13 ± 6.2 mg/24 h, $p < 0.05$) when feeding on *A. halimus* (Fig. 2). Thus, it is apparent that less oxalate ingested in the *C. album* diet was soluble.

Dry food intake in the fat sand rats was similar for both diets (18.79 ± 1.55 g/24 h vs. 18.54 ± 2.20 g/24 h, $p = 0.69$), but since *A. halimus* contains more ash than *C. album*, ash intake was significantly higher (6.76 ± 0.56 g/24 h vs. 5.01 ± 0.59 g/24 h, $p < 0.001$) on the *A. halimus* diet (Table 1). This was also reflected in the significant differences in urine osmotic concentration and urine [Cl⁻] ($p < 0.001$ for both) between animals eating the two diets (Table 1). Seeking to interpret why the difference, we performed a relationship analysis of apparent oxalate degradation vs. the amount of inorganic plant matter (ash) ingested (Fig. 3), which gave a significant relationship ($R^2 = 0.42$; $p = 0.012$), suggesting that oxalate degradation is augmented by larger NaCl intake.

Since *C. album* contains more water per unit wet mass, sand rats consuming *C. album* ingested significantly more water than rats feeding on *A. halimus* ($p < 0.001$), and excreted significantly more water in urine ($p < 0.001$),

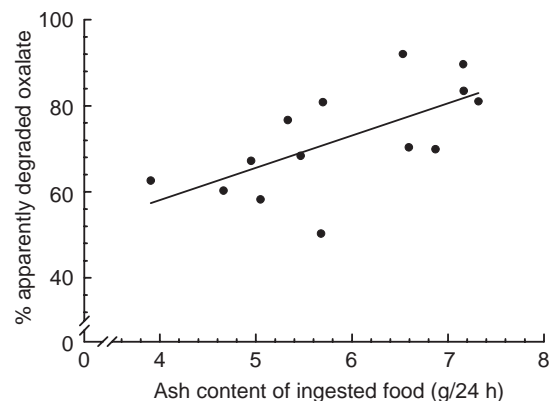


Fig. 3. A scatter-plot of apparent oxalate degradation vs. the amount of inorganic plant matter (ash) ingested; correlation coefficient is 0.65, with $p = 0.012$ for the correlation coefficient being higher than 0.

although water excretion in feces did not differ significantly between the two diets (Table 1).

For one animal, we occasionally experienced difficulties in obtaining enough urine for analysis. When this was so, sample size was six and not seven.

4. Discussion

In the wild, fat sand rats feed on a diet that is very rich in oxalates and in our experiments they ate at least 300 mg oxalate daily (Fig. 1). For both diets, *A. halimus* and *C. album*, the oxalate/calcium ratio was high, indicating that most of the oxalate was soluble, and that all calcium was in the sparingly soluble form of calcium oxalate; thus the food plants had very poor calcium bioavailability. Apparent oxalate degradation (oxalate ingested–oxalate excreted) was substantial on both diets, supporting the assertion that bacterial degradation of oxalate is the primary means of oxalate removal in fat sand rats.

Shirley and Schmidt-Nielsen (1967) indirectly demonstrated that fat sand rats, in fact, rely on symbiotic bacteria to degrade oxalate. When sand rats were fed a “bread” containing ^{14}C labeled calcium oxalate (sparingly soluble), 20% of the ^{14}C activity was recovered in expired CO_2 , while when fed ^{14}C sodium oxalate (soluble), nearly a 100% of the activity was recovered, providing indirect evidence that bacterial degradation of oxalate does take place, and suggesting that the symbiotic oxalate-degrading bacteria are particularly efficient in degrading soluble oxalate. This mechanism of intestinal oxalate degradation seems important not only in preventing soluble oxalate from entering the body, but also in eliminating oxalate that is transported back from the plasma into the intestinal lumen. Indeed, Shirley and Schmidt-Nielsen (1967) found evidence that ^{14}C from labeled oxalic acid injected into the plasma of fat sand rats is recovered in expired CO_2 , and more recently, in rabbits, proteins that transport oxalate from the circulatory system into the intestinal lumen were discovered (Hatch and Freel, 1995).

The means of calcium acquisition from an exclusively chenopod diet has yet to be elucidated. Chenopods are considered to have the lowest calcium bioavailability of nearly all edible plants because they have such a high oxalate/calcium ratio, and consequently almost no ionized calcium (Noonan and Savage, 1999). Any plant with an oxalate/calcium ratio higher than 2.0 (when both oxalate and calcium are expressed in mEq) is a poor source of calcium for humans (Noonan and Savage, 1999), and chenopods such as *Chenopodium* spp. and *Atriplex* spp. have an oxalate/calcium ratio of 4.0 and higher (Noonan and Savage, 1999). The oxalate/calcium ratio we found in this study was 4.5 for *A. halimus* and 6.5 for *C. album*, making both plants very poor calcium sources for mammals. Yet, even though fat sand rats fed solely on them, they did not apparently lack calcium (Fig. 2), and the oxalate/calcium

ratio in their urine was 0.45 for fat sand rats feeding on *A. halimus* and 1.5 in fat sand rats feeding on *C. album*. How, then, do fat sand rats maintain calcium balance?

We propose that large ingested quantities of NaCl increase availability of calcium ions and calcium availability. The K_{sp} for calcium oxalate in water at 25 °C is 2.3×10^{-9} (Kolthoff et al., 1969). In tissue culture media at 37 °C, the equilibrium concentration products, $[\text{Ca}^{2+}][\text{C}_2\text{O}_4^{2-}]$ are 10 times higher than that due to the higher ionic strength of the solution (Belliveau and Griffin, 2001). Thus, under physiological conditions, calcium oxalate solubility is expected to be higher than in water. Yet, still only a minute fraction of the calcium is soluble and available for absorption. The sap of saltbush plants eaten by sand rats contains more than 3% NaCl; calculated from Kam and Degen (1988), with corrections made for salt-scraping by the rats prior to ingestion. In a solution containing high [NaCl], calcium oxalate becomes more soluble (Belliveau and Griffin, 2001). If this high [NaCl] does not equilibrate with blood plasma before the consumed food reaches the upper duodenum, then the calcium in calcium-oxalate may become more available for absorption in the gut, possibly with a concurrent increase in epithelial calcium transporters. Indeed, absorption of calcium in laboratory rats was shown to occur in the upper duodenum (Bronner and Pansu, 1999). Whether this is also the case in fat sand rats deserves further examination.

In addition, we suggest that if these animals practice coprophagy, it could account for increased calcium availability. Fat sand rats feed on *A. halimus* in which the oxalate/calcium ratio is 4.5:1, yet they excrete feces with an oxalate/calcium ratio of 1.25:1 because most of the soluble oxalate is degraded, probably by intestinal bacteria. If reingested, this reduced oxalate/calcium ratio in the feces would increase calcium availability to a level higher than in the food plants.

Significantly more oxalate was degraded when the fat sand rats consumed *A. halimus* (Fig. 1) than when they ate *C. album*. There is a statistically significant correlation between plant inorganic material intake and oxalate degradation (Fig. 3). Whether increased salt intake does augment calcium absorption in fat sand rats also deserves further study.

Thus, the presence of oxalate degrading bacteria together with a mechanism yet to be determined of calcium acquisition allows fat sand rats to exploit a resource that is abundant in their habitat yet unavailable to other animals.

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