

**CALCIUM AND PHOSPHORUS BIOAVAILABILITY
OF DESERT TORTOISE FOOD PLANTS**

FINAL REPORT

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Calcium And Phosphorus Bioavailability Of Desert Tortoise Food Plants

Abstract

Exotic plants can comprise a major component of the diet for some desert tortoises (*Gopherus agassizii*) in the Mojave Desert. Introduced plants may not be as nutritious as native plants, and thereby may influence the growth and welfare of neonate and juvenile tortoises. Nutrient availability in a native grass (*Achnatherum hymenoides*), an introduced grass (*Schismus barbatus*), a native forb (*Malacothrix glabrata*) and an introduced forb (*Erodium cicutarium*) were measured in one to two year old juvenile desert tortoises. We fed tortoises measured amounts of chopped foods daily for ~130 days (dry grass) or ~90 days (green forb). Orts and feces were collected daily and dried to constant mass, and calcium, phosphorus, and magnesium contents of food and feces were measured. Apparent calcium digestibilities (as %, not accounting for endogenous fecal losses) did not differ significantly among foods. The amount of calcium obtained from foods (mg obtained/g dry food) did not differ between grass species or between forb species, but was significantly lower for grasses than for forbs, because the grasses were lower in calcium content. Apparent phosphorus digestibility was relatively high for the forbs, but was negative for both grasses. Tortoises lost small amounts of phosphorus while feeding on the grasses, but gained much phosphorus while eating forbs. Apparent magnesium digestibility and magnesium obtained were generally higher for forbs than for grasses. Net nutrient availability was better explained by the type of food (forb vs. grass) than by its geographic origin (native vs. exotic); however, if exotic grasses are replacing native forbs rather than native grasses, the nutritional quality of the food available to the tortoises could be decreasing. Comparison of nutrient availability to estimated requirements for growth by

juveniles and egg production by adult females suggests that phosphorus is the more limiting nutrient in both cases, and that calcium may pose a significant osmotic challenge for excretion in this desert species. Management practices that promote availability of forbs could increase growth rates and shell ossification, which would enhance predator resistance of juvenile tortoises.

Introduction

The desert environment poses many challenges for herbivorous reptiles. Water and food availability fluctuate widely between seasons and between years, and both are often in limited supply or are simply not available. Relative amounts of nutrients may vary; for example many desert plants are high in calcium, but may be low in phosphorus (Nagy et al. 1976). Further, the bioavailability of nutrients (amount absorbed relative to amount ingested) can be affected by other compounds in the plants (e.g. oxalate, phytate), and can vary seasonally and between plant species (Robbins 1993). Against this tapestry of a variable supply of essential resources, the resource needs of desert reptiles fluctuate as well, especially due to the rapid growth of the young and the reproductive efforts of adult females in spring. Mortality of young tortoises is high. Their soft shells do not fully mineralize until they are 5-7 years old, leaving them vulnerable to predation (Wilson et al. 1994). Shell ossification, and therefore overall growth, may be limited by availability of calcium and/or phosphorus; growth may also be limited by availability of other major nutrients including energy and nitrogen. Magnesium, which makes up 0.3-0.75% of bone ash mass (Robbins 1991) is also critical to bone growth. Excess calcium may pose an osmoregulatory challenge if water is limited.

We predict that availability of calcium, phosphorus, and magnesium will vary among different foods, especially between forbs and grasses, and possibly between native and exotic species. These differences may affect calcium, phosphorus, and magnesium balance in juvenile desert tortoises, and therefore possibly shell mineralization and overall growth rate. We examined calcium, phosphorus, and magnesium digestibility in four desert tortoise food plants, and compared amounts of nutrients obtained from these foods to estimated requirements for growth and egg production.

Methods

Study animals

Juvenile desert tortoises (one to two years old) were obtained from members of the California Turtle and Tortoise Club and from Dr. David Morafka of California State University, Dominguez Hills. Animals were kept at the University of California, Los Angeles in an approved vivarium room. Tortoises were kept in individual plastic bins (39.4 cm x 25.4 cm x 14 cm) fitted with 25 W spotlights at one end that were on for 14 h each day to allow tortoises to thermoregulate. Room temperature was always about 24 °C, and the daytime thermal gradient in the bins ranged from 27-32 °C. Fluorescent "full-spectrum" ultraviolet lights (ZooMed) were mounted 25-30 cm above the bins and were also on for 14 h each day. Tortoises were maintained on fresh greens (primarily chopped kale leaves) when not participating in a feeding trial.

Diets

Tortoises feed primarily on annual wildflowers (forbs) in the spring, then switch to dried senescent grasses in the summer (Nagy *et al.* 1998). Four foods were studied: *Achnatherum hymenoides* (Indian ricegrass, a native bunch grass), *Schismus barbatus* (split grass, an exotic grass), *Malacothrix glabrata* (desert dandelion, a native forb) and *Erodium cicutarium* (redstem filaree, an exotic forb). Wild tortoises in the Mojave desert eat all of these foods.

The two grasses were collected in desert tortoise habitat in the Mojave Desert during the summer when dry and senescent. All above-ground parts of the grasses were collected and cleaned of dirt and debris. Grasses were then chopped in a Wiley mill and sifted to a relatively uniform size (2-4 mm lengths). Dry grass was stored at room temperature and dried at least overnight at 60 °C just before feeding. The forbs were collected in the field during the spring while still green. Both forbs had a mix of flowers and mature fruit/seeds on them when collected. Those parts of the plants that we judged tortoises would eat in the field (leaves, flowers and thin stems) were cleaned and finely chopped, then refrigerated or frozen until used.

Feeding trials

Juvenile tortoises were offered each food in turn, and food intake and feces production were measured. Nutrient contents of the food and feces were measured and digestibilities calculated from these data. To determine growth rate, tortoises were periodically weighed and shell dimensions (carapace length, shell width between the 4th and 5th marginal scutes and maximal shell height at the same distance from the anterior end; Nagy *et al.*, submitted) were measured. Both mass and shell “volume” (length x width x height, all in cm) changes were used as measures of growth.

Tortoises were initially divided into two groups of ten; one group was fed the grass *S. barbatus* while the other was fed the grass *A. hymenoides*. Two tortoises in the *S. barbatus* group were in poor health and died shortly after the trial began; two others refused the food and were dropped from the study, with a resulting final group size of six. Animals were kept on the grasses for 133 days, after which all animals (n = 16) were switched to the forb *E. cicutarium*. This trial continued for 90 days. Animals were then fed fresh kale leaves for 20 days until the forb *M. glabrata* became available in the field. Tortoises were then offered *M. glabrata* for 90 days.

In all trials tortoises were offered weighed amounts of food daily. Samples of green forbs were taken periodically for measurement of water content (drying at 60 °C to constant mass) so that the dry matter amounts of food offered could be calculated. Grasses were stored at 60 °C before feeding and were presumed to contain no water when weighed and offered. Orts (uneaten food) were collected every 10 days and dried at 60 °C. The amount of food eaten was therefore calculated every ten days as food offered minus Orts, on a dry matter basis. Feces were collected daily, dried at 60 °C, and pooled over 10-day intervals. To reduce problems such as coprophagy and contamination of feces by uneaten food, rectangular trays made from plastic-coated paper cold-drink cups were taped to the plastron of each tortoise to catch feces. Trays were replaced when soiled. Tortoises were placed in water pans and allowed to drink ad libitum before each trial began, but then they were not given water again for the first 50 days of the grass trials, or the first 70 days of the forb trials. After this, drinking water was provided for about an hour at 10-day intervals.

Accurate dry matter digestibility (DMD; g retained/g ingested) measurements are critical, because digestibilities of all other nutrients are calculated from these numbers. Because tortoises

may only defecate every few days, longer trial periods improve the accuracy of the measurement of rate of feces output. For the grasses, we determined that trials of about 125 days were needed to obtain consistent and reliable results. For the forbs *E. cicutarium* and *M. glabrata*, the last 40 days of the 90-day trials gave consistent results and were used for DMD measurements.

We initially used the "pulse" method to measure digestibility. We force-fed six 1 mm x 3-5 mm pieces of plastic tape to each tortoise every ten days, varying color each time, and started new feces collection vials when the next color tape was found in the feces. Feces collection periods were therefore not necessarily congruent with ort collection periods. This method provides a direct measure of the amount of food retained by the animal between markers. However, it relies on the assumption that the markers provide a reliable indicator of food passage (that is, they move through the gut at the same rate as the food). Plastic tape markers did not prove to be a reliable indicator of food passage, and sometimes appeared to become trapped in the gut for extended periods. For example, in one instance the marker from one trial came out before the marker of the preceding trial, and in another instance the marker did not emerge for over 80 days. We therefore found it necessary to change our method of measuring digestibility to the "steady-state" method. In this method, the amount of food eaten in a given period is measured, and the feces produced during that same time period are collected. This requires the assumption that the animals have not become constipated or developed diarrhea, and thus have not produced more or less feces than normal, and have not grown substantially. An increase in size during the trial would lead to an overestimate of DMD, because of an increase in the total amount of feces stored in the animal. This method also requires that the feces being collected are from the food type being tested. To insure this, we fed test diets for at least 30 days before beginning sample collections.

During the grass trials, steady-state digestibility was measured from the day the second or third marker was recovered from each tortoise (start of a new fecal vial) to the last day grass was fed, January 18, 2000. The total length of the period therefore varied among tortoises (125 ± 7 days). Food intake for the first few days of the trial was prorated based on the 10-day intake rate measured during that period.

Analysis of calcium, magnesium, and phosphorus

All food and fecal samples were dried to constant mass at 60 °C and finely ground using a Spex mixer/mill before nutrient analysis. Only those feces that were free of contamination by urates or uneaten food were used for nutrient analysis. Dried, ground samples of food and feces (approximately 0.25 g) were digested in a Hach Digesdahl digester (sulfuric acid and hydrogen peroxide digestion) and diluted to a standard volume of 100 ml. Aliquots of this solution were stored frozen or at room temperature in polypropylene freezer vials until analyzed. Calcium, magnesium, and phosphorus were analyzed using Hach Co. colorimetric assays modified for use with a Coleman Junior II spectrophotometer. Digested samples were diluted as necessary to bring the sample concentration within the range of the tests. Duplicate digestions of some samples showed low error in the digestion step relative to the measurement error in the colorimetric tests, so for most samples a single digestion was analyzed in duplicate or triplicate.

In the phosphorus assay (Hach method 8048; Hach Co. 1997), phosphorus reacted with molybdate and ascorbic acid to produce molybdenum blue; absorbance was read at 840 nm and compared to a standard curve prepared daily. In the calcium and magnesium assay (Hach method 8030; Hach Co. 1997), calcium and magnesium reacted with calmagite to produce a red color, then sequentially chelated calcium and magnesium using EGTA (ethylene glycol-bis(β -

aminoethyl ether)-N, N, N', N'-tetraacetic acid) and EDTA (ethylenediaminetetraacetic acid). Absorbance at each stage was read at 522 nm and compared to daily standard curves for calcium and magnesium. For both assays, samples were run in duplicate or triplicate and were rerun if duplicates were not within 5% of each other. Phosphorus, calcium and magnesium in the food and fecal samples (mg/g dry sample) were then calculated using the sample concentration, dilution factor, and mass of digested sample.

Adult tortoise trials

Adults tortoises had been fed the same four foods in a previous study (Meienberger et al. 1993; Nagy et al 1998), using similar methods. Food and fecal samples for *M. glabrata* and *A. hymenoides* trials on adults were still available and were analyzed for calcium, magnesium, and phosphorus using the same procedures as for the juvenile tortoise samples. In addition, previously unreported data for calcium are presented here for adults eating *S. barbatus* and *E. cicutarium*.

Calculations

The term apparent digestibility is used here to distinguish our values from "true digestibility" (also called mineral "availability") because of the possibility of gut secretion, as well as uptake, of minerals (Maynard et al. 1979). Distinguishing between secreted and undigested minerals in the feces would require endogenous turnover measurements that were beyond the scope of this study.

Apparent digestibilities were calculated using the nutrient contents of the food and feces and the dry masses (g) of food ingested and feces produced for each animal as follows:

$\text{Nutrient}_{\text{ingested}} \text{ (in mg)} = \text{Nutrient content of food (in mg/g)} \times \text{food ingested during the measurement period (in g)}$

$\text{Nutrient}_{\text{feces}} \text{ (in mg)} = \text{Nutrient content of feces for each individual (in mg/g)} \times \text{feces produced (in g)}$

$\text{Net nutrient}_{\text{retained}} \text{ (in mg)} = \text{Nutrient}_{\text{ingested}} - \text{Nutrient}_{\text{feces}}$

$\text{Apparent nutrient digestibility} = (\text{Nutrient}_{\text{retained}} / \text{Nutrient}_{\text{ingested}}) \times 100\%$

Nutrient obtained was also calculated for each animal as follows:

$\text{Nutrient obtained (in mg/g dry food)} = \text{Nutrient content of food (in mg/g)} \times (\text{Nutrient digestibility}/100)$

Statistics

Statistics were calculated using SigmaStat 2.0 for Windows (Jandel Scientific Software). Where possible, percentages were normalized by arcsine square root transformation (Zar 1984); however, some nutrients had negative digestibilities, so this was not possible for all cases. A probability value of 0.05 or less indicated statistically significant differences. Where multiple tests were conducted, a sequential Bonferroni correction was applied within a set of tests (Rice 1989).

Results

Food intake rates and growth rates for juvenile tortoises

Food consumption rates (mg dry food/day) were significantly correlated with body mass for all four foods (Fig. 1). Y-intercepts for all four were not significantly different from zero. We therefore were able to evaluate mass-specific intake rates without biases due to allometric effects of mass. Mass-specific intake rates were higher for forbs than for grasses, but did not differ within those categories (Table 1).

Growth rates (g gained or lost/day) were significantly correlated with body mass of tortoises eating *Erodium* and the same was true for *Malacothrix* when two outlier points were left out of the calculation (Fig. 2). Intercepts were not significantly different from zero, so the relationship between body mass and growth rate was directly proportional, not allometric. Growth rates in the grass feeding trials were not significantly correlated with body mass ($p = 0.15$ for *Achnatherum* and 0.15 for *Schismus*; Fig. 2). Tortoises eating the grasses did not grow, but instead shrank slightly (mean change rates in mass and in shell volume for each grass diet were negative and significantly different from zero rate of change; Table 1). Tortoises grew while eating both forbs; mean growth rates for these foods did not differ from one another (Table 1). Pooling all four diets, mass-specific growth rate was positively correlated with mass-specific dry matter intake rate (Figure 3).

Dry matter digestibility (DMD) was not significantly correlated with growth rate for the forbs ($p = 0.727$, both foods pooled), so our assumption of steady state in calculating DMD values probably did not bias these results substantially. DMD was slightly but significantly correlated with growth rate for the grasses ($R^2 = 0.281$, $p = 0.035$, for both grasses pooled), but

the correlation is low, and probably does not reflect a serious violation of the assumption of steady-state.

Calcium, magnesium and phosphorus

The two grasses had substantially less calcium, phosphorus, and magnesium than the two forbs (Table 2). Within the forbs, *M. glabrata* tended to have higher calcium and magnesium content, but lower phosphorus content, than *E. cicutarium*. The foods fed to adult tortoises had similar amounts of Ca, Mg and P as the same foods fed to juvenile tortoises (Table 2).

Apparent calcium digestibility did not differ significantly among foods for either juveniles or adults (Table 2). However, calcium obtained (mg retained/g dry food eaten) did differ. For juveniles, calcium obtained was lowest for the two grasses (which did not differ from one another), higher for *E. cicutarium*, and highest for *M. glabrata*. For adults, calcium obtained was significantly higher for *M. glabrata* than for the other three foods, which did not differ from one another.

Apparent phosphorus digestibility and obtainable phosphorus for both juveniles and adults differed between food types (Table 2). Juvenile tortoises eating grasses lost more phosphorus in the feces than was present in the food, and therefore had negative values for digestibility and P obtained. Apparent phosphorus digestibility and P obtained for adult tortoises feeding on *A. hymenoides* did not differ significantly from zero. Tortoises eating forbs gained phosphorus. There were no differences between native and exotic grasses or between native and exotic forbs; phosphorus digestibility and P obtained were significantly lower for the two grasses than for the two forbs.

All four foods contained substantially more calcium than phosphorus, and animals obtained more calcium than phosphorus from the foods (Table 2). For forbs, the ratio of calcium to phosphorus obtained was typically lower than the ratio present in the food. For example, the *Malacothrix* fed to juvenile tortoises contained 9 times as much calcium as phosphorus, but juvenile tortoises obtained only 4 times as much calcium as phosphorus. Due to their very low phosphorus content, grasses had a very high ratio of calcium to phosphorus (about 20:1), but because tortoises eating grasses had a net loss of phosphorus, the ratio of calcium to phosphorus obtained was negative. For example, when feeding on *Achnatherum*, juvenile tortoises gained six units of calcium for every unit of phosphorus lost (Table 2).

For juvenile tortoises, apparent magnesium digestibility and magnesium obtained were much lower for *S. barbatus* than the other three foods, and higher for *E. cicutarium* than for *A. hymenoides* and *M. glabrata*, which did not differ from one another (Table 2). For adults, however, *A. hymenoides* and *M. glabrata* did differ.

Discussion

Tortoises lost mass and volume when eating grasses, but gained mass and volume when eating forbs. This is partly due to the lower availability of nutrients in grass foods (calcium, magnesium, and phosphorus (Table 2), and also energy and nitrogen, Shemanski et al. ms. in prep). However, voluntary intake rates for grasses were far lower than rates for forbs (Table 1), and it may be that the tortoises were simply not eating enough dry matter to maintain body mass. When the data for growth and intake rates for all diets are pooled (Fig. 3), grasses and forbs appear to fall on the same line. Intake rates of less than ~2.5 mg/g day generally resulted in negative growth rates, regardless of the food type.

Calcium digestibilities for other desert herbivores feeding on green annuals are similar to the values reported here. All are apparent digestibilities; no calcium turnover studies have been done on these species. Chuckwallas feeding on green annuals have a calcium digestibility of 68%, and obtain 18.9 mg Ca/g dry food eaten (Nagy 1977). Jackrabbits feeding on spring annuals retain 73% of the calcium in their diet, or 17.2 mg/g dry food eaten (Nagy et al. 1976). Calcium values for both species are similar to those for tortoises feeding on *M. glabrata* (Table 2), though higher than values for the other foods. Chuckwallas gain 2.4 mg magnesium/g dry food, again similar to the amount tortoises obtained from forbs.

Calcium:phosphorus

Studies on mammals have revealed that assimilation of both calcium and phosphorus is optimal when both are present in roughly equal amounts (Ca:P of 2:1 to 1:1; Robbins 1993). If one is present in excess, it may interfere with uptake of the other, due to the formation of insoluble (and therefore indigestible) calcium phosphate compounds. This effect may have contributed to the low (negative) apparent digestibility of phosphorus for the two grasses; there was about twenty times as much calcium as phosphorus in these foods. In contrast, the Ca:P ratios in the forbs were closer to equality, and the ratios actually obtained from these foods were even closer to optimal (Table 2) because of the higher apparent digestibility of phosphorus. A similar effect was observed for tortoises feeding on *Schismus* and a different forb, *Sphaeralcea ambigua* (Barboza 1995). Ca:P in the foods were 1.9:1 and 14.5:1, respectively, but tortoises obtained calcium and phosphorus at ratios of 1.6:1 for *Schismus* and 3.5:1 for *Sphaeralcea*. Tortoises gained phosphorus while eating *Schismus* in this study; however, the *Schismus* used

was green (not dry and senescent), and may have differed in composition from that used in the present study.

Calcium, phosphorus, and growth

Rapid growth of juvenile tortoises to a “predator-resistant” size is critical to the survival of these animals. Therefore, tortoises should be acting to maximize growth rate and shell ossification. For bone development and shell ossification, both calcium and phosphorus are needed to form hydroxyapatite, the acellular bone matrix. The ratio of calcium to phosphorus in bone varies somewhat across species and ages, but is typically around 2:1 (Robbins 1993). This is similar to the Ca:P ratio obtained by juvenile tortoises feeding on *M. glabrata* and *E. cicutarium* (Table 2). Tortoises lost phosphorus while eating both grasses, possibly preventing bone growth. This at least partially explains the lack of overall growth on these foods (Table 1), though other nutrients such as nitrogen could also be limiting. It is possible that tortoises could increase in size without increasing bone mass, but this would leave them with thinner, more fragile shells.

Loss of phosphorus while feeding on dry grasses could potentially explain the juvenile tortoises’ reluctance to eat these foods. Adult tortoises can gain energy from feeding on these diets, and do so during the summer, gaining energy while losing water and nitrogen (Nagy and Medica 1986; Henen 1999). For juvenile tortoises, energy gain may be less important than avoidance of phosphorus loss. Captive juveniles fed dry grass *ad lib.* ate much less of it than they did of the two forbs (Table 1), despite the fact that they could probably consume more grass than forb (on a dry matter basis) if they chose. The forbs had a high water content, reducing the amount of dry matter that could fill the gut, whereas the grasses were eaten dry and therefore

more matter could potentially be eaten. It is possible that, unlike adults, juveniles do not normally feed heavily on dry grasses in the summer.

Using published data on tortoise growth rates, food intake and body composition, combined with nutrient uptake rates for the four foods used in this study, we compared estimated calcium, phosphorus, and magnesium requirements for growth during the second year of life to the amounts of those nutrients obtained during that year. During the second year of a desert tortoise's life, total mass increases from 35 to 54 g, and dry mass increases from 6.1 to 12.6 g (Nagy et al. 1997). Data for body composition of juvenile desert tortoises are not available, but other chelonians have been studied. Hatchling soft-shelled turtles (*Trionyx spiniferus*) contain, on a dry matter basis, 2.79% Ca, 0.26% Mg, and 1.74% P (calculated from data in Packard and Packard 1991). Desert tortoises, which have eggs that are similar in structure and composition to those of soft-shelled turtles (Congdon and Gibbons 1985) are likely to have hatchlings that are similar in composition as well. While these percentages increase in larger juveniles and adults, which have proportionally heavier shells (Naegle 1976), values for the second year are probably more similar to hatchling values than adult values. Total body content of Ca, Mg and P would therefore increase by 183, 17, and 114 mg, respectively (Table 3), as a tortoise grows from 35 to 54 g wet mass during year 2 of life. During that year, a young tortoise eats an estimated 96.8 g of food, on a dry matter basis (Nagy et al. 1997). Approximately 66% of this food is eaten during the 3 months in the spring when green food is available, and the remaining 34% is eaten during the remainder of the year and is likely to be primarily dried grasses. Using the nutrient availability for the four foods used in this study (mg nutrient obtained/g dry food; Table 2), we estimated Ca, Mg, and P intake during the second year (Table 3). We first compared tortoises feeding only on native species (*M. glabrata* and *A. hymenoides*) with tortoises feeding only on

exotic species (*E. cicutarium* and *S. barbatus*). The total annual nutrient intakes were very similar, so we then pooled diets, assuming an equal ratio of native and exotic foods (Table 3), and compared estimated intake with estimated needs. All intake values were higher than the amount needed for growth, but the relative excesses differed between nutrients. Tortoises obtained four times as much calcium as needed, 7.5 times as much magnesium, and only 1.6 times as much phosphorus as necessary based on estimated growth. Again, phosphorus was the more limiting nutrient. Actual annual requirements for minerals may be higher than the amounts needed for building new bone if there is an endogenous turnover of calcium and phosphorus associated with maintenance of existing tissues (Klasing 1998).

Calcium and reproduction

Once tortoises reach adult size growth slows considerably, and dietary mineral requirements should decrease. An exception to this is egg production by females. It has been proposed that during egg production, female tortoises may become deficient in calcium, as can birds (Klasing 1998). Female Texas tortoises (*G. berlandieri*) have higher mortality rates than males, apparently due in part to bone loss as a result of a high reproductive rate (Hellgren et al. 2000). We estimated calcium, magnesium, and phosphorus contents of desert tortoise eggs based on existing data for *G. polyphemus* (Linley and Mushinsky 1994) and *Trionyx spiniferus* (Packard and Packard 1991), both of which are expected to be similar in composition to *G. agassizii* (Congdon and Gibbons 1985). Desert tortoise eggs average about 33 g (Henen 1997), and are probably about 11.1 g dry matter. *T. spiniferus* eggs contain 9.9% calcium, 0.99% phosphorus, and 0.17% magnesium on a dry matter basis, so each desert tortoise egg contains approximately 1100 mg Ca, 110 mg P, and 18 mg Mg. Annual dry matter intake for female desert tortoises is

1072 g (Henen 1997); we estimated from his data that 654 g are springtime forbs and 418 g are dried grasses eaten in the summer. Using our values for amount of nutrient obtained/g dry food for adult tortoises feeding on native foods (Table 2), we calculated annual calcium, phosphorus and magnesium intakes, and estimated how many eggs could be produced with that amount of nutrient, assuming that all of the nutrient obtained is used for egg production (Table 4). Relative to calcium and phosphorus, magnesium is clearly not a limiting nutrient for egg production. Calcium and phosphorus yielded similar numbers of eggs. Eggs contain about 10 times as much calcium as phosphorus, and the Ca:P ratio obtained by adults eating *M. glabrata* was 11:1 (Table 2). Later in the season, however, females lose phosphorus while feeding on dry grasses, and the overall Ca:P ratio for the season is 15:1. So phosphorus is potentially more limiting than calcium, though the numbers here are comparable (enough phosphorus to make 7 eggs, vs. enough calcium to make 10 eggs). Both values are slightly higher than the actual number of eggs typically produced in a year (3-6 eggs per year, Henen 1997), but suggest that both calcium and phosphorus could potentially limit egg production. Data for magnesium and phosphorus were not available for adults feeding on the two exotic species, but calcium data were available. Tortoises feeding exclusively on *E. cicutarium* and *S. barbatus* would only gain enough calcium to make about 4 eggs (Table 4), less than half the eggs which could be produced on the native diets. This suggests that the exotic foods could constrain egg output due to lower calcium availability. However, these estimates of egg production were made using existing data for adults. The nutritional content of plant foods is variable and would have a significant impact on these estimates. For example, the *Erodium* fed to juvenile tortoises in this study contained 22.7 mg calcium/g (Table 2), nearly twice as much as the *Erodium* fed to adults (12.4 mg calcium/g), and juveniles obtained more calcium than adults eating that diet (7.7 vs. 5.1 mg calcium/g dry

food). If adults had been fed this higher-calcium *Erodium*, they might be able to obtain more calcium from it, and therefore potentially produce more eggs.

Calcium and osmoregulation

For all four diets, and especially the grasses, it appears that calcium is being consumed in excess relative to phosphorus. If no phosphorus is available for bone growth, there is probably no way to store the calcium taken in, and it must be excreted. Even if phosphorus is present, calcium in excess of the required Ca:P ratio must also be excreted. Does this calcium excretion pose an osmoregulatory challenge for juvenile tortoises?

Using the estimates for juvenile tortoise nutrient requirements and intake during the second year of life (see above), and water intake by juveniles for that same time period (Nagy et al. 1997), we estimated excretion of calcium, magnesium, and phosphorus relative to water excretion. Juvenile tortoises take in approximately 168.4 ml of water during their second year (Nagy et al 1997). 12.3 ml of this water goes into new tissue as tortoises grow from 35 to 53.8 g. The remainder must be lost via feces, urine, and evaporative losses. Fecal water loss of 119 ml was calculated from dry matter intake (63.9 g forb and 32.9 g grass) and overall digestibility (51.4%; Nagy et al. 1997) and fecal water content (forbs: 2.9 ml/g dry, grasses 1.8 ml/g dry, Hazard unpublished data). Ignoring evaporative water loss, this leaves a maximum of 37.1 ml of water that could be excreted in the urine. Juvenile tortoises apparently assimilate calcium, phosphorus and magnesium from their diet in excess of their growth requirements (559, 68, and 111 mg, respectively, during year 2; Table 3). If these surplus minerals are deposited in the liquid urine, the resulting solution would contain 377 mM calcium, 60 mM phosphorus, and 124 mM magnesium. These numbers are very high relative to plasma osmotic concentration (282-

355 mosM; Nagy and Medica 1986), and suggest that these nutrients may pose a significant osmotic challenge to growing tortoises. Even if it is assumed that all phosphorus obtained is used for growth, and that twice as much calcium as phosphorus is needed for growth (an overestimate; hatchling tortoises have a total body Ca:P of 1.6:1), the resulting urine would still contain 255 mM calcium. Some calcium could be excreted as insoluble precipitates with uric acid; urinary precipitates of desert tortoises may contain ~1.4% Ca on a dry matter basis (Barboza 1995). This would reduce the osmotic stress imposed by calcium excretion.

Implications for management and conservation of tortoises

There are few differences between native and exotic foods within food type (grass, forb). Both *M. glabrata*, the native forb, and *E. cicutarium*, the exotic forb, have favorable Ca:P ratios and provide similar amounts of phosphorus, the more limiting nutrient. Both grasses are very poor phosphorus sources, and tortoises fail to grow on these diets. Adults, with their well-ossified shell, appeared to have less of a problem with phosphorus when feeding on *A. hymenoides*, and may be more tolerant of slight losses of phosphorus because of the large P reserves in existing shell. For juveniles, management actions that maximize long-term availability of forbs (native or exotic) in the spring and later in the year may enhance growth and survival of the species.

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Table 1. Voluntary food intake rates and growth rates (mean \pm S.D.) for juvenile desert tortoises eating natural foods. Growth rate is percent change in body mass per day. Within columns, diets sharing letters do not differ significantly from one another ($p > 0.05$). Negative growth rates for *A. hymenoides* and *S. barbatus* were significantly different from zero growth.

Diet	Intake rate (mg/g day)	Growth rate (% mass/day)	Growth rate (% volume/day)
<i>A. hymenoides</i>	1.65 \pm 0.46 a	-0.045 \pm 0.031 a	-0.027 \pm 0.038 a
<i>S. barbatus</i>	1.43 \pm 0.37 a	-0.043 \pm 0.025 a	-0.040 \pm 0.019 a
<i>M. glabrata</i>	4.05 \pm 1.13 b	0.139 \pm 0.128 b	0.126 \pm 0.124 b
<i>E. cicutarium</i>	5.22 \pm 1.82 b	0.162 \pm 0.133 b	0.164 \pm 0.131 b

Table 2. Nutrient content (mg/g dry food), apparent digestibility (%) and nutrient obtained (mg/g dry food) for four foods eaten by juvenile and adult desert tortoises. Values are means (with s.d. in parentheses). Dry matter digestibilities for juveniles are from Shemanski et al. (in prep) and for adults are from Nagy et al. 1998. Calcium results for adults feeding on *Schismus* and *Erodium* are unpublished data from a previous study (C. Meienberger, I. Wallis and K. Nagy, pers. comm.). P-values are for the ANOVA comparing values for all four diets. Within age classes, diets sharing a letter are not significantly different (ANOVA and Tukey's t-tests). "*" indicates that juvenile and adult means differ significantly for that diet (two-tailed t-tests with sequential Bonferroni correction within nutrient).

	Age	p	<i>Achnatherum</i>	<i>Schismus</i>	<i>Malacothrix</i>	<i>Erodium</i>
Dry matter digestibility (%)	juvenile	<0.001	42.3 (10.4) a	44.6 (6.3) a	55.6 (4.2) b	71.1 (3.9) c
	adult	<0.001	46.9 (5.2) a	49.7 (8.4) a	70.1 (2.6)* c	63.3 (5.0)* b
Ca in food (mg/g)	juvenile		6.23 (1.08)	7.82 (1.58)	34.0 (1.27)	22.7 (0.65)
	adult		9.11 (2.75)	5.1	31.6 (1.58)	12.4
Ca digestibility (%)	juvenile	0.065	34.9 (23.9)	50.5 (9.2)	36.5 (7.9)	33.9 (12.0)
	adult	0.431	47.8 (16.8)	49.2 (11.4)	44.6 (6.0)*	40.8 (8.9)
Ca obtained (mg/g)	juvenile	<0.001	2.19 (1.50) a	3.95 (0.72) a	12.38 (2.69) c	7.69 (2.72) b
	adult	<0.001	4.36 (1.53)* a	2.51 (0.58) a	14.09 (1.89)* b	5.06 (1.11)* a
P in food (mg/g)	juvenile		0.284 (0.028)	0.427 (0.048)	3.88 (0.09)	4.94 (0.23)
	adult		0.688 (0.021)	n/a	2.21 (0.25)	n/a

P digestibility (%)	juvenile	<0.001	-128.6 (62) a	-112.8 (60.7) a	73.8 (4.8) b	65.8 (9.8) b
	adult	0.007	-39.4 (87.7)* a	n/a	52.5 (21.1)* b	n/a
P obtained (mg/g)	juvenile	<0.001	-0.36 (0.17) a	-0.48 (0.26) a	2.86 (0.19) b	3.25 (0.48) b
	adult	<0.001	-0.27 (0.604) a	n/a	1.30 (0.52)* b	n/a
Ca:P in food	juvenile		22:1	18:1	9:1	5:1
	adult		13:1	n/a	14:1	n/a
Ca:P obtained	juvenile		6:-1	8:-1	4:1	2:1
	adult		16:-1	n/a	11:1	n/a
Mg in food (mg/g)	juvenile		1.57 (0.09)	1.77 (0.16)	6.47 (1.73)	5.54 (0.47)
	adult		1.29 (0.35)	n/a	4.27 (0.21)	n/a
Mg digestibility (%)	juvenile	<0.001	17.2 (10.4) b	1.6 (8.1) a	20.9 (8.1) b	37.2 (11.2) c
	adult	<0.001	0.49 (15.3)* a	n/a	35.1 (9.3)* b	n/a
Mg obtained (mg/g)	juvenile	<0.001	1.08 (0.65) b	0.029 (0.144) a	1.35 (0.52) b	2.06 (0.62) c
	adult	<0.001	0.006 (0.20)* a	n/a	1.50 (0.40) b	n/a

Table 3. Calcium, phosphorus and magnesium intake and requirements during the second year of life for a juvenile desert tortoise. Values calculated using data from the current study, Nagy et al. 1997 and Packard and Packard 1991. See text for details.

	Calcium (mg)	Phosphorus (mg)	Magnesium (mg)
Content in a 35 g tortoise (6.06 g dry mass)	169	105	16
Content in a 53.8 g tortoise (12.6 g dry mass)	352	219	33
Increase during second year	183	114	17
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Intake during second year, feeding on native species			
Spring (<i>Malacothrix</i>)	791	183	86
Summer (<i>Achnatherum</i>)	72	-12	36
Total intake in second year	863	171	122
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Intake during second year, feeding on exotic species			
Spring (<i>Erodium</i>)	491	208	132
Summer (<i>Schismus</i>)	130	-16	1
Total	621	192	133
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Intake during second year, feeding on native and exotic species (assuming 1:1 native:exotic)			
Spring	641	196	109
Summer	101	-14	19
Total	742	182	128

Table 4. Calcium, phosphorus and magnesium intake and requirements for egg formation by female tortoises. Values calculated using data from the current study, Nagy et al. 1997, Henen 1997 and Packard and Packard 1991. See text for details. A dash indicates data not available.

	Calcium (mg)	Phosphorus (mg)	Magnesium (mg)
Content in a 32.9 g egg (11.1 g dry mass)	1099	110	18.4
<u>Annual intake, feeding on native species</u>			
Spring (<i>Malacothrix</i>)	9215	850	982
Summer (<i>Achnatherum</i>)	1822	-113	2.5
Total intake	11037	737	985
Number of eggs possible from diet nutrient	10	6.7	54
<u>Annual intake, feeding on exotic species</u>			
Spring (<i>Erodium</i>)	3309	-	-
Summer (<i>Schismus</i>)	1049	-	-
Total	4358	-	-
Number of eggs possible from diet nutrient	4	-	-

Figure 1. Linear relationships between food intake rates (g dry food/day) and body mass for juvenile desert tortoises eating four foods. All slopes were significantly different from zero, and all intercepts were not significantly different from zero. Native forb: *Malacothrix glabrata*. Native grass: *Achnatherum hymenoides*. Exotic forb: *Erodium cicutarium*. Exotic grass: *Schismus barbatus*.

Figure 2. Linear relationships between growth rate (g gained or lost/day) and body mass for juvenile tortoises eating four foods. Slopes for the grasses were not significantly different from zero, but the slope for *Erodium* was. The slope for *Malacothrix* was significant when two outliers were removed. Native forb: *Malacothrix glabrata*. Native grass: *Achnatherum hymenoides*. Exotic forb: *Erodium cicutarium*. Exotic grass: *Schismus barbatus*.

Figure 3. Relationship between growth rate and food intake rate for juvenile tortoises. Regression line represents all four foods combined. Native forb: *Malacothrix glabrata*. Native grass: *Achnatherum hymenoides*. Exotic forb: *Erodium cicutarium*. Exotic grass: *Schismus barbatus*.





