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Gut Loading to Enhance the Nutrient Content of Insects As Food for Reptiles: A Mathematical Approach

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A variety of commercially raised insects are fed to insectivorous reptiles, but information concerning appropriate diets used to feed these insects is limited. In the present study, house crickets (*Acheta domesticus* adults and nymphs), mealworms (*Tenebrio molitor* larvae), and silkworms (*Bombyx mori* larvae) were fed diets containing graded levels of calcium (Ca) and/or vitamin A–nutrients that are low or absent in most insects. Diets and insects were analyzed for moisture, Ca, phosphorus (P), and vitamin A. For adult crickets and cricket nymphs, body Ca and vitamin A concentrations increased in a linear fashion with increasing levels of dietary Ca or vitamin A. Ca concentrations of silkworms also increased in a linear fashion with increasing levels of dietary Ca. For mealworms, body Ca and vitamin A concentrations increased in a nonlinear fashion with increasing levels of dietary Ca or vitamin A. These regression equations, in conjunction with insect nutrient composition, allow for the calculation of the optimum nutrient concentration for gut-loading diets. Final recommendations were based on National Research Council (NRC) requirements for rats, adjustments for the energy content of the insects, and nutrient overages as appropriate. Gut-loading diets for crickets (adults and nymphs) should be supplemented to contain the following nutrients, respectively: Ca (51 and 32 g/kg), vitamin A (8,310 and 5,270 μg retinol/kg), vitamin D (300 and 190 μg cholecalciferol/kg), vitamin E (140 and 140 mg RRR-α-tocopherol/kg), thiamin (31 and 21 mg/kg), and pyridoxine (20 and 10 mg/kg). Gut-loading diets for mealworms should be supplemented to contain the following nutrients: Ca (90 g/kg), iron (51 mg/kg), manganese (31 mg/kg), vitamin A (13,310 μg retinol/kg), vitamin D (460 μg cholecalciferol/kg), vitamin E (660 mg RRR-α-tocopherol/kg), thiamin (5 mg/kg), vitamin B_{12} (650 μg/kg), and methionine (29 g/kg). Gut-loading diets for silkworms should be supplemented to contain the following nutrients: Ca (23 g/kg), iodine (0.7 mg/kg), vitamin D (140 μg cholecalciferol/kg), vitamin E (70 mg RRR-α-tocopherol/kg), and vitamin B_{12} (226 μg/kg). Zoo Biol 22:147–162, 2003. © 2003 Wiley-Liss, Inc.

Key words: Ca; vitamin A; gut loading; crickets; mealworms; silkworms

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INTRODUCTION

Insects are an important food source for many animals commonly kept in zoos. While nutrient requirements for most insectivores have not been quantitatively determined, in captivity the reliance on only one or two species of insects likely makes them more prone to nutritional deficiencies. Examples of nutritional deficiencies of animals fed a diet consisting solely of insects have been documented [Modzelewski and Culley, 1974; Allen, 1997; Toft and Wise, 1999]. While most reports involve calcium (Ca) deficiency, other nutrient deficiencies have been described as well [Ferguson et al., 1996; Mayntz and Toft, 2001; Miller et al., 2001].

Two methods are commonly used to enhance the nutritional value of insects. The first involves using a powder to coat the insect with the appropriate nutrient (usually Ca) just before it is fed to the animal. While effective, this method can provide variable results because the amount that adheres to the insect depends on the characteristics of the powder, the insect species being fed, and the ability of the insect to groom itself and remove the nutrient supplement [Trusk and Crissey, 1987]. Other potential problems are the possibility that the dust will change the palatability of the insect, and that certain nutrients may not be available in a form suitable for dusting.

A more common method is to feed the insect a nutrient-dense diet so that the food contained in the gastrointestinal tract supplements the nutrients contained in the insect's body, thereby providing a more balanced diet for the animal being fed. The importance of gut contents in providing supplemental Ca to young thrushes and blackbirds was first shown by Bilby and Widdowson [1971]. To date, this method has been well researched with regards to Ca, using crickets [Allen and Oftedal, 1989; Anderson, 2000; Hunt et al., 2001], mealworms [Martin et al., 1976; Anderson, 2000; Klasing et al., 2000; Hunt et al., 2001], and to a lesser degree waxworms [Strzelewicz et al., 1985]. There are also reports of the use of gut loading to enhance the Ca, vitamin A, and vitamin D content of crickets when fed to the panther chameleon (*Chamaeleo pardalis*) [Ferguson et al., 1996], and the Ca, vitamin A, and vitamin D content of crickets when fed to leopard geckos (*Eublepharis macularius*) [Allen, 1997]. These studies provide valuable information regarding Ca loading for insects, but little information as to the level of other nutrients that should be present in diets designed for gut loading. Recently the complete nutrient composition of a number of invertebrates was reported, which provides a baseline nutrient concentration of these species and identifies nutrients that may be deficient when they are fed as a sole diet [Finke, 2002]. The purpose of these experiments is to develop a series of equations to estimate the gastrointestinal tract contents of each species. By the use of these equations, the dietary concentration of any nutrient can be calculated, and the insect can be supplemented such that the desired nutrient composition of the crickets, mealworms, and silkworms can be obtained.

MATERIALS AND METHODS

Animals

Adult house crickets, house cricket nymphs (*Acheta domesticus*), and mealworms (*Tenebrio molitor* larvae) were obtained from Timberline Industries (Marion, IL) and used as received. Silkworm eggs (*Bombyx mori*) were obtained from Mulberry Farms (Fallbrook, CA). The silkworm larvae were fed an artificial

diet until they weighed approximately 1,000 mg (about 20–25% of their maximum size), at which time they were used in these experiments.

Diet

The base diet for crickets was Timberline Cricket Power Food, and for mealworms it was wheat bran. For both crickets and mealworms, six diets were made with increasing Ca content by the addition of 0, 48, 91, 130, 167, and 200 g/kg of calcium carbonate ($CaCO_3$) (FMC Corporation, Chicago, IL) to the base diet. The $CaCO_3$ was added to the base diet and mixed thoroughly. Vitamin A content was increased using Rovimix A-500W (178,500 μg retinyl acetate/g, equal to 150,000 μg retinol equivalents/g; Roche Vitamins, Inc., Parsippany, NJ). A solution of vitamin A (300 μg retinol equivalents/ml distilled water) was made, sprayed on the base diet, and mixed thoroughly. The diets containing added vitamin A were then dried at 70° C for 30 min to remove the added moisture. The base diet for the silkworm larvae was Mulberry Farms Silkworm Chow. The Ca content was increased by the addition of 0, 48, 91, 130, 167, and 200 g/kg of $CaCO_3$ to the dry-powder base diet, and mixed thoroughly. The powdered diet was then mixed with water (3 g water to 1 g powdered diet), heated, and then allowed to cool, forming a solid, high-moisture gel diet. The diet was kept refrigerated prior to feeding. Vitamin A fortification of the silkworm diet was not studied, because it was previously shown [Finke, 2002] that silkworms fed this diet contain adequate vitamin A.

Feeding Experiments

All experiments were carried out at a temperature of 22–24°C with a photoperiod of 10:14 (light:dark). Two hundred crickets (adults (average weight 408 mg) and nymphs (average weight 68 mg)) were placed into each of 22 plastic rectangular containers (26.5 cm long × 15.9 cm wide × 17.1 cm high) with ventilated plastic tops (Kritter Keeper; Lee's Pet Products, San Marcos, CA). Cardboard egg cartons were stacked in each container to increase surface area and provide cover. Food was provided in dry powdered form in small dishes (6.1 cm diameter × 1.2 cm high). Distilled water was also provided in the same-size dishes, and small pieces of paper towel were placed in the water dishes to prevent the crickets from drowning. Dishes were cleaned and refilled daily. Mealworm larvae (150, average weight 135 mg) were placed in each of 11 plastic circular containers (17.8 cm diameter × 15.9 cm high) with ventilated plastic tops (Kritter Keeper, Lee's Pet Products) filled with 1 kg of the respective diet. Silkworm larvae (40, average weight 1,251 mg) were placed into each of six plastic rectangular containers (26.5 cm long × 15.9 cm wide × 17.1 cm high) (Kritter Keeper; Lee's Pet Products). For the silkworm larvae, a plastic-wrap top was used to maintain high humidity and minimize moisture loss in the diets. Food was provided in slices (approximately 0.5 cm × 0.5 cm × 2.0 cm) and changed daily. All insects were fed the diets for 48 hr, after which the insects were frozen. The time period of 48 hr was used because it was previously shown to be a sufficient time for both crickets and mealworms to maximize Ca content when fed high-Ca diets [Allen and Oftedal, 1989; Anderson, 2000; Klasing et al., 2000; Hunt et al., 2001].

Chemical Analysis

Diets and insects were kept frozen until analyzed for moisture, vitamin A, Ca, and phosphorus (P). Vitamin A was extracted using AOAC official method 974.29 [AOAC International, 1995] and quantified by high-performance liquid chromatography (HPLC) with detection at 325 nm. Calcium was analyzed using inductively coupled plasma-atomic emission spectrometry [Dahlquist and Knoll, 1978]. Phosphorus was analyzed using a colorometric assay (method 984.27 [AOAC International, 1995]). Moisture was determined by vacuum drying (method 925.09 [AOAC International, 1995]). For the diets, each data point represents the mean $\pm$ SEM of three separate analyses. For the insects, each data point represents the mean $\pm$ SEM of two separate analyses. Data for Ca, P, and vitamin A content of both diets and insects are shown on an "as is" basis.

Statistical Analysis

Insect Ca, Ca:P ratio, and vitamin A concentration were analyzed as a function of dietary Ca or vitamin A concentration using Statgraphics Plus for Windows 5.0 (Statistical Graphics Corp., Rockeville, MD).

RESULTS

The crickets were fed six experimental Ca-fortified diets containing 10.3–85.8 g Ca/kg. The effect of dietary Ca concentration on the Ca concentration of adult crickets and cricket nymphs is shown in Figure 1. There are no data for adult crickets fed the diet containing 58.2 g/kg Ca, because the samples were lost prior to analysis. For adult crickets and cricket nymphs, a linear effect of diet Ca concentration on cricket Ca concentration (adult crickets F=1,069; P=0.001; cricket nymphs F=153; P=0.0002) was observed.

Cricket P concentration was not affected by dietary treatment (adult crickets P=0.386; cricket nymphs P=0.182; data not shown). Because cricket Ca increased linearly and cricket P did not, cricket Ca:P ratios increased linearly with increasing cricket Ca concentration (adult cricket Ca:P=0.057 + [0.156 $\times$ diet Ca]; F=1070; P=0.0001; cricket nymph Ca:P=0.153 + [0.201 $\times$ diet Ca]; F=220; P=0.0001; data not shown). The steeper slope (0.201) observed in cricket nymphs compared to adult crickets (0.156) is a result of both the greater Ca concentration (Fig. 1) and the lower P concentration of cricket nymphs relative to that of adult crickets (nymphs=2.41 g P/kg cricket; adults=2.89 g P/kg cricket; t=13.5, P=0.0000). Ca concentrations of 60.5 g Ca/kg diet for adult crickets, and 42.2 g Ca/kg diet for cricket nymphs would result in a Ca:P ratio of 1:1.

The crickets were fed six experimental vitamin A-fortified diets that contained 5,700–32,900 μg retinol equivalents/kg diet. As shown in Figure 2, there was a linear relationship between diet vitamin A concentration and cricket vitamin A concentration for both adult crickets (F=101; P=0.021) and cricket nymphs (F=157; P=0.0002).

The mealworms were fed six experimental Ca-fortified diets that contained from 0.9–75.0 g Ca/kg diet. As shown in Figure 3, there was a nonlinear relationship between diet Ca concentration and mealworm Ca concentration (F=905; P=0.0001), which could best be described by a second-order polynomial

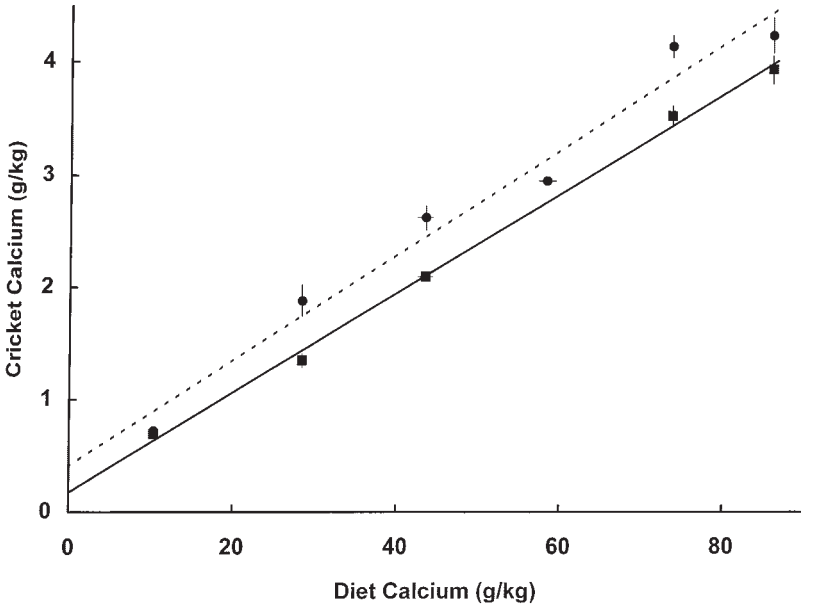

Fig. 1. Effect of dietary Ca content on adult cricket (■) and cricket nymph (●) Ca content. Values are means ± SEM. Regression equation mean ± SE for adult crickets $Y=0.173\pm 0.076+0.045\pm 0.001\times X$; r=0.9986. For cricket nymphs $Y=0.404\pm 0.215+0.047\pm 0.004\times X$; r=0.9872.

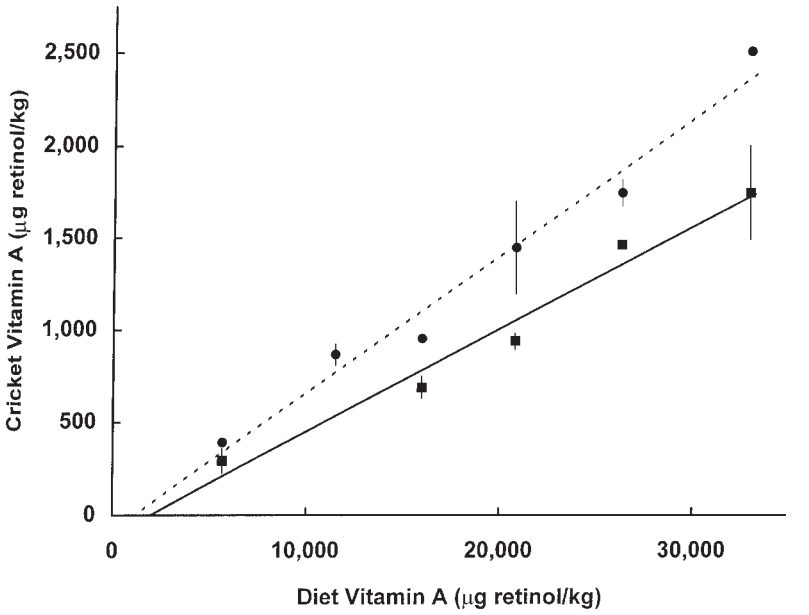

Fig. 2. Effect of dietary vitamin A content on adult cricket (■) and cricket nymph (●) vitamin A content. Values are means ± SEM. Regression equation mean ± SE for adult crickets $Y=-111\pm 125+0.056\pm 0.006\times X$; r=0.9854. For cricket nymphs $Y=-91\pm 126+0.75\pm 0.006\times X$; r=0.9875.

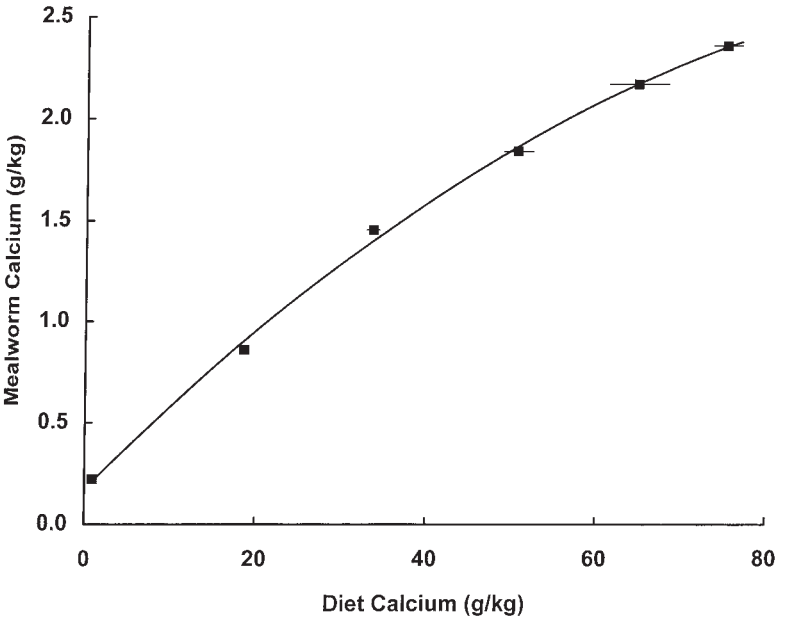

Fig. 3. Effect of dietary Ca content on mealworm Ca (■) content. Values are means ± SEM. Regression equation mean ± SE $Y=0.170\pm0.042+0.043\pm0.002\times X-0.0002\pm0.0001\times X^2$; $r^2=0.9983$.

equation. Mealworm P concentration was affected by dietary treatment ($P=0.036$), although concentrations ranged only from 3.2 to 3.3 g P/kg mealworm across all treatments. As a result, mealworm Ca:P ratios mirrored the nonlinear pattern seen for mealworm Ca concentration (mealworm Ca:P = 0.05 + [0.134 × diet Ca] + [–0.006 × diet Ca^2]; F = 1562; $P=0.0000$; data not shown).

The mealworms were fed five experimental vitamin A–fortified diets that contained 0–25,800 μg retinol equivalents/kg diet. The effect of dietary vitamin A concentration on the vitamin A concentration of mealworms is shown in Figure 4. As was seen in mealworms fed the Ca-fortified diets, there was a nonlinear relationship between diet retinol concentration and mealworm retinol concentration (F = 551; $P=0.002$), which could best be described by a second-order polynomial equation.

The silkworms were fed six experimental Ca-fortified diets that contained 2.4–22.7 g Ca/kg diet. Unlike the diets for crickets and mealworms, these gel diets contained on average 737 g water/kg diet; thus, on a dry weight (DW) basis the Ca content ranged from 9.2 to 86.4 g/kg diet. The effect of dietary Ca concentration on the Ca concentration of silkworms is shown in Figure 5. For silkworms there was a linear relationship between diet Ca concentration and silkworm Ca concentration (F = 318; $P=0.0001$). Silkworm P concentration was unaffected by dietary treatment, hence silkworm Ca:P ratios increased in a linear fashion with increasing dietary Ca concentration (silkworm Ca:P = 0.267 + [0.106 × diet Ca]; F = 221; $P=0.0001$; data not shown). A concentration of 6.9 g Ca/kg diet would result in silkworms with a Ca:P ratio of 1:1.

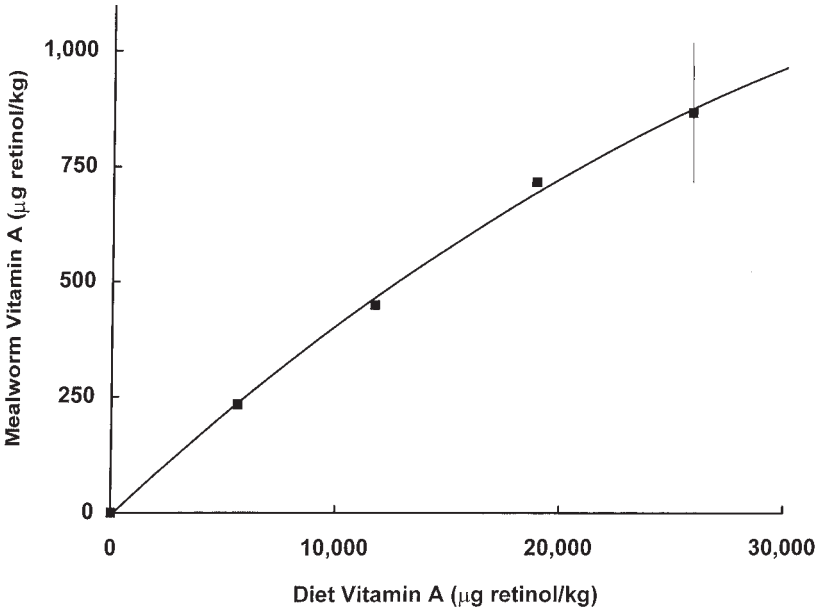

Fig. 4. Effect of dietary vitamin A content on mealworm (■) vitamin A content. Values are means ± SEM. Regression equation mean ± SE $Y = -5 \pm 20 + 0.045 \pm 0.004 \times X - 0.000004 \pm 0.000001 \times X^2$; $r^2 = 0.9982$.

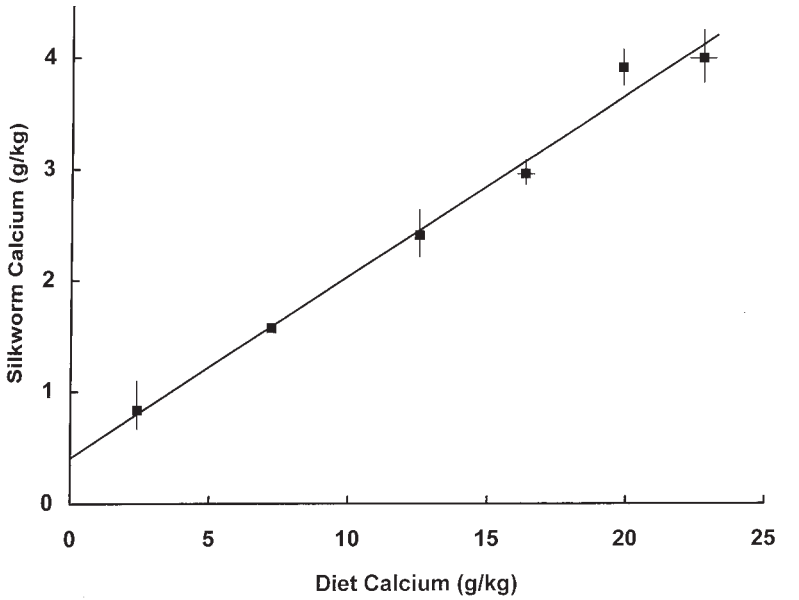

Fig. 5. Effect of dietary Ca content on silkworm Ca (■) content. Values are means ± SEM. Regression equation mean ± SE $Y = 0.403 \pm 0.140 + 0.164 \pm 0.009 \times X$; $r^2 = 0.9938$.

DISCUSSION

These data show that the nutrient composition of crickets, mealworms, and silkworms can be modified by diet. By feeding these insect species diets containing graded levels of nutrients (in this case, Ca and vitamin A), a series of regression equations were constructed. The slope of these equations represents insect gastrointestinal tract contents, which can be used to develop diets to modify insect nutrient content.

Two previous studies also demonstrated that cricket Ca content could be modified by diet. Anderson [2000] fed crickets one of eight different commercial diets ranging from 14.5 to 353 g Ca/kg diet and observed an increase in cricket Ca concentration, but no consistent pattern between diet Ca concentration and cricket Ca concentration. Food consumption varied among dietary treatments, which suggests wide variations in the palatability of the diets used, and emphasizes the importance of adequate food intake when using diets for gut loading. In addition to diet composition, the physical form of the diet may have had an effect on palatability, since some of the diets were in powder form while others were pelleted. Allen and Oftedal [1989] reported higher food consumption of crickets fed powdered diets compared to crickets fed pelleted diets.

Using $CaCO_3$ to adjust the Ca content of a base diet, Allen and Oftedal [1989] reported an increase in cricket Ca content when fed six diets ranging from 29.2 to 114 g/kg Ca (DW basis). The crickets used in their study were similar in size to those used in the present study (320 and 408 mg, respectively). After adjusting dietary Ca content to 8% moisture (the average in this study), an analysis of their data shows a significant linear effect ($F=51$; $P=0.002$) between cricket Ca concentration and diet Ca concentration. The slope of the regression line from their data (mean $\pm$ SE -0.039 ± 0.005) is similar to that obtained in these experiments for adult crickets (0.044 ± 0.001). The similarity in the results of these two studies, despite differences in experimental conditions and base diets, suggests that the use of these regression equations in formulating gut-loading diets may have wide applications.

There are no comparable data for crickets fed diets containing graded levels of vitamin A. However, the results from regression analysis of crickets fed vitamin A-supplemented diets confirm the validity of this technique, because the slopes are similar to those seen for crickets fed diets containing graded levels of Ca. For adult crickets the slopes for the regression lines were 0.056 ± 0.006 (vitamin A) and 0.044 ± 0.001 (Ca). The slopes for the regression lines for cricket nymphs were 0.075 ± 0.006 (vitamin A) and 0.047 ± 0.004 (Ca).

Cricket nymphs tended to have higher levels of Ca and vitamin A than adult crickets when fed the same diet. This suggests that for crickets, gastrointestinal tract contents, as a percent of body weight, decrease with increasing body size. This agrees with the data of Hunt et al. [2001], who showed that for crickets fed a high-Ca diet, dry matter (DM) Ca content (as a percent of DW) increased with decreasing cricket size. These data are also consistent with the observation by Ferguson et al. [1996] that smaller crickets contain more vitamin A per unit body weight than larger crickets fed the same diet.

Two previous studies [Zwart and Rulkens, 1978; Anderson, 2000] attempted to manipulate the Ca content of mealworms by feeding them a variety of commercial supplements, but exact dose-response relationships were not quantified. Zwart and

Rulkens [1978] reported that mealworms fed a wheat bran diet or supplements containing 117, 106, or 407 g Ca/kg diet resulted in mealworms with Ca contents of 1.3, 8.4, 5.6, and 5.4 g/kg (DW basis), respectively. Similarly, Anderson [2000] fed mealworms one of eight different diets ranging from 4.4 to 353 g/kg Ca. Only one diet (containing 353 g/kg Ca) produced mealworms with Ca contents greater than 6.0 g/kg (DW basis), and a Ca:P ratio greater than 1:1 (1.4:1). In both studies the diets used were commercial supplements differing in form (powders and pellets) as well as base ingredients.

In contrast, Klasing et al. [2000] fed mealworms diets containing graded levels of Ca in either a chicken starter mash or wheat bran base. Ca was added as $CaCO_3$, and dietary Ca contents ranged from 0 to 130 g/kg diet. When mealworms were fed graded levels of Ca in the chicken starter mash, there was a linear increase in the Ca concentration of mealworms with increasing levels of dietary Ca. The Ca concentration of mealworms also increased when they were fed graded levels of Ca from wheat bran-based diets, but the response did not appear to be linear.

In the current study, mealworm Ca and vitamin A content also responded to increasing levels of dietary Ca and vitamin A, respectively, in a consistent nonlinear fashion. As was seen for crickets, the slope of the regression line for mealworms fed the Ca-fortified diets (0.043 ± 0.003) was similar to that seen for mealworms fed vitamin A-fortified diets (0.045 ± 0.004). The reason for the nonlinear response in mealworms fed the Ca- and vitamin A-fortified diets in this study, and in mealworms fed the wheat bran-based diets [Klasing et al., 2000] is unknown. Due to the difference in densities between the $CaCO_3$ and the wheat bran, the burrowing of the mealworms through the diet could have created some separation of the $CaCO_3$ and the wheat bran, and may help explain the nonlinear response. Since the vitamin A was applied as a liquid, separation of dietary components does not appear to be a likely explanation for the nonlinear response observed for mealworms fed the vitamin A-fortified diets. Despite dietary Ca levels as high as 75 g/kg, the inability of any diet to produce Ca levels high enough to meet requirements, or to produce a Ca:P ratio greater than 1:1, is consistent with the data of Klasing et al. [2000]. In a separate experiment, a liquid calcium chloride ($CaCl_2$) solution was also applied to a wheat bran-based diet in an effort to reduce the dustiness of the diet and to prevent separation between the wheat bran and the Ca supplement. Using $CaCl_2$ as the Ca source resulted in only a slight increase ($Y = 0.05 + [0.0158 \times \text{diet Ca}]$; $F = 19.0$; $P = 0.0121$) in mealworm Ca concentration, presumably due to the poor palatability of the diets (Finke, unpublished data).

There are no published studies concerning gut loading using silkworm larvae. The only study of gut loading with any species of lepidopteran larvae is that of Strzelewicz et al. [1985]. In that study, waxworms (larvae of the waxmoth (*Galleria mellonella*)) were fed four diets containing 5, 35, 57, and 79 g Ca/kg diet (DW basis) and analyzed for Ca after being fed the diet for 1–3 days. At each level of dietary Ca, waxworm Ca content increased from day 1 to day 3. An analysis of the day 2 data shows a linear relationship (waxworm $Ca = 0.060 + [0.024 \times \text{diet Ca}]$; $F = 119.8$; $P = 0.008$) between waxworm Ca content and diet Ca content that weakened significantly by day 3 (waxworm $Ca = 0.090 + [0.029 \times \text{diet Ca}]$; $F = 9.6$; $P = 0.091$). It appears that the response shown by waxworms to increasing dietary Ca was similar to that observed in silkworms; however, based on only four levels of dietary Ca and a single analysis at each time and treatment, it is difficult to draw any firm conclusions.

These regression equations can be used to develop gut-loading diets to provide a more nutritionally complete prey item for insectivores. In all of the following calculations the slope of the Ca fortification experiments were used, for two reasons. First, the observed standard errors for Ca analysis were smaller than those for vitamin A, due to the lower analytical variance for the Ca assay. Second, any gut-loading diet would require high levels of Ca, which could have a negative effect on palatability. This would be taken into account by using the Ca regression equations. Using these regression equations, the nutrient composition of fasted insects [Finke, 2002], an estimate of reptile nutrient requirements based on the NRC recommendations for rats [National Research Council, 1995], an adjustment for the energy density of the insects [Finke, 2002], and an adjustment (overage) to provide an additional safety factor, the optimum concentration of nutrients for gut-loading diets can be estimated. The requirements for rats were used, since most of the nutrient requirements for rats have been determined by experimental studies. In contrast, many of the nutrient requirements for poultry or fish are based on practical diets and thus do not represent true minimums [National Research Council, 1993, 1994]. An example of the calculation for Ca in adult crickets is shown below. The following parameters were used in the calculations:

NRC Ca requirement=5 g/kg diet;
Overage=50%.
Energy density of NRC requirement=3,950 kcal ME/kg;
Energy density of adult crickets=1,402 kcal ME/kg.
Fasted adult cricket Ca content=0.407 g/kg;
Regression slope=0.0446.
Step 1: Calculate the ME-adjusted Ca requirement with a 50% overage (ME-adjusted Ca requirement=5 g/kg $\times$ 1.5 $\times$ (1,402 kcal ME/3,950 kcal ME)= 2.662 g/kg).
Step 2: Calculate the Ca required in the GI tract after correction for the body Ca content (calcium required in GI tract=2.662–0.407=2.255 g/kg).
Step 3: Calculate the Ca required in the gut-loading diet to fill the GI tract to meet the ME-adjusted requirement (calcium required in gut-loading diet=2.255/ 0.0446=50.6 g/kg).

Thus, in this example a diet containing 50.6 g Ca/kg diet would result in an adult cricket that contains 1.58 g Ca/1,000 kcal ME, which is equal to the NRC requirement with a 50% overage.

The nutrient content, as determined by these calculations, of diets required to gut load adult crickets, cricket nymphs, mealworms, and silkworms fed diets similar to those used here is shown in Table 1. The adjustment of nutrient requirements to provide a safety factor, and of the values to account for the high-energy density of certain insect species means that more nutrients were identified as being appropriate for supplementation than were identified previously [Finke, 2002].

A number of authors [Zwart and Rulkens, 1978; Allen and Oftedal, 1989; Allen, 1997; Barker et al., 1998; Anderson, 2000; Finke, 2002] have demonstrated the necessity of Ca supplementation in insects. The values (80 g Ca/kg diet) suggested by Allen and Oftedal [1989] for gut-loading crickets are higher than those calculated here, but were based on obtaining crickets with 1% Ca (DW basis) and a positive

TABLE 1. Dietary nutrient concentrations of insect diets required for gut loading calculated from regression equations

Nutrient	Overage (%)	Adult[a] crickets	Cricket[a] nymphs	Mealworms[a]	Silkworms[a]
Calcium (g/kg)	50	51	32	90[b]	23
Iron (mg/kg)	25	A	A	51	A
Manganese (mg/kg)	25	A	A	31	A
Iodine (mg/kg)	25	A	A	A	0.7
Vitamin A (μg retinol equivalents/kg)	50	8,310	5,270	13,310	A
Vitamin D_3 (μg cholecalciferol/kg)	50	300	190	460	140
Vitamin E (mg RRR-α-tocopherol/kg)	200	140	140	660	70
Thiamin (mg/kg)	25	31	21	5	A
Pyridoxine (mg/kg)	50	20	10	A	A
Vitamin B_{12} (μg/kg)	25	A	A	650	226
Methionine (g/kg)	0	A	A	29	A

[a]Calculated on an 8% moisture basis.
[b]Dietary concentration could not be calculated from these studies and is an estimate based on the data of Klasing.
A, Insect contains sufficient nutrient as not to require dietary supplementation.

Ca:P ratio. Using the current regression equations and those criteria, estimates for dietary Ca levels are 65.8 g/kg (1% Ca, DW basis) and 60.5 g/kg (Ca:P=1) for adult crickets, and 44.4 g/kg (1% Ca, DW basis) and 42.2 g/kg (Ca:P=1) for cricket nymphs. Using these criteria, the values for adult crickets are similar to those recommended by Allen and Oftedal [1989] for similar-size crickets.

The dietary Ca levels for mealworms could not be calculated from this study when a polynomial equation with a 50% overage was used; however, when no overage is used, a dietary Ca content of 79 g/kg is suggested for mealworms. Using the data for wheat bran-based diets published by Klasing et al. [2000], and fitting a polynomial equation to the data allows for a better estimate of dietary Ca concentrations required for gut-loading diets for mealworms. Using these data and a 50% overage, a concentration of 90 g Ca/kg diet is suggested for mealworms fed wheat bran-based diets.

Allen [1997] showed excellent Ca retention in leopard geckos fed crickets containing 2.7–6.1 g Ca (DM basis), indicating that the Ca was readily available. Similarly, Klasing et al. [2000] determined that the Ca in gut-loaded mealworms was 76–89% as available (depending on the response criteria) as oyster shell Ca when fed to young chicks. Given the high apparent availability of Ca in the insect species tested to date, care should be exercised when fortifying diets with Ca. While Ca deficiencies are a common problem in reptiles, excessive Ca supplementation should be avoided because Ca has been shown to interfere with the absorption of magnesium, zinc, manganese, and possibly copper [National Research Council, 1995].

Based on these regression equations, trace mineral supplementation is only necessary for diets fed to mealworms. Depending on the base diet used, supplementation may not be necessary. Wheat bran contains 128 mg/kg iron and

125 mg/kg manganese [National Research Council, 1985], indicating that only iodine supplementation may be necessary when mealworms are fed wheat bran-based diets.

Data from several studies suggest that supplementation of diets with vitamin A may be necessary [Pennino et al., 1991; Ferguson et al., 1996; Barker et al., 1998; Miller et al., 2001; Finke, 2002]. Insects can convert various carotenoids to either retinal or 3-hydroxyretinal for use as a visual pigment [Seki et al., 1998; von Lintig and Vogt, 2000]. Since retinoids are found almost exclusively in the insects' eyes, and the levels are relatively low, insects are unlikely to be a significant source of preformed vitamin A for most insectivores [Smith and Goldsmith, 1990; Seki and Vogt, 1998]. The exception may be herbivorous lepidopteran larvae (including *Bombyx mori*), which have been shown to contain significant amounts of vitamin A [Dreyer and Wehmeyer, 1982; Kodondi et al., 1987; Finke, 2002].

Ferguson et al. [1996] reported signs of vitamin A deficiency and increased mortality of chameleons fed crickets whose diet contained only 1,700 μg retinol equivalents/kg from carotenoids. Assuming that chameleons can convert these carotenoids to vitamin A, it is estimated that this diet would result in crickets containing 30–50% (depending on the size of the cricket) of the NRC requirement of vitamin A for rats [National Research Council, 1995]. In contrast, no mortality or signs of vitamin A toxicity were observed in chameleons fed crickets whose diet contained 16,295 or 30,890 μg retinol equivalents/kg. Using the regression equations developed in the current work, it is estimated that these levels would provide the chameleons with crickets containing three to eight times the vitamin A requirement, based on NRC requirements for rats [National Research Council, 1995]. The medium vitamin A diet (16,295 μg retinol equivalents/kg) is about twice that recommended for gut-loading adult crickets (Table 1). The overage used for vitamin A (50%) is higher than that for other nutrients because vitamin deficiency has been described in reptiles, and because of the high level of vitamin E recommended. In other species, high levels in vitamin E have been shown to interfere with vitamin A absorption [National Research Council, 1995].

For vitamin D, a 50% overage is recommended because of reports of poorly mineralized bones and/or bone fractures in captive reptiles. Chemical analysis suggests that vitamin D supplementation is necessary [Finke, 2002]. For lizards exposed to little if any ultraviolet light, cholecalciferol supplementation has been shown to have a positive effect on bone mineralization in leopard geckos (*Eublepharis macularius*), but not in giant day geckos (*Phelsuma madagascariensis*) [Allen et al., 1996]. Supplemental cholecalciferol was also ineffective at improving reproductive success in the panther chameleon, and only high-intensity UV light improved egg hatchability [Ferguson et al., 1996]. Although it may not be effective for all species, vitamin D supplementation would appear to be prudent given the wide variation in the quantity and quality of light sources for captive reptiles [Allen et al., 1996; Jones et al., 1996; Ferguson et al., 1999, 2002].

For vitamin E, a 200% overage is recommended because of the high fat content of these insects (80 g/kg for silkworms to 350 g/kg for mealworm larvae) vs. the 55 g fat/kg diet (all values on a DW basis) recommended by the NRC for diets fed to rats [National Research Council, 1995; Finke, 2002]. Additionally, the high unsaturated fat content of most insects also means that a higher vitamin E content is necessary [Jones et al., 1972; Martin et al., 1976; Finke, 2002]. Dierenfeld [1989, 1994] reported vitamin E deficiency in zoo animals (insectivorous reptiles were not studied) and

suggested that their diets should contain 50–200 mg vitamin E/kg of diet. Wild-caught insects appear to contain higher levels of vitamin E than those reported for cultured insects (Pennino et al., 1991; Oyarzun et al., 1996; Barker et al., 1998; Cedra et al., 2001; Finke, 2002).

While no data are available regarding supplementation of water-soluble vitamins, the addition of thiamin (crickets and mealworms), pyridoxine (crickets), and vitamin B_{12} (mealworms and silkworms) is indicated based on chemical analysis [Finke, 2002]. Increasing the dietary carbohydrate content in rats increases the thiamin requirement [National Research Council, 1995]. Since the NRC requirement for thiamin is based on diets containing 63–72% carbohydrate, and insects contain little carbohydrate, it is unclear whether animals fed insects require additional thiamin. For both rats and poultry, the pyridoxine requirement increases with increasing levels of dietary protein [National Research Council, 1994, 1995]. For this reason, an overage of 50% is used for pyridoxine, given the high protein content of these insects. It is unlikely that the natural ingredients in most diets supply B vitamins at the levels recommended here.

The only amino acid supplementation suggested from chemical analysis is for total sulfur amino acids (methionine plus cystine) in mealworms [Finke, 2002]. These results are consistent with those obtained in three previous feeding trials [Goulet et al., 1978; Finke et al., 1987; Onifade et al., 2001], in which methionine was shown to be the first limiting amino acid when young growing rats were fed a purified diet containing freeze-dried mealworm larvae, Mormon cricket meal, or dried housefly larvae. No additional overage was used for methionine, for several reasons. First, mealworms have been shown to contain high levels of choline (mealworms: 900 mg choline/1,000 kcal ME vs. NRC requirements for rats: 190 mg choline/1,000 kcal ME) [Finke, 2002], and choline via betaine can function as a methyl donor, thereby decreasing the need for methionine [National Research Council, 1995]. Second, if the maintenance requirement (rather than the growth requirement) for total sulfur amino acids of rats is used, no amino acid supplementation is necessary. Third, methionine is among the most toxic of the amino acids, so over-supplementation should be avoided. Arginine may also be important when fed to uricotelic species, such as birds and reptiles, since they have a limited ability to synthesize arginine via the urea cycle [Finke, 1984]. However, all three insect species tested (adult crickets 8.9 g/1,000 kcal ME, cricket nymphs 9.9 g/1,000 kcal ME, mealworms 4.7 g/1,000 kcal ME, and silkworms 4.0 g/1,000 kcal ME) have previously been shown to contain sufficient arginine to meet the requirements of broiler chicks (3.9 g/1,000 kcal ME) [Finke, 2002; National Research Council, 1994].

In addition to the required nutrients, wild-caught insects also contain a variety of carotenoids, including β-carotene, lutein, and zeaxanthin, sequestered from their foodplants [Goodwin, 1949; Feltwell, 1974; Carroll et al., 1997]. The carotenoids in wild-caught insects may provide a source of vitamin A for some insectivores, and may play a role in immune function, coloration, and protection from ultraviolet light-induced damage [Latscha, 1990; Roll, 2000]. In a previous study [Raila et al., 2002] of β-carotene supplementation in the diet of the herbivorous lizard (*Iguana iguana*), no β-carotene was detected in the plasma and no change was observed in plasma retinol. This suggests that iguanas do not absorb β-carotene, although other carotenoids appear to be readily absorbed. Ferguson et al. [1996], citing unpublished

work by Talent, suggested that dietary β-carotene could prevent vitamin A deficiency in insectivorous lizards (*Sceloporus* sp). While the addition of carotenoids to gut-loading diets may be beneficial, the appropriate carotenoids and levels for supplementation have not yet been determined.

Little is known about the nutrient requirements of reptiles and amphibians, but the few studies available regarding Ca requirements suggest that extrapolation of their requirements from other species provides reasonable estimates. Allen [1997] reported differences in bone ash and Ca content in juvenile leopard geckos (*Eublepharis macularius*) fed high- vs. low-Ca crickets. She also reported that geckos fed high-Ca crickets for 8 months had well-mineralized bone, whereas geckos fed low-Ca crickets had poorly mineralized bone, with fractures evident by radiography. In a second experiment (Allen et al., 1997), in which young leopard geckos were fed crickets containing 2.7–8.5 g Ca/kg (DM basis), Ca retention data suggested requirements to be 6.1–8.5 g Ca/kg (DM basis). Assuming an energy content of 4,144 kcal ME/kg (DM basis) for these crickets [Finke, 2002], the leopard gecko's Ca requirement would be 1.47–2.05 g Ca/1,000 kcal ME. These values are only slightly higher than the Ca requirements recommended for growing rats (1.27 g Ca/1,000 kcal ME), but less than those for broiler chicks and trout (3.12 g Ca/1,000 kcal ME, and 2.78 g Ca/1,000 kcal DE, respectively) [National Research Council, 1993, 1994]. The 50% overage used in these calculations would result in a value of 1.90 g Ca/1,000 kcal ME, a value similar to the estimated requirement for leopard geckos.

Using lizards fed mealworms and capsules containing $CaCO_3$, van der Wardt et al. [1999] estimated the Ca requirement of crag lizards (*Pseudocordylus melanotus*) by Ca balance. This research showed that lizards maintained Ca balance at dietary Ca levels ranging from 3.6 to 55.6 mg Ca/14 days. After removing the data for the two lizards that ate no or only one mealworm during the 14-day balance period, a regression analysis shows a significant linear affect between Ca intake and Ca balance (Ca balance [mg/14 days]=$-0.257 + [0.173 \times$ Ca intake]; $F=19.6$; $P=0.011$). From this equation a zero Ca balance would be obtained at 1.42 mg Ca/14 days. With an average mealworm intake of 2.05 g/14 days, and assuming an energy content of 2.05 kcal ME/g for mealworms [Finke, 2002], this calculates to approximately 0.33 g Ca/1,000 kcal ME diet.

CONCLUSIONS

1. The recommendations calculated from these experiments appear to provide reasonable guidelines for the development of gut-loading diets for reptiles. These recommendations assume that the form and palatability of the diet enable adequate consumption.

2. More research into the factors affecting the palatability of diets for mealworms may help explain the different dose-response curves seen by Klasing et al. [2000] and overcome some of the limitations of the regression equations developed here.

3. In addition to their use in nutrient supplementation, these equations might also be used for other purposes. Knowing the weight of the insect and the amount of food in its gastrointestinal tract might allow zoo nutritionists and veterinarians to use insects to deliver a wide range of other compounds, such as carotenoids, and medicines, such as anthelmintics, to captive insectivores.

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