PRACTICAL INVESTIGATION OF CRICKET DUST SUPPLEMENTS COMMONLY USED TO ENHANCE DIETS PROVIDED TO INSECTIVORE SPECIES UNDER HUMAN CARE

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Abstract

Amphibians and reptiles commonly managed under human care are commonly fed farmed feeder crickets (*Acheta domesticus*) that are deficient in calcium. Calcium deficiency can lead to the development of nutritional metabolic bone disease in animals consuming the crickets; therefore, feeder crickets are commonly supplemented with calcium by either dusting the crickets' exoskeleton or by providing crickets with a calcium enriched diet. Experiments evaluating the efficacy of dusting provide variable results, so we sought to evaluate the nutrient content of feeder crickets commonly fed to insectivores at Disney's Animal Kingdom after dust supplementation. Our objectives were to determine which of three dust supplements adhered the best to crickets and optimized the cricket nutrient content at various time points, while mimicking the practical dusting method used at Disney's Animal Kingdom. Three dust supplements, including the product prepared by Disney's Animal Nutrition Center and two commercially available dusting supplements (Repashy and Rep-Cal), were tested for adherence at 4 time points (0, 30, 90, 180 minutes). Nutrient composition of the dusted crickets were then compared across time points and to un-dusted crickets. Based on assessment of cricket weight, all dust treatments had the greatest adherence when measured at time point 0 min. As crickets spent more time in the shaker, dust adherence decreased. All dusted crickets had significantly greater concentrations of Ca ($p \leq 0.05$) when compared to un-dusted control crickets, and Ca concentrations decreased over time for all dusted crickets. Compared to the other two supplements, Repashy achieved the greatest Ca concentration and vitamin A concentration, as it was the only supplement tested containing the vitamin. Repashy also appeared grossly stickier that the Disney and Rep-Cal products. This study assessed the importance of measuring both adherence of dust supplements and resulting nutrient content of feeder crickets over time, using practical application methods, in order to select a product that will achieve the desired cricket nutrient content for insectivore diets.

Introduction

Amphibians and reptiles are commonly managed under human care, either privately owned or as part of a larger collection maintained at a zoo, aquarium, educational facility, or conservation program. It is well-described that farmed feeder crickets (*Acheta domesticus*) commonly fed to these species under human care are deficient in calcium, particularly in relation to a relatively

greater phosphorous content (Allen & Oftedal 1989; Finke 2002; Oonincx & Dierenfeld 2012). This inverse ratio of calcium to phosphorous (Ca:P) leads to nutritional metabolic bone disease which results in stunted growth, osteomalacia, skeletal fractures, neurologic symptoms, and death (Modzelewski et al. 1974; Dierenfeld et al. 1995; Ferguson et al. 1996; Miller et al. 2001; Mader 2006; Hoby et al. 2010).

In order to prevent nutritional metabolic bone disease in amphibians and reptiles under human care, the diet is commonly supplemented with calcium to increase the Ca:P to 1:1 - 2:1 (Allen & Oftedal 1989). Supplementation practices, however, present an ongoing challenge (Trusk & Crissey, 1987; Allen & Oftedal, 1989; Finke 2003; Sabatini et al. 1998; Coslik et al. 2009; Sullivan et al. 2009). There are two common methods by which insects are supplemented with calcium. The first entails feeding insects a nutrient-rich diet (termed gut-loading), and the second involves applying a powder (or dust) that adheres to the outside of the insect. Both forms of supplementation are administered to insects immediately prior to delivery as prey. Gut-loading has been well-studied and has proven successful in its ability to increase the calcium content of insects (Allen & Oftedal 1989; Sabatini et al. 1998; Finke 2003; Schlegel et al. 2005; Coslik et al. 2009). Experiments evaluating the efficacy of dusting, however, are sparser and results are variable (Sabatini et al. 1998; Li et al. 2009; Sullivan et al. 2009; Michaels et al. 2014). Live crickets are reported to clean over 50% of the supplement off of their bodies within 2.5 minutes of being dusted, and may only retain 10% of the dust before being consumed by the animal (Li et al. 2009; Sabatini et al. 1998). The most recent study was conducted in the United Kingdom and used three dusting supplements: Vetark Professional Nutrobal, Exo Terra Calcium + D3, and Zoo Med Repti Calcium with D3 (Michaels et al. 2014). No difference was found in the Ca:P ratio of dusted black field crickets (*Gryllus bilmaculatus*) and silent crickets (*G. assimilis*) among the three supplements used, and a Ca:P of greater than 1:1 in the crickets was achieved up to 5.5 hours following dust application. Michaels *et al.* (2014) concluded that dusting, irrespective of supplement brand, was a successful method to provide calcium supplementation to feeder crickets fed to amphibians and reptiles, but that time, species, and instar stage impacted cricket calcium content.

We sought to evaluate the nutrient content of the species and life stage of feeder crickets commonly fed to amphibians and reptiles at Disney's Animal Kingdom after supplementation with the dust prepared by the Animal Nutrition Center and two common dust supplements commercially available in the United States. Our objectives were to determine which of the three dust supplements adhered the best to crickets and optimized the cricket nutrient content. We hypothesized that initial adherence would vary among supplements but that adherence of all three treatments would decrease over time. We also hypothesized that each of the three supplements would achieve a different nutrient profile in the feeder crickets based on their varied nutrient compositions.

Materials & Methods

Two of the dusting supplements tested for adherence and ability to improve the nutrient profile of feeder crickets were commercially available: Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056) and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031). The third supplement tested was a proprietary reptile blend formulated by the nutritionist at the Disney's Animal Nutrition Center (Disney's Animal Kingdom, Lake Buena Vista, FL 32830). All dusting supplements were analyzed for gross energy, proximate,

and mineral analysis (Dairy One, Ithaca, NY 14850), and vitamins A and E (Covance Inc., Princeton, NJ 08540). Separate groups (Rep 1 n=6, Rep 2 n=12) of adult feeder crickets (¾" size, The Gourmet Rodent, Newberry, FL 32669) were weighed and placed into Cricket Shakers (Rep-Cal Research Labs) with each of the three dust treatments. To replicate standard operating procedures of the Disney's Animal Kingdom herptile team, Cricket Shakers were used to dust crickets, and the experiment was conducted at ambient temperature (75.7-81.1□F) and humidity (48-53%).

Twelve Cricket Shakers, each with 12 g of crickets per shaker, were used for each dusting treatment. The starting weight of the dust in the shaker holding cup was recorded. Dust was applied by inverting the shaker 10 times until crickets were well-coated. Crickets were retained in the shaker for four distinct time periods: 0 min (immediate), 30 min, 90 min, and 180 min. The designated time periods were selected based on observations by the Disney's Animal Kingdom herptile team and reflected the number of minutes that may elapse between dust application and consumption of the insect by an amphibian or reptile. When the given time period elapsed, dusted crickets were transferred into a tared quart size ziplock freezer bag to obtain the final weight on a gram scale. The dust remaining in the cup was also weighed and recorded. For each treatment, crickets from the 12 shakers were pooled into one Ziplock back that was immediately placed into a -20□C freezer. Frozen crickets were shipped overnight with icepacks for gross energy, proximate, and mineral analyses (Dairy One), and for vitamin A and E analyses (Covance). This experiment was done over 4 weekdays within a 2 week period, in order to account for any environmental variability and to accommodate for limitations with supply ordering and manpower.

The differences in nutrient concentration between control crickets and treated crickets were calculated, and those differences were compared both within treatment and within time-point (proc glimmix, SAS University © 2012-2016). Nutrient composition of dust supplements were described based on one analysis performed. Due to variability not accounted for in the initial power analysis performed, significant differences in dust adherence and the effect of time could not be determined, so differences were also described.

Results

The nutrient analysis of dusting supplements varied in crude protein, fat, calcium, phosphorous, and vitamins A and E concentration (Table 1). Disney's proprietary blend dust supplement had the greatest crude protein and fat content but lowest calcium concentration compared to the other two supplements. On the other hand, Rep-Cal had the lowest crude protein and fat contents but the greatest calcium concentration, with negligible phosphorous and vitamin E concentrations and no vitamin A. The crude protein, fat, and calcium contents of Repashy fell between that of Rep-Cal and Disney's proprietary blend, but Repashy had the greatest vitamin A and E concentrations compared to the other two supplements.

Dust adherence measurements were highly variable. Based on assessment of cricket weight, all treatments had the greatest adherence when measured at time point 0 min (Figure 1). As crickets spent more time in the shaker, dust adherence decreased, but assessment in latter time-points was less reliable based on cricket weight measurement alone. Alternatively, the weight of the dust remaining in the cup appeared to be a more reliable measure (Figure 2). Based on gross observation, the Repashy supplement was stickier than the other two products. Repashy also had

the greatest adherence compared to Rep-Cal and Disney's proprietary blend dust supplements at every time point, with the exception of time 0. For this time point, Rep-Cal had slightly greater adherence than the other two dust supplements, but Rep-Cal also was the most variable in its adherence as measured over time.

Nutrient concentration differences between dusted crickets and control (un-supplemented) crickets varied based on supplement (Table 2) and time. All dusted crickets had greater ($p \leq 0.05$) gross energy, neutral detergent fiber (NDF) and calcium (Ca) than control crickets (Table 2). Crickets dusted with Disney's proprietary blend and Repashy supplements had similar vitamin E concentrations that were greater ($p \leq 0.05$) than control crickets. Repashy also had greater ($p \leq 0.05$) dry matter (DM) and vitamin A concentrations than control crickets. Time-point zero and time-point 180 min. had greater ($p \leq 0.05$) DM than control crickets, and time-point zero also had greater ($p \leq 0.05$) crude protein (CP) concentrations compared to control crickets. The only supplement that had a greater ($p \leq 0.05$) concentration of vitamin A than control crickets was Repashy at all time-points (Repashy $\mu = 3.5 \pm$ stdev 1.5 IU/g vit A as fed, vs Control not detectable). Concentrations of Ca and vitamin A decreased over time, as expected, for all dusted crickets (Table 3).

Discussion

One way to improve the calcium content of feeder crickets fed to amphibians and reptiles is to supplement crickets with application of a topical dust containing calcium. The efficiency of nutrient intake by the animals consuming the dusted crickets is variable and relies largely on the inherent stickiness of and nutrient concentrations within the dust product, environmental temperature and humidity, and the time between dust application and consumption by the animal. We analyzed the adherence of three dust supplements and resulting cricket nutrient content at several time-points in order to replicate the practical minimum and maximum amount of time it may take from dust application to consumption.

Crickets dusted at time-point zero had the greatest amount of dust adhered and greatest nutrient content over crickets dusted at all other time-points. Cricket weights, however, were an unreliable measure of dust adherence with greater time lapsed, possibly because of cannibalism and loss of body parts through the Cricket Shaker sieve. Weighing the dust remaining in the cup after shaking and time lapsed was a better measure of adherence. We saw the greatest loss of dust from the system at time-point zero, which means the most dust was adhered to the crickets at that time-point. The loss of dust then decreased with increasing time-points. The 180 minute time-point, however, had more variable results, potentially due to increased cannibalism and loss of body parts that fell through the sieve into the cup holding the remaining dust.

Nutrient concentration differences among dusted crickets and control crickets, however, irrespective of time-point, validate the benefit of dust supplementation, even if 3 hours has lapsed between dust application and consumption. All dusted crickets had significantly greater concentrations of Ca ($p \leq 0.05$) when compared to un-dusted control crickets. Repashy appeared to be grossly stickier and achieved the greatest Ca and vitamin A contents compared to the two products tested.

Although nutrient requirements of reptiles and amphibians have yet to be well-described, we believe it is important to ensure feeder crickets are supplemented with Ca and vitamin A to help

prevent occurrence of the more commonly described herptile diseases. This study assessed the importance of measuring both adherence of dust supplements and resulting nutrient content of feeder crickets over time, using practical application methods, in order to select a product that will achieve the desired cricket nutrient content for insectivore diets. Further studies may be warranted as other products emerge on the commercial market to determine which dust supplement may be preferred based on adherence and nutrient content.

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Table 1. Nutrient analysis of three dust supplements: Disney's Animal Kingdom proprietary blend and two commercially available products.

Dust Supplement[1]	GE[2] kcal/100g as fed	DM[2] % as fed	CP[2]	NDF[2]	CF[2]	Ca[2]	P[2]	Ca:P	Vitamin A IU/g as fed	Vitamin E mg/kg as fed
			% dry matter (g/100g dry matter)							
Proprietary blend	1.7	91.1	14.0	4.9	1.9	27.0	0.23	117	69	11900
Repashy	1.4	97.1	7.8	8.9	1.7	28.5	0.36	79	313	3930
Rep-Cal	1	99.4	3.8	15.1	0.3	39.5	0.03	1317	ND[1]	6

[1] Proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830); Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056); and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031)

[2] GE, gross energy; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; CF, crude fat; Ca, calcium; P, phosphorous; ND, not detectable

Table 2. Gross energy and nutrient concentrations of crickets dusted at initial time-point zero and un-dusted control crickets.

Dust Supplement[1]	GE[2] kcal/100g as fed	DM[2] % as fed	CP[2]	NDF[2]	CF[2]	Ca[2]	P[2]	Ca:P	Vitamin A IU/g as fed	Vitamin E mg/kg as fed
			% dry matter (g/100g dry matter)							
Proprietary blend	5.6[A]	25.9	71.7	19.7[A]	16.2	1.4[A]	0.94	1.5	ND[1]	188[B]
Repashy	5.1[A]	26.0[C]	64.8	19.7[A]	14.9	5.1[A]	0.90	5.7	5.6[C]	115[B]
Rep-Cal	5.1[A]	25.1	66.7	18.9[A]	14.9	4.3[A]	0.88	4.9	ND	21
Control	5.9	22.6	76.2	24.5	16.2	0.1	1.04	0.1	ND	18

[1] Proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830); Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056); and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031)

[2] GE, gross energy; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; CF, crude fat; Ca, calcium; P, phosphorous; ND, not detectable

[A] Nutrient concentrations differed ($p \leq 0.05$) between all dusted crickets and un-dusted control crickets.

[B] Nutrient concentrations differed ($p \leq 0.05$) between only proprietary blend and Repashy dusted crickets and un-dusted control crickets.

[C] Nutrient concentrations differed ($p \leq 0.05$) between only Repashy dusted crickets and un-dusted control crickets.

Table 3. Gross energy and nutrient concentrations of dusted crickets analyzed at each of four time-points compared with un-dusted control crickets at initial time-point zero.

Dust Supplement[1]	Time (min)	GE[2] kcal/100g as fed	DM[2] % as fed	CP[2]	NDF[2]	CF[2]	Ca[2]	P[2]	Ca:P	Vitamin A IU/g as fed	Vitamin E mg/kg as fed
				% dry matter (g/100g dry matter)							
Proprietary blend	0	5.6	25.9	71.7	19.7	16.2	1.4	0.94	1.5	ND[1]	188
Proprietary blend	30	5.6	23.6	73.6	23.6	14.1	1.5	0.97	1.5	ND	212
Proprietary blend	90	5.7	23.5	72.9	18.4	14.5	1.4	1.01	1.4	ND	245
Proprietary blend	180	5.6	25.1	73.2	26.3	14.9	1.5	0.98	1.5	ND	280
Repashy	0	5.9	26.0	64.8	19.7	14.9	5.1	0.90	5.7	5.6	115
Repashy	30	5.9	24.4	70.4	21.0	12.7	3.8	0.96	4.0	3.1	70
Repashy	90	5.1	25.0	69.4	24.3	15.3	3.1	0.93	3.3	2.3	73
Repashy	180	5.2	25.7	68.3	25.2	13.8	3.8	0.92	4.1	3.0	79
Rep-Cal	0	5.5	25.1	66.7	18.9	14.9	4.3	0.88	4.9	ND	21
Rep-Cal	30	5.3	23.4	74.1	20.9	14.5	1.5	0.98	1.5	ND	23
Rep-Cal	90	5.1	23.0	72.7	19.6	15.9	2.1	0.98	2.1	ND	24
Rep-Cal	180	5.6	24.6	70.9	22.0	12.7	3.3	0.92	3.6	ND	14
Control	0	5.9	22.6	76.2	24.5	16.2	0.1	1.04	0.1	ND	18

[1] Proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830); Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056); and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031)

[2] GE, gross energy; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; CF, crude fat; Ca, calcium; P, phosphorous; ND, not detectable

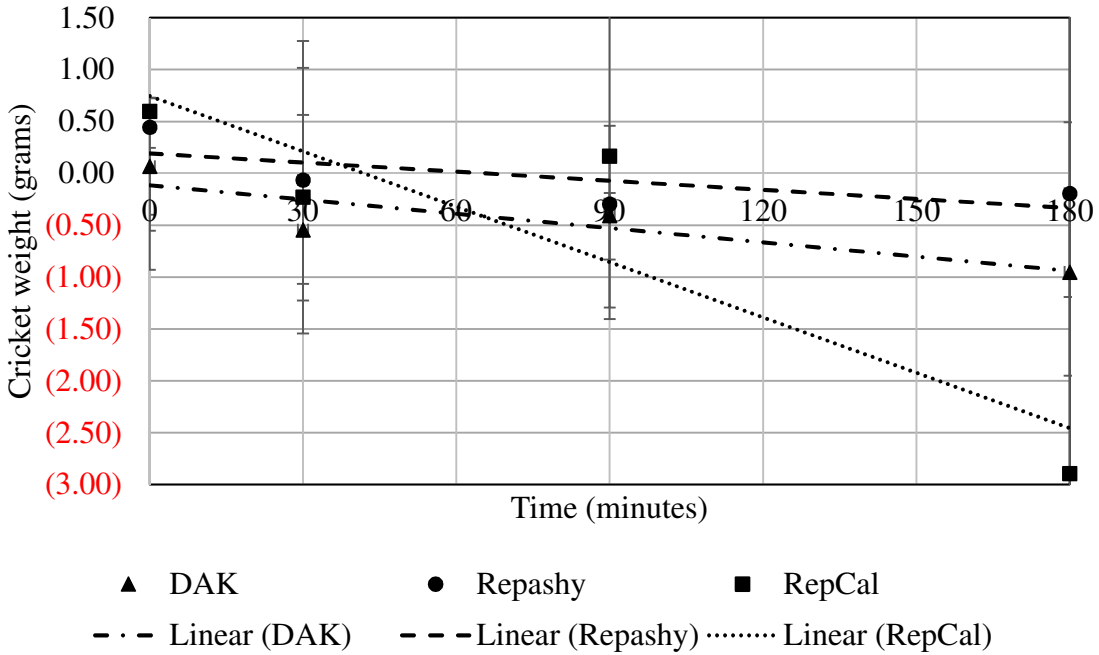

Figure 1. Weight measurements (g) of crickets dusted with supplements, proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830), Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056), and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031), at time-points 0, 30, 90, and 180 minutes. All treatments had the greatest adherence when measured at time point 0 min, and as crickets spent more time in the shaker, dust adherence decreased. Nevertheless, assessment at the latter time-points was less reliable based on cricket weight measurement alone.

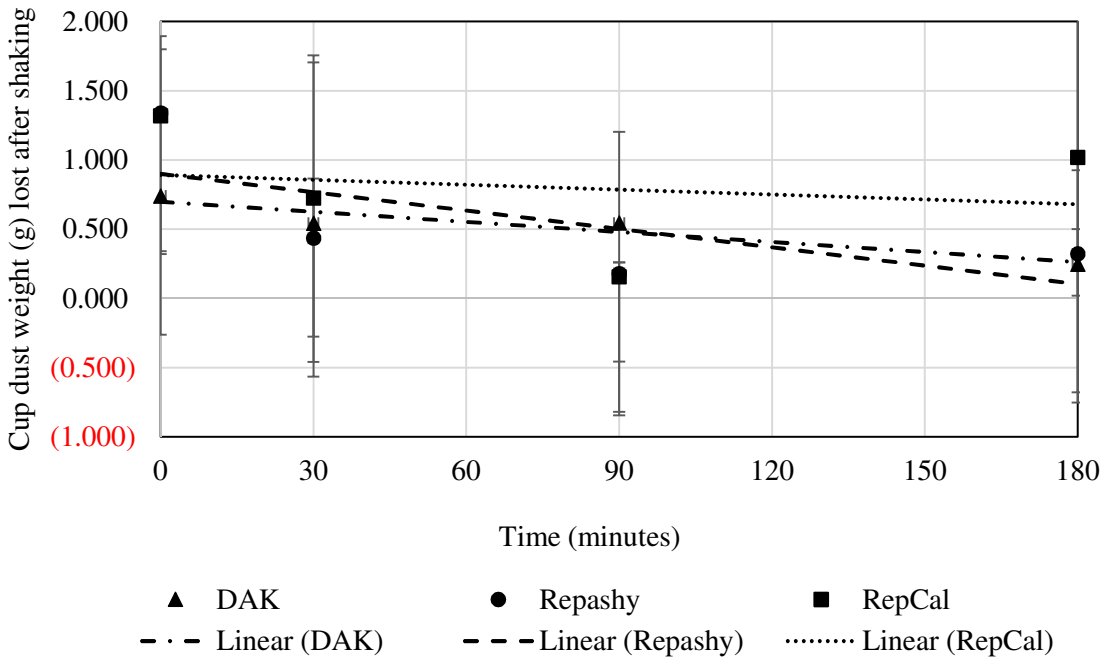

Figure 2. Weight measurements (g) of dust remaining in the Cricket Shaker cup for each of the three dust supplements, proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830), Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056), and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031), at time-points 0, 30, 90, and 180 minutes. The greatest loss of dust (g) from the system was observed at time-point zero, which means that the most dust was adhered to the crickets at that time-point. The loss of dust then decreased with increasing time-points. The 180 minute time-point, however, had more variable results, potentially due to increased cannibalism and loss of body parts that fell through the sieve into the cup holding the remaining dust.