

# CALCIUM GUT LOADING OF MEALWORMS (*TENEBRIO MOLITOR*) AND THE BENEFIT OF GEL WATER OVER APPLE SLICES FOR MAXIMUM CALCIUM GUT LOADING

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## Abstract

The importance of gut loading insects with calcium is a critical component of an insectivorous animal's diet. The insect used in this study for calcium supplementation was the regular mealworm (*Tenebrio molitor*). The standard operating procedure for most gut loading protocols includes feeding high-water produce items (i.e., apples, leafy green vegetables, etc.) in conjunction with a high calcium insect supplement. The purpose of this study was to compare the effect of apple slices and gel water on supplement intake and resulting calcium concentrations in mealworms. Mealworms were separated into two treatment groups with three replicates in each group. On a daily basis, one treatment group received 100 g of apple slices and the other group received 100 g of gel water. Both groups received 75 g of insect supplement daily. An initial 30gmealworm sample was taken at 0 hours. Additional 30 g samples were collected on days 1 and 2 with a 60 g sample collected on day 3. Results were analyzed using paired t-tests across treatments and paired observations within treatments. The results indicate the mealworms offered gel water had higher calcium concentrations at days 1, 2, and 3 than those offered water through apple slices. Within treatments, calcium concentrations were higher ( $P<0.05$ ) on day 3 than on days 1 or 2.

## Introduction

In the wild, insectivores are able to ingest calcium from a variety of insects and from their habitat.<sup>1</sup> When insectivorous species are raised in captivity the practice of gut loading insects with calcium is essential to promote healthy animals. In the article, "Feeding Captive Insectivorous Animals: Nutritional Aspects of Insects as Food" the authors reject the practice of coating insects with calcium supplement due to the insect tasting less appetizing.<sup>2</sup> A second concern is that the insect can rub or shake off the calcium supplement.<sup>3</sup> A problem for insectivorous animal collections worldwide is that insects, including mealworms (*Tenebrio molitor*), have low calcium and high phosphorous concentrations, which can promote metabolic bone disease.<sup>4</sup> Due to the wide use of mealworms for insectivorous collections gut loading with calcium is essential. Most procedures for maintaining or gut loading insects requires providing the mealworms with produce items high in water, such as apples or leafy green vegetables.<sup>4</sup> The high fiber content, caloric density, and presumed higher palatability of these items may discourage the mealworm from consuming higher amounts of the less palatable insect supplement. It is proposed that using cricket gel water as a hydration source for mealworms instead of produce will encourage greater consumption of insect supplement and lead to increased calcium concentrations in gut loaded mealworms.

## Methods

Two treatments, each containing three replicates, were established to test the hypothesis. Regular, adult mealworms, weighing approximately 0.056 grams each, were housed in square polycarbonate containers (1.9 liter, 18.5 cm x 18.5 cm x 10 cm; Cambro Manufacturing Company, Huntington Beach, CA 92647 USA). The mealworms were received seven days prior to the study and were kept in wheat bran and housed at approximately 4.4°C prior to use. Mealworms were sifted in a mesh strainer to dispose of feces and wheat bran. Thirty grams of mealworms were collected as a sample for day 0 and frozen. Two hundred grams of mealworms were placed in each container. Each container received 75 g of Calcium Fortified Insect Supplement (Marion Zoological, Plymouth, Minnesota 55441 USA). The mealworms and supplement were mixed to disperse the supplement evenly throughout the mealworms. Each container in the apple treatment group received 100 g of thinly sliced, red delicious apples. Each apple quarter was cut into four slices; seeds and peels remained intact.

Water was added to concentrated water gel (Cricket Water Bites, Nature Zone, Chico, California 95928 USA), comprised of as per instructions to create a polymerized starch gel. Each container in this treatment group received 100 g of water gel. The apple slices and cricket water gel pieces were distributed evenly across the top of the mealworm and supplement mixture according to their assigned containers.

Remaining apple material and gel water pieces were removed each day. Mealworms receiving gel water were carefully scrutinized each day for gel/insect supplement residue that was attached to the mealworms; all residues were physically removed by hand from individual worms that had gel adhered to them. All mealworm treatments were then sifted to remove any residual supplement and fecal material prior to sampling. Thirty grams from each replicate were sampled on days 1 and 2 and placed in a -22°C freezer until analysis. Fresh insect supplement and apple or gel water was provided daily for the mealworms. On day three after apple or gel pieces were removed and mealworms were sifted, a 60 g sample was collected and frozen for future analysis. Samples of insect supplement, apples, and gel water were sampled and frozen for analysis.

Daily room temperatures were monitored using a digital thermometer and recorded. A household electric heater was used to ensure proper room temperature was maintained. Recorded room temperatures ranged from 18 – 27.3°C. The lower room temperature of 18°C

At the completion of the study, all samples were packed in dry ice and sent to Dairy One Forage Laboratory (730 Warren Road, Ithaca, New York 14850 USA) for calcium and phosphorus analysis. Results within groups were averaged. Differences across treatments were analyzed using a student's t-test. Differences across days within treatments were analyzed using paired observations t-test.

## Results and Discussion

The calcium and phosphorus concentrations of items fed to the mealworms are reported in Table 1. The gel water did not add any appreciable amounts of calcium to the diet. The gel water claims to provide 0.1-0.12% calcium. However, based on our results of 0.01% calcium on an as

fed basis, the guaranteed analysis concentrations are most likely reporting the pure gel form of the product (i.e., prior to water being added).

When comparing treatments across days, the mealworms fed gel water consistently had higher concentrations of calcium ( $P < 0.05$ ) (Table 2). When comparing across days, but within treatment groups, the data are not as clear. When using apples or gel water as a water source, it is better to gut load the mealworms for at least two days with no added benefit from feeding them for a third day. Using gel water in combination with a calcium-fortified insect supplement results in greater calcium concentrations than the apple slices.

It appeared, but not objectively measured, that using gel water for mealworms resulted in a higher rate of mortality than those fed apple slices. The authors strongly believe that this is not due to any toxic ingredients in the gel water. Instead, when larger pieces of gel combined with the insect supplement it became tenacious and appeared that this mixture might have asphyxiated the mealworms. Another potential explanation may be that too much gel water was offered in relation to the amount of mealworms being fed. The authors purposefully overfed water sources and insect supplement to not have intake limited by low availability. With smaller gel pieces and lower proportions of gel water offered, the authors believe the mortality rate will be reduced.

#### LITERATURE CITED

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**Table 1.** Calcium (Ca) and phosphorus (P) composition of items fed for gut loading mealworms.

Item	Insect Supplement <sup>a</sup>	Apple	Gel Water <sup>b</sup>
Moisture, %	7.40	87.9	98.7
Ca, % of dry matter	9.19	0.05	0.80
P, % of dry matter	0.84	0.10	0.00
Ca:P ratio	10.9:1	0.5:1	not applicable

<sup>a</sup>Calcium Fortified Insect Supplement (Marion Zoological, Plymouth, Minnesota 55441 USA)

<sup>b</sup>Cricket Water Bites, Nature Zone, Chico, California, 95928 USA; Concentrations are estimated due to laboratory's inability to determine dry matter concentration.

**Table 2.** Calcium (Ca), phosphorus (P), and Ca:P ratio (mean  $\pm$  SEM) changes over time in gut loaded mealworms using apples or gel water as water sources.

Item		Day 0	Day 1	Day 2	Day 3
Ca, % of DM	Apple	0.15 $\pm$ 0.05 <sup>a</sup>	0.38 $\pm$ 0.003 <sup>*,b</sup>	0.39 $\pm$ 0.04 <sup>*,b,c</sup>	0.49 $\pm$ 0.01 <sup>*,c</sup>
	Gel water <sup>d</sup>	0.15 $\pm$ 0.05 <sup>a</sup>	0.49 $\pm$ 0.003 <sup>*,b</sup>	0.58 $\pm$ 0.06 <sup>*,b,c</sup>	0.74 $\pm$ 0.06 <sup>*,c</sup>
P, % of DM	Apple	0.95 $\pm$ 0.06 <sup>a,c</sup>	0.82 $\pm$ 0.003 <sup>*,a</sup>	0.80 $\pm$ 0.01 <sup>*,b</sup>	0.80 $\pm$ 0.003 <sup>*,b,c</sup>
	Gel water <sup>d</sup>	0.95 $\pm$ 0.06 <sup>a,b</sup>	0.86 $\pm$ 0.003 <sup>*,a</sup>	0.83 $\pm$ 0.01 <sup>*,b</sup>	0.82 $\pm$ 0.003 <sup>*,b</sup>
Ca:P ratio	Apple	0.16 $\pm$ 0.06 <sup>a</sup>	0.47 $\pm$ 0.002 <sup>*,b</sup>	0.48 $\pm$ 0.04 <sup>*,b,c</sup>	0.62 $\pm$ 0.01 <sup>*,c</sup>
	Gel water <sup>d</sup>	0.16 $\pm$ 0.06 <sup>a</sup>	0.57 $\pm$ 0.03 <sup>*,b</sup>	0.69 $\pm$ 0.06 <sup>*,b,c</sup>	0.91 $\pm$ 0.07 <sup>*,c</sup>

\*Means within a column within item categories differ (P<0.05).

<sup>a,b,c</sup>Means within a row with unlike superscripts differ (P<0.05).

<sup>d</sup>Cricket Water Bites, Nature Zone, Chico, California 95928 USA