Effects Of A High Calcium Diet On Gut Loading In Varying Ages Of Crickets (*Acheta domestica*) And Mealworms (*Tenebrio molitor*)

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Insects are a poor source of calcium. Consequently, it is a common practice for zoos to supplement them in order to provide a nutritionally balanced diet to many species. Current methods of supplementation include dusting and gut-loading. The experiment was conducted in order to determine the effect of a high calcium diet on gut loading in varying ages of crickets (*Acheta domestica*) and mealworms *(Tenebrio molitor)*. An eight percent calcium diet was fed to four replicates of each of the following insects: week-old (pinheads), half-grown, and adult crickets; and small, medium, and large mealworms. Initial gut contents were evacuated from the insects and they were started on the experimental diet (day zero). Crickets and mealworms were maintained on the experimental diet for eight or seven days, respectively and were sampled at days zero, two, four, and eight (crickets); or zero, two, four, and seven (mealworms). Samples were analyzed for calcium and phosphorus content. Small and medium mealworms reached the desired 1:1 Ca:P ratio at day two, other treatments did not. Temperature may have played a role in the dietary intake of the insects.

Key words: invertebrates; supplement; environmental temperature

INTRODUCTION

Many captive animal diets consist solely of invertebrates such as arachnids, annelids, crustaceans, and insects. Invertebrates lack an internal skeleton and therefore these diets often fail to meet the animal's mineral, specifically calcium, requirements. In its natural habitat, an animal may supplement low-mineral invertebrates by varying its diet to include high-mineral species or by ingesting soil along with the insect. Captive animals, however, do not have these options. A typical cricket has a Ca:P ratio of only 0.15:1 whereas, a dietary ratio of 1:1 – 2:1 is the usual recommendation for birds and mammals [Allen and Oftedal, 1989]. Therefore, if supplementation does not occur, captive insectivorous animals may be afflicted with diseases such as rickets, osteomalacia, and metabolic bone disease.

Two methods are currently used for mineral supplementation. Insects can be dusted with a high concentration mineral powder or gut-loaded with a high calcium diet. When dusted, the insect must be consumed right away. If not, the insect will begin to groom itself, thereby removing the powder and leading to inconsistent supplementation. Gut-loading is an alternative method. The insect is fed a high calcium diet with the purpose of filling its gastrointestinal tract and increasing the concentration of calcium in the consumed insect. Though there have been several studies comparing the differences of dusting vs. gut-loading in adult crickets, very little research has been completed to show the efficiency of gut loading in various ages and sizes of crickets and mealworms. Therefore, the objectives of this study were to: 1. Obtain at least a 1:1 Ca:P ratio in week old (pinhead), half-grown, and adult crickets (*Acheta domestica*) and small, medium, and large mealworms (*Tenebrio molitor*) by feeding an eight percent calcium diet and 2. Determine if the rates of calcium uptake differ among various sizes of insects fed an 8% calcium diet.

MATERIALS AND METHODS

Crickets were obtained from the Armstrong Cricket farm, West Monroe, LA and mealworms were obtained from GRUBCO, Inc., Hamilton, OH. Upon arrival, approximately 40 g of week old crickets, and 75 g of all other insects were placed in one of 24 plastic terraria covered with plastic mesh lids. The experiment consisted of four replicates of each of the following treatments: WOCR (week old crickets), HGCR (half-grown crickets), ADCR (adult crickets), SMMW (small mealworms), MDMW (medium sized mealworms), and LGMW (large mealworms). Several squares of egg carton provided shelter and two petri dishes containing paper towels soaked with ROPURE deionized water served as the water source. Terrariums were placed so that each enclosure rested on a 7.5 cm wide strip of Herp-Heat. This established a thermal gradient of 23-41°C (73-106°F).

Crickets and mealworms were fasted for the first 48 h in order to evacuate their gut contents. At this point, a sample of 2 g wet mass (pinheads) and approximately 15 g wet mass of all other treatments was taken for subsequent dry matter and mineral analysis. This was considered day zero of the study. Terrariums were cleaned and 104 $\pm$ 4 g of a Zeigler 8% calcium ground diet (Product No. 53900000) was placed on the floor of each terrarium as substrate. Laboratory analysis of the diet was conducted to determine actual diet content of calcium and phosphorus (9.1% and 1.0%, respectively). Crickets and mealworms were maintained on the experimental diet for eight or seven days, respectively, and were sampled at days two, four, and eight (crickets) or two, four, and seven (mealworms) according to the sampling weights mentioned above. All samples were immediately frozen in a -70°C freezer.

Laboratory analysis

Samples were dried in a Fisher Isotemp Oven at 60°C to determine dry matter content and subsequently rinsed with ROPURE to remove any diet residue. After rinsing, the samples were dried and ground into a homogenous mixture in a Krups coffee grinder. All samples were analyzed in duplicate. Samples of approximately 0.5 g were removed from the whole ground sample and dried overnight at 80°C for determination of a dry matter correction factor. Samples of approximately 0.15 g were removed from the whole ground sample and digested in 6 mL of nitric acid in a CEM microwave digestion system (MDS) 2000. Digested samples were diluted to 60 mL with ROPURE deionized water and then analyzed for mineral content using a Varian Inductively Coupled Plasma (ICP) spectrometer according to standard AOAC methods [1996].

Statistical Analysis

The statistical software Systat (Version 6.0.1, © 1996, SPSS, Inc.) was used to analyze all data. Repeated measures analysis of variance (ANOVA) was performed on dry-matter based calcium and phosphorus data and Ca:P ratios. Post-hoc tests (Bonferroni, $\propto = 0.0125$) were performed for day two Ca:P ratio data sets. Single factor ANOVA's (Microsoft Excel, Version 2000) were performed on dry-matter concentrations, dry-matter based calcium and phosphorus levels, and Ca:P ratios on individual sizes to determine treatment effects. Levels of significance were set at the five percent level.

RESULTS

Crickets

There were no significant differences in the dry matter content of WOCR and HGCR throughout the eight-day period. There was a significant decrease in the dry matter of the ADCR from day zero to day two. There were no significant changes after day two.

Phosphorus levels did not significantly change in HGCR or ADCR throughout the eight-day period, however, in WOCR there was a significant decrease in the level of phosphorus from day zero to day two. There were no significant changes after day two.

Overall, time and size had a significant effect on the uptake of calcium and the Ca:P ratio in crickets throughout the eight-day period (Fig. 1). Week old crickets (WOCR) and HGCR took up a significantly greater amount of calcium than ADCR by day two, however, the required 1:1 ratio was not obtained by any of the three sizes. WOCR had the highest Ca:P ratio of 0.88 at day four, whereas HGCR and ADCR reached the highest ratios (0.67 and 0.48) at day two. The Ca:P ratio increased significantly in WOCR by day two and was maintained until day eight. In HGCR and ADCR the Ca:P ratio increased significantly from both day zero to day two and day two to day four and was maintained throughout day eight.

Mealworms

There was a significant increase ($P < 0.05$) in the dry matter content of all sizes of mealworms throughout the experiment.

In SMMW and MDMW, there was a significant increase in phosphorus by day four or two (respectively), however, the levels returned to the original level by day eight. There were no significant differences in the phosphorus content of LGMW throughout the experiment.

Overall, time and size had a significant effect on the uptake of calcium in mealworms throughout the eight-day period (Fig. 2). SMMW and MDMW took up a significantly greater amount of calcium than LGMW by day two. The calcium content and Ca:P ratio increased significantly with each two day period for the eight-day sampling period. In LGMW the calcium content and Ca:P ratio increased significantly by day two and was then maintained. SMMW and MDMW reached the desired Ca:P ratio (0.98 and 1.10, respectively) at day two whereas LGMW reached the highest Ca:P ratio at day four (0.86:1).

DISCUSSION

Dry matter did not increase significantly in any of the insects in this study. Allen and Oftedal [1989] found that dry matter of adult crickets increased significantly with greater uptake of the diet. It is most probable that the insects in this study did not consume enough of the diet to trigger the increase in dry matter content.

The ideal Ca:P ratio was not reached by any of the crickets on the experimental diet. Previously, adult crickets reached a 1:1 ratio after two days on an eight percent calcium diet [Allen and Oftedal, 1989]. In contrast to the 26-29°C temperature range crickets were exposed to in that experiment, crickets in the current study were exposed to temperatures between 23-41°C. The high end of this temperature range may have been excessive to the point of decreasing the intake of the crickets. Other studies have also had difficulty reaching the 1:1 Ca:P ratio in crickets [Trusk and Crissey, 1987; Sabatini et al., 1998]. Further research will be conducted to determine the effect of temperature on calcium uptake in crickets. Indeed, small and medium sized mealworms maintained at the same temperatures did reach the desired ratio.

A higher uptake of calcium and a more significant increase in the Ca:P ratio occurred in the smaller sizes of both crickets and mealworms. If the gastrointestinal tract is larger relative to the body in smaller insects, they would have a greater capacity to gut load the diet. Alternatively, the smaller insects may have filled their gut to maximum capacity whereas the larger insects may not have eaten to full capacity.

Similar to Allen and Oftedal [1989], phosphorus levels did not change significantly in half-grown or adult crickets throughout the study. In week old crickets, phosphorus decreased significantly from day zero to day two and was then maintained. Because the guts were evacuated prior to feeding the experimental diet, it is not clear why this occurred.

An important aspect of gut-loading is the timing involved in feeding the insects out. According to these results, small and medium mealworms reach a 1:1 Ca:P at day two and dropped significantly after that. Allen and Oftedal [1989] show that Ca levels are maintained from 48 – 120 h. In this study, levels were not maintained, however, this could be due to the higher terrarium temperature. Most likely, crickets and mealworms should be gut loaded and fed out between two and four days after being subjected to the diet.

CONCLUSIONS

1. Small and medium mealworms reached a 1:1 Ca:P ratio after two days on an eight percent calcium diet.
2. There was a significant increase in the Ca:P ratio of large mealworms and week-old, half-grown, and adult crickets on an 8% calcium diet, however, a 1:1 Ca:P ratio was not obtained.
3. A higher uptake of calcium and a more significant increase in the Ca:P ratio occurred in the smaller sizes of both crickets and mealworms.
4. Ideally, crickets and mealworms should be gut loaded and fed out between two and four days after being subjected to the diet.

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TABLE 1. Mean dry matter (DM), calcium (Ca), and phosphorus (P), concentrations (%) and Ca:P ratios (dry matter basis) ± standard errors in week-old (WOCR), half-grown (HGCR), and adult (ADCR) crickets fed an eight percent calcium diet for eight days

Treat-ment		Day 0	Day 2	Day 4	Day 8
WOCR	%DM	$24.4^a \pm 1.4$	$24.4^a \pm 0.9$	$28.2^a \pm 2.4$	$28.1^a \pm 1.4$
	%Ca	$0.10^a \pm 0.02$	$0.68^b \pm 0.04$	$0.74^b \pm 0.09$	$0.47^c \pm 0.08$
	%P	$1.22^a \pm 0.01$	$1.11^b \pm 0.04$	$1.12^b \pm 0.04$	$0.89^b \pm 0.11$
	Ca:P	$0.08^a \pm 0.02$	$0.61^b \pm 0.04$	$0.66^b \pm 0.07$	$0.53^b \pm 0.04$
HGCR	%DM	$24.3^a \pm 1.0$	$23.6^a \pm 0.3$	$26.3^a \pm 2.6$	$25.1^a \pm 0.2$
	%Ca	$0.08^a \pm 0.00$	$0.58^b \pm 0.03$	$0.46^c \pm 0.03$	$0.42^c \pm 0.04$
	%P	$0.94^a \pm 0.05$	$0.93^a \pm 0.04$	$0.91^a \pm 0.04$	$0.84^a \pm 0.02$
	Ca:P	$0.09^a \pm 0.00$	$0.63^b \pm 0.01$	$0.51^c \pm 0.05$	$0.50^c \pm 0.03$
ADCR	%DM	$42.4^a \pm 1.6$	$33.6^b \pm 0.6$	$30.7^b \pm 2.6$	$36.9^b \pm 1.8$
	%Ca	$0.11^a \pm 0.00$	$0.45^b \pm 0.02$	$0.27^c \pm 0.01$	$0.27^c \pm 0.02$
	%P	$0.96^a \pm 0.03$	$0.96^a \pm 0.03$	$0.84^a \pm 0.05$	$0.92^a \pm 0.04$
	Ca:P	$0.12^a \pm 0.00$	$0.47^b \pm 0.03$	$0.32^c \pm 0.01$	$0.29^c \pm 0.03$

[abcd] Means with any identical letters are not significantly different at the 5% level by single factor ANOVA.

TABLE 2. Mean dry matter (DM), calcium (Ca), and phosphorus (P) concentrations (%) and Ca:P ratios (dry-matter basis) ± standard errors in small (SMMW), medium (MDMW), and large (LGMW) mealworms fed an eight percent calcium diet for seven days

Treat-ment		Day 0	Day 2	Day 4	Day 7
SMMW	%DM	$33.1^{a} \pm 0.8$	$40.7^{b} \pm 0.4$	$43.4^{c} \pm 0.5$	$39.9^{b} \pm 0.9$
	%Ca	$0.09^{a} \pm 0.00$	$1.16^{b} \pm 0.09$	$0.86^{c} \pm 0.03$	$0.36^{d} \pm 0.01$
	%P	$1.06^{ab} \pm 0.16$	$1.17^{b} \pm 0.05$	$1.06^{ab} \pm 0.03$	$0.86^{a} \pm 0.01$
	Ca:P	$0.09^{a} \pm 0.01$	$0.99^{b} \pm 0.04$	$0.82^{c} \pm 0.02$	$0.41^{d} \pm 0.01$
MDMW	%DM	$35.3^{a} \pm 0.5$	$41.0^{b} \pm 0.2$	$42.3^{c} \pm 0.2$	$42.3^{c} \pm 0.5$
	%Ca	$0.06^{a} \pm 0.00$	$1.03^{b} \pm 0.05$	$0.79^{c} \pm 0.10$	$0.47^{d} \pm 0.02$
	%P	$0.82^{ab} \pm 0.03$	$0.93^{c} \pm 0.01$	$0.89^{bc} \pm 0.04$	$0.76^{a} \pm 0.02$
	Ca:P	$0.07^{a} \pm 0.01$	$1.11^{b} \pm 0.05$	$0.88^{c} \pm 0.09$	$0.61^{d} \pm 0.04$
LGMW	%DM	$38.9^{a} \pm 0.4$	$38.3^{a} \pm 3.1$	$42.4^{b} \pm 0.1$	$40.1^{ab} \pm 2.1$
	%Ca	$0.06^{a} \pm 0.01$	$0.47^{b} \pm 0.04$	$0.48^{b} \pm 0.07$	$0.30^{b} \pm 0.09$
	%P	$0.76^{a} \pm 0.05$	$0.73^{a} \pm 0.01$	$0.76^{a} \pm 0.03$	$0.73^{a} \pm 0.02$
	Ca:P	$0.08^{a} \pm 0.03$	$0.63^{b} \pm 0.05$	$0.63^{b} \pm 0.09$	$0.41^{b} \pm 0.13$

[abcd] Means with any identical letters are not significantly different at the 5% level by single factor ANOVA.

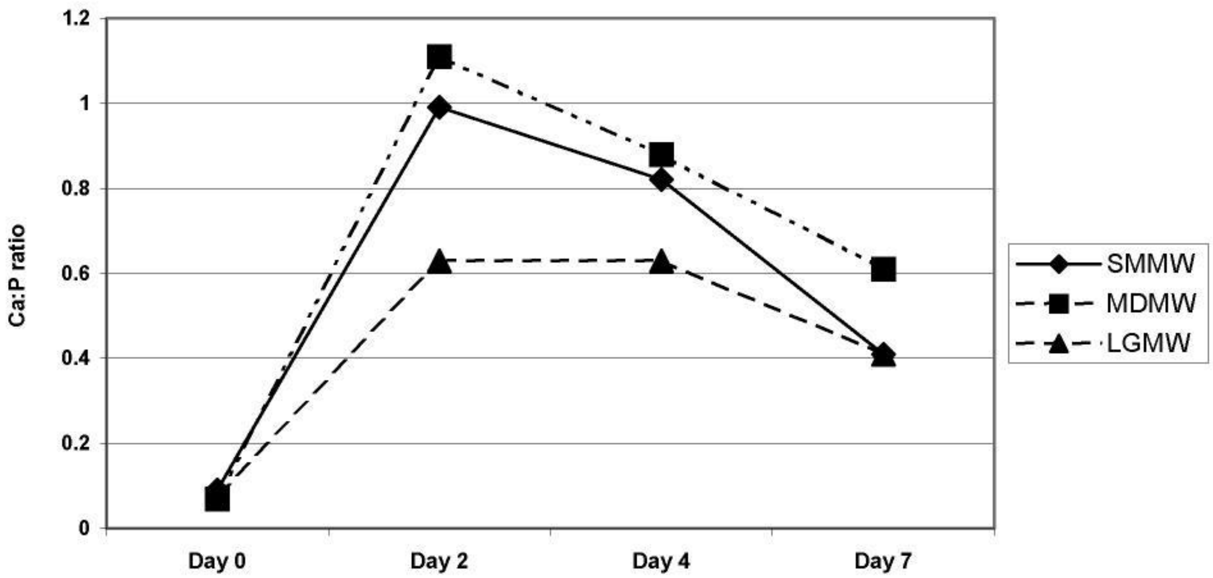

Fig. 2. Ca:P ratios in small (SMMW), medium (MDMW), and large (LGMW) mealworms fed an eight percent calcium diet for seven days.