THE EFFECT OF TERRARIUM SIZE, EGG CRATE AREA, STOCKING DENSITY, AND TIME ON CRICKET MORTALITY AND THE ABILITY TO ACHIEVE A 1:1 CALCIUM TO PHOSPHORUS RATIO.

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Abstract

In order to provide a nutritionally adequate diet for captive insectivorous animals, invertebrates such as crickets and mealworms must be supplemented with calcium. Current methods of supplementation include dusting and gut-loading. The size of the terrarium, the area of egg crates within the terrarium, cricket stocking density, and length of time on the gut loading diet, may all play a role in cricket survivability and the ability to achieve the desired 1:1 calcium to phosphorus ratio. Two experiments were conducted to determine the effect of these factors. None of the treatments significantly affected the percent of cricket death, though the calcium level did increase significantly from the baseline. Only one replicate of the crickets collected live reached the 1:1 calcium to phosphorus ratio, however, the crickets collected dead had a significantly greater calcium to phosphorus ratio than the crickets collected live. Further research should be directed at formulating a diet that can maintain the health of the insect in conjunction with increasing the calcium to phosphorus ratio.

Introduction

In order to provide a nutritionally adequate diet for captive, insectivorous animals, invertebrates such as crickets and mealworms must be supplemented with calcium. The calcium to phosphorus (Ca:P) ratio for a typical commercially available cricket is 0.15:1 whereas a dietary ratio of 1:1-2:1 is recommended for birds, reptiles, and mammals.[1] Insectivorous animals fed insects with insufficient calcium and/or a Ca:P ratio of less than 1:1 are at risk of developing diseases such as rickets, osteomalacia, and metabolic bone disease. Methods commonly used to supplement insects include dusting with a highly concentrated calcium powder or gut loading with a high calcium diet. With dusting, the exact concentration ingested by the target animal is difficult to quantify due to insect grooming and/or the properties of the powder. For example, the particle size of the powder and its electrostatic properties may affect how well it adheres to the insect.[7] Supplementation via calcium supplemented gel-like water sources has yet to be proven successful, whereas gut loading the insect with a high calcium diet has proven to be a valid, yet inconsistent, method.[1,5]

At the Fort Worth Zoo, quality control analysis of insects from several animal areas, held under varied environments has indicated that gut loading alone is not successful in producing insects with 1:1 Ca:P ratios. Consequently, studies were undertaken to improve gut loading techniques by attempting to develop a practical and successful husbandry method. Previous research showed factors such as temperature, stocking density, size of cricket, particle size of diet, and actual calcium concentration impacted the success of gut loading techniques.[3,5,7]

Materials and Methods

Two experiments were conducted at the Fort Worth Zoo. Experiment one tested the effect of terrarium size and egg crate surface area, and stocking density on survivability of adult crickets fed Mazuri® Hi-Ca Cricket diet. Success was measured as the survivability of crickets to seven days. Experiment two was designed to test the effect of time on calcium and phosphorus levels in crickets fed the same diet. Terrarium size and stocking density in experiment two were based on the most successful and/or practical treatment from experiment one.

For both experiments, crickets were obtained from the Armstrong Cricket farm, West Monroe, LA. On arrival, a sample of approximately 100g wet mass was removed for baseline and control dry matter and mineral analysis. Chick feeders filled with deionized water served as the water source and Mazuri® Hi-Ca Cricket diet (analyzed Ca: 10.48%) was offered in petri dishes at 1.35 grams of food per one gram of insects. Room temperature was 27°C ± 2°C in both experiments.

Experiment 1

Crickets were randomly allocated into one of twelve treatments, which were a combination of surface areas and grams of crickets (Table 1). Each treatment contained three replicates. Stocking densities were determined by dividing the surface areas of the containers and the egg carton(s) by the weight of crickets. The surface area of the small terrarium was 2361cm^2 and the large terrarium was 5861cm^2. The egg crates contributed addition surface area as follows: egg crate area A: 1841cm^2, B: 3683cm^2, C: 2762cm^2 and D: 5524cm^2. All crickets were maintained in this way for seven days. On day seven, crickets were removed, dead crickets were separated from live, and all crickets were immediately frozen in a –80°C freezer.

Experiment 2

Crickets were split into 18 large terrariums, with a stocking density of 0.023 crickets per 1cm^2. The following treatments were randomly allocated to each of the terrariums (3 replicates per treatment): day two (D2), day three (D3), day four (D4), day six (D6), day eight (D8), and day ten (D10). The treatment designated the day at which the terrarium would be removed from the experiment. Upon removal, the dead crickets were separated from live. All crickets were immediately frozen in a -80°C freezer.

Laboratory analysis

Samples were dried in a Fisher Isotemp Oven at 60°C to determine dry matter content. Once dry, they were rinsed with ROPURE to remove any diet residue. After rinsing, the samples were dried at 60°C and ground into a homogenous mixture (size = 1mm). Samples of approximately 0.50 grams were removed from the whole ground sample and dried at 80° to a constant weight for determination of a dry matter correction factor. Samples of approximately 0.15 grams were digested using nitric acid in a CEM microwave digestion system 2000. Digested samples were analyzed for calcium and phosphorus using a Varian Inductively Coupled Plasma (ICP) spectrometer according to the Association of Analytical Chemists (AOAC) Official Methods of Analysis.[2]

Statistical Analysis:
Single factor ANOVA's (Microsoft Excel, Version 2000) were performed on percent death, dry-matter concentrations, dry-matter based calcium and phosphorus levels, and Ca:P ratios to determine treatment effects. Data are presented as mean $\pm$ standard error (SEM). Level of significance was set at five percent.

Results

Experiment 1
Although there were no significant differences in the percent death (Table 2) between any of the treatments, as stocking density increased, it appeared that cricket death increased. The overall average percent death was 33.28, whereas in the container with the highest stocking density (0.029 crickets per cm^2) cricket death was 46.18%. Likewise, the container with the lowest stocking density (0.007 insects per cm^2) had a percent death of 28.19%. There were no significant differences in the percent dry matter of live or dead crickets (among treatments), however, the difference between live and dead crickets was significant (29.89% ± 3.21 and 55.81% ± 12.78, respectively) (Table 2). Calcium levels increased significantly in all treatments over that of the baseline, however the desired 1:1 Ca:P ratio was only reached in the large terrarium with stocking density of 0.013 insects per cm^2 (LTD75). Interestingly, the dead crickets reached the desired Ca:P ratio in all of the large terrarium treatments (Table 3). Phosphorous levels did not increase significantly from baseline for the living crickets, but were observed to have significantly increased in the dead crickets. The Ca:P ratio was significantly greater for all treatments when comparing live versus dead (0.72 ± 0.22; 1.31 ± 0.25) respectively. There was a significantly greater increase in the Ca:P ratio in the large terrarium treatments (0.72 ± 0.22) over that of the small terrarium treatments (0.49 ± 0.15) ($P<0.05$).

Experiment 2
There was an initial significant increase in the level of calcium from baseline to day two, however past two days calcium did not increase significantly (Table 4). Calcium concentration was highest at day 6 (0.93%) and then dropped to 0.67% and 0.64% by days eight and ten, respectively. Although there was a significant increase in calcium, the desired Ca:P ratio of 1:1 was not reached with any of the treatments. There were significant differences in the Ca:P ratios between treatments with the Ca:P ratio decreasing significantly over time (Table 4). Except for day six, there were no significant differences in the percent death over the experimental period. The reason for this change is not clear. Environmental parameters were not recorded daily, which may have affected survivability. The average Ca:P ratio (mean ± SEM) for dead crickets (1.88 ± 0.39) was significantly greater than that of the live crickets (0.77 ± 0.10). The dry matter and phosphorous content of the adult crickets did not change significantly over the ten-day period.

Discussion

As with other published data, this experiment showed gut loading adult crickets is difficult and inconsistent. The first objective of this study was to determine the effect of terrarium size and stocking density on cricket survivability to seven days. It appeared that the densities chosen for

this experiment were not high enough to cause a significant increase in death. Neither the egg crate area nor the number of crickets significantly affected percent death.

Although the desired Ca:P ratio was met in the LTD75 group, the standard error of the mean (SEM) for the calcium concentrations among the three replications was large (1.06 ± 0.29), and only one of the three replicates achieved the ratio. Also, this treatment appeared to have the second largest percent death observed. Given that there was little difference between the survivability across treatments, the decision to use the LTC100 treatment for the second experiment was based on the fact that the larger terrariums appeared to have a smaller percent death and the crickets collected live had a significantly higher Ca:P ratio than those collected from the small terrariums.

In experiment two, there was a significant increase in the level of calcium from baseline to day two. Although calcium concentrations appeared to increase up to day six, at no point did the Ca:P ratio reach the desired 1:1 ratio. The Ca:P ratio then decreased again post day six to levels similar to those observed in the live crickets after seven days in experiment one. The significantly lower percent death that occurred in the replicates that were removed on day six was not clear or may be an example of the effect of environmental conditions on survivability. Humidity and/or light cycle were not recorded during the study period, which may affect survivability. Overall percent death was lower in experiment two than experiment one for the same treatment. This may be a factor of differences in environment or initial health of the crickets between shipments.

Though it is not known when cricket death occurred, it is interesting to note that in both experiments, the collected dead crickets reached the 1:1 Ca:P ratio on a dry matter basis. Additionally, there was also a significant difference in the dry matter between the crickets collected live and those collected dead. This is to be expected given desiccation over time; however, the difference in dry matter may also be due to increased diet consumption. Previous research found that the dry matter of adult crickets increased significantly with greater uptake of the diet.[1] Therefore, the crickets collected dead may have consumed more diet and reached the 1:1 ratio, but did not survive. It is also possible that although particle size of the diet did not appear to inhibit consumption, it may have compromised the gastrointestinal tract of the insect.

It appears that there is a threshold of six days on calcium accumulation for crickets maintained on the Mazuri® Hi-Ca Cricket diet. The development of a protocol for a consistent method of reaching the desired Ca:P ratio of 1:1 requires further research. Further research should include dietary formulation, with the primary objective being the development of a high calcium diet that will sustain a cricket's life for seven days.

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Table 1: Treatments, terrarium size, egg crate area, grams of crickets and stocking density used in experiment 1 to determine the effect of stocking density on survivability of adult crickets for seven days.

Treatment	Terrarium Size	Egg crate surface area	Crickets (g)	Stocking density (insect/cm^2)*
STA20	Small (Surface area = 2361 cm^2)	A 1841 cm^2	20	0.010
STA40			40	0.019
STA60			60	0.029
STB20		B 3683 cm^2	20	0.007
STB40			40	0.013
STB60			60	0.020
LTC75	Large (Surface area = 5861 cm^2)	C 2762 cm^2	75	0.017
LTC85			85	0.020
LTC100			100	0.023
LTD75		D 5524 cm^2	75	0.013
LTD85			85	0.015
LTD100			100	0.018

* insect number based on estimated weight of an adult cricket (1 cricket = 0.5g).

Table 2: A comparison of stocking density, mean percent death and dry matter content (DM) of crickets housed in small terrariums (ST-2361 cm^2) or large terrariums (LT-5861 cm^2), with varying available surface areas and grams of crickets. ¥

Treatment	Stocking density (insect/cm^2)*	% Death	%DM (live)	% DM (dead)
STA20	0.010	23.09 ± 13.06	31.09 ± 1.70	-ψ
STA40	0.019	31.34 ± 8.72	30.18 ± 1.53	-
STA60	0.029	46.18 ± 5.76	30.29 ± 0.80	-
STB20	0.007	28.19 ± 4.48	31.24 ± 2.82	-
STB40	0.013	30.25 ± 14.02	30.19 ± 2.28	-
STB60	0.020	37.25 ± 9.53	26.10 ± 5.00	-
LTC75	0.017	26.68 ± 14.77	33.81 ± 0.10	76.64 ± 4.39
LTC85	0.020	34.24 ± 16.05	33.24 ± 0.34	76.25 ± 4.29
LTC100	0.023	29.88 ± 18.03	33.16 ± 0.16	77.45 ± 2.51
LTD75	0.013	44.88 ± 13.08	32.94 ± 0.69	73.47 ± 1.49
LTD85	0.015	34.05 ± 11.38	33.37 ± 0.48	67.03 ± 5.06
LTD100	0.018	33.34 ± 15.69	33.35 ± 0.29	73.61 ± 3.05

¥Values expressed as mean + SEM. Differences in surface area were achieved by adding egg crates to terrariums: A: 1841cm^2, B: 3683cm^2, C: 2762cm^2 and D: 5524cm^2. Grams of crickets in terrariums were 20, 40, 60, 75, 85, or 100

* insect number based on estimated weight of an adult cricket (1 cricket = 0.5g).

ψ sample contamination precluded dry matter analysis.

Table 3: Mean calcium (Ca) and phosphorus (P) concentrations and Ca:P ratios in small terrariums (ST-2361 cm^2) or large terrariums (LT-5861 cm^2), with varying available surface areas and grams of crickets.[¥]

	Stocking density	Calcium (%DM)		Phosphorous (%DM)		Ca:P (%DM)
Trt.	(insect/cm^2)*	Alive	Dead	Alive	Dead	Alive
BASE		0.14^a	0.14^a	0.97^a	0.97^a	0.14^a
STA20	0.010	0.58 ± 0.14^b	-[ψ]	1.01 ± 0.02^a	-[ψ]	0.57 ± 0.14^b
STA40	0.019	0.53 ± 0.13^b	-	1.03 ± 0.05^a	-	0.52 ± 0.15^b
STA60	0.029	0.42 ± 0.04^b	-	1.04 ± 0.04^a	-	0.41 ± 0.05^b
STB20	0.007	0.50 ± 0.12^b	-	1.07 ± 0.01^a	-	0.46 ± 0.11^b
STB40	0.013	0.58 ± 0.11^b	-	1.01 ± 0.04^a	-	0.57 ± 0.11^b
STB60	0.020	0.41 ± 0.05^b	-	1.03 ± 0.01^a	-	0.39 ± 0.05^b
LTC75	0.017	0.68 ± 0.05^b	1.45 ± 0.11^b	0.95 ± 0.01^a	1.11 ± 0.01^b	0.71 ± 0.04^b
LTC85	0.020	0.62 ± 0.06^b	1.22 ± 0.39^b	1.02 ± 0.01^a	1.06 ± 0.03^b	0.61 ± 0.06^b
LTC100	0.023	0.63 ± 0.12^b	1.68 ± 0.13^b	0.98 ± 0.02^a	1.12 ± 0.02^b	0.64 ± 0.13^b
LTD75	0.013	1.06 ± 0.29^c	1.35 ± 0.04^b	0.98 ± 0.06^a	1.18 ± 0.04^b	1.06 ± 0.23^c
LTD85	0.015	0.63 ± 0.08^b	1.53 ± 0.03^b	0.97 ± 0.06^a	1.05 ± 0.06^b	0.66 ± 0.11^b
LTD100	0.018	0.62 ± 0.08^b	1.52 ± 0.14^b	1.03 ± 0.01^a	1.16 ± 0.01^b	0.61 ± 0.09^b

[¥]Values expressed as mean + SEM. Differences in surface area were achieved by adding egg crates to terrariums: A: 1841cm^2, B: 3683cm^2, C: 2762cm^2 and D: 5524cm^2. Grams of crickets in terrariums were 20, 40, 60, 75, 85, or 100

[ψ]sample contamination precluded mineral analysis.

[abc] Means found in columns with identical letters are not signficantly different by single factor ANOVA (P=0.05).

Table 4: Mean percent death, dry matter (DM), calcium (Ca), and phosphorous (P), concentrations (%) and Ca:P ratios (dry matter basis) ± standard errors in adult crickets fed Mazuri® Hi-Ca Cricket Diet calcium diet for ten days.

DAY	Death (%)	DM	Ca	P	Ca:P
0	0	30.25[a]	0.16[a]	1.04[a]	0.15[a]
2	17.19 ± 3.45[a]	30.69 ± 0.47[a]	0.84 ± 0.06[b]	1.04 ± 0.01[a]	0.81 ± 0.07[bcde]
3	22.24 ± 2.70[a]	30.69 ± 0.17[a]	0.88 ± 0.06[b]	1.05 ± 0.03[a]	0.84 ± 0.08[bde]
4	19.58 ± 6.53[a]	31.08 ± 0.22[a]	0.81 ± 0.18[b]	1.07 ± 0.01[a]	0.76 ± 0.16[bcde]
6	5.91 ± 3.61[b]	30.93 ± 0.76[a]	0.93 ± 0.15[b]	1.12 ± 0.11[a]	0.82 ± 0.04[d]
8	17.58 ± 6.56[a]	31.33 ± 0.30[a]	0.69 ± 0.04[b]	1.04 ± 0.02[a]	0.67 ± 0.05[ef]
10	19.60 ±7.12[a]	30.40 ± 0.67[a]	0.64 ± 0.02[b]	1.01 ± 0.08[a]	0.64 ± 0.04[cf]

[abcdef] Means, within columns, with any identical letters are not significantly different by single factor ANOVA (P=0.05).