THE EFFECT OF A PRODUCE BASED GUT LOAING DIET ON MINERAL, VITAMIN AND CAROTENOID CONTENT OF ADULT CRICKETS (*ACHETA DOMESTICA*)

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Abstract

Adequate supplementation of calcium and other nutrients including vitamin A is integral to successfully holding insectivorous amphibians and reptiles in captivity. Dusting and gut loading insects are two commonly used methods of supplementation. A produce based gut loading diet may be more palatable and thus may gut load more successfully and for an extended period of time. Adult crickets (*Acheta domestica*) were held on four produce based high calcium diets or a calcium carbonate diet for four days. Intake and survivability were recorded and insects were analyzed for mineral, vitamin, and carotenoid content. Survivability was high, only three out of approximately 900 crickets died over the study period. Intake did not change significantly within diet over time. The desired 1:1 calcium:phosphorus ratio was not achieved, however, the ratio was significantly higher in the apple, carrot, and sweet potato treatments over that of the calcium carbonate and papaya treatments. A very wet diet (13-21% dry matter) may have precluded the ability of the crickets to consume enough to achieve the high calcium content. Retinol concentrations stayed consistently low throughout the study period (no detection or < 333 iu/kg, dry matter basis), however, there was a significant increase in the level of beta carotene of adult crickets consuming the carrot and sweet potato diets. More research is needed to determine if beta carotene can be used as a source of vitamin A for insectivorous amphibians and reptiles and also to determine if a dry produce based diet would lead to successful gut loading.

Introduction

Invertebrate insects lack vital nutrients including calcium[1], and vitamin A.[4] Several studies have attempted to supplement insects in order to reach a 1:1 calcium:phosphorus (Ca:P) ratio.[1,2,4-11,13,15] Dusting the insect with a calcium-based powder and gut loading with a high (8%) calcium diet are two sporadically successful methods of calcium supplementation.[1,9,10,15] Numerous factors affect the ability to achieve the desired 1:1 Ca:P ratio by gut loading[1,9] and, in general, dusting is greatly inconsistent.[15] Previous research has investigated factors such as age, stocking density, time on diet, water source, temperature, and particle size of the diet on the success of gut-loading.[7-11]

It is possible that palatability of the diet may also have an effect on successful gut-loading. Survivability of the insect may be compromised when feeding a non-palatable high calcium diet. Gut loading is most effective between days two and four of feeding.[1] Considering the fact that many insects are held for up to seven days, a diet that supports a readily available 1:1 Ca:P ratio would be most practical for keeper management. The objective of this study was to create a palatable gut loading diet and to test its effect on intake and survivability along with mineral, vitamin, and carotenoid concentrations of crickets.

Methods

Crickets (*Acheta domestica*) were obtained from the Armstrong Cricket farm, West Monroe, LA. Upon arrival, approximately 200 g of crickets were removed, fasted for 24 hours, and frozen at -80ºC for subsequent baseline and control dry matter, mineral, vitamin, and carotenoid analysis. Remaining crickets were divided into 15 terrariums (11.5" x 5.5" x 8.5"), with ventilated plastic tops (Penn-Plax©, Incorporated, Hauppauge, NY), 30 grams per terrarium. Several squares of egg cartons provided shelter and distilled water was offered fresh daily in 2*2*2 boats with marbles to prevent drowning.

Crickets were fasted for 24 hours to evacuate their gut contents and subsequently randomly assigned to one of five dietary treatments. The experiment consisted of three replicates of each of the following treatments: apple, papaya, carrot, sweet potato, or calcium carbonate. Calcium carbonate was offered as is, however, each of the other food items was blended with calcium carbonate to create an approximately eight % calcium (dry matter basis) slurry diet. All diets were formulated using the Animal Nutritionist (N-squared, Inc. Durango Software, Silverton, OR, USA). The diet was pre-made in bulk and a 200 gram sample removed and frozen at -80ºC for subsequent baseline dry matter, mineral, vitamin, and carotenoid analysis. The remaining slurry diet was carefully weighed as 15 gram portions into two*two*two labeled and tared weigh boats.

Each day the remaining diet was removed and a fresh 15 gram portion offered. The remaining diet was dried at 60ºC and weighed in order to calculate daily dry matter intake. The experiment lasted four days. A 15 gram sample of crickets was removed at days two and four and frozen at -80ºC for subsequent analysis. Over the four day period temperature and deaths were recorded.

Laboratory analysis

Insect and diet samples were pulverized (size = one mm) using liquid nitrogen and a mortar and pestle. All samples were split into two portions, one to be dried for mineral analysis and the other frozen at -80°C for vitamin and carotenoid analysis.

Mineral analysis

Samples were dried in a Fisher Isotemp Oven at 60°C to determine dry matter content. Once dry, they were reground using a coffee grinder (Mr. Coffee® coffee mill, Model 3164, Cleveland, OH) into a homogenous mixture (size = one mm). Samples of approximately a half gram were dried at 80° to a constant weight for determination of a dry matter correction factor. Samples of approximately a half gram were digested using nitric acid in a CEM™ MARS-X® (Microwave Accelerated Reaction System). Digested samples were analyzed for calcium, phosphorus, magnesium, sodium, and iron using a Varian™ Inductively Coupled Plasma atomic emission spectrometer according to the Association of Analytical Chemists (AOAC) Official Methods of Analysis.[3]

Vitamin and carotenoid analysis

Approximately two grams of each sample was homogenized with a 2% pyrogallol methanol solution and then alloquoted at 1.5 grams each into 3 test tubes. Samples were saponified in a 60% v/v potassium hydroxide solution and a 70ºC water bath for 60 minutes. Following saponification, samples were extracted according to methods previously described[14] and analyzed for retinol, alpha- and gamma-tocopherol, beta carotene, lutein, canthaxanthin, echinenone, beta-cryptoxanthin, and zeaxanthin using a Waters™ 2695® separations module HPLC, with a Grace™ Vydac 201TP54® column and a Waters™ 2487® dual wavelength absorbance detector.

Statistical analysis

One-way ANOVA's for independent samples were conducted to determine if there were differences in the calcium:phosphorous ratio and/or mineral, vitamin or carotenoid concentrations as a result of different dietary treatments. A one-way ANOVA for independent samples was conducted to see any significant differences in intake between treatments (diets) and a one-way ANOVA for correlated samples was conducted to see if there was a change in intake over time within treatment. Data are presented as mean $\pm$ standard error (SEM). Level of significance was set at five percent. (VasserStats: Website for Statistical Computation ©Richard Lowry, 1998-2007, Poughkeepsie, NY).

Results

Adult crickets on the calcium carbonate diet treatment consumed significantly less food over the period of four days than those on the apple, carrot, sweet potato and papaya diet treatments (Table 2). There were no statistically significant differences in daily intake within diet treatments over time (Table 3). Crickets did not reach the optimal 1:1 Ca:P ratio within the four day study period. The Ca:P ratio, however, was significantly higher at days two in the apple and carrot treatments and at day four in the apple, carrot and sweet potato treatments than that of the calcium carbonate and papaya treatments. There was a significant decrease in the Ca:P ratio from day zero (baseline) to day two in the calcium carbonate, papaya and sweet potato treatments, however, by day four there was not a significant difference in the sweet potato treatment Ca:P ratio. Mineral concentrations varied over the four day period, most decreasing significantly from day zero to days two and four (Table 4).

Retinol concentrations stayed consistently low throughout the study period (no detection or $<$ 333 iu/kg cricket dry matter basis). There was a significant decrease in the level of alpha tocopherol from day zero (baseline) to two in the papaya treatment, however the papaya treatment at day two was the only treatment that did not have a significant decrease in the level of gamma tocopherol. All other treatments had a significant decrease in the level of gamma tocopherol by day two. Significant increases occurred in the beta carotene levels of adult crickets consuming the carrot and sweet potato diets. The concentration of beta cryptoxanthin significantly decreased in all treatments with the exception of papaya and there was a significant increase in the level of lutein solely in the carrot treatment (Table 5).

Only three crickets of approximately 900 crickets died over the study period, all on the calcium carbonate diet treatment. Temperature remained constant at 76ºF over three days, dropping slightly to 74ºF on day four.

Discussion

The desired 1:1 Ca:P ratio was not achieved in this study. It is likely that this occurred because of the high water content of the diets. A typical gut loading diet has a dry matter content of approximately 90% whereas the fruit and vegetable diets used in this study had dry matters varying between 13% and 21%. There was, however, no decrease in intake over time and therefore the diets were most likely palatable. It is likely that the crickets' guts became full with water and, though the diets were formulated for approximately eight percent calcium on a dry matter basis, the crickets never consumed enough dry matter to achieve the high calcium levels.

The palatability of a fruit or vegetable based diet may be an integral part of keeping crickets alive and gut loaded over an extended period of time. Very few deaths occurred during this four day period in contrast to those on a typical cricket feed.[9] The three deaths that did occur were crickets on the calcium carbonate treatment. It would be interesting to see if a dry fruit or vegetable diet would be as palatable as a wet diet, and if so, if the crickets would achieve the 1:1 Ca:P ratio.

The analyzed mineral concentrations of feeder crickets in this study that met minimum carnivore requirements were phosphorus (0.26%),[12] magnesium (0.04%),[12] and sodium (0.068%).[1] Neither calcium (0.29%),[12] iron (80 ppm),[12] nor vitamin E (100 iu/g)[12] met minimum requirement levels. The concentration of vitamin A in feeder crickets in this study as well as others[4,5] did not meet minimum carnivore requirements (5000–9000 iu/kg, maintenance, growth/reproduction).[12] It is therefore necessary to supplement not just calcium but also vitamins A and E. No significant changes in the concentration of vitamin A occurred between treatments in this study; however there was a significant increase in beta carotene in the carrot and sweet potato treatments over that of the other treatments. It is unknown, however, whether amphibians and reptiles can utilize beta carotene as a source of vitamin A. Additional research is necessary in this area.

In conclusion, neither a calcium carbonate diet nor four wet fruit and vegetable based diets successfully gut loaded adult crickets. More research is needed to determine the best way to create a palatable diet that will increase survivability of the cricket while successfully gut loading calcium and vitamin A.

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Table 1: Dry matter, mineral, and vitamin content of high calcium apple, carrot, papaya, sweet potato slurry diets or calcium carbonate (CaCO3) diet fed to adult crickets over a period of four days. Mineral and vitamin concentrations are on a dry matter basis.

Apple	Apple	Papaya	Sweet potato	Carrot	CaCO3
Dry matter, %	20.15	14.64	20.50	13.26	99.73
Calcium, %	6.71	7.59	7.77	8.59	38.95
Phosphorous, %	0.03	0.11	0.07	0.14	0.01
Potassium, %'	0.15	0.65	0.58	0.88	0.01
Magnesium, %	0.10	0.18	0.14	0.19	0.72
Sodium, %	0.01	0.03	0.02	0.35	0.03
Iron, ppm	55.82	72.37	78.99	77.13	592.00
Manganese, ppm	12.90	13.51	18.77	21.82	239.00
Zinc, ppm	10.64	7.78	10.08	12.52	20.00
Retinol, iu/kg	< 333	< 333	< 333	< 333	< 333
Alpha Tocopherol, iu/kg	266.39	490.15	265.72	645.04	13.37
Gamma Tocopherol, ppm	53.31	87.85	47.89	112.67	2.14
Beta Carotene, ppm	6.72	21.78	17.28	59.89	nd
Lutein, ppm	< 0.1	< 0.1	< 0.1	< 0.1	nd
Beta Cryptoxanthin, ppm	2.95	8.29	5.67	11.87	nd
Zeaxanthin, ppm	< 0.1	6.56	3.70	15.31	nd

Table 2: Average intake (four days, dry matter basis) per gram of adult cricket fed a high calcium apple, carrot, papaya, sweet potato slurry diet or calcium carbonate .

Calcium carbonate	Apples	Carrots	Sweet Potato	Papaya
0.0113 ± 0.0022*[a]	0.0317 ± 0.0045[b]	0.0413 ± 0.0016[b]	0.0348 ± 0.0012[b]	0.032 ± 0.0018[b]

* Values expressed as mean ± standard error of the mean (SEM).

[abc]Means found in rows with identical letters are not significantly different by single factor ANOVA (P<0.05).

Table 3: Average daily intake (n = three, grams ± standard error of the mean) on a dry matter basis per gram of adult cricket fed a high calcium apple, carrot, papaya, sweet potato slurry diet or calcium carbonate. There were no statistically significant differences within diet over time.

Day	Calcium carbonate	Apples	Carrots	Sweet Potato	Papaya
1	0.014 ± 0.0029	0.055 ± 0.0019	0.046 ± 0.0023	0.039 ± 0.0020	0.046 ± 0.0035
2	0.005 ± 0.0009	0.024 ± 0.0028	0.038 ± 0.0040	0.031 ± 0.0024	0.038 ± 0.0023
3	0.008 ± 0.0019	0.030 ± 0.0021	0.044 ± 0.0015	0.034 ± 0.0027	0.041 ± 0.0023
4	0.017 ± 0.0072	0.017 ± 0.0012	0.038 ± 0.0020	0.035 ± 0.0009	0.038 ± 0.0020

Table 4: Mean (n = three) Calcium:Phosphorous ratio and mineral (dry matter basis) concentration of adult crickets fed a high calcium apple, carrot, papaya, sweet potato slurry or calcium carbonate diet at days zero (baseline), two and four of intake.

	Day	Apples	CaCO3	Carrots	Papaya	Sweet potato
Calcium: Phosphorus	0			0.15 ± 0.017		
	2	0.13 ± 0.020[ac]	0.05 ± 0.000[b]	0.13 ± 0.013[ac]	0.04 ± 0.006[b]	0.07 ± 0.020[bc]
	4	0.13 ± 0.003[a]	0.06 ± 0.009[b]	0.18 ± 0.022[a]	0.07 ± 0.009[b]	0.11 ± 0.037[a]
Calcium, %	0			0.11 ± 0.007		
	2	0.07 ± 0.013[ab]	0.03 ± 0.000[b]	0.09 ± 0.007[a]	0.03 ± 0.003[b]	0.04 ± 0.013[ab]
	4	0.07 ± 0.006[ac]	0.04 ± 0.006[c]	0.11 ± 0.015[ab]	0.04 ± 0.006[c]	0.09 ± 0.003[ab]
Phosphorous, %	0			0.75 ± 0.043		
	2	0.57 ± 0.010[b]	0.61 ± 0.012[b]	0.64 ± 0.018[a]	0.60 ± 0.035[b]	0.58 ± 0.012[b]
	4	0.56 ± 0.012[ac]	0.60± 0.029[abc]	0.66 ± 0.015[b]	0.56 ± 0.022[ac]	0.56 ± 0.010[ac]
Magnesium, %	0			0.11 ± 0.007		
	2	0.03 ± 0.000[b]	0.02 ± 0.003[b]	0.04 ± 0.003[b]	0.02 ± 0.003[b]	0.03 ± 0.003[b]
	4	0.03 ± 0.003[b]	0.03 ± 0.007[b]	0.04 ± 0.003[b]	0.02 ± 0.003[b]	0.03 ± 0.003[b]
Sodium, %	0			0.37 ± 0.012		
	2	0.08 ± 0.003[bc]	0.07 ± 0.000[c]	0.11 ± 0.006[b]	0.06 ± 0.003[c]	0.08 ± 0.007[bc]
	4	0.09 ± 0.006[bc]	0.07 ± 0.003[bc]	0.12 ± 0.006[c]	0.07 ± 0.006[b]	0.09 ± 0.000[b]
Iron, ppm	0			51.33 ± 0.333		
	2	35.53 ± 2.443[bc]	26.52 ± 5.317[bc]	45.37 ± 5.555[ac]	16.3 ± 8.210[b]	30.97± 1.764[bc]
	4	37.21 ± 2.660[abd]	18.27 ± 3.301[c]	50.52 ± 5.351[b]	33.13 ± 1.167[ad]	36.37 ± 1.491[abd]

* Values expressed as mean ± standard error of the mean.

[abc] Means found in rows with identical letters are not significantly different by single factor ANOVA ($P<0.05$).

Table 5: Mean (n = three) vitamin and carotenoid concentrations on dry matter basis of adult crickets fed an high calcium apple, carrot, papaya, sweet potato slurry or calcium carbonate diet at days zero (baseline), two and four of intake.

	Day	Apples	CaCO3	Carrots	Papaya	Sweet potato
Retinol, iu/kg	0	< 333				
	2	nd¥	< 333	< 333	< 333	nd
	4	nd	< 333	< 333	nd	< 333
Alpha Tocopherol, iu/kg	0	29.99 ± 3.274				
	2	30.90 ± 1.999^{ab}	35.61 ± 0.685^{a}	32.52 ± 0.184^{ab}	23.64 ± 4.530^{b}	24.58 ± 1.912^{ab}
	4	28.54 ± 1.118^{ab}	34.31 ± 1.996^{a}	35.67 ± 2.931^{a}	18.22 ± 4.639^{b}	24.82 ± 1.746^{b}
Gamma Tocopherol, ppm	0	8.08 ± 0.274				
	2	6.19 ± 0.041^{b}	5.60 ± 0.299^{b}	6.01 ± 0.095^{b}	7.11 ± 1.506^{ab}	5.90 ± 0.560^{b}
	4	5.93 ± 0.415^{b}	5.96 ± 0.444^{b}	6.13 ± 0.430^{b}	6.30 ± 1.286^{b}	5.68 ± 0.569^{b}
Beta Carotene, ppm	0	< 0.10				
	2	< 0.10^{a}	< 0.10^{a}	3.68 ± 1.131^{b}	< 0.10^{a}	1.83 ± 0.965^{c}
	4	< 0.10^{a}	< 0.10^{a}	4.82 ± 0.665^{b}	< 0.10^{a}	3.14 ± 0.315^{b}
Beta cryptoxanthin, ppm	0	1.54 ± 0.145				
	2	< 0.10^{b}	< 0.10^{b}	0.52 ± 0.027^{c}	1.47± 0.439^{a}	0.45 ± 0.059^{c}
	4	< 0.10^{b}	< 0.10^{b}	0.68 ± 0.066^{c}	1.65 ± 0.318^{a}	0.89 ± 0.094^{c}
Lutein, ppm	0	< 0.10				
	2	< 0.10^{a}	nd	0.75 ± 0.19^{b}	< 0.10^{a}	< 0.10^{a}
	4	< 0.10^{a}	nd	1.06 ± 0.35^{b}	< 0.10^{a}	< 0.10^{a}
Zeaxanthin	no detection					
Canthaxanthin	no detection					
Echinenone	no detection					

* Values expressed as mean ± standard error of the mean.

¥ nd = not detectable

^{abc} Means found in rows with identical letters are not significantly different by single factor ANOVA (P<0.05).