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DIETARY MANIPULATION OF THE CALCIUM CONTENT OF FEED CRICKETS

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Abstract: Insects used in zoo feeding programs are poor sources of calcium (Ca) and have inverse calcium : phosphorus (Ca:P) ratios. An experiment was conducted to determine if feeding diets high in dietary Ca to adult crickets would cause an increase in whole-body Ca content. Experimental diets were formulated to contain 2, 4, 6, 8, 10, and 12% Ca and were provided to the crickets for 12, 24, 48, 72, 96, and 120 hr. Crickets were analyzed for dry matter, Ca, and phosphorus. Dietary treatment and duration of treatment were found to have significant effects on Ca content and Ca:P ratio of crickets, but not on P content. The Ca content and Ca:P ratio of crickets increased during the initial period of feeding, but after 48 hr these mineral levels remained stable. Calcium content and Ca:P ratio of crickets were a function of dietary Ca, reaching maximal values of 1.4% and 1.7:1, respectively, when diets containing 12% Ca were fed. Radiographs revealed radiopaque material in the gastrointestinal tracts (GIT) of most crickets fed diets high in calcium (8, 10, or 12% Ca) but not in the GIT of crickets fed diets low in calcium. It is concluded that in order to obtain crickets with a Ca:P ratio of 1:1 or higher it is necessary to feed diets containing at least 8% Ca. Other factors which might influence the calcium content of crickets are discussed.

Key words: Crickets, *Acheta domestica,* calcium, calcium : phosphorus ratio, insectivory, insect prey, zoo animal nutrition.

INTRODUCTION

Crickets (*Acheta domestica*) and mealworm larvae (*Tenebrio molitor*) are the two most commonly used species of insect prey in U.S. zoos. They are fed to insectivorous and omnivorous mammals, birds, reptiles, and amphibians. Some captive animals, such as frogs, geckos, flycatchers, and tarsiers, may be obligately insectivorous, so that insects or invertebrates constitute their sole source of nutrients. There has been little research on the suitability of crickets and mealworm larvae as the complete diet for zoo animals. Limited information on nutrient composition indicates that these insects are poor sources of calcium (Ca), have high concentrations of nitrogen (protein), and, in the case of mealworm larvae, contain high concentrations of fat.[2,6–8,11,12]

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Metabolic bone disease is seen in a number of species of zoo animals[4] and is usually the result of dietary imbalances of Ca, phosphorus (P), and/or vitamin D. Zoo animals fed a diet solely of crickets or mealworm larvae may show signs of poorly mineralized bones.[1,8] In an attempt to avoid this problem, zoo personnel frequently supplement insects with a Ca source. For example, $CaCO_3$ powder or vitamin and mineral preparations may be dusted on insects just prior to feeding. Although dusting insects with a Ca source can increase Ca concentrations,[2] the effect will depend on the Ca content of the supplement as well as the amount that is adhering at the time the insect is eaten. If a cricket is not consumed within a short period of time, it may use its appendages to groom the dust from its body. It is not uncommon for crickets to be eaten hours after they have been placed in a zoo cage, at which time little if any of the supplement may remain on the crickets.

Another approach is to use insects that have been previously maintained on high-calcium feed materials.[2,12] Bilby and Widdowson suggested that it was the calcium content in the guts of invertebrates which

Table 1. Ingredients used in the formulation of a cricket (8% calcium) diet.

Ingredient	% by weight
Corn, ground	8.3
Alfalfa, dehydrated	10.0
Soybean meal, 48% CP	28.7
Wheat, ground	27.0
Calcium carbonate	20.0
Dicalcium phosphate	2.0
Salt, granular	0.5
Vitamin premix[a]	0.25
Mineral premix[b]	0.25
Soy oil	3.0

[a] The vitamin premix contained the following nutrients per g: 28,000 IU vitamin A, 2,800 IU vitamin D_3, 132 IU vitamin E, 0.6 mg vitamin K, 6.0 μg vitamin B_{12}, 7.1 mg vitamin B_1, 2.0 mg riboflavin, 35.6 mg niacin, 9.5 mg pantothenic acid, 2.0 mg pyridoxine, 1.5 mg folic acid, 99 μg biotin, 190 mg choline.

[b] The mineral premix contained the following nutrients per g: 144 mg calcium, 0.04 mg phosphorus, 4.3 mg magnesium, 0.60 mg potassium, 84.2 mg iron, 83.3 mg zinc, 81.1 mg copper, 119 mg manganese, 0.08 mg selenium, 0.32 mg iodine.

provided sufficient calcium for nestling blackbirds and thrushes.[3] In a preliminary study, we demonstrated that both the Ca content and the Ca:P ratio of crickets could be altered by manipulation of the Ca content of the food eaten by the crickets.[2] The changes in mineral levels are probably a result of changes in the composition of gastrointestinal contents. The present study was designed to confirm these results and to define the quantitative relationship between diet composition and resultant cricket composition. We were particularly interested in establishing:

1. The level of dietary Ca that is necessary to produce a cricket with a Ca:P ratio of approximately 1:1.
2. The length of time that crickets need to be maintained on experimental diets in order to effect this change in Ca concentrations.
3. The site at which Ca is accumulated in crickets fed high Ca diets.

MATERIALS AND METHODS

Crickets were fed six different diets (three replicate groups per dietary treatment) and sampled at sequential time intervals. A basal high-Ca (8% Ca) diet (Table 1), which had been developed and tested in cooperation with Zeigler Bros., Inc. (Gardners, Pennsylvania 17325, USA), was modified to produce a series of experimental diets containing 2, 4, 6, 8, 10, and 12% Ca. Calcium levels in the diets were manipulated by replacing ground corn with varying amounts of limestone ($CaCO_3$) and were manufactured by Zeigler Bros., Inc. Diets were ground to pass through a 2.4-mm-mesh screen. Samples of each diet were saved for subsequent analysis.

The experimental cages consisted of white plastic bins (approximately 30 cm wide × 20 cm high × 40 cm long) with securely fitting lids. The lids included openings (20 × 30 cm) that were covered with metal screening to allow ventilation and illumination for the crickets. The crickets were exposed to a 12:12 photoperiod, and ambient temperature ranged from 26° to 29°C. Six to eight pieces of cardboard egg carton, cut into 8-cm square pieces, were placed in each cricket bin to provide surfaces for climbing and resting. In each bin, feed was presented in a 23-cm (diameter) metal pan with 2.5 cm high sides which was placed on the bottom of the cage. Every 24 hr, feed was replaced and pans were cleaned. Distilled, deionized water was presented in a 15-cm (diameter) plastic petri dish lined with paper towel. The water supply of each bin was contained in a 250-ml Berzelius beaker inverted over the petri dish. The flow rate of water was controlled by placing a 3-mm-wide rubber band under the lip of the beaker. The rubber band served as a wick, directing the water into the petri dish.

Adult crickets were purchased from a commercial supplier (Jiminy Cricket, Richmond, Virginia 23260, USA). An allotment of approximately 500 g of crickets (mean weight = 0.32 g ± 0.015 SE, n = 30) was placed into each of the 18 bins. Initial (0 time) cricket samples of 50 g were taken from each bin prior to introduction of the feed pans. Each dietary treatment was ran-

Table 2. Calcium and phosphorus concentrations of cricket diets.[a]

Diet	Calcium (%)	Phosphorus (%)
2%	2.92	0.76
4%	5.59	0.78
6%	7.69	0.70
8%	8.95	0.66
10%	10.57	0.72
12%	11.44	0.70

[a] Values are based on duplicate analyses and are expressed on a dry matter basis.

domly assigned to three bins. The crickets had free access to the feed pans and were observed feeding within 1 hr of food presentation. Subsequent cricket samples of approximately 50 g were removed from each of the bins at 12, 24, 48, 72, 96, and 120 hr after introduction of the feed pans. The crickets were placed in plastic bags and immediately frozen at −10°C.

Duplicate 5–10-g samples of crickets were removed from each of the bags and weighed to the nearest 0.001 g into tared 250-ml Phillips beakers. The samples were digested by nitric (10 ml) and perchloric (3 ml) acids under a perchloric acid fume hood. Calcium determinations were performed on the wet ash, with duplicate readings per sample, by flame atomic absorption spectrophotometry. Phosphorus was measured colorimetrically, with duplicate readings per sample, according to Gomorri.[5] Dry matter was determined on duplicate 5–10-g samples by drying to constant weight in a vacuum oven at 100°C for 2 days. Feeds were assayed for dry matter, Ca, and P by these same methods.

Sites of Ca accumulation were identified by radiography of frozen crickets (approximately 25 crickets per dietary treatment at 72 hr) with a Hewlett Packard Faxitron X-ray unit (Palo Alto, California 94303, USA). Crickets were exposed for 0.2 min at 2 ma and 35 kvp using Kodak NMB-1 film (Eastman Kodak, Rochester, New York 14650, USA).

Data were analyzed using a PC SAS (SAS Institute, Cary, North Carolina 27511, USA) statistical program for two-way analysis of variance (ANOVA). A probability level of 0.05 was chosen for determining statistical significance of observed differences. Means were compared using the Least Significant Difference procedure (LSD) controlling the comparisonwise error rate at 0.05. More conservative means comparison tests were also employed but did not substantially alter the conclusions and hence are not reported.

RESULTS

The assayed Ca concentrations of the experimental diets are presented in Table 2. Although there was some deviation from target Ca levels, the analyses approximate the expected range of Ca levels (2.9–11.4% Ca). The deviations may stem from some settling and segregation of ingredients during shipping, causing sampling error. Since diets were mixed prior to placement in feed pans, deviation of offered feed from target Ca levels is apt to be less than the analyses in Table 2 indicate. For the sake of convenience, we will refer to diets by their target Ca levels. The P concentrations of all diets ranged from 0.66 to 0.78%.

Both duration of treatment and type of dietary treatment had significant effects on Ca content and on Ca:P ratio of crickets (Fig. 1; Table 3). The significant interaction of dietary treatment and duration of treatment indicates that the change in Ca content over time differed among treatment groups. There were no significant dietary treatment, treatment duration, or interaction effects for P.

The effect of treatment duration

Table 4 presents the means for dry matter (DM), Ca, P, and Ca:P ratio as functions of the duration of treatment. The length of time crickets were maintained on diets appeared to have an effect on dry matter content. In general, crickets fed for longer periods appeared to be lower in dry matter content.

Calcium concentration in crickets also dif-

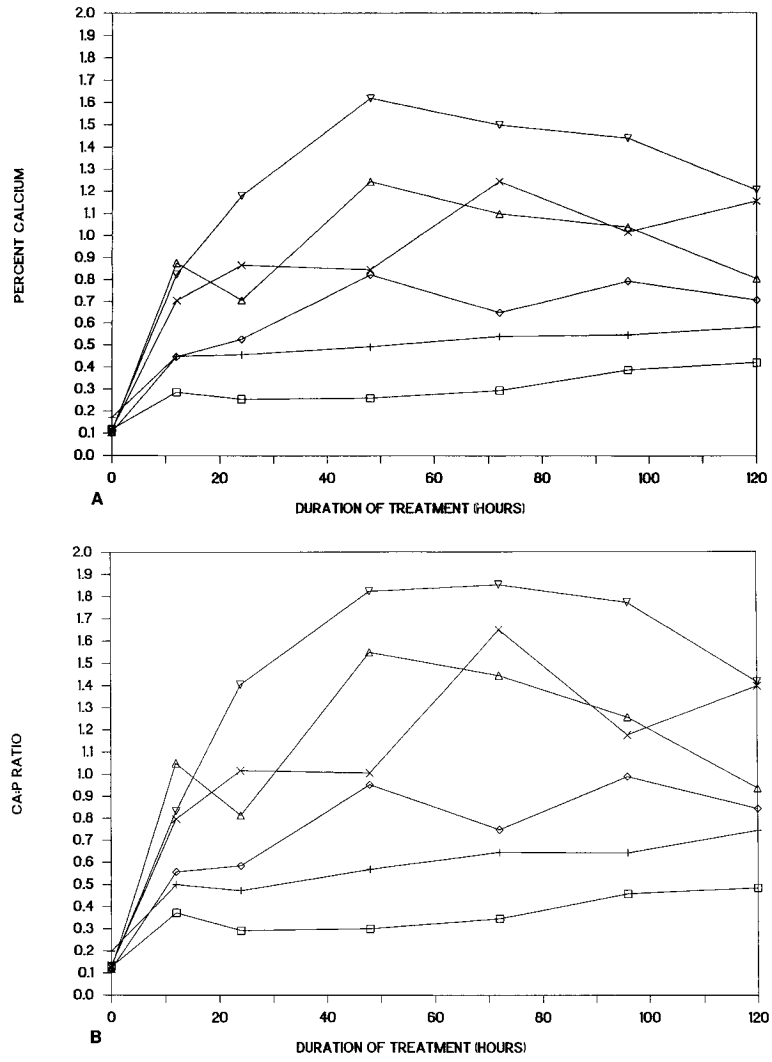

Figure 1. **a.** Effect of dietary calcium level on the calcium content of adult crickets. Duration of treatment refers to the length of time that crickets consumed experimental diets prior to sampling. Symbols refer to experimental diets as follows: □ = 2% Ca, + = 4% Ca, ◇ = 6% Ca, △ = 8% Ca, × = 10% Ca, ▽ = 12% Ca. **b.** Effect of dietary calcium on the calcium : phosphorus ratio of adult crickets. Symbols as in Figure 1a.

fered according to duration of treatment. Crickets maintained for 48–120 hr had higher Ca levels than did those fed for 12–24 hr. Phosphorus concentrations were not affected by treatment duration. Differences among Ca:P ratio means were similar to those among Ca means; the ratio improved with duration of treatment from 0 to 48 hr; thereafter mean Ca:P ratio did not change significantly. We conclude that crickets must be fed experimental diets for 48 hr to reach stable Ca:P levels.

The effect of diet composition

Table 5 presents the means and standard errors for dry matter, Ca, P, and Ca:P of crickets fed diets ranging from 2 to 12% Ca for the time periods 48–120 hr. Crickets that had fed for only 12 or 24 hr were excluded as they had yet to reach stable calcium and

Table 3. Analysis of variance of dry matter and mineral levels in crickets fed experimental diets for 0–120 hr.

Parameter	df[a]	Dry matter		Calcium		Phosphorus		Ca:P	
		F[b]	P[c]	F	P	F	P	F	P
Dietary treatment	5	2.60	0.0309	34.13	0.0001	0.24	0.9442	21.09	0.0001
Duration	6	8.27	0.0001	24.33	0.0001	0.58	0.7419	24.33	0.0001
Interaction[d]	30	0.74	0.8264	1.93	0.0100	0.61	0.9379	1.40	0.1100

[a] Degrees of freedom.
[b] F-value.
[c] Probability.
[d] Dietary treatment × treatment duration.

Ca:P levels. For this data set, analysis of variance revealed no significant differences attributable to duration of treatment for dry matter ($P = 0.394$), Ca ($P = 0.923$), P ($P = 0.864$), or Ca:P ratio ($P = 0.823$). Diet had no significant effect on either dry matter ($P = 0.319$) or phosphorus ($P = 0.995$).

In general, as dietary Ca increased, the concentration of Ca in crickets increased (Table 5). Crickets that received 12% Ca diets for 48–120 hr had a mean Ca value of 1.44%, which was significantly higher than that of any other group. Crickets fed 10 and 8% Ca diets had means of 1.06 and 1.04% Ca, respectively, which were significantly lower than the 12% group but higher than the 6, 4, or 2% Ca crickets. Phosphorus concentrations did not differ among groups (range, 0.85–0.87%).

The Ca:P ratio of the 12% Ca group (1.72:1) was significantly higher than that of any other treatment group (Table 5). There was no difference in the ratio between the 10 and 8% Ca groups (1.31:1 vs. 1.30:1), but the 6, 4, and 2% Ca groups had significantly lower ratios.

Radiographic evaluation

Radiography revealed that radiopaque material accumulated in the gastrointestinal tracts (GIT) of crickets fed high Ca diets. Crickets fed 2, 4, or 6% Ca diets had less radiodense material in the GIT, and in most cases it was impossible to visualize the GIT (Fig. 2a). The GIT of most crickets fed diets containing 8, 10, or 12% Ca appeared as well-defined structures (Fig. 2b).

DISCUSSION AND CONCLUSIONS

This study has demonstrated that the Ca concentration of adult crickets can be effectively increased by feeding diets containing

Table 4. Comparison of the dry matter (%) and mineral contents[a] of crickets in relation to the duration of time that diets were fed.

Time (hr)	n	Dry matter		Calcium		Phosphorus		Ca:P	
		Mean[b]	±SE[c]	Mean	±SE	Mean[d]	±SE	Mean	±SE
0	18	32.033^{A}	0.272	0.120^{A}	0.006	0.8745	0.017	0.138^{A}	0.007
12	18	30.694^{B}	0.417	0.597^{B}	0.063	0.8750	0.027	0.685^{B}	0.072
24	18	29.739^{BC}	0.345	0.665^{B}	0.076	0.8931	0.022	0.764^{BC}	0.095
48	18	29.867^{BC}	0.498	0.879^{C}	0.118	0.8643	0.022	1.032^{CD}	0.145
72	18	29.294^{C}	0.268	0.886^{C}	0.118	0.8357	0.024	1.114^{D}	0.174
96	18	28.911^{C}	0.270	0.868^{C}	0.106	0.8572	0.022	1.049^{D}	0.138
120	18	29.517^{C}	0.417	0.824^{C}	0.093	0.8544	0.020	0.970^{D}	0.122

[a] Expressed as a percent of dry matter.
[b] Means in a column with different superscripts are significantly different at the 0.05 probability level (LSD test).
[c] Standard error.
[d] Since phosphorus showed no significant diet effect by ANOVA, means were not compared by the LSD procedure.

Table 5. Comparison of the dry matter (%) and mineral contents[a] of crickets fed experimental diets[b] of various calcium concentrations.

		Dry matter		Calcium		Phosphorus		Ca:P	
Diet	*n*	Mean[c]	±SE	Mean[d]	±SE	Mean	±SE	Mean	±SE
2%	12	30.242	0.452	0.340^{A}	0.024	0.859	0.019	0.344^{A}	0.029
4%	12	29.600	0.510	0.539^{AB}	0.039	0.850	0.024	0.649^{AB}	0.059
6%	12	28.625	0.460	0.761^{B}	0.051	0.853	0.022	0.883^{B}	0.081
8%	12	29.458	0.469	1.044^{C}	0.100	0.840	0.031	1.296^{C}	0.153
10%	12	29.333	0.470	1.062^{C}	0.106	0.852	0.035	1.307^{C}	0.160
12%	12	29.125	0.364	1.439^{D}	0.115	0.865	0.031	1.716^{D}	0.176

[a] Expressed as a percent of dry matter.

[b] Based on treatment durations of 48–120 hr.

[c] Since dry matter and phosphorus showed no significant diet effect by ANOVA, means were not compared by the LSD procedure.

[d] Means in a column with different superscripts are significantly different at the 0.05 probability level (LSD test).

high levels of Ca if fed for a sufficient period of time. The optimal diet to use for feeding crickets will depend on the desired level of Ca (and desired Ca:P ratio) and the length of time the crickets are to be maintained on the diet. While the Ca requirements of insectivorous animals are unknown, a dietary Ca:P ratio of 1:1–2:1 is the usual recommendation for birds and mammals.[9,10] Higher levels may be desirable for birds producing large numbers of eggs.[10] It appears that crickets must be fed a diet containing at least 8% Ca and that it should be fed for at least 48 hr to achieve a Ca:P ratio of 1:1 or higher.

The diets used in this study were formulated to produce change in whole-body calcium levels of the insects, not to provide optimal nutrient levels for cricket growth or reproduction. Very high concentrations of dietary Ca were achieved by inclusion of large amounts (up to 30%) of limestone. As it may be difficult for crickets to obtain sufficient energy and nutrients from such diets, we do not recommend that high Ca diets be fed to crickets for prolonged periods of time.

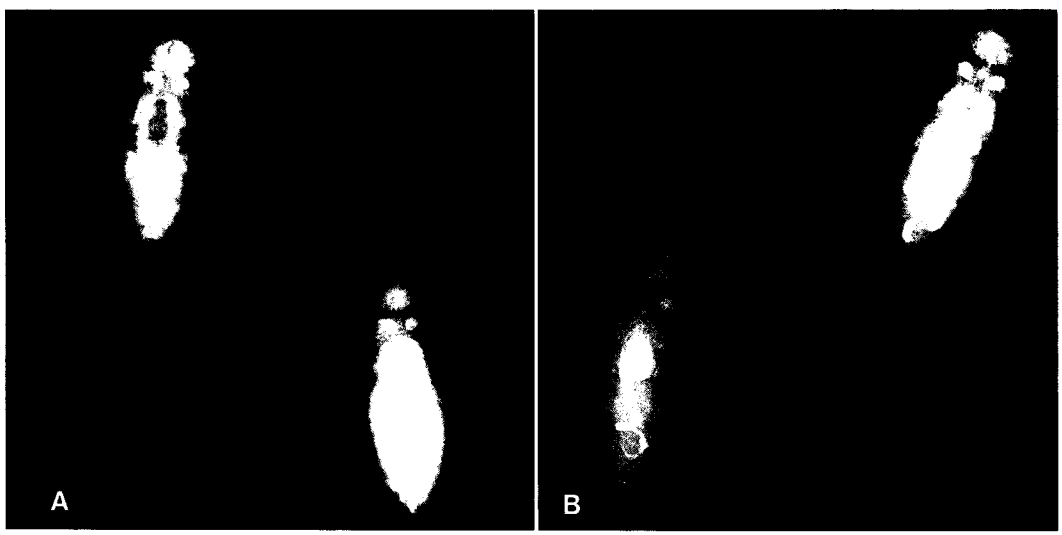

Figure 2. a. Radiograph of crickets fed 2% calcium diet for 72 hr. The gastrointestinal tract is poorly defined. **b.** Radiograph of crickets fed 12% calcium diet for 72 hr. The gastrointestinal tract is outlined as a radiopaque structure along the midline of the cricket. The radiopacity is presumably due to calcium contained in the gut.

It is also questionable whether very young crickets (e.g., "pinheads") will survive for very long on such diets. Studies are needed on the effect of high dietary Ca on growth and survival of young crickets.

A source of clean water may be an important factor in consumption of food by crickets, especially with diets high in dry matter and containing such high levels of limestone. Crickets offered cut pieces of orange and apple in addition to an 8% Ca diet appear to feed on the fruit more frequently than on the 8% Ca diet (M. Allen, pers. obs.). The common zoo practice of offering fruit and vegetables to crickets as a supplemental source of water and food may undermine the benefits of diets high in Ca. The crickets in this study only had access to the experimental diets and water.

The physical form of the diet may also be important to food consumption by crickets. In a preliminary study we determined that crickets eat twice as much of either 2% or 8% Ca diets when ground than when presented in pelleted form ($\frac{3}{16}$-inch pellets). The effects of factors such as water source, diet form, temperature, and cricket size on food intake of crickets needs further investigation.

As illustrated in Figure 2b, the ingested Ca becomes concentrated in the digestive tract. Thus the extent of GIT fill in the crickets may be an important factor in determining whole-body Ca content. Gastrointestinal tract fill is apt to be influenced by a number of behavioral and physiological factors, including pattern of feeding, transit time of ingesta, and fecal excretion. Gastrointestinal tract capacity may also differ among diverse species of insects, so the relationship of dietary Ca and Ca in the bodies of insects may be species specific.

Crickets dusted with a Ca source are apt to provide little Ca for a predator if consumed many hours later. Similarly, crickets fed high Ca diets prior to introduction into the cages of predators will eventually excrete Ca from the GIT. One solution for this problem has been investigated at the National Zoo. Feed pans containing an 8% Ca cricket diet are placed in enclosures housing tarsiers (*Tarsius bancanus*) as part of routine husbandry. Crickets are introduced into the enclosures periodically, and consume the high Ca diet, which the tarsiers ignore. Random samples of crickets caught at various locations in these enclosures had mean Ca:P ratios of 1.64:1 ± 0.26 SE (n = 10). Thus, although the tarsiers forage periodically rather than consume food at discrete meal times, the ingested crickets contain appropriate Ca:P ratios.

In the final analysis, the best method for cricket supplementation must be judged by effects on growth, reproduction, and health of the insectivorous animals. Allen et al.[1] examined the effects of feeding hatchling leopard geckos (*Eublepharis macularius*) with crickets that had been kept on either low (1.3%) or high (8.9%) Ca diets. After 7 mo, geckos fed high Ca crickets had significantly greater bone ash (61.0%) and bone Ca (21.6% of dry fat-free bone) than did geckos receiving low Ca crickets (27.7% ash and 17.8% Ca). Bone integrity, as evaluated radiographically and histologically, was abnormal in geckos fed low Ca crickets compared to geckos fed high Ca crickets[1] (M. Allen, unpubl.). Weight gains were also significantly greater in geckos fed high Ca crickets. Similar studies are needed with other insectivorous species.

It is not clear whether crickets fed on high Ca diets provide sufficient quantities of all nutrients required by insectivorous species. Nutrients such as trace minerals, amino acids, and vitamins could still be limiting, but knowledge of the nutrient composition of insects and invertebrates is very incomplete. An insectivorous predator in its natural habitat normally selects from a wide variety of invertebrate species. Zoos that maintain insectivorous animals have an obligation to expand the variety of invertebrate prey used in feeding programs as well as to investigate ways to improve further the nutrient concentrations of the few insect species presently available.

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