JZAR JOURNAL OF ZOO AND AQUARIUM RESEARCH
www.jzar.org

Research article

Manipulation of the calcium content of insectivore diets through supplementary dusting

Christopher J. Michaels, Rachael E. Antwis and Richard F. Preziosi

Faculty of Life Sciences, University of Manchester, UK.
** Correspondence: Christopher J Michaels; c.j.michaels44@gmail.com*

Keywords:
calcium supplementation, captive husbandry, cricket, feeder insects, nutritional metabolic bone disease

Article history:
Received: 29 January 2014
Accepted: 22 July 2014
Published online: 31 July 2014

Abstract

Insects fed to captive insectivores are deficient in calcium with inverse calcium to phosphorous ratios (Ca:P), and supplementation is required to avoid nutritional metabolic bone disease (NMBD). One method of improving the nutritional value of feeder insects is by "dusting" with powdered supplements, although it is often suggested that these are rapidly shed from prey insects. Here we analysed the calcium content of hatchling, second, fourth and adult instars of black field crickets and silent crickets at increasing time intervals after dusting, as well as comparing three commercially available brands of supplement in fourth instar black field crickets. Our data show these brands do not differ from one another in terms of calcium delivery, despite differences in calcium content. We also show that dusting can be used to increase Ca:P ratios above 1:1 in crickets up to 5.5 hours after dusting, with the exception of adult black field crickets, and thus dusting is a useful method of calcium supplementation.

Introduction

Insectivorous animals, particularly amphibians and non-avian reptiles, are widely maintained in captivity as part of education, research and conservation programmes, as well as in private homes as pets. Particularly of note are *ex-situ* breeding programmes for amphibians that have been established as a critical part of global amphibian conservation interventions (Gascon et al. 2007). It is well known that insects commonly used as captive diets are deficient in calcium, particularly in relation to their high phosphorus content (Allen and Oftedal 1989; Allen et al. 1993; Frye 1997; Barker et al. 1998; Finke 2002; Oonincx and Dierenfeld 2012). A diet low in calcium can lead to nutritional metabolic bone disease (NMBD), whereby a lack of ionic calcium in blood leads to the mineral being drawn out of the bone in response to parathyroid hormone (or never being laid down in developing animals) to provide calcium for essential physiological functions (Allen et al. 1993; Mader 2006; Antwis and Browne 2009; Hoby et al. 2010). Additionally, a diet with an inverse calcium: phosphorus (Ca:P) ratio can lead to NMBD via secondary hyperparathyroidism, where excess ionic phosphorus in the blood combines with ionic calcium causing a fall in blood calcium concentrations, thereby inducing the release of parathyroid hormone (Frye 1997). NMBD is considered the most prevalent nutritional problem associated with captive amphibians, and the result is varying degrees of osteomalacia, skeletal deformity and multiple fractures, lethargy, neurological symptoms and death (Mader 2006; Hoby et al. 2010).

In order to avoid NMBD, the calcium content of feeder insects can be artificially increased to provide both higher levels of calcium and a more appropriate Ca:P ratio of 1:1 to 2:1 (Allen and Oftedal 1989; Allen et al. 1993). There are two common methods of doing this; "gut-loading", whereby insects are fed a calcium-rich diet shortly before being fed themselves to target animals (Allen and Oftedal 1989; Allen et al. 1993), and "dusting", whereby crickets are covered with a fine powder containing calcium. The effects of gut-loading on the nutritional content of insects have been reasonably well studied (Allen and Oftedal 1989; Allen et al. 1993; Ogilvy et al. 2012) and this method is frequently employed to enrich the calcium content of diets. Dusting remains a highly popular supplementation

Table 1. Calcium, phosphorus and Ca:P ratios of the three supplement powders compared in this study. Data are from brand websites.

	Mineral content (min. %)		
Product (Brand)	Calcium	Phosphorus	Ca:P
Nutrobal (Vetark Professional)	20.8	0.45	46:1
Calcium + D3 (Exo Terra)	35	–	–
Repti Calcium with D3 (ZooMed)	38	–	–

method and is often used alongside gut-loading, despite a lack of evidence as to its efficacy (C. Michaels and R. Antwis, pers. obs.). Feeder insects typically exhibit cleaning behaviour after dusting, leading to speculation that supplement powders persist on insects for such a short time as to render them useless if insects are not eaten immediately (Allen and Oftedal 1989; King et al. 2011). There are currently no data on the effects of dusting on the calcium content or Ca:P ratio of feeder insects, or the speed at which the mineral supplement is lost. Furthermore, little is understood about any differences between species and instars of feeder insects, and between commercially available brands of supplement in terms of their suitability for supplementing dietary calcium.

In this study we compared the performance of three common brands of supplement in a single instar of black field crickets (*Gryllus bimaculatus)*. We also investigated the initial calcium/phosphorus content and rate of calcium loss in several instars of two common feeder cricket species, *G. bimaculatus* and silent crickets (*G. assimilis*) dusted with one of the calcium supplements.

Methods

Comparison of supplement brands

Three commercially available powder supplements – Nutrobal (Vetark), Calcium D3 (ExoTerra) and Repti Calcium D3 (ZooMed) – were compared for calcium retention on fourth instar black field crickets (*G. bimaculatus*). All three supplements contain calcium as the principal component (Table 1). *G. bimaculatus* were fasted for 24 hours to remove any residual gut contents (Ogilvy et al. 2012) and split into three groups, with each dusted with one of the supplements. Crickets were released into separate ExoTerra Faunariums (460 x 300 x 160 mm) containing a layer of damp paper towel covering the floor, similar to the design of many amphibian enclosures. Crickets were released at densities similar to a heavy feeding of a real animal enclosure: hatchling crickets, approximately 3 insects/cm^2; second and fourth instars, approximately 0.5 insects/cm^2; adults, approximately 0.3 insects/cm^2. Ambient temperature was 23^o C throughout sampling. Additionally, control crickets were released without dusting into identical trial enclosures and sampled in the same way at the same time points. Crickets were sampled at 0, 0.5, 1.5, 3.5 and 5.5 hours after dusting, with five samples of 0.15–0.3g (single adult crickets, multiple individuals of smaller instars as necessary) collected per time-point. Crickets were transferred via forceps from the trial enclosures to acid-washed scintillation tubes (see below) and immediately frozen at -80°C in a freezer (Sanyo). This method ensured that all supplement powder that was on the cricket at sample collection remained in the tube for analysis.

Comparison of cricket species and instars

The retention of Nutrobal (VetArk) on hatchling, second, fourth and adult instars of black field crickets (*G. bimaculatus*) and silent crickets (*G. assimilis*) was analysed. Crickets were fasted, dusted and sampled under the same conditions as above, including control samples for each time-point. The data for fourth instar *G. bimaculatus* dusted with Nutrobal collected in the brand comparison assay were re-used for this analysis.

Mineral analysis

Methods follow Woodburn et al. (2011). All equipment and consumables were acid washed in 9:1 ultrapure water: 70% ultrapure nitric acid (H_2NO_3) for 24 hours before use to remove trace heavy metals. Frozen cricket samples were air dried at 60° C until there was no further weight change. Dry mass of crickets was recorded by subtracting the mass of empty scintillation tubes from the total mass of the tube plus the dried cricket samples.

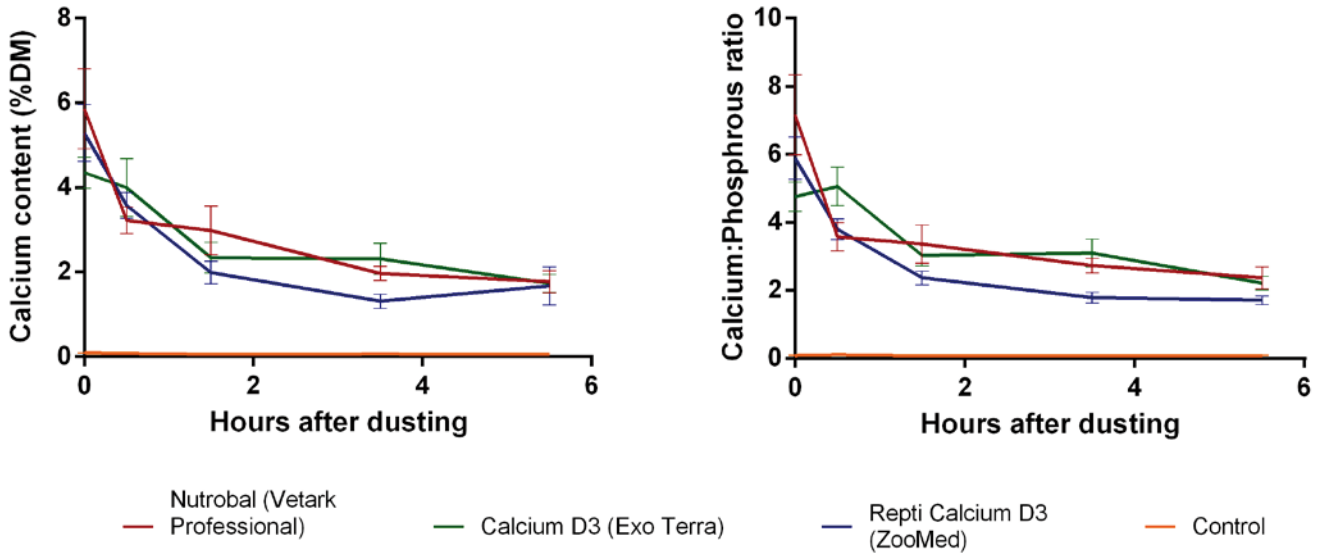

Figure 1. Changes over time in calcium content (left) and Ca:P ratio (right) in fourth instar *G. bimaculatus* crickets dusted with three commercially available calcium supplements, alongside controls. Note differences in y-axes between graphs. Error bars indicate SEM.

Samples were digested for 48 hours using 70% ultrapure nitric acid (Sigma, UK). Once crickets were entirely digested, 40% ultrapure hydrogen peroxide (Sigma, UK) was added to neutralise the acid over a further 48 hours. Digests were then diluted 1:9 with ultrapure water for analysis using inductively coupled plasma atomic emission spectrometry (ICP-AES). Measurements were conducted on a Perkin-Elmer Optima 5300 dual view ICP-AES machine. Calcium concentration was measured at 317.927 nm and phosphorus concentration at 213.620 nm. Calibration standards (0, 1, 5, 10, 100 mg/l) were run every 20 samples to ensure measurements were accurate and to facilitate construction of calibration curves. To obtain the actual calcium and phosphorus contents of each samples, the average calcium and phosphorus content of the digest blanks was subtracted from their respective reported concentrations for each sample.

Statistical analyses

Data were analysed in SPSS v.20 (IBM Statistics). Data were normally distributed and general linear models (GLMs) were used to determine differences in calcium content, phosphorus content and Ca:P ratio for the data sets according to the following parameters (where relevant): time after dusting, species, instar, supplement brand, control/dusted and all possible interactions. Non-significant terms ($p>0.05$) were removed from the models until only significant predictors remained, and only salient significant predictors are reported in the results. For brand comparisons, data were analysed initially including control data, and then excluding control data, in order to compare brands without the control data affecting the analysis. For mineral analyses, all data were analysed together, including controls. Species were also analysed separately without controls, apart from phosphorus data, where there was no difference between control and dusted crickets (see below).

Results

Comparison of supplement brands

There was a significant effect of dusting, such that there was a difference between dusted and control crickets in terms of calcium content ($F_{2,96}$ = 112.712, $p<0.001$) and Ca:P ratio ($F_{2,96}$ = 133.711, $p<0.001$). Having removed control data from the model, time after dusting was a significant predictor of both calcium content ($F_{1,72}$= 56.959, $p<0.001$) and Ca:P ratio ($F_{1,72}$ = 55.160, $p<0.001$), with a decrease in both over time (Figure 1). We found no effect of the three brands used in terms of calcium content of dusted crickets ($F_{1,72}$ = 0.624, p = 0.539) or the Ca:P ratio ($F_{1,72}$= 1.873; p = 0.161) (Figure 1). As only Nutrobal contains phosphorus, we did not compare brands for this mineral; see below for analysis of phosphorus content of crickets dusted with Nutrobal.

Comparison of cricket species and instars dusted with Nutrobal

Analysing all data together, cricket phosphorus levels remained stable over time in both *G. assimilis* and *G. bimaculatus* ($F_{3,192}$ = 0.062, p=0.803; Figure 2) and there was no significant increase in phosphorus between control and dusted crickets ($F_{3,192}$ =1.662; p=0.198; Figure 2). There was a significant difference between species (post-hoc LSD pairwise comparison, $p<0.000$), but this was very small and largely due to an anomalously variable increase in phosphorus levels in second instar *G. bimaculatus* at the start of the trial (Figure 2).

Analysing all data together, calcium levels and Ca:P ratios were significantly higher in dusted than control crickets ($F_{3,384}$ =310.8, $p<0.001$; $F_{3,384}$ =192.741; $p<0.001$). Analysing species separately and without controls, calcium content and Ca:P ratios of crickets declined with time in both *G. assimilis* ($F_{2,96}$ = 27.857; $p<0.001$; $F_{2,96}$ = 21.207, $p<0.001$, respectively) and *G. bimaculatus* ($F_{2,96}$ = 38.533, $p<0.001$; $F_{2,96}$ = 49.424, $p<0.001$, respectively).

For both species, all dusted crickets achieved a Ca:P ratio greater than 1:1 at all time points (Figure 2). Overall, adult *G. bimaculatus* were the least effective at delivering calcium, with Ca:P barely exceeding 2:1 immediately after dusting and eventually declining to just below 1:1, whereas hatchlings of both species were the most effective, with Ca:P ratios remaining above 4:1 after 5.5 hours. Other instars of both species were intermediate (Figure 2).

Discussion

Dusting with Nutrobal significantly increased the calcium content and maintained a Ca:P ratio greater than 1:1 for all instars of both cricket species for 5.5 hours after dusting. However, in adult *G. bimaculatus* dusting barely maintained Ca:P ratios higher than 1:1 for about 4 hours. Dusting increased the calcium content of all instars of both species in all instars at all time-points. This elevated it to values above the minimum necessary content estimated for growing carnivore and poultry species of 0.4–1.2% dry mass (DM) (NRC 1994, 2006; Oonincx and Dierenfeld 2012), and much higher than values in control crickets (mean of all control crickets of both species and all instars = 0.13%DM). In adult black crickets, however, mean values approached 0.4%DM by the end of the study, with some samples containing as little as 0.35%DM calcium.

The most marked increase in calcium content was in hatchlings, which attained calcium concentrations immediately after dusting of around 56 times (9.8%DM, *G. bimaculatus*) and 86 times (11.6%DM, *G. assimilis*) the content of control hatchlings and also retained high calcium contents for at least 5.5 hours (the duration of this study). At the end of the study, hatchlings still retained high calcium contents of 3.8%DM (*G. assimilis*) and 4.8%DM (*G. bimaculatus*). Although this may seem attractive, it is important to note that while excess calcium can usually be tolerated (Barker et al. 1998), elevated calcium levels can have long-term health impacts in some organisms (e.g. humans: Whiting and Wood 1997). Furthermore, other nutritional compounds included in mixed supplements, including vitamin D_3, can lead to toxicosis if fed in excess (Wallach and Hoessle 1966; Li et al. 2009). It may be important to bear the potential for overdose in mind when feeding newly dusted hatchling crickets to insectivores. Adult black crickets showed markedly lower calcium levels than all other instars of both species, around a tenth of the content of fourth and second instars immediately after dusting. It is likely that this difference is due to the structure of the cuticle in imago crickets. In *G. bimaculatus,* the adult cuticle is particularly hard and shiny and the supplement powder may not adhere as well as to the softer, rougher cuticle of nymphs. These data suggest that the use of large nymphs (fourth or fifth instars) dusted with calcium supplements may be more effective at delivering supplements than adult crickets for feeding larger insectivores when using *G. bimaculatus* as a feeder species.

Despite Nutrobal containing phosphorus, we found no significant increase in P levels between dusted and control crickets (Figure 1). This may be because the phosphorus content of Nutrobal is low (0.45%) and the mass of powder adhering to insects is low in comparison to the mass of the insect, which in combination make the phosphorus contribution of Nutrobal to a dusted cricket, particularly given its high intrinsic phosphorus content, negligible.

All control crickets contained very low levels of calcium and thus exhibited highly inappropriate Ca:P ratios of close to zero. The low Ca:P ratios in undusted adult, second and fourth instar crickets are similar to values reported from other studies (Bernard et al. 1997; Barker et al. 1998; Allen and Oftedal 1989; Finke 2002) with overall mean values of 0.253:1 (*G. assimilis*) and 0.136 (*G. bimaculatus*). For hatchling crickets, our findings (*G. assimilis*: 0.184%DM; *G. bimaculatus*: 0.149%DM) are in contrast with Barker et al. (1998),

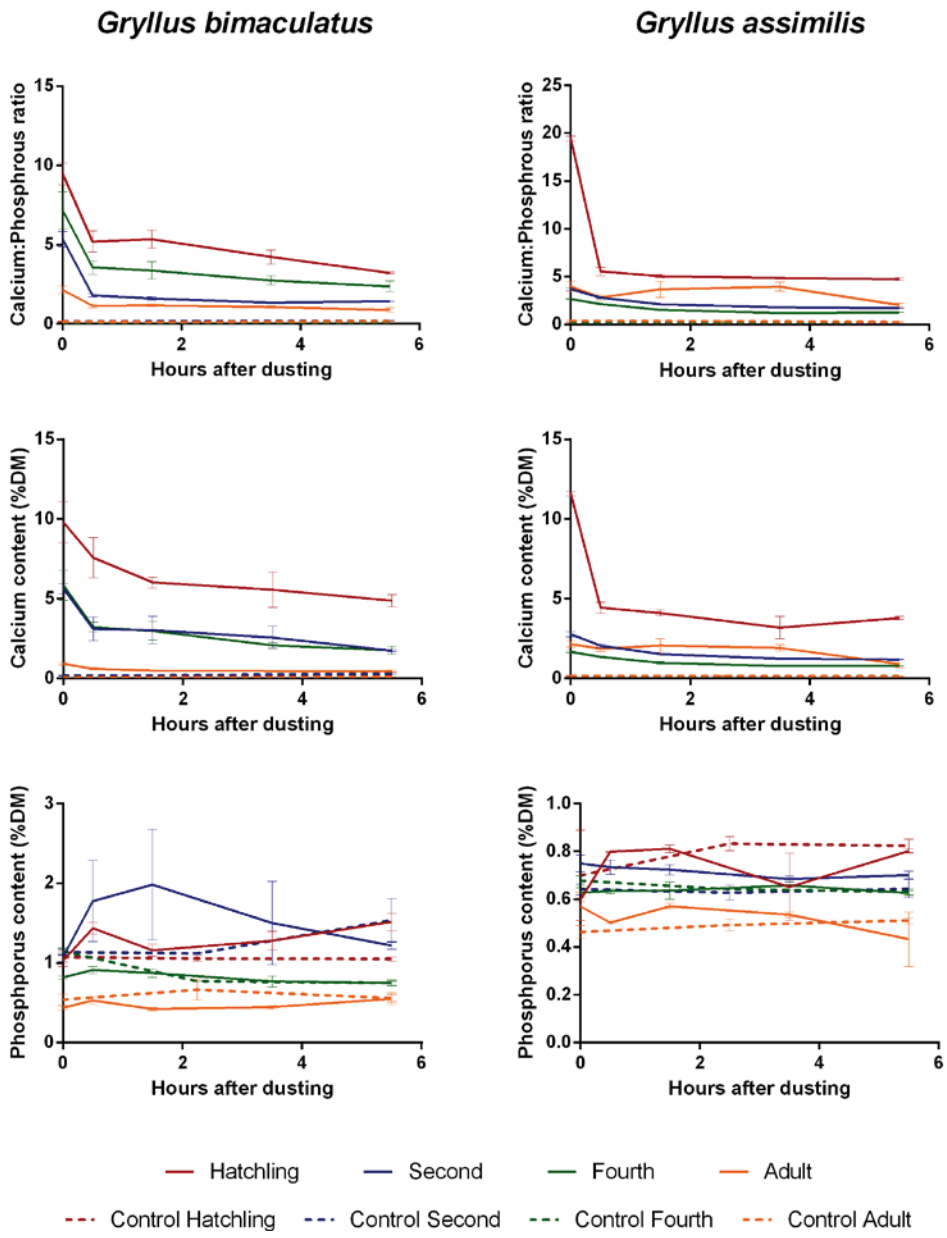

Figure 2. Changes over time in Ca:P ratios (top row), calcium content (middle) and phosphorus content (bottom) in dusted and control hatchling, second, fourth and adult instars of *G. bimaculatus* (left) and *G. assimilis* (right) crickets. Note differences in y-axes between graphs. Error bars indicate SEM.

who found very high levels of calcium in hatchling brown crickets (*Acheta domestica*), but are more akin to values reported by Bernard et al. (1997), who found low calcium contents in both adults and hatchlings of the same species. The reasons for this discrepancy are unclear, but it is possible that diets fed to crickets before analysis increased calcium content in Barker et al.*'s* (1998) study, where insects were analysed without a fasting period in order to analyse the content of crickets fed directly from the box to insectivores. The relatively low Ca:P ratios of dusted second instar black crickets in the present study, compared with their calcium content, may be due to slightly anomalous phosphorus readings, which are higher in the beginning and middle of the trial for this instar than for others and also show greater variation (Figure 2). Based on calcium content alone, second and fourth instar crickets are more or less identical in their nutritious value.

We found no differences in the efficacy of the brands tested in elevating and maintaining calcium content and Ca:P ratios, at least in fourth instar black crickets, despite the fact that Nutrobal contains less calcium than ExoTerra Calcium D3 and ZooMed Repti Calcium D3. Nutrobal has a different powder structure that the other two brands, which may explain the similar performance despite different calcium contents (see Table 1). These results suggest that brand choice is unimportant in supplying calcium to animals via supplementary dusting, although we did not test dusted crickets for other nutritional compounds apart from calcium and phosphorous.

Our data suggest that supplementing calcium through dusting crickets successfully increases calcium content of feeder crickets to levels that provide appropriate Ca:P ratios to captive insectivores. Furthermore, they suggest that calcium supplements may persist

on the cuticle of prey insects for longer than has been speculated (Allen and Oftedal 1989) and may therefore represent a suitable calcium delivery route even should insectivores not immediately consume prey. Our results also indicate that supplementary dusting can achieve Ca:P ratios to a level at least equal to and, in some instars, far exceeding levels achieved through gut loading by Allen and Oftedal (1989), who were able to produce adult crickets with a maximum ratio of around 2:1 using a very high calcium diet. It must be borne in mind that calcium content due to gut-loading will also almost certainly decrease after crickets are introduced to an enclosure as the calcium-rich material in their gut is voided (Allen and Oftedal 1989). Dusting is also an instant supplementation method and does not require the preparation of gut-loading diets, nor necessitates long periods of time required for insects to successfully gut load; up to 48 hours to attain the highest Ca:P ratios (Allen and Oftedal 1989). Dusting also allows easy supplementation of vitamin D_3, which is lacking in most feeder insects (Finke 2002). Given that gut-loading can be a successful route for supplementing other nutrients, including carotenoids (Ogilvy et al. 2012), it may be possible to supplement calcium using the dusting method while using gut loading to simultaneously enrich prey insects in other respects. As calcium-rich diets may kill crickets or not be eaten at all by small nymphs (Allen and Oftedal 1989), dusting may represent a superior method of calcium supplementation in early instars.

Our results also highlight the fact that extremely high levels of calcium can be delivered to animals eating insects immediately after dusting. Given that some other nutritional components of common supplements can easily cause toxicosis and that long-term exposure to very high levels of dietary calcium may be harmful (Whiting and Wood 1997), it may be important to be aware of the quantity of powder adhering to crickets before they are consumed. It is important to note, however, that our experiment used an approximation of a simple amphibian enclosure, with damp paper towel substrate. Our data do not address the effect of more complex enclosures, such as those including soil and leaf litter, which may provide insects with a greater means to remove supplements more rapidly from their cuticle. Furthermore, predation behaviour including time until capture and incidental and deliberate removal of supplement powders on capture may influence the nutritional quality of dusted prey insects. Further studies investigating these issues would be useful in developing a better understanding of correct calcium supplementation for insectivores.

Acknowledgements

The authors would like to thank Karlina Ozolina for her help in the laboratory, Paul Lythgoe for ICP-AES analysis and the anonymous reviewers for their comments. This work was supported by a National Environment Research Council studentship (CJM) and a Biotechnology and Biological Sciences Research Council studentship (REA).

References

Allen M.E., Oftedal O. T. (1989) Dietary manipulation of the calcium content of feed crickets. *Journal of Zoo and Wildlife Medicine* 20: 26–33.

Allen M.E., Oftedal O.T., Ullrey, D E. (1993) Effect of dietary calcium concentration on mineral composition of fox geckos (*Hemidactylus garnoti*) and Cuban tree frogs (*Osteopilus septentrionalis*). *Journal of Zoo and Wildlife Medicine* 24: 118–128.

Antwis R., Browne R. (2009) Ultraviolet radiation and vitamin D_3 in amphibian health, behaviour, diet and conservation. *Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology* 154: 184–190.

Barker D., Fitzpatrick M.P., Dierenfeld, E.S. (1998). Nutrient composition of selected whole invertebrates. *Zoo Biology* 17: 123–134.

Bernard J.B., Allen M.E., Ullrey D.E. (1997) Feeding captive insectivorous animals: Nutritional aspects of insects as food. *Nutrition Advisory Group Handbook, Fact Sheet* 3: 1–7.

Finke M.D. (2002) Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology* 21: 269–285.

Frye F.F. (1997) The importance of calcium in relation to phosphorus, especially in folivorous reptiles. *Proceedings of the Nutrition Society* 56: 1105–1118.

Gascon C., Collins J.P., Moore R.D., Church D.R., McKay J.E., Mendelson J. R. III (Eds.) (2007). *Amphibian Conservation Action Plan.* Gland: IUCN/ SSC Amphibian Specialist Group.

Hoby S., Wenker C., Robert N., Jermann T., Hartnack S., Segner H., Aebischer C., Liesegang A. (2010) Nutritional metabolic bone disease in juvenile veiled chameleons (*Chamaeleo calyptratus*) and its prevention. *Journal of Nutrition* 140: 1923–1931.

King J.D., Muhlbauer M.C., James A. (2011) Radiographic diagnosis of metabolic bone disease in captive bred mountain chicken frogs (*Leptodactylus fallax*). *Zoo Biology* 30: 254–259.

Li, H., Vaughan M.J., Browne R.K. (2009) A complex enrichment diet improves growth and health in the endangered Wyoming toad (*Bufo baxteri*). *Zoo Biology* 28: 197–213.

Mader D.R. (2006). Metabolic bone diseases. In: Mader D.R. (ed.). *Reptile Medicine and Surgery*. 2nd edn. St. Louis, Missouri: Saunders Elsevier, 841–851.

NRC (1994) *Nutrient Requirements of Poultry.* Washington, DC: National Academy Press.

NRC (2006). *Nutrient Requirements of Dogs and Cats.* Washington, DC: National Academy Press.

Ogilvy V., Fidgett A.L., Preziosi R.F. (2012) Differences in carotenoid accumulation among three feeder-cricket species: implications for carotenoid delivery to captive insectivores. *Zoo Biology* 31: 470–478.

Oonincx D.G.A.B., Dierenfeld E.S. (2012) An investigation into the chemical composition of alternative invertebrate prey. *Zoo Biology* 31: 40–54.

Wallach J.D., Hoessle C. (1966) Hypervitaminosis D in green iguanas. *Journal of the American Veterinary Medical Association* 149: 912–914.

Whiting S.J., Wood R. J. (1997) Adverse effects of high-calcium diets in humans. *Nutrition Reviews* 55: 1–9.

Woodburn K., Walton R., McCrohan C., White K. (2011) Accumulation and toxicity of aluminium-contaminated food in the freshwater crayfish, *Pacifastacus leniusculus. Aquatic Toxicology* 105: 535–542.