A ZOO-WIDE EVALUATION INTO THE CURRENT FEEDER INSECT SUPPLEMENTATION PROGRAM AT THE BROOKFIELD ZOO

Roy D. McClements BS, [1,2] *Barbara A. Lintzenich MS,* [2] *and Jennifer Boardman*[2]

[1]*Faculty of Veterinary Sciences, University of Sydney, Sydney, Australia;* [2]*Daniel F. and Ada L. Rice Conservation Biology and Research Center, Chicago Zoological Society, Brookfield Zoo, Brookfield, Illinois, USA*

Abstract

Commercially raised insects are an important food source for captive animals. For those animals that are purely insectivorous, the nutrient concentrations of the food source are vitally important for the health and welfare of the animal, particularly the Ca to P ratio. In the summer of 2002, a zoo-wide evaluation of the current methods of insect supplementation was conducted at the Brookfield Zoo. The data were collected to determine whether individual animal holding facilities, using insects as a source of food, were achieving the desired mineral concentrations and Ca-to-P ratio. Crickets (*Acheta domestica*) and mealworms (*Tenebrio molitor*) of various sizes were delivered to the animal holding facility on day one of the data collection period. For three days, all feeder insects were placed in containers containing Marion™ insect meal and a water source, following the current insect supplementation protocol, then 100 grams of each feeder insect was collected. Additionally, a further 100 grams of feeder insect was collected if a secondary supplementation procedure occurred e.g. dusted with one of four dusting agents. Insect samples were analyzed for a variety of nutrients and minerals. Other data collected included building/container temperatures, handling procedures and watering schedules. Nutrient composition of the insects varied substantially across locations. Although there is a standard zoo-wide handling protocol, individual animal holding facilities have different interpretations and techniques, which may have led to variability in the results. To determine the significance of these findings additional data collections should be undertaken.

Introduction

Insectivorous animals in their free-living environment will consume a wide range of insect species. However, many insects are poor sources of minerals.[1] Thus to ensure that the animal maintains it's own homeostasis, it may supplement its diet by consuming soil, varying its diet to include high-mineral containing insects and/or consuming insects that were feeding on mineral rich sources.[2] In captivity, animals may not have these options and to ensure that nutritional deficiencies do not develop, zoological institutions will use supplementation techniques to increase the mineral concentrations of these feeder insects. In addition, a problem with feeding commercially grown insects is the narrow focus of insect species. Whereas in the wild the assortment of insect species is numerous, for many captive programs the reliance on two insect species, crickets (*Acheta domestica*) and mealworms (*Tenebrio molitor*), are very common.

There are few documented studies on insect supplementation in relation to vitamin and mineral levels. Although other minerals have been researched, emphasis has been placed on Ca and P, and obtaining the required 1:1 ratio.[1,3,7] The two primary methods used to enhance mineral content within insect species are dusting and gut-loading. Many institutions, including Brookfield Zoo, will use a combination of the two to enhance the Ca-to-P ratio and ensure adequate supplementation. Dusting involves covering the insect with a powder containing a mineral-rich substance. A problem associated with dusting, requires the insect to be consumed immediately to ensure that the supplement is not removed by the grooming performed by the insect.[7] Other potential problems include the possibility of the dust changing the palatability of the insect and that certain nutrients may not be available in a form suitable for a dust.[3] The alternative method is gut-loading with the purpose of filling the gastrointestinal tract with a Ca-rich source prior to offering to the intended animal. This method has been heavily researched in recent times[2,5,6] with the emphasis being on achieving the 1:1 Ca-to-P ratio. Hunt[7] demonstrated that achieving a 1:1 Ca-to-P ratio with an 8% Ca content in the diet was extremely difficult, even under laboratory conditions.

Within the current protocol of the Brookfield Zoo, gut-loading is the primary supplementation technique. The protocol involves the feeding of both crickets and mealworms on an insect supplementation diet containing 8% Ca (DMB) for 48-72 hours prior to offering them to the target animal. The secondary supplementation, when required, is the use of a high-Ca powder, dusted on prior to feeding. Brookfield Zoo has eleven animal holding facilities that used crickets and mealworms as dietary sources. Of most importance, are those facilities that feed insects as the exclusive dietary items.

The objective of this study was to:

1. Evaluate the current zoo-wide protocol in relation to the supplementation program in place at the Brookfield Zoo.
2. Determine the nutrient content of the feeder insects used as dietary items and determine sources of variation.
3. From this information, make recommendations in relation to the zoo-wide protocol in an attempt to decrease the variation and increase the mineral contents and ratios.

Methods

In the summer of 2002, a zoo wide analysis of the current insect supplementation program was conducted at the Brookfield Zoo. Crickets (adults, half & quarter-inch) and mealworms (giant & regular) were obtained from Timberline Industries, Marion IL and distributed to each of the animal holding facilities. In accordance with zoo-wide protocol the insects were placed in bins, given a moisture/water source and a quantity of Marion™ Insect Meal for a 72-hour period. At the completion of this time frame, one hundred grams of each insect sample was collected. If further supplementation was required, in the form of dusting, an additional one hundred grams was obtained. Dusting was achieved using one of three agents, Rep-Cal® (Rep-Cal Research Laboratories, Los Gatos, CA), Bone-meal or a 50% Theralin mix (in-house supplement). All insects were then frozen at -70°C and shipped frozen to Covance Laboratories (Madison, WI) for analysis. Analyses included fat, protein and an array of micro- and macro-minerals. Data were also collected on building and container temperatures, handling procedures and watering schedules. These environmental factors were used to determine whether there were any non-treatment factors that may have effected gut-loading potential.

The statistical software SPSS for Windows (Version 10.0.5, copyright © SPSS Inc, 1999) was used to analyze all the data. Analyses of the means and multiple dependant analysis of variance (ANOVA) were performed on dry matter, protein, fat, Ca and P. These variables were analyzed against six independent variables, including size, dusting, dusting agent, temperature, handling procedures, and holding facility. A 5% level of significance was used to test statistical difference.

Results and Discussion

Dry matter

Mean dry matter was calculated at 29.8%, 25.8% and 24.8% for adults, half- and quarter-inch crickets and 41.2% and 36.9% for giant and regular mealworms respectively (Table 1). Moisture was significantly higher in mealworms offered a wet cloth as the moisture source over fresh produce. (P=0.012, n=26). For crickets, there were no significant differences found in relation to non-treatment factors, including comparisons between those insects gut-loaded and those that were gut-loaded and dusted. Proximate analysis of dry matter, for both crickets and mealworms were similar to those of previously published data. The moisture content of quarter- and half-inch crickets was larger than the moisture level of adult crickets, which is similar to observations made by Finke[5] and Hunt,[7] yet is different to those levels published by Barker.[4] Although Barker's observations were different than those seen in this experiment, those observations were made on a small sample size, with a coefficient of variation percentage of approximately 17%. Very little data is available for mealworms. Hunt[7] showed that DM increases in regular mealworms over an 8-day experimental period, whereas giants do not significantly increase. The levels seen in this experiment after 3-days are equivalent to those seen in the Hunt[7] experiment.

Protein and Fat

Among the three sizes of crickets, significant differences were found in relation to protein and fat levels. In general as cricket size increased, protein increased and fat decreased. A similar, but inverse relationship was observed with the mealworms, where increasing size decreased protein content and increased fat. Of the other non-treatment factors, none were shown to have significant effects on protein and/or fat. Protein concentration did not change significantly when comparisons were made with previously published cricket baseline data.[4,5] On average, during the testing period, it was observed that adult crickets increase in protein concentration by approximately 3%. It can be deduced that this was as a consequence of the diet, since there was no statistically significant dusting effect on protein concentration. There is no published data for comparison for mealworms in relation to protein. Observations by Finke[5] illustrated that as cricket size increases, fat increases linearly, from 14% in quarter-inch through 20% for adult crickets. Thus the results showing a dramatic 50% decrease in fat concentration for adult crickets (10.28% ± 0.287) over the 3-day interval is notable, although interestingly this significant decrease was not seen in the smaller crickets. As a consequence of this relationship, the diet was analyzed and was found to contain approximately 4% fat DMB. One of the major concerns facing animal-holding facilities is an increased rate of death in adult crickets' post 3-days on supplementary feeding. If this dramatic decrease in fat were to continue as a result of the dietary supplement, the rapidly declining fat content could contribute to an increase death rate.

Calcium

Ca concentration for both insect species was incredibly variable. Size (P=0.000), dusting (P=0.013), and dusting agents (P=0.012) were shown to be significant contributors to the variability observed in the crickets. Ca purely from gut-loading was both the most variable and in lowest concentration in the adults. Dusted crickets of all sizes were significantly higher in Ca than non-dusted crickets (P=0.035 and P=0.014) respectively. Rep-cal™ and the 50% Theralin mix were responsible for the most significant increases in Ca levels when crickets were dusted. When Ca concentration was measured as a function of external environmental temperature, there was no significant difference in Ca concentration across all cricket sizes. However when adults were analyzed independently, temperatures greater than 80°F, had significantly greater Ca concentrations than those crickets housed in temperatures of less than 80°F (P=0.09, n=23). Ca levels were a result of the Marion™ insect meal diet. From previously published data, adult crickets contain on average between 0.13-0.21% Ca (DMB) and after 3-days adults contained, on average 0.72% Ca (DMB)[4]. Mealworm and Ca concentration were highest when comparisons were made between size (P=0.012) and dusting (P=0.000). Regular mealworms had significantly higher mean Ca concentrations when compared against giant mealworms after 3-days gut-loading. Although dusting was shown to be a significant contributor, the lack of an adequate sample size may have skewed these results. Results from this experiment are similar to other published data, where smaller crickets have greater gut-loading capacity than their adult counterparts. Finke[6] suggested that this maybe due to the decreasing size of the gastrointestinal tract, as a percentage of body weight, with increasing body size. Hunt[7] obtained an exponential increase in Ca with regular mealworms after two days supplementation, but was unable to repeat the findings in giant mealworms. In this trial, similar exponential results were not obtained in regular and/or giants. Although dusting had significant effects on Ca content of analyzed crickets and mealworms, it must be iterated that once dusting was completed the insects were frozen immediately, thus the chances of these insects being able to remove much of the dust prior to death is small. Under real conditions, time between dusting and consumption is variable. Analysis of individual buildings, showed that buildings that have the greatest percentage of insectivorous animals, were also those that had the lowest Ca concentrations. Although this statistic was not significant and may have been skewed due to small sample sizes, it is an important statistic because of the absolute significance of Ca in the metabolic pathways of insectivorous animals.

Phosphorus

In relation to phosphorus concentrations, statistical significance was observed in the cricket sizes (P=0.004, n=38), with smaller crickets have greater percentage of P on a dry matter basis than those of larger crickets. Temperature was the only environmental factor that showed a statistical significance, where temperatures above 80°F increased the levels of phosphorus (P=0.000, n=38). Size (P=0.000) and handling (P=0.012) had significant effects on the P levels within mealworms. Regular mealworms were significantly higher in concentration in P than giant mealworms. When handling was used as a non-treatment effect, mealworms which are added to old stock have significantly higher P concentrations (P=0.012, n=22)

Ca-to-P Ratio

Of the 11 animal buildings using insect meal and gut-loading to achieve the Ca-to-P ratio of 1:1, in relation to crickets, only one building achieved better than that ideal ratio and only one other building got within 5% of achieving the ideal ratio. When these adult crickets were then dusted with a Ca dense agent, 4 out of 9 achieved the ratio. In half- and quarter-inch crickets achieving this ratio was much more common, although gut-loading alone did not always suffice in achieving the desired ratio. All quarter-inch crickets that were gut-loaded and then dusted achieved better than the 1:1 ratio. For mealworms, none of the buildings achieved the desired ratio using gut-loading alone as the source of Ca. When combined with a dusting agent there was a significant increase in success, with all achieving a better than 1:1 ratio. The majority of the published data is involved in attempting to obtain the 1:1 Ca-to-P ratio. Hunt[7] demonstrated that supplementary feeding of crickets with a high Ca diet for 2-days increased Ca concentration by 400%, although they were unable to obtain the desired 1.1 ratio. Finke[6] found that a level of 50.6g/kg Ca for 2-days allowed the Ca-to-P ratio to reach >1 under experimental conditions, the specific concentration that was achieved was 0.4% Ca DMB. However due to the intrinsic P concentrations of the crickets obtained at Brookfield Zoo, the Ca concentration would need to be closer to 1% to reach the 1:1 ratio. Anderson[3] used "Cricket Diet" (Zeigler Bros, PA) containing 7.15% Ca. Within the first 24 hours the maximum concentration of Ca was achieved and desired Ca-to-P ratio reached. The information that can be deduced from these published data sets highlight the difficulty of achieving the desired Ca-to-P ratio. Therefore the variability that was seen across the animal holding facilities was not unexpected. Dusting in a majority of the cases, had the desired effect of increasing the Ca content of the crickets and improving Ca-to-P ratio. Apart from the problems associated with the insect grooming itself, keeper involvement also increases variability.

Conclusions

1. The randomness observed in Ca-to-P ratio when comparing different animal handling facilities can not be completely attributed to the insects' own metabolic capacity. Many of the non-treatment factors had significant effects. Although data from this experiment are inconclusive to make confident recommendations about non-treatment factors, further research should be undertaken.
2. The considerable drop in fat concentration as seen in the adult crickets after 3-days supplementation, is a very notable statistic and one that requires further investigation.
3. The Ca-to-P ratio of 1:1 can be obtained through gut-loading, although achieving it regularly under any condition is difficult. Even though dusting achieved increased Ca concentrations, due to the variability observed in this experiment, it is not recommended as an alternative. However prior to making definite zoo-wide recommendations in regards to the insect supplementation protocol, further investigation and supplementary surveys must be performed.

LITERATURE CITED

1. Allen M.E. 1997. From blackbirds and thrushes…. To the gut-loaded cricket: a new approach to zoo animal nutrition. Brit. J Nutr. 78(sup.2) S135-S143.

2. Allen M.E., O.T. Oftedal, and D.E. Ullrey. 1993. Effect of dietary calcium concenbtration on mineral composition of fox geckos (*Hemidactylus garnoti*) and Cuban tree frogs (*Osteophilus septentrionalis*). J Zoo. Wild Med. 24(2) 118-128

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

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

5. Finke M.D. 2002. Complete Nutrient Composition of commercially raised invertebrates used as food for insectivores. Zoo. Biol. 21:269-285

6. 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

7. Hunt A.S, A.M. Ward, and G. Ferguson . 2001. Effects of a High Calcium Diet on Gut Loading in Varying Ages of Crickets (*Acheta domestica*) and Mealworms (*Tenebrio molitor*) *In*: Proc. NAG 4th Conference on Zoo & Wild Nutr. 94-91 (Ed. Edwards MS, Lisi KJ, Schlegel ML Bray RE)

8. Trusk A.M., S. Crissey . 1987 Comparison of calcium and phosphorus levels in crickets fed a high-calcium diet versus those dusted with supplement. Proc 7th Dr. Scholl Conf. Nutr. Captive Wild Animals, 93-99

Table 1: Selected nutrient composition of crickets and mealworms, DMB (mean ± SEM).

	N	Dry matter %	Protein %	Fat %	Calcium, %	Phosphorus %
Adult Cricket	23	29.84 ± 0.23	68.73 ± 0.72	10.28 ± 0.29	0.72 ± 0.07	1.00 ± 0.02
½-inch cricket	5	25.80 ± 0.75	65.36 ± 1.66	16.54 ± 0.77	1.07 ± 0.03	1.06 ± 0.02
¼-inch cricket	10	24.88 ± 0.56	60.23 ± 1.78	14.11 ± 0.41	2.04 ± 0.05	1.11 ± 0.03
Giant mealworm	11	41.32 ± 0.29	49.13 ± 0.97	37.60 ± 0.34	0.26 ± 0.05	0.57 ± 0.02
Regular mealworm	15	35.93 ± 0.97	53.90 ± 0.55	28.10 ± 0.58	0.48 ± 0.59	0.94 ± 0.02