EVALUATING GUT-LOADING DIETS AND DUSTING TO IMPROVE THE CALCIUM CONCENTRATION OF PIN-HEAD AND ADULT CRICKETS (*Acheta domesticus*)

Michael L. Schlegel, PhD, PAS[1,2]*, Alejandra Renjifo BS[2], and Eduardo V. Valdes, PhD[2]

[1]Department of Animal Sciences, University of Florida, Gainesville, FL 32611, USA and [2]Disney's Animal Kingdom, PO Box 10000, Lake Buena Vista, FL 32830 USA

Abstract

Four experiments were conducted to evaluate the effectiveness of dusting crickets with calcium carbonate versus feeding a custom or commercial high-calcium diet on the calcium and phosphorus concentration of pin-head (13.7 mg) and juvenile (320.9 mg) crickets. Crickets were housed in 37.9 L aquariums, provided with water and egg crates for hiding, maintained on a 12 h light: 12 h dark cycle, and temperatures ranging from 18.1 to 33.3 °C. Experiments 1 and 2 demonstrated that dusting crickets with calcium carbonate, applied at a rate of 10% of the cricket weight, increased ($P < 0.05$) Ca concentration and Ca:P ratio of the crickets more than 27 times. The commercial diet fed for 48 h increased ($P < 0.05$) the Ca content of pin-head crickets 4.4 times and both the custom and commercial diets fed for 48 h increased ($P < 0.05$) Ca content and the Ca:P ratio 3 to 4 times in adult crickets. Experiment 3 evaluated extending feeding time of the custom diet to 96 and 168 h. Extending feeding time increased ($P < 0.05$) cricket Ca content and Ca:P ratio 58% over control crickets, but the increase was less than demonstrated in Experiment 1 and 2. In experiment 4, the use of the custom or commercial high-Ca diets ground through a 1 mm screen increased ($P < 0.05$) the Ca and Ca:P ratio more than 7 times in juvenile crickets. Although both dusting and feeding high-calcium diets increased Ca content of pin-head and juvenile crickets, only dusting increased the Ca:P ratio above 1:1. However, unless dusted crickets are consumed shortly after dusting, the added Ca may be lost. It appears that factors such as palatability, temperature, particle size, or additional nutrients may be impacting consumption of the high calcium diets by the crickets.

Introduction

Previous articles have discussed the medical issues observed in highly insectivorous animals receiving a predominately invertebrate diet.[1,2] Farm raised invertebrates, such a house crickets (*Acheta domesticus*), fed to mammalian, avian, reptilian, and amphibian insectivores are low in Ca concentration with a higher P concentration resulting in a Ca:P ratio less than 1:1.[1,2,4,6-9] Protocols have been established using Ca-enriched diets to feed crickets for 24 – 72 h to increase the Ca content of house[1,2,7,8] and Jamaican (*Gryllus assimmilis*)[5] crickets and provided a Ca:P ratio greater than 1:1. This strategy fills the gastrointestinal tract of the crickets with the Ca-enriched diet and is referred to as 'gut-loading.' Although the effectiveness of these protocols have been documented, there were an equal number of documented experiments and high calcium cricket diets which do not achieve the desire results of increasing the Ca content and the Ca:P ratio above 1:1.[2, 7,8,10,11]

An alternative to 'gut-loading' is to dust crickets with a Ca supplement which adheres to the invertebrate when consumed. While desired Ca concentrations and Ca:P ratios are achieved after dusting, the crickets must be consumed within minutes since the crickets begin cleaning themselves to removed the added dust[12]. In the process of changing to a lower priced high-Ca cricket diet, the revised 'gut-loading' protocol needed to be evaluated. The objective of these series of studies was to evaluate a custom prepared and a commercial high-Ca cricket diet by 1) evaluating the effectiveness of dusting or gut-loading pin-head and adult crickets, 2) evaluating the length of time needed to gut-load crickets, and 3) evaluating the effect of diet particle size on gut-loading effectiveness.

Materials and Methods

Crickets and Husbandry

House crickets (Lucky Lure Cricket Farm, Leesburg, FL 34749-0956) were received the morning of experiment start date. Crickets (n = 20) not used for the experiments were used to determine average cricket weight and length (head to tail, Pocket caliper, No. 132me, General Tools Mfg. Co. LLC., New York, NY) (Table 1).

Six 37.9 L aquarium tanks fitted with screen lids were used for each experiment (3 replicates per treatment). Diets were provided on the floor of the tank. The amount of diet provided was based on daily immature cricket intake (at least 110 mg diet/g of cricket) determined by Anderson.[2] A tap-water soaked sponge, placed in two petri dishes, was changed twice daily to supply fresh water. Four partial egg crates were placed into each tank to provide hiding places. High and low ambient temperatures (HI9063, Hanna instruments USA, Woonsocket, RI 02895) were recorded daily during each experiment (Table 2). The room which housed the crickets was on a 12 h light: 12 h dark cycle.

Experiment 1: Evaluating dusting and high-Ca cricket diets to gut-load pin-head crickets.

Approximately thirty-seven thousand pin-head crickets (Table 1) were combined into a 37.9 L aquarium to provide a bulk container from which to sample. Three replicates of 35 g (actual 36 g) of crickets were removed from the bulk container and frozen to provide a control (0 h). To evaluate the effect of dusting on Ca concentration of crickets, three replicates of 35 g (actual 35.4 g) of crickets were removed from the bulk container and placed in a 0.95 L resealable plastic bags and mixed with 3.5 g of calcium carbonate (40% Ca; Spectrum Chemical Mfg. Corp. New Brunswick, NJ 08901) frozen for 30 min, and sifted to remove excess supplement, and refrozen. Into each tank, 30 g of either a custom or commercial high-Ca cricket diet (Mazuri, Richmond, IN 47374) was placed. Ninety grams of crickets (Table 2) were added to each tank to be gut-loaded. After 48 to 50 h of gut-loading, 30 g of living crickets (range: 20.6 – 34.2 g) were collected and frozen (-24 °C) for analysis.

Experiment 2: Evaluating dusting and high-Ca cricket diet to gut-load juvenile crickets.

Three thousand juvenile crickets (three-quartered grown, Table 1) were separated into three replicates. Each replicate (1000 crickets) was placed into a 37.9 L aquarium to provide a bulk container from which to sample. From each replicate, 35 g (actual 40.3 g) of crickets were removed from the bulk container and frozen to provide a control, 35 g (actual 35.2 g) of crickets were removed form the bulk container and placed in a 0.95 L resealable plastic bag and mixed with 3.5 g of calcium carbonate, frozen for 30 min, and sifted to removed excess supplement, and refrozen. Into each tank, 30 g of either a custom or commercial high-Ca cricket diet was placed along with 90 g (Table 2) of adult crickets to be gut-loaded. After 48 to 50 h of gut-loading, all living crickets were collected and frozen (-24 °C) for analysis.

Experiment 3: Evaluating the length of time (96 vs 168 h) to gut-load juvenile crickets using a custom high-Ca diet.

Three thousand juvenile crickets (three-quarter grown, Table 1) were separated into three replicates. Each replicate (1000 crickets) was placed into a 37.9 L aquarium to provide a bulk container from which to sample. From each replicate, 35 g (actual 36.2 g) of crickets were removed from the bulk container and frozen to provide a control. Into each tank were placed 140.2 g of a custom high-Ca cricket diet (Table 3) and 130 g (Table 2) of juvenile crickets. After 96 and 168 h of gut-loading, all living crickets were collected, weighed, and frozen (-24 °C) for analysis.

Experiment 4: Evaluating grinding a custom and commercial high-Ca cricket diet to gut-load juvenile crickets.

Three thousand juvenile crickets (three-quarter grown, Table 1) were separated into three replicates. Each replicate (1000 crickets) was placed into a 37.9 L aquarium to provide a bulk container from which to sample. From each replicate, 35 g (actual 37.8 g) of crickets were removed from the bulk container and frozen to provide a control. The custom and commercially available diets used in Experiments 1-3 were ground through a 1 mm screen (Model ZM100; F. Kurt Retsch Gmbh and Co. KG, Haan, Germany). Into each tank were placed 50 g of a custom or a commercial diet (Table 3) and 90 g (Table 2) of juvenile crickets. After 48 to 50 h all living crickets were collected, weighed, and frozen (-24 °C) for analysis.

Feed particle size (one replicate of each feed whole and ground) was determined using a Ro-Tap RX-29 test sieve shaker (W.S. Tyler, Mentor, Ohio 44060 USA) with the diets shaken for 10 min.[3] Feed samples and crickets were analyzed at a commercial laboratory (Dairy One, Ithaca NY 14850) for DM, Ca, P, crude protein, and fat. Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC 27513 USA). Heterogeneous variance was tested using the f_{max} test.[9] Data were transformed (square root or log) as needed to eliminate the heterogeneous variance. Means were separated using Tukey's t-test.

Results

Experiment 1 and 2

Dusting both pin-head and juvenile crickets with 10% calcium carbonate, by weight, increased ($P < 0.05$) Ca concentration and the Ca:P ratio of the of the crickets more than 27 times compared with control crickets (Table 4 and 5). The commercial diet increased ($P < 0.05$) pin-head cricket Ca content and Ca:P ratio 4.4 times (Table 4) compared with control crickets. In juvenile crickets, the custom and commercial diets both increased ($P < 0.05$) Ca content and the Ca:P ratio 3 and 4 times, respectively, compared with control crickets (Table 5). Cricket survival rate was similar for both diets.

Experiment 3

Crickets which were fed the custom high-Ca cricket diet for 96 and 168 h had 8-9% greater ($P < 0.05$) DM and 14-19% lower ($P < 0.05$) fat than control crickets (Table 6). Crickets fed for 168 h had 4.5% greater ($P < 0.05$) crude protein content than control crickets. Crickets fed for 96 and 168 h had 61 and 58% greater ($P < 0.05$) calcium and 61 and 54% greater ($P < 0.05$) Ca:P ratio than control crickets, respectively. Cricket survival rate was 15% lower ($P < 0.05$) when fed for 168 h compared with crickets fed for 96 h.

Experiment 4

Crickets fed the ground custom high-Ca cricket diet had lower ($P < 0.05$) DM than the control crickets. Calcium content and the Ca:P ratio of the crickets fed both ground diets were more than 7 times greater ($P < 0.05$) than control crickets. Crickets fed the ground commercial diet had 5% greater P content than control crickets. Cricket recovery was similar for both diets.

Discussion

Dusting crickets with calcium carbonate (10% of the cricket weight) was effective to increase Ca concentration and Ca:P ratio of pin-head and juvenile crickets. Trusk and Crissey[12] failed to increase the Ca concentration of dusted crickets using a 11% Ca supplement applied at a rate of 1.8% of the weight of the crickets. If all the calcium carbonate adhered to the crickets, the theoretical Ca concentration of the crickets would have been 12.6 and 10.8% for pin-head and juvenile crickets, respectively. Based on the resulting Ca concentration of the dusted crickets, only 39 and 30% of the calcium carbonate adhered, respectively. To achieve at least a 1:1 Ca:P ratio, calcium carbonate would have to be applied at a rate of at least 0.63 and 0.57% of the cricket weight. One concern with dusting crickets is that the palatability of crickets may change reducing intake or as determined by Trusk and Crissey,[12] the Ca concentration of dusted crickets decreases after 3 h due to the the crickets cleaning themselves.

In all four experiments, the use of either the custom or commercial high-Ca cricket diets with 8 to 9.5% Ca failed to increase the cricket Ca content sufficiently to provide a Ca:P ratio~~n~~ greater than 1:1. This observation agrees with the study by Hunt et al.,[10] but disagrees with the studies of Allen and Oftedal[1], Anderson[2], Finke[7], and Finke et al.[8] The range in temperatures used in the four studies (18.1 to 33.3 °C, Table 2) was similar to the temperatures used by Allen and Oftedal (26-29 °C)[1] and Finke et al (25-28°C)[8] which demonstrated effective gut-loading procedures. Although McClements et al.[11] determined that adult crickets had greater Ca content when housed at 26.7 °C or higher than below this temperature, this relation was not observed across all cricket sizes.

Experiment 3 was conducted to evaluate the length of time needed to achieve sufficient Ca content of the crickets, by extending the feeding time beyond 48 h to 96 or 168 h. Increasing gut-loading time did not achieve the desire affect. Both Allen and Oftedal[1] and Hunt et al.[10] observed an increase in cricket Ca up to 48 h, but did not see additional increases in Ca when crickets were fed longer.

When experiments 1-3 were ineffective to increase the Ca:P ratio to 1:1, one of the concerns was the particle size of the diet. Allen and Oftedal[1] ground the diets in their study through a 2.4 mm-mesh screen. The average particle sizes of the custom and commercial diets in the current study were 0.773 mm and 0.418 mm, respectively. This would suggest that the diets were ground sufficiently, but Experiment 4 was conducted to evaluate the effect of grinding the cricket diets through a 1 mm screen on increasing cricket Ca content. The ground diets had an average particle size of 0.144 and 0.211 mm for the custom and commercial diet, respectively. Grinding the cricket diets increased the Ca content and Ca:P ratio of the crickets to a greater extent then the unground diets, but these ground diets still did not achieve the 1:1 Ca:P ratio desired. One of the observations made during Experiment 4 was that the finely ground diets adhered to the juvenile crickets to a greater extent than when unground. When the crickets were sampled, an attempt was made to sift the crickets prior to sampling. Additionally, it was observed that the crickets seem to 'drown' in the fine diet resulting in the lowest live cricket recovery of experiments 2-4. Finke et al.[8] demonstrated that both a finely ground and an extruded cricket diet increased the Ca content and provided a 1:1 Ca:P ratio.

~~0~~

It is important to evaluate the effectiveness of invertebrate 'gut-loading' programs to ensure highly insectivorous animals are receiving proper nutrition. Under the conditions tested in the series of experiments present in this paper, gut loading crickets increased their Ca content, but not to the desired concentration to provide a Ca:P ratio greater than 1:1. Dusting crickets was an effective method to increase the Ca concentration of the crickets and achieve greater than a 1:1 Ca:P ratio, but unless the crickets are consumed shortly after dusting, the added Ca may be lost. It appears that factors such as palatability, temperature, particle size, or additional nutrients may be impacting consumption of the high-Ca diets by the crickets. ~~As stated by previous authors, additional research is needed to evaluate these variables.~~

Acknowledgements

The authors would like to thank Jamie Sincage, zoological manager, and Disney's Animal Kingdom invertebrate keepers for their assistance with setting up the study area for each experiment.

Literature Cited

1. Allen, M.E. and O.T. Oftedal. 1989. Dietary manipulation of the calcium content of feed crickets. J. Zoo Wildl. Med. 20: 26-33.

2. Anderson, S.J. 2000. Increasing calcium levels in cultured insects. Zoo Biol. 19: 1-9.

3. Baker, S. and T. Herrman. 2002. Evaluating particle size. Kansas State Univ., Manhattan, Kansas.

4. Barker, D., M.P. Fitzpatrick, and E.S. Dierenfeld. 1998. Nutrient composition of selected whole invertebrates. Zoo Biol. 17: 123-134.

5. Eidhof, K., D. Venema, D. Kuiper, T.R. Huisman. 2005. Increasing calcium content in Jamaican field crickets (Gryllus assimilis). 4th European Zoo Nutrition Mtg. Abstract Book. Leipzig, Germany P. 64 (Abstr.).

6. Finke, M.D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol. 21: 269-285.

7. Finke, M.D. 2003. Gut loading to enhance the nutrient content of insects as food for reptiles: A mathematical approach. Zoo Biol. 22: 147-162.

8. Finke, M.D., S.U. Dunham, C.A. Kwabi. 2005. Evaluation of four dry commercial gut loading products for improving the calcium content of crickets, *Acheta domesticus*. J. Herp. Med. Surg. 15: 7-12.

9. Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences, vol. 1. The Iowa State Univ. Press, Ames, Iowa.

10. Hunt, A.S., A.W. Ward, and G.W. Ferguson. 2001. Effects of a high calcium diet on gut loading in varying ages of crickets (*Acheta domestica*) and mealworms (*Tenebrio molitor*). Proc. 4th Conf. Zoo Wildl. Nutr. Pp. 94-99.

11. McClements, R.D., B.A. Lintzenich, and J. Boardman. 2003. A zoo-wide evaluation into the current feeder insect supplementation program at the Brookfield Zoo. Proc. Nutr. Advisory Group 5th Conf. Zoo Wildl. Nutr. Pp. 54-59.

12. Trusk, A.M., and S. Crissey, 1987. Comparison of calcium and phosphorus levels in crickets fed a high calcium diet versus those dusted with supplement. Proceedings of the 6th and 7th Dr. Scholl Conferences on the Nutrition of Captive Wild Animals, Pp. 93-99.

Table 1. Cricket (*Acheta domesticus*) weight and length used in four experiments.

			Weight		Length	
Experiment	Cricket size	n	mg	SD	mm	SD
1	Pin-head	20	13.7	3.9	6.1	0.91
2	Juvenile[a]	20	345.2	101.3	24.6	4.98
3	Juvenile[a]	20	287.5	88.1	18.3	1.33
4	Juvenile[a]	20	330.1	52.0	18.2	1.40

[a]Three-quarter grown.

Table 2. Cricket (*Acheta domesticus*) weight, diet offered, and temperature ranges of four experiments evaluating dusting or gut-loading crickets to increase calcium content.

				Temperature range, °C	
Experiment	Total cCricket weight, g	Total diet offered, g	Daily diet (mg) per cricket (g)	Low	High
1	90.8	30.0	165[a]	23	31
2	89.3	30.0	167[a]	23	28
3	132.4	140.2	265[b] / 151[c]	18.1	33.3
4	90.6	50.0	276[a]	20	25

[a]48 h study.
[b]96 h study.
[c]168 h study.

Table 3. Selected nutrient composition of a custom and a commercial high-calcium cricket diet.

Item	Custom Diet	Commercial Diet
Moisture, %	10.7	8.6
Crude protein, % of DM	20.2	20.7
Ca, % of DM	8.0	9.5
P, % of DM	0.78	0.68
Ca:P ratio	10.3	14.0

Table 4. Influence of dusting or feeding a custom or commercial high-calcium cricket diet for 48 hours on the calcium and phosphorus content of pin-head crickets (*Acheta domesticus*).

	Treatments				
Item	Control	Dusting	Custom diet	Commercial diet	SEM
n	3	3	3	3	
DM, %	23.23^{y}	26.10^{x}	22.40^{y}	22.50^{y}	0.582
Ca, % of DM	0.20^{z}	5.40^{x}	0.25^{z}	0.88^{y}	0.130[a]
P, % of DM	1.26^{x}	0.84^{y}	1.27^{x}	1.25^{x}	0.051
Ca:P ratio	0.16^{z}	6.47^{x}	0.20^{z}	0.71^{y}	0.199[b]

[a]Square root transformed SEM = 0.038.
[b]Square root transformed SEM = 0.021.
[xyz]Means within a row with unlike superscripts differ ($P < 0.05$).

Table 5. Influence of dusting or feeding a custom or commercial high-calcium cricket diet for 48 hours on the crude protein, fat, calcium and phosphorus content and recovery of juvenile crickets (*Acheta domesticus*).

	Treatments				
Item	Control	Dusting	Custom diet	Commercial diet	SEM
n	3	3	3	3	
DM, %	28.20^{y}	30.47^{x}	28.67^{xy}	29.17^{xy}	0.417
CP, % of DM	63.80^{y}	58.70^{z}	65.43^{x}	64.13^{xy}	0.298
Fat, % of DM	27.53^{x}	24.10^{y}	24.07^{y}	24.20^{y}	0.369
Ca, % of DM	0.12^{z}	4.02^{x}	0.38^{y}	0.49^{y}	0.184[a]
P, % of DM	0.81^{x}	0.83^{x}	0.81^{x}	0.83^{x}	0.018
Ca:P ratio	0.15^{z}	4.86^{x}	0.47^{yz}	0.59^{y}	0.228[b]
Live cricket recovery, %	-	-	73.0	72.3	5.5

[a]Log transformed SEM = 0.077.
[b]Square root transformed SEM = 0.074.
[xyz]Means within a row with unlike superscripts differ ($P < 0.05$).

Table 6. Influence of feeding a custom high-calcium cricket diet for 96 or 168 hours on the crude protein, fat, calcium, and phosphorus content and recovery of juvenile crickets (*Acheta domesticus*).

	Treatments			
Item	Control	96 h	168 h	SEM
n	3	3	3	
DM, %	27.30[y]	29.50[x]	29.77[x]	0.459
Crude protein, % of DM	66.43[y]	67.27[xy]	69.43[x]	0.558
Fat, % of DM	22.70[x]	19.57[y]	18.47[y]	0.530[a]
Ca, % of DM	0.12[y]	0.19[x]	0.19[x]	0.006
P, % of DM	0.91[x]	0.92[x]	0.95[x]	0.010
Ca:P ratio	0.13[y]	0.21[x]	0.20[x]	0.006
Live cricket recovery, %	-	85.4[x]	72.5[y]	1.5

[a]No appropriate transformation was found to correct heterogeneous variance.
[xy]Means within a row with unlike superscripts differ ($P < 0.05$).

Table 7. Influence of feeding a ground custom or commercial high-calcium cricket diet fed for 48 h on the crude protein, fat, calcium, and phosphorus content and recovery of juvenile crickets (*Acheta domesticus*).

	Treatments			
Item	Control	Ground custom diet	Ground commercial diet	SEM
n	3	3	3	-
DM, %	74.0[x]	72.6[y]	73.0[xy]	0.28
Ca, % of DM	0.10[y]	0.70[x]	0.78[x]	0.0236[a]
P, % of DM	0.85[y]	0.88[xy]	0.89[x]	0.0069[b]
Ca:P ratio	0.11[y]	0.80[x]	0.88[x]	0.028
Live cricket recovery, %	-	60.0	62.0	4.0

[a]Square root transformed SEM = 0.014.
[b]Square root transformed SEM = 0.015.
[xy]Means within a row with unlike superscripts differ ($P < 0.05$).