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Effect of Commercial Diets on the Nutritional Value and Mortality Rates of Dubia Roaches (*Blaptica dubia*)

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Abstract

Dubia roaches (*Blaptica dubia*) are a popular feeder insect but, like most other insects, have an inverse calcium to phosphorous (Ca:P) ratio. A plethora of insect diets claim to correct this inverse ratio, but limited evidence-based data exists. The goals of this study were to determine whether diets claiming to correct the Ca:P ratio can do so, to determine the effect of diet on the nutritional value and mortality of dubia roaches, and to determine the fasting period required to empty the gut of dubia roaches. In phase one, nymphal roaches were fasted (24 h) and randomly divided into a control group ($n = 6$, baseline) and two experimental groups ($n = 6$ each). Each cohort represented approximately 75–80 roaches. Roaches in the experimental groups were fed either 50 g of a high calcium diet or low calcium diet (24 h). The Ca:P ratio was significantly higher ($P < 0.001$) in the high calcium group compared with the control and low calcium diet groups. In phase two, adult roaches (25.4–31.8 mm) were fasted (24 h) and then gut-loaded for 24 or 168 h on a high calcium or low calcium diet ($n = 6$/group). The Ca:P ratio was significantly higher ($P < 0.001$) in the high calcium group compared with the low calcium diet group, regardless of time. No significant difference (all $P > 0.06$) in mortality occurred across diets or overtime. In phase three, adult roaches were fasted (24 h), gut-loaded on the high calcium diet (24 h), and then fasted for 24, 48, or 72 h ($n = 3$). The Ca:P ratio did not decrease over time ($P = 0.092$). These results confirm that a high calcium diet can increase the Ca:P ratio in dubia roaches; however, not all gut-loading diets can alter the Ca:P ratio.

Keywords: *Blaptica dubia*, calcium, dubia roach, gut-loading, nutrition, reptile

Introduction

A wide variety of commercially available insects are available to use as a source of food and nutrition for captive reptiles, including house crickets (*Acheta domesticus*), mealworms (*Tenebrio molitor*), silkworms (*Bombyx mori*), superworms (*Zophobas morio*), waxworms (*Galleria mellonella*), and black soldier fly larvae (*Hermetia illucens*; Finke, 2002; Boykin *et al*., 2020b). However, a common theme among most of these insects is that they naturally have an inverse calcium to phosphorus ratio (Ca:P; ≤0.28), with the black soldier fly larvae being the single exception with a positive Ca:P ratio of 2.5 (Finke, 2002; Boykin *et al*., 2020b). Numerous studies have been completed to improve the nutritional value of these insects for reptiles, with most studies focused on correcting the calcium concentration of the insect to create a positive Ca:P ratio (Finke, 2003; Finke *et al*., 2005; Boykin *et al*., 2021).

A recent feeder insect to emerge on the market is the dubia roach (*Blaptica dubia*). This insect has gained in popularity because of its ease of rearing, long lifespan, and naturally high protein content (Wu, 2013; Pei Yee *et al*., 2018). Both nymphal (194.5 g/kg) and adult dubia roaches (202.6 g/kg in adult males, 203.8 g/kg in adult females) have relatively high crude protein (CP) concentrations on a dry matter (DM) basis compared with the previously noted insects (≤187 g/kg on a DM basis; Finke, 2003; Pei Yee *et al*., 2018; Boykin *et al*., 2020b). The only insects with comparable protein content are superworms (197 g/kg CP on DM basis) and adult house crickets (205 g/kg on DM basis; Finke, 2003). However, similar to most other insects, dubia roaches have an inverse Ca:P ratio (0.35 in nymphs, 0.26 in adults; Cerreta *et al*., 2021). Although studies have been conducted to measure the nutritional value of dubia roaches (Yi *et al*., 2013; Oonincx *et al*., 2015; Kulma *et al*., 2016; Pei Yee *et al*., 2018, Cerreta *et al*., 2021), only a single study completed by the authors evaluated the effect of a high calcium diet (8% DM) on the Ca:P ratio of dubia roaches (Barras *et al*., 2024b). In Barras *et al*. (2024b), the research found that an 8% calcium diet could correct the inverse Ca:P ratio of the roaches but did not measure the effects of the high calcium diet on the roach longevity. Moreover, the other studies measuring the nutrition of dubia roaches focused on non-commercially available diets, leaving a need to evaluate commonly accessible commercial diets to measure their consistency (Yi *et al*., 2013; Oonincx *et al*., 2015; Kulma *et al*., 2016; Pei Yee *et al*., 2018; Cerreta *et al*., 2021).

As noted previously, the longevity of dubia roaches is one reason they have gained in popularity as feeder insects. The nymph stage for these roaches can range from 180–380 days, and their entire life span can extend to 530 days (Koehler *et al*., 1990). For comparison, the adult house cricket lives for about 120 days, black soldier flies have a life cycle of 40 days, and mealworms remain in the larval stage for approximately 112 days (Martin *et al*., 1976; Lyn *et al*., 2011; Boykin and Mitchell, 2021).

Although the long-life span of dubia roaches is appealing, feeding a gut-loading diet can come with uncertainties. For example, increased mortality in insects has been associated with feeding high calcium gut-loading diets. Gut-loading diets normally contain high amounts of DM, and a water source may not be provided to reduce the likelihood of satiety (Boykin *et al*., 2021; Aguilar *et al*., 2022; Bitter *et al*., 2022). However, it is thought that dehydration, due to being offered only a high DM diet, can lead to mortality (Boykin *et al.,* 2021). Latney *et al*. (2017) found that there was a 100% mortality in superworms and 30.7% mortality in mealworms when gut-loaded on a high calcium diet for 48 h if the diet was used as a bedding substrate; however, mortality dropped to 0.83% and 0.31%, respectively, when the diet was offered in a feeding dish. Because of the increased risk of mortalities associated with high calcium gut-loading diets, it is essential to measure mortality rates in dubia roaches offered these diets. Additionally, evaluating the mortality rates of roaches fed diets with naturally higher moisture contents could be used to determine the risk of dietary moisture content for dubia roaches.

The specific aims of this study were to 1) determine whether commercial diets with claims of being balanced diets for insects provide a comparable level of nutrition; 2) determine whether commercial insect diets that claim to correct calcium concentrations and an inverse Ca:P ratio can do so; 3) determine whether commercial diets affect the mortality rate of dubia roaches over a 7 day feeding period; 4) determine whether the age of the dubia roach affects their nutritional composition based on the commercial diet offered; 5) determine the fasting period required to empty the digestive tract of dubia roaches; and 6) determine how dubia roaches reared on historical diets compare with the diets used in these studies.

The specific objectives were to measure the protein, fat, moisture, and mineral contents of dubia roaches fed two different commercial diets over 24 h and 7 days and to measure the mortality rates of dubia roaches offered these diets for 7 days. An additional objective was to determine the fasting period needed to clear the digestive tract in dubia roaches. The final objective was to compare the nutritional composition of dubia roaches from the peer-reviewed literature to dubia roaches from the present study. The specific hypotheses being tested included that there would be differences in the nutritional status of dubia roaches at different ages and when fed different commercial diets, especially as it relates to the calcium content and Ca:P ratio of the dubia roaches, and that mortality rates would be low for both diets and age groups. Additionally, gut-loaded dubia roaches that converted to a positive Ca:P ratio would have an inverse Ca:P ratio after 72 h of fasting. The final hypothesis was that significant differences would occur in the nutritional composition of dubia roaches when comparing roaches fed historical non-commercial diets to fasted roaches and roaches fed either the high calcium or low calcium diet.

Materials and Methods

Diet nutritional analysis: Three 30-g samples of both a high calcium diet (Fluker's High Calcium Dubia Roach Diet, Fluker Farms, Port Allen, LA, USA) and the low calcium diet (Nature Zone's Total Bites Diet, Nature Zone, Chico, CA, USA) were sampled from their original store containers. Samples were placed in Nasco Whirl-Pak bags (Whirl-Pak, Madison, WI, USA) and placed inside a −80°C (−112°F) freezer until they were sent off for analysis.

Moisture loss of diet: Six 50-g samples of the high calcium diet and six 50-g samples of the low calcium diet were placed in clear, plastic, round, 2-L containers with a vented lid (Josh's Frogs, Owosso, MI, USA). Containers were covered with a vented lid and housed in the same area as the dubia roaches to mimic the feeding study. Weights of each diet were measured at 24 h (1 day) and 168 h (7 days) to assess moisture loss over time.

Insects and housing: For phase one, 2,000 nymphal dubia roaches (16 mm in length) were obtained from a commercial breeder (DubiaRoaches.com, Wichita, KS, USA). Roaches were randomly divided into 24 cohorts ($n = 24$), with each cohort of roaches weighing between 16.6–18.8 g. The cohort weights were based on the minimum weight (15 g) needed for sample nutritional analysis. Each group was placed in the same plastic containers used for assessing moisture loss in the diet. A large piece of cardboard egg carton was placed in each container for shelter. Egg cartons were obtained from the roach shipping containers. Roaches were acclimated to their environment for 5 days. During this time, roaches were provided pieces of sweet potato for nutrition and moisture. Roaches were maintained at ambient room temperature and humidity to more accurately reflect how they might be raised by pet owners. Temperature and humidity were measured throughout the duration of the study using an EL-USB-2 temperature and humidity data logger (LASCAR electronics, Erie, PA, USA). Average temperature and humidity for the study were 22 ± 0.6°C (71.6°F) and 50.5 ± 1.1%, respectively.

For phase two, 600 adult dubia roaches (25.4 to 31.8 mm in length) were obtained from a commercial breeder (Dubia Roaches.com). Adult roaches were not acclimated before fasting because they had been maintained in a 132L dark blue plastic maintenance bin (82.6 cm × 50.2 cm × 47.3 cm; Sterilite, Townsend, MA, USA) with holes drilled into the top for aeration for a reptile research colony for 2 months. Roaches in the maintenance bin were fed the low calcium diet *ad libidum* for nutrition and moisture. Adult roaches were randomly divided into 30 cohorts ($n = 30$), with each cohort of roaches weighing between 18.6–20.7 g. Roaches were housed using the same containers described in phase one. Roaches were maintained at ambient room temperature and humidity. Temperature and humidity were measured throughout the duration of the study as stated in phase one. Average temperature and humidity for the adult and nymph studies were 22.5 ± 0.9°C (72.5°F) and 50.8 ± 0.7%.

For phase three, 300 adult dubia roaches (25.4 to 31.8 mm in length) were obtained from a commercial breeder (Dubia Roaches.com). Adult roaches were acclimated the same as adult roaches from phase two. Adult roaches were randomly divided into 12 cohorts ($n = 12$), with each cohort of roaches weighing between 17.9–23.2 g. Roaches were housed using the same conditions described for phase one. Roaches were maintained at ambient room temperature and humidity, and temperature and humidity were measured throughout the study as described in phase one. Average temperature and humidity were 23.1 ± 0.3°C (73.6°F) and 49.7 ± 2.0%.

Phase one: diet comparison of nymph dubia roaches: All roach groups were weighed using a VWR P-Series portable balance (VWR International, LLC, Radnor, PA, USA) before the 24 h fast, after the 24 h fast, and after access to their respective diets for 24 h. Containers housing the roaches were divided into four groups: a baseline group (roaches after 24 h fast, $n =$ six containers), a control group (roaches fasted 48 h, $n =$ six containers), and two experimental groups (roaches fasted 24 h and fed one of two commercial diets for 24 h, $n =$ six containers each group). After being fasted for 24 h, the dubia roaches in the baseline group were weighed and humanely euthanized according to American Veterinary Medical Association 2020 guidelines (Miller *et al.*, 2020). In brief, roaches were overdosed on isoflurane (Fluriso, Vet One, Boise, ID, USA) by placing an isoflurane-soaked cotton ball into a sealed plastic bag (Great Value, Walmart, Bentonville, AR, USA) with the roaches. Once all movement ceased (approximately 5 min), each group was transferred to a Nasco Whirl-Pak and immediately placed inside a −80°C (−112°F) freezer. At the begining of the feeding trial, one experimental group received 50 g of the high calcium diet per container ($n = 6$; Fluker's High-Calcium Dubia Roach Diet), and the other experimental group received 50 g of the low calcium diet per container ($n = 6$; Total Bites). These diets were offered to the roaches for 24 h. The control group was fasted for an additional 24 h. After this 24 h feeding or fasting (48 h total fast for control group), the dubia roaches were again weighed and humanely euthanized, as previously described. Samples were stored at −80°C (−112°F) until being analyzed. Mortality counts were performed for each container at the time of collection.

Phase two: diet comparison of adult dubia roaches: All roach groups were weighed using a VWR P-Series portable balance before a 24 h fast, after the 24 h fast, and after access to their respective diets for 1 or 7 days. Containers housing the roaches were divided into five groups: a control group (roaches after 24 h fast, $n =$ six containers) and four experimental groups (roaches fasted 24 h and fed either the high calcium diet or low calcium diet for 1 day or 7 days, $n =$ six containers each). After being fasted for 24 h, the dubia roaches in the control group were weighed and humanely euthanized, as described previously. At the same time, two experimental groups received 50 g of the high calcium diet per container ($n = 12$; Fluker's High-Calcium Dubia Roach Diet), and the other experimental groups received 50 g of the low calcium diet per container ($n = 12$; Total Bites). These diets were offered to the different cohorts of roaches for up to 1 or 7 days ($n = 12$; 1 day or 7 days). After the roaches were fed the diets for the predetermined feeding period, they were weighed again and humanely euthanized, as previously described. Samples were stored at −80°C (−112°F) until being analyzed. Mortality counts were performed for each container at the time of collection.

Phase three: dubia roach digestive tract emptying time: All roach groups were weighed using a VWR P-Series portable balance before a 24 h fast, after the 24 h fast, after access to the high calcium diet for 24 h, and after fasting for 24, 48, or 72 h post–gut-loading. Containers housing the roaches were divided into four groups: a control group (roaches gut-loaded for 24 h on the high calcium diet, $n =$ three containers) and three experimental groups (roaches fasted for 24, 48, or 72 h after gut-loading on the high calcium diet for 24 h, $n =$ three containers each). After being gut-loaded on the high calcium diet for 24 h, dubia roaches in the control group were weighed and humanely euthanized, as previously described. At the same time, the three experimental groups were removed from the diet and fasted ($n = 9$ total). Roaches were weighed and humanely euthanized following the 24 h ($n = 3$), 48 h ($n = 3$), or 72 h ($n = 3$) fasting periods. Samples were stored at −80°C (−112°F) until being analyzed. Mortality counts were performed for each container at the time of collection.

Nutritional analysis: All the samples were removed from the −80°C (−112°F) freezer and transported on frozen gel packs to the Zooquarius Laboratory Services (affiliate of Dairy One Laboratory, Ithaca, NY, USA) for chemical analysis. Diet and dubia roach samples were analyzed for percent moisture, DM, CP, crude fat (CF), ash, calcium, phosphorus, magnesium, potassium, sodium, sulfur, iron, zinc, copper, manganese, and molybdenum content. Diets and dubia roaches were first homogenized and dried in a forced air oven at 60°C (140°F) for at least 4 h to obtain DM content (and thus respective moisture content) using National Forage Testing Association Method 2.2.1.1 (Undersander *et al.*, 1993). Samples were then placed in a drying oven at 135°C (275°F) for 2 h following AOAC Official Method 930.15 (AOAC, 2019a). For all minerals, the diets and roaches were predigested using HNO_3, HCl, and H_2O_2 and digested using a CEM Microwave Accelerated Reaction System (MARS6; CEM Corporation, Matthews, NC, USA). All mineral concentrations were then analyzed using a Thermo iCAP Pro XP Inductively Coupled Plasma Radial Spectrometer (Thermo Scientific, Waltham, MA, USA; (Dairy One Forage Laboratory, 2022). To measure CF content, the diets and dubia roaches were homogenized and then analyzed using the Acid Hydrolysis Filter Bag Technique on the $\text{ANKOM}^{\text{HCL}}$ Hydrolysis system (ANKOM Technology, Macedon, NY, USA), where samples are weighed into XT4 filter bags and hydrolyzed with HCl in a sealed Teflon vessel. Final extraction was performed using a solvent made of petroleum ether, diethyl ether, and ethanol via the ANKOM XT15 Extractor. Total fat content was determined by the difference in weight between samples. For ash content of both the diets and roaches, samples were ground in a porcelain crucible and placed in a temperature-controlled furnace preheated to 600°C (1,112°F) and held at this temperature for 2 h. Samples were transferred to a desiccator, cooled, weighed immediately, and reported following the formula provided by the AOAC Official Method 942.05 (AOAC, 2019b). For CP, both the diets and roaches were ground and then analyzed by combustion using a CN628 Carbon/Nitrogen Determinator (Leco Corporation, St. Joseph, MI, USA; AOAC, 2019c).

Statistical analysis: Distributions of the data were evaluated for normality using the Shapiro-Wilk test, skewness, kurtosis, and q–q plots. Data that were normally distributed are reported by the mean, standard deviation, and minimum–maximum (min–max) values, whereas non-normally distributed data are reported by the median, 25–75% (or 10–50% if 25–75% was not available), and min–max values. Any data that were not normally distributed were log transformed for parametric statistics; however, if the data could not be normalized (diets: manganese and molybdenum; phase one: iron and manganese; phase two: manganese), non-parametric statistics were used. For normally distributed data, an independent sample *T*-test was used to evaluate differences in nutritional values between the two diets. The Levene's test was used to test for homogeneity of variance. If Levene's test was violated, a corrected *T*-test was used. A Mann-Whitney *U*-test was used to compare dietary differences for non-normally distributed data. A mixed linear model was used to assess the moisture loss in the diets over time.

For phase one, a repeated measures ANOVA was used to evaluate the difference in the nutritional analysis of nymphal dubia roaches fed different gut-loading diets. Mauchly's test of sphericity was used to evaluate homogeneity of variance. If sphericity was violated, a Greenhouse Geiser correction was used to report both the *F* statistic and *P* value. *Post hoc* Bonferroni tests were used to characterize differences between groups.

For phase two, a linear mixed model was used to evaluate the difference in the nutritional analysis of adult dubia roaches fed different diets and gut-loaded for different periods of time. Roach was added to the model as the random factor and time and group were fixed factors. *Post hoc* Bonferroni tests were used to characterize differences between groups.

For phase three, a linear mixed model was used to evaluate the difference in the nutritional analysis of adult dubia roaches fed the high calcium diet and then fasted for different time periods. Roach was added to the model as the random factor and time and group were fixed factors. *Post hoc* Bonferroni tests were used to characterize differences between groups. Single sample *T*-tests (normalized data) or single sample Wilcoxon signed rank tests (non-normalized data) were used to compare the present results to the historic peer-reviewed nutritional results. SPSS 28.0 (IBM Statistics, Armonk, NY, USA) was used to analyze the data. A $P \leq 0.05$ was used to determine statistical significance.

Results

Diet nutritional analysis: All of the nutrient values were significantly different between high and low calcium diets, except for manganese and molybdenum ($P = 0.1$; Table 1). The DM ($P < 0.0001$), CP ($P < 0.0001$), CF ($P = 0.002$), ash ($P = 0.001$), calcium ($P = 0.003$), phosphorus ($P = 0.002$), Ca:P ratio ($P = 0.02$), magnesium, ($P < 0.0001$), iron ($P < 0.0001$), zinc ($P < 0.0001$), and copper ($P = 0.004$) were significantly higher in the high calcium diet compared with the low calcium diet (Table 1), whereas moisture ($P < 0.0001$), potassium ($P < 0.0001$), sodium ($P < 0.0001$), and sulfur ($P < 0.0001$) were significantly higher in the low calcium diet. Specific differences between diets can be found in Table 1.

Moisture loss of diet: Significant differences were found in diet weight, and thus moisture loss, over time and between diets (all $P < 0.001$; Table 2).

Mortality: No significant difference in mortality was found between diets, time, or the interaction term diet and time (all $P \geq 0.06$). The number of mortalities ranged between zero to four at each collection period for nymphs (Table 3) and zero to one for adults (Table 4).

Phase one: diet comparison of nymph dubia roaches: Dubia roach moisture ($P < 0.0001$), DM ($P < 0.0001$), CP ($P = 0.012$), calcium ($P < 0.0001$), phosphorus ($P = 0.001$), Ca:

Table 1. Nutritional contents of two insect diets, HC and LC. All values other than moisture and dry matter are presented on a percent dry matter basis. Normally distributed data are reported by the mean, SD, and min–max values, whereas non-normal data are reported by the median, 25–75%, and min–max values. Significant differences are noted by $P < 0.05$.

Independent samples *T*-test

Nutrient	Diet	Mean	SD	Min–Max	*P* value
Moisture (%)	HC	7.8	0.06	7.8–7.9	<0.0001
	LC	90.5	0.26	90.2–90.7	
Dry matter (%)	HC	92.2	0.06	92.1–92.2	<0.0001
	LC	9.5	0.26	9.3–9.8	
Crude protein (%)	HC	15.5	0.12	15.4–15.6	<0.0001
	LC	6.1	0.36	5.8–6.5	
Crude fat (%)	HC	7.2	0.67	6.8–8.0	0.002
	LC	3.8	0.53	3.2–4.2	
Ash (%)	HC	27.7	2.72	24.9–30.3	0.001
	LC	13.7	0.46	0.16–0.18	
Calcium (%)	HC	8.3	0.78	7.4–8.8	0.003
	LC	0.17	0.01	0.16–0.18	
Phosphorus (%)	HC	1.02	0.07	0.98–1.1	0.002
	LC	0.05	0	0.05–0.05	
Ca:P ratio	HC	8.2	1.24	6.7–8.9	0.02
	LC	3.4	0.2	3.2–3.6	
Magnesium (%)	HC	0.41	0.01	0.4–0.42	<0.0001
	LC	0.15	0.006	0.15–0.16	
Potassium (%)	HC	1.2	0.04	1.15–1.23	<0.0001
	LC	3.2	0.1	3.1–3.3	
Sodium (%)	HC	0.07	0.01	0.06–0.07	<0.0001
	LC	0.73	0.02	0.71–0.76	
Sulfur (%)	HC	0.26	0.01	0.25–0.27	<0.0001
	LC	1.67	0.07	1.6–1.7	
Iron (ppm)	HC	257	10.5	246–267	<0.0001
	LC	47	2	45–49	
Zinc (ppm)	HC	71.3	0.6	71–72	<0.0001
	LC	5	0	5	
Copper (ppm)	HC	14.3	1.5	13–16	0.004
	LC	1	0	1	

Mann-Whitney *U*-test

Nutrient	Diet	Median	10–50%	Min–Max	*P* Value
Manganese (ppm)	HC	91	90–91	90–100	0.1
	LC	4	3–4	3–4	
Molybdenum (ppm)	HC	1.9	1.7–1.9	1.7–2.0	0.1
	LC	0.001	0.001–0.001	0.001–0.1	

HC, high calcium dubia roach–specific diet; LC, low calcium insect diet; SD, standard deviation; min–max, minimum–maximum values; ppm, parts per million.

P ratio ($P < 0.0001$), sulfur ($P = 0.003$), copper ($P = 0.006$), iron ($P < 0.0001$), and manganese ($P = 0.004$) were significantly different between the two dietary groups. Crude fat ($P = 0.786$), ash ($P = 0.147$), magnesium ($P = 0.083$), potassium ($P = 0.958$), sodium ($P = 0.307$), zinc ($P = 0.869$), and molybdenum ($P = 0.499$) were not significantly different based on diet. Specific differences in the nutritional contents of the dubia roaches between diets can be found in Table 5.

Table 2. Moisture loss in diets over time. All data is presented as the means, SD, and min–max values of the weights of the diets over time. Weight of diet was measured in grams. Significant differences are noted by $P < 0.05$.

Diet	Time	Mean	SD	Min–Max	*P*
HC	D0	50.00	0	50.00	All $P < 0.001$
	D1	49.83	0.009	49.82–49.83	
	D7	49.34	0.01	49.33–49.36	
LC	D0	50.00	0	50.00	
	D1	48.45	0.07	48.38–48.52	
	D7	37.84	0.2	37.47–38.14	

SD, standard deviation; min–max, minimum–maximum values; HC, high calcium diet; LC, low calcium diet; D, day.

Phase two: diet comparison of adult dubia roaches: Dubia roach ash, calcium, phosphorus, Ca:P ratio, magnesium, iron, and manganese were significantly (all $P < 0.009$) different by diet, time, and the interaction term time × group. Moisture ($P < 0.001$), DM ($P < 0.001$), CP ($P < 0.001$), potassium ($P < 0.001$), sodium ($P < 0.001$), sulfur ($P < 0.001$), and copper ($P < 0.001$) were significantly different between the diets. Dubia roach moisture ($P = 0.003$), DM ($P = 0.003$), CP ($P = 0.002$), and sulfur ($P = 0.006$) were also significantly different for the interaction term diet × time. Specific differences in the nutritional contents of the dubia roaches based on diet, time, and the interaction of diet × time can be found in Tables 6, 7, and 8, respectively.

Phase three: dubia roach digestive tract emptying time: Dubia roach moisture, DM, calcium, phosphorus, and Ca:P ratio were not significantly different over time (all $P \geq 0.091$). The nutritional composition of the dubia roaches over the 24–72 h fasting periods can be found in Table 9.

Historical comparison of the nutritional composition of dubia roaches: Moisture, DM, CP, CF, calcium, phosphorus, and Ca:P ratio of nymphs that were fasted, fed the high calcium diet for 24 h, or the low calcium diet for 24 h were significantly different (all $P \leq 0.024$) when compared with the nutritional composition of dubia roaches fed historical diets (Pei Yee *et al.*, 2018; Cerreta *et al.*, 2021). The diet

Table 3. Number of nymphal dubia roach mortalities based on diet and time. All data is presented as the mean, SD, and min–max values of the number of nymph roach mortalities per container. Significant differences are noted by $P < 0.05$.

Diet	Time	Mean	SD	Min–Max	*F*	*P*
Baseline	24 h fast	0.67	0.82	0–2	Diet = 0.998	Diet = 0.405
Control	24 h fast	1.83	1.47	0–4		
	48 h fast	0.67	0.82	0–2	Time = 3.784	Time = 0.06
HC	24 h fast	1.17	1.33	0–3		
	Post-GL	0.67	0.82	0–2	Diet × time =	Diet × time =
LC	24 h fast	1.00	0.89	0–2	0.552	0.581
	Post-GL	0.67	0.82	0–2		

SD, standard deviation; min–max, minimum–maximum values; HC, high calcium diet; LC, low calcium diet; GL, gut-load.

Table 4. Number of adult dubia roach mortalities based on diet and time. All data is presented as the mean, SD, and min–max values of the number of adult roach mortalities per container. Significant differences are noted by $P < 0.05$.

Diet	Time	Mean	SD	Min–Max	*F*	*P*
Control	0D	0.08	0.11	0	Diet = 0.939	Diet = 0.399
HC	1D	0.16	0.16	0–1	Time = 0.666	Time = 0.52
	7D	0.17	0.16	0–1		
LC	1D	0.33	0.16	0–1	Diet × time =	Diet × time =
	7D	0.33	0.16	0–1	0.074	0.929

SD, standard deviation; min–max, minimum–maximum values; 0D, day 0; 1D, day 1; 7D, day 7; HC, high calcium; LC, low calcium.

offered in the study by Pei Yee *et al.* (2018) comprised kitchen waste, and the diet offered by Cerreta *et al.* (2021) comprised dog food and fish flakes. Ash of nymphs that were fasted or fed the low calcium diet were not significantly different ($P \geq 0.072$) from the dubia roaches fed the historical diet by Cerreta *et al.* (2021) but were significantly different when compared with dubia roaches fed the historical diet provided by Pei Yee *et al.* (2018; all $P < 0.001$). Moisture, DM, CP, CF, calcium, phosphorus, and the Ca:P ratio of the adult dubia roaches that were fasted, fed the high calcium diet for 24 h, or fed the low calcium diet for 24 h were significantly different (all $P \leq 0.042$) from the dubia roaches fed the historical diets (Yi *et al.*, 2013; Oonincx *et al.*, 2015; Pei Yee *et al.*, 2018; Cerreta *et al.*, 2021). Again, the diets fed by Pei Yee *et al.* (2018) and Cerreta *et al.* (2021) were kitchen waste and dog food and fish flakes, respectively. The diet fed by Oonincx *et al.* (2015) comprised several combinations of high protein, high fat, low protein, and/or low fat food sources. The control diet was provided by Kreca VOF (Kreca Ento-Feed BV, Ermelo, Netherlands). The diet fed in Yi *et al.* (2013) comprised carrots and chicken mash. Nutritional components that did not differ between these groups included moisture and DM of adult dubia roaches fed the high calcium diet for 24 h and adult male dubia roaches fed the historical diet provided by Cerreta *et al.* (2021); CP of fasted dubia roaches, roaches fed the high calcium diet, and roaches fed the low calcium diet compared with dubia roaches fed the historical diet provided by Oonincx *et al.* (2015); CF of fasted dubia roaches, dubia roaches fed the high calcium diet, and dubia roaches fed the low calcium diet compared with the adult male dubia roaches fed the historical diet provided by Cerreta *et al.* (2021); and ash of fasted dubia roaches and roaches fed the low calcium diet compared with adult male and female dubia roaches fed the historical diet provided by Cerreta *et al.* (2021). Oonincx *et al.* (2019) was excluded from CF comparison because in that study fat was calculated based on the total fatty acid content rather than CF. Thus, the overall fat concentration would be lower in that study. Cerreta *et al.* (2021) was excluded from CP comparison because protein was calculated differently from the authors' studies, and we believe that percent CP comparison would not be accurate. For more information regarding the nutritional composition of nymphal or adult dubia roaches compared with the historical literature, refer to Tables 10 and 11, respectively.

Discussion

The results of this study proved the hypotheses set by the authors. Nutritional differences were found between the diets, age groups, and sampling times, and mortality rates were low for all groups. These findings are noteworthy because both the lay literature and textbooks recommend gut-loading insects, but they do not discuss potential differences between diets. For example, when vet approved gut-loading is entered into a Google search engine, one of the first result to populate after the gut-loading diet ad is, "Gut Loading Feeder Insects for Reptiles" by Allan's Pet Center. Although the post highlights the significance of including calcium in an insect's diet and the length of time insects should be gut-loaded, it does not provide any specifics regarding what diets should be and it provides a wide range of how much calcium should be included in the gut-loading diets (Gut Loading Feeder Insects for Reptiles, 2021).

Veterinarians making recommendations to zoological staff or private owners of reptiles and amphibians should read the nutritional labels on these diets to ensure the diets are meeting the expected nutritional requirements of the animals in their care. It is also critical to understand that package labels for these types of products are not held to the same standard as other human or pet (e.g., dog, cat) food products, so claims may be made that are not justified. Finke *et al.* (2004) inspected several calcium-fortified, high-moisture commercial cricket diets and found that the diets did not contain enough calcium to correct the naturally inverse Ca:P ratio of insects. Although several products claimed they would help "shift the Ca:P ratio of insects closer to the ideal of 2:1," the diets did not have a significant effect on cricket calcium content with Ca:P ratios of 0.34 in control crickets and 0.31 to 0.32 in crickets gut-loaded for 48 h with the calcium-fortified diets (Finke *et al.*, 2004). The low calcium diet (Total Bites) used in the present studies claimed to be an "all-in-one diet for both pet insects and feeder insects" and has "balanced nutrition with amino acids (proteins), vitamins, and minerals"; however, closer inspection during these studies proves otherwise.

Moisture loss from a diet can affect the nutritional composition of the diet by concentrating certain components in the diet (Bluestein and Labuza, 1988), and because the low calcium diet primarily comprised water, the authors believed it imperative to quantify the amount of moisture lost from the diets over time. Significant differences occurred in the weight of both diets over 24 h and 7 days and when comparing the high calcium diet to the low calcium diet. The high calcium diet lost 0.34% and 1.32% of its weight after 1 and 7 days, respectively. In contrast, the low calcium diet lost 3.1% and 24.3% of its weight over the same 1 and 7 days, respectively. This moisture loss is not entirely surprising considering the amount of moisture in the low calcium diet; however, the label on the low calcium

Table 5. Results of phase two of the study: nutritional contents of nymph dubia roaches gut-loaded on either a HC roach-specific diet, LC diet, or fasted for 24 h (C) or 48 h (F) presented on a percent dry matter basis. Normally distributed data are reported by the mean, SD, and min–max values, whereas non-normal data are reported by the median, 10–50%, and min–max values. Significant differences are noted by $P < 0.05$.

Normal data

Nutrient	Diet	Mean	SD	Min–Max	*P* value
Moisture (%)	C	74.5	0.5	73.6–74.9	0.004
	F	74.6^{a}	0.5	74–75.4	
	HC	$72.3^{b,c}$	0.7	71.5–73.2	
	LC	74.7^{a}	0.5	73.9–75.2	
Dry matter (%)	C	25.5	0.5	25.1–26.4	0.005
	F	25.4^{a}	0.5	24.6–26	
	HC	$27.7^{b,c}$	0.7	26.8–28.5	
	LC	25.3^{a}	0.5	24.8–26.1	
Crude protein (%)	C	76.9	2.1	72.6–78.4	<0.0001
	F	76.7^{a}	2.3	73.4–79.4	
	HC	69.6^{b}	3.5	66.6–74.6	
	LC	74.1	1.9	70.4–75.6	
Crude fat (%)	C	17.8	0.9	17–19.1	0.786
	F	18.2	1.2	16.8–19.9	
	HC	17.1	1.1	16–18.7	
	LC	17.8	1.7	15.6–19.8	
Ash (%)	C	5.9	0.3	5.6–6.5	0.147
	F	5.4	0.5	4.7–6.2	
	HC	7.5	0.9	6.1–8.3	
	LC	5.5	0.3	5.1–5.9	
Calcium (%)	C	0.13	0.01	0.12–0.15	<0.0001
	F	0.13^{a}	0.01	0.11–0.14	
	HC	$1.2^{b,c}$	0.1	1–1.4	
	LC	0.16^{a}	0.02	0.13–0.18	
Phosphorus (%)	C	0.79	0.05	0.72–0.84	0.001
	F	0.89^{c}	0.04	0.84–0.96	
	HC	0.83	0.03	0.8–0.87	
	LC	0.81^{b}	0.02	0.78–0.83	
Ca:P ratio	C	0.17	0.01	0.16–0.18	<0.0001
	F	0.14^{a}	0.01	0.13–0.15	
	HC	$1.5^{b,c}$	0.2	1.2–1.7	
	LC	0.19^{a}	0.03	0.16–0.23	
Magnesium (%)	C	0.18	0.02	0.16–0.2	0.083
	F	0.19	0.01	0.18–0.2	
	HC	0.21	0.01	0.2–0.23	
	LC	0.18	0.01	0.16–0.19	
Potassium (%)	C	1.5	0.1	1.4–1.6	0.958
	F	1.6	0.06	1.6–1.7	
	HC	1.5	0.03	1.4–1.5	
	LC	1.5	0.04	1.4–1.6	
Sodium (%)	C	0.7	0.03	0.61–0.69	0.307
	F	0.7	0.05	0.65–0.78	
	HC	0.6	0.02	0.57–0.63	
	LC	0.6	0.03	0.6–0.68	
Sulfur (%)	C	0.42	0.03	0.36–0.45	0.003
	F	0.47	0.02	0.44–0.49	
	HC	0.43	0.01	0.42–0.44	
	LC	0.43	0.02	0.4–0.46	
Zinc (ppm)	C	243	25	203–265	0.869
	F	260	14	237–277	
	HC	233	12	212–248	
	LC	238	18	213–257	
Copper (ppm)	C	9.3	2.1	6–12	0.006
	F	12.8	1	12–14	
	HC	10.3	1.2	9–12	
	LC	10	0.6	9–11	
Molybdenum (ppm)	C	1.6	0.1	1.4–1.8	0.499
	F	1.6	0.3	1.3–2	
	HC	1.7	0.2	1.4–1.9	
	LC	1.7	0.2	1.5–1.9	

Non-normal data

Nutrient	Diet	Median	10–50%	Min–Max	*P* value
Iron (ppm)	C	63	57.8–64	51–64	<0.0001
	F	61.5^{a}	58.5–67.8	57–70	
	HC	$87^{b,c}$	83.5–94.5	82–96	
	LC	59^{a}	54.8–62.3	54–63	
Manganese (ppm)	C	10.5	9.8–10.3	9–14	0.004
	F	10^{a}	8.8–11	8–11	
	HC	$18^{b,c}$	17.8–19.5	17–24	
	LC	9^{a}	7.8–10.5	7–12	

HC, high calcium; LC, low calcium; SD, standard deviation; min–max, minimum–maximum values; ppm, parts per million.

[a] Values differ significantly from the value for the roaches fed the high calcium diet.

[b] Values differ significantly from the value for the fasted roaches.

[c] Values differ significantly from the value for roaches fed the low calcium diet.

diet recommends that the total bites are to be eaten in 1 day. The significant loss in moisture of the low calcium diet after both 1 and 7 days could affect the nutritional composition of the diet and thus the nutritional composition of dubia roaches when gut-loaded on the diet for 7 days.

Significant differences between the DM and moisture contents of the diets were found. The high calcium diet comprised 92.2% DM and 7.8% moisture, whereas the low calcium diet comprised 9.5% DM and 90.5% moisture. These findings were not unexpected because the first ingredients in the high and low calcium diets are wheat bran and water, respectively. By their definition, high moisture diets tend to be nutritionally less complete (e.g., lower concentrations of DM), and it is more challenging to increase other nutrients because of the high moisture content. Dietary moisture content in insect prey is critical for the reptiles consuming these diets because this is a primary source of fluids for them (Mitchell, 2006), and the naturally high moisture content of the dubia roaches (74.5% for nymphs and 71.3% for adults) ensures that sufficient moisture is available. An approximate 3% difference was found between the moisture contents of nymphal dubia roaches fed the high and low calcium diets, with all roaches having

Table 6. Differences of nutritional contents of adult dubia roaches gut-loaded on either a HC diet or a LC diet. Data presented on a percent dry matter basis. Normally distributed data are reported by the mean, SD, and min–max values, whereas non-normal data are reported by the median, 25–75%, and min–max values. Significant differences are noted by a $P < 0.05$.

Normal data

Parameter	Diet	Mean	SD	Min–Max	F	P
Moisture (%)	Control	71.3^{a}	0.8	70.0–71.9	665.5	<0.001
	HC	$63.8^{b,c}$	1.0	61.6–65.0		
	LC	72.0^{a}	0.9	70.7–73.5		
Dry matter (%)	Control	28.8^{a}	0.8	28.1–30.0	665.5	<0.001
	HC	$36.2^{b,c}$	1	35.0–38.4		
	LC	28^{a}	0.9	26.5–29.3		
Crude protein (%)	Control	72.9^{a}	3.6	70.2–80.0	46.1	<0.001
	HC	$62.8^{b,c}$	4.2	55.9–71.0		
	LC	72^{a}	3.9	65.1–79.0		
Ash (%)	Control	7.2^{a}	0.8	6.4–8.2	215.8	<0.001
	HC	$10.4^{b,c}$	2.1	7.5–13.1		
	LC	6.4^{a}	0.5	5.6–7.1		
Calcium (%)	Control	1.2^{a}	0.4	0.7–1.6	290.7	<0.001
	HC	$3.1^{b,c}$	0.8	1.8–4.1		
	LC	0.8^{a}	0.24	0.5–1.1		
Phosphorus (%)	Control	0.72^{c}	0.04	0.65–0.76	24.7	<0.001
	HC	0.71^{c}	0.03	0.67–0.78		
	LC	$0.65^{a,b}$	0.05	0.57–0.72		
Ca:P ratio	Control	1.6^{a}	0.6	0.8–2.3	280	<0.001
	HC	$4.3^{b,c}$	1	2.5–5.3		
	LC	1.2^{a}	0.3	0.8–1.8		
Magnesium (%)	Control	$0.16^{a,c}$	0.008	0.15–0.17	195.1	<0.001
	HC	$0.2^{b,c}$	0.03	0.16–0.24		
	LC	$0.14^{a,b}$	0.01	0.12–0.16		
Potassium (%)	Control	1.36	0.07	1.28–1.47	18.2	<0.001
	HC	1.41^{c}	0.09	1.18–1.44		
	LC	1.28^{a}	0.08	1.23–1.56		
Sodium (%)	Control	0.69^{a}	0.05	0.66–0.79	65.2	<0.001
	HC	$0.53^{b,c}$	0.04	0.47–0.61		
	LC	0.68^{a}	0.04	0.58–0.75		
Sulfur (%)	Control	0.53^{a}	0.03	0.49–0.57	126.4	<0.001
	HC	$0.42^{b,c}$	0.01	0.41–0.44		
	LC	0.57^{a}	0.05	0.49–0.65		
Iron (ppm)	Control	75.3^{a}	7.9	68–89	272.6	<0.001
	HC	$120.3^{b,c}$	22.5	90–151		
	LC	67.3^{a}	7.4	58–79		
Copper (ppm)	Control	15.3	0.8	14–16	15.8	<0.001
	HC	16^{c}	0.9	15–17		
	LC	14.4^{a}	1.2	13–16		

Non-normal data

Parameter	Diet	Median	SD	25–75%	F	P
Manganese (ppm)	Control	13^{a}	3.5	9.8–16.5	240.1	<0.001
	HC	$28^{b,c}$	8.3	22.3–37.0		
	LC	10.0^{a}	1.6	8.0–11.0		

HC, high calcium; LC, low calcium; SD, standard deviation; min–max, minimum–maximum values; ppm, parts per million.

[a] Values differ significantly from the value for the roaches fed the high calcium diet.

[b] Values differ significantly from the value for the control (fasted) roaches.

[c] Values differ significantly from the value for roaches fed the low calcium diet.

Table 7. Differences of nutritional contents of adult dubia roaches gut-loaded on either a HC or LC diet for 1 or 7 days. Data presented on a percent dry matter basis. Normally distributed data are reported by the mean, SD, and min–max values, whereas non-normal data are reported by the median, 25–75%, and min–max values. Significant differences are noted by $P < 0.05$.

Normal data

Parameter	Diet	Mean	SD	Min–Max	F	P
Ash (%)	Control	7.2^{a}	0.8	6.4–8.2	53.1	<0.001
	1D	$9.4^{b,c}$	3	5.9–13.1		
	7D	7.4^{a}	1.3	5.6–9.7		
Calcium (%)	Control	1.2^{a}	0.4	0.7–1.6	38.6	<0.001
	1D	$2.3^{b,c}$	1.5	0.6–4.1		
	7D	1.5^{a}	0.9	0.5–2.9		
Phosphorus (%)	Control	0.72^{c}	0.04	0.65–0.76	20.9	<0.001
	1D	0.71^{c}	0.04	0.61–0.78		
	7D	$0.65^{a,b}$	0.04	0.57–0.70		
Ca:P ratio	Control	1.6^{a}	0.6	0.9–2.3	27.9	<0.001
	1D	$3.2^{b,c}$	2	1.0–5.3		
	7D	2.3^{a}	1.3	0.8–4.2		
Magnesium (%)	Control	0.16^{a}	0.008	0.15–0.17	57.5	<0.001
	1D	$0.19^{b,c}$	0.04	0.13–0.24		
	7D	0.15^{a}	0.02	0.12–0.18		
Iron (ppm)	Control	75.3^{a}	7.9	68–89	49.86	<0.001
	1D	$105.1^{b,c}$	37.5	58–151		
	7D	82.4^{a}	20.1	59–114		

Non-normal data

Parameter	Diet	Median	SD	25–75%	F	P
Manganese (ppm)	Control	13^{a}	3.5	9.8–16.5	18.9	<0.001
	1D	$22^{b,c}$	14.3	11.0–37.0		
	7D	16^{a}	6.7	8.3–22.3		

HC, high calcium; LC, low calcium; SD, standard deviation; min–max, minimum–maximum values; ppm, parts per million; 1D, 1 day; 7D, 7 days.

[a] Values differ significantly from the value for the roaches gut-loaded for 1 day.

[b] Values differ significantly from the value for the control (fasted) roaches.

[c] Values differ significantly from the value for roaches gut-loaded for 7 days.

moisture content ≥71.5%. An 8.2% difference occurred between the moisture content of adult dubia roaches fed the high and low calcium diets, with all roaches having moisture contents ≥63.1%. Time did not affect the moisture content of adult dubia roaches.

The moisture and DM content of the diets did not appear to have a significant clinical effect on the roaches fed either diet. Although a significant difference was found in the percentage of CP (DM basis) measured in the nymphal dubia roaches (69.6% CP in roaches fed the high calcium diet compared with 74.1% CP in roaches the low calcium diet), the DM content of the CP for the high calcium diet (192.5 g/kg) and low calcium diet (187.1 g/kg) was opposite of their DM%. This is not surprising because the DM content was higher in the high calcium diet compared with the low calcium diet. Thus, the overall CP on a DM basis will be affected. This also occurred in the adult dubia roaches

Table 8. Differences of nutritional contents of adult dubia roaches gut-loaded for 1 or 7 days on either a HC or LC diet. Data presented on a percent dry matter basis. Normally distributed data are reported by the mean, SD, and min–max values, whereas non-normal data are reported by the median, 25–75%, and min–max values. Significant differences are noted by $P < 0.05$.

Normal data

Parameter	Diet	Time	Mean	SD	Min–Max	*F*	*P*
Moisture (%)	Control	0D	71.3	0.8	70.0–71.90	11	0.003
	HC	1D	64.5	0.7	63.2–65.0		
		7D	63.1	0.8	61.6–63.7		
	LC	1D	71.7	0.8	70.7–72.3		
		7D	72.3	1	70.8–73.5		
Dry matter (%)	Control	0D	28.8	0.8	28.1–30.0	11	0.003
	HC	1D	35.5	0.7	35.0–36.8		
		7D	36.9	0.8	36.3–38.4		
	LC	1D	28.3	0.6	27.7–29.3		
		7D	27.7	1	26.5–29.2		
Crude protein (%)	Control	0D	72.9	3.6	70.2–80.0	12.4	0.002
	HC	1D	59.6	2.4	55.9–63.2		
		7D	65.9	3	62.5–71.0		
	LC	1D	73.6	2.9	70.9–79.0		
		7D	70.4	4.4	65.1–77.4		
Ash (%)	Control	0D	7.2	0.8	6.4–8.2	42	<0.001
	HC	1D	12.2	0.7	11.1–13.1		
		7D	8.5	0.8	7.5–9.7		
	LC	1D	6.5	0.4	5.9–6.9		
		7D	6.3	0.5	5.6–7.1		
Calcium (%)	Control	0D	1.2	0.4	0.7–1.6	19.5	<0.001
	HC	1D	3.8	0.3	3.4–4.1		
		7D	2.4	0.4	1.8–2.9		
	LC	1D	0.9	0.2	0.6–1.1		
		7D	0.7	0.3	0.5–1.1		
Ca:P ratio	Control	0D	1.6	0.6	0.9–2.3	16	<0.001
	HC	1D	5.1	0.3	4.7–5.3		
		7D	3.4	0.6	2.5–4.2		
	LC	1D	1.3	0.2	1.0–1.6		
		7D	1.1	0.4	0.8–1.8		
Magnesium (%)	Control	0D	0.16	0.008	0.15–0.17	19.3	<0.001
	HC	1D	0.22	0.01	0.21–0.24		
		7D	0.17	0.008	0.16–0.18		
	LC	1D	0.15	0.01	0.13–0.16		
		7D	0.13	0.01	0.12–0.14		
Sulfur (%)	Control	0D	0.53	0.03	0.49–0.57	9	0.006
	HC	1D	0.43	0.008	0.42–0.44		
		7D	0.42	0.008	0.41–0.43		
	LC	1D	0.54	0.02	0.52–0.56		
		7D	0.6	0.06	0.49–0.65		
Iron (ppm)	Control	0D	75.3	7.9	68–89	29.7	<0.001
	HC	1D	140.3	7.4	132–151		
		7D	100.2	9.4	90–114		
	LC	1D	69.8	7.9	58–79		
		7D	64.7	6.4	59–76		

Non-normal data

Parameter	Diet	Time	Median	SD	25–75%	*F*	*P*
Manganese (ppm)	Control	0D	13	3.5	9.8–16.5	8	0.009
	HC	1D	36	4.4	33.5–41.5		
		7D	22.5	1.4	21.5–23.3		
	LC	1D	11	1.5	8.8–11.3		
		7D	8.5	1.8	8.0–11.3		

HC, high calcium; LC, low calcium; SD, standard deviation; min–max, minimum–maximum values; ppm, parts per million; 0D, 0 days; 1D, 1 day; 7D, 7 days.

Table 9. Nutritional analysis of high calcium gut-loaded adult dubia roaches after a 72 h fasting period. Data is presented on a percent dry matter basis. Normally distributed data are reported by the mean, SD, and min–max values. Significant differences are noted by $P < 0.05$.

Parameter	Time	Mean	SD	Min–Max	*F*	*P*
Moisture (%)	Control	66.1	0.7	65.3–66.6	3.071	0.091
	24 h fast	67.0	0.7	66.5–67.8		
	48 h fast	66.8	1.2	65.9–68.2		
	72 h fast	68.2	0.7	67.4–68.6		
Dry matter (%)	Control	33.9	0.7	33.4–34.7	3.071	0.091
	24 h fast	33.0	0.7	32.2–33.5		
	48 h fast	33.2	1.2	31.8–34.1		
	72 h fast	31.8	0.7	31.4–34.7		
Calcium (%)	Control	3.3	1.1	2.3–4.6	2.705	0.116
	24 h fast	2.3	0.2	2.1–2.6		
	48 h fast	2.1	0.2	1.9–2.2		
	72 h fast	2.1	0.4	1.7–2.3		
Phosphorus (%)	Control	0.67	0.04	0.64–0.71	1.672	0.249
	24 h fast	0.67	0.02	0.66–0.69		
	48 h fast	0.65	0.01	0.63–0.67		
	72 h fast	0.63	0.03	0.62–0.64		
Ca:P ratio	Control	4.9	1.5	3.5–6.4	3.054	0.092
	24 h fast	3.4	0.3	3.1–3.7		
	48 h fast	3.2	0.2	3.0–3.4		
	72 h fast	3.3	0.5	2.7–3.7		

SD, standard deviation; min–max, minimum–maximum values.

(CP: high calcium – 59.6% [211.1 g/kg], low calcium – 62.9% [178.2 g/kg]). Roaches fed all the diets had CP concentrations above the recommendations for CP for mice (180 g/kg) and growth in rats (150 g/kg; Benevenga *et al.*, 1995). All roaches (≥59.6 CP on DM% basis), regardless of the diet, also exceeded the CP recommendations for herbivorous (18–22% CP), omnivorous (20–25% CP), and carnivorous reptiles (30–50% CP; Nijboer, 2020). Note that Nutritional Resource Council recommendations are presented in g/kg or ppm on a DM basis (Benevenga *et al.*, 1995), whereas Merck Veterinary Manual recommendations are presented on a percent DM basis or ppm as this is how these sources referenced their data (Nijboer, 2020; Tables 12 and 13).

All insects (12.9–18.2% CF on DM basis) exceeded the recommendations for CF in omnivorous reptiles (3–6% CF on DM basis); no recommendations are available for carnivorous or herbivorous reptiles (Nijboer, 2020). However, all (36.5–47.4 g/kg) but the adult dubia roaches gut-loaded on the high calcium diet for at least 24 h (53.9 g/kg) fell short of the recommendations for mice and growth in rats (50 g/kg; Benevenga *et al.*, 1995). These values appear to fall short for some recommendations and exceed others, and it is necessary to recognize that the recommendations for reptiles are not referenced in the Merck Veterinary Manual and should be considered subjective (qualitative) rather than evidence based (quantitative; Nijboer, 2020).

Table 10. Historical comparison of the nutritional composition of nymphal dubia roaches on a percent dry matter basis. The diet fed in Pei Yee *et al.* (2018) comprised kitchen waste; the diet fed in Cerreta *et al.* (2021) comprised dog food and fish flakes. Significant differences are noted by $P < 0.05$.

Nutrient	Study	Mean	Fast	HC	LC
Moisture (%)	Barras		74.5	72.3	74.7
	Pei Yee	59.1	<0.001	<0.001	<0.001
	Cerreta	67.5	<0.001	<0.001	<0.001
Dry matter (%)	Barras		25.5	27.7	25.3
	Pei Yee	40.9	<0.001	<0.001	<0.001
	Cerreta	32.5	<0.001	<0.001	<0.001
Crude protein (%)	Barras		76.9[a]	69.6	74.1[a]
	Pei Yee	47.5	0.028	<0.001	0.028
Crude fat (%)	Barras		17.8[a]	17.1	17.8
	Pei Yee	44.2	0.027	<0.001	<0.001
	Cerreta	29.8	0.027	<0.001	<0.001
Ash (%)	Barras		5.9	7.5	5.5
	Pei Yee	2.5	<0.001	<0.001	<0.001
	Cerreta	5.6	0.072	0.003	0.406
Calcium (%)	Barras		0.13	1.25	0.16
	Cerreta	0.22	<0.001	<0.001	<0.001
Phosphorus (%)	Barras		0.79	0.83	0.81
	Cerreta	0.62	<0.001	<0.001	<0.001
Ca:P ratio	Barras		0.2[a]	1.5	0.2
	Cerreta	0.4	0.024	<0.001	<0.001

HC, high calcium diet; LC, low calcium diet.

[a] Non-normal data, single sample Wilcoxon signed rank test used instead of a single sample *t*-test.

When evaluating the nutrient quality, quantity, and overall diet for captive animals, it is valuable to also consider the energy expenditure of the animals. Captive reptiles and amphibians receive less exercise than their wild counterparts and likely put minimal effort into obtaining their prey (i.e., roaches in a cage would be analogous to "fish in a barrel"). Thus, a diet with a lower protein and fat content may benefit captive reptiles and amphibians by being less calorically dense and reducing the likelihood of obesity and other challenges to their health (Kirkwood, 1991; Kristensen *et al.*, 2022).

Significant differences were found in the calcium concentrations of the diets and the dubia roaches after being fed the respective diets. The high calcium diet (76.5 g/kg, 8.3% diet) had 478 times more calcium than the low calcium diet (0.16 g/kg, 0.17% diet) on a DM basis. Allen and Oftedal (1989) found that house cricket diets should include at least 8% calcium on a DM basis to ensure conversion to a positive Ca:P ratio. The findings in the present study were consistent with the recommendations for crickets because nymphal dubia roaches fed the high (8.3% calcium) and low calcium diets (0.17%) were found to have positive and inverse Ca:P ratios after 24 h of gut-loading, respectively. Adult dubia roaches fed the high calcium diet for 1 and 7 days also had positive Ca:P ratios (5.1 and 3.4, respectively), but adult roaches fed the low calcium diet had a positive Ca:P ratio after 24 h (1.3) and an inverse ratio after 7 days (0.8). It should be noted that the fasted adult dubia roaches had a Ca:P ratio of 1.6. This is contrary to what other research has found: that adult dubia roaches have a naturally inverse Ca:P ratio of 0.04 to 0.40 (Kulma *et al.*, 2016; Cerreta *et al.*, 2021). The authors suspect that the adult dubia roaches were positive as controls after 24 h because they were removed from a maintenance bin where some roaches may have been previously gut-loaded with the high calcium diet. Roaches collected from the maintenance bin were being used for a digestibility study for blue tongue skinks (*Tiliqua scincoides*; Barras *et al.*, 2024a). During the digestibility study, roaches were fasted for 24 h and then randomly assigned to two groups: gut-loaded for 24 h on a high calcium diet or low calcium diet. The roaches were offered to the skinks, but any roaches not eaten within a 24 h period were returned to the maintenance bin to be used later for the digestibility study. Prior studies conducted on insects indicate that a 24 h fasting period should be used for gut-emptying in cockroach nymphs (Finke, 2013); however, the results from this current study with adult dubia roaches maintaining a positive Ca:P ratio up to 72 h post–gut-loading suggests that this time period is not sufficient for adult dubia roaches. Additionally, the decline in the Ca:P ratio between days 1 and 7 in dubia roaches fed the low calcium diet indicates that the low calcium diet is insufficient at maintaining a positive Ca:P ratio.

Because we found that dubia roaches require longer than 24 h to completely empty their gut, the authors believed it necessary to evaluate how the fasting period can affect the nutritional composition of dubia roaches. It was expected that moisture would increase over time and that DM would decrease over the 72 h fasting period due to roaches passing feces. However, although this did occur, it was not statistically significant ($P = 0.091$ for each). Additionally, it was proposed that the percent calcium and Ca:P ratio would significantly decrease over time; however, at 72 h of fasting, no significant difference occurred (Ca, $P = 0.116$; Ca:P, 0.092), and roaches fed the high calcium diet maintained positive Ca:P ratio at 72 h (3.3 ± 0.5, min–max: 2.7–3.7). One possible reason that dubia roaches maintained this positive Ca:P ratio despite being fasted was because no moisture source was provided. Moisture was not provided so that their nutritional composition would not be altered if roaches ate the vehicle containing the moisture, such as a paper towel. Lack of moisture in the diet can lead to dehydration and constipation in humans (Casey, 2014). It is possible that lack of moisture in the roaches caused the gut to slow down or even stop, preventing calcium from being excreted. Another possibility is that fasting prevented roaches from receiving the stimulus to elicit gastrointestinal emptying. Rabbits need fiber to stimulate cecocolic motility via distension effect or by formation of volatile fatty acids that promote peristalsis (Oglesbee and Lord, 2020). Direct comparisons cannot be made between the two species, but roaches do have an alimentary canal that has its own microbiome that produces volatile fatty

Table 11. Historical comparison of the nutritional composition of adult dubia roaches on a percent dry matter basis. The diet fed in Pei Yee *et al.* (2018) comprised kitchen waste; the diet fed in Cerreta *et al.* (2021) comprised dog food and fish flakes. The diet fed in Oonincx *et al.* (2015) comprised several combinations of either a high protein, high fat, low protein, and/or low fat food source. The control diet was provided by Kreca VOF (Kreca Ento-Feed BV, Ermelo, Netherlands). The diet fed in Yi *et al.* (2013) comprised carrots and chicken mash. Significant differences are noted by $P < 0.05$.

Nutrient	Study	Sex/diet	Mean	Fast	HC	LC
Moisture (%)	Barras	—	—	71.3	64.6[a]	71.7
	Pei Yee	Male	62.7	<0.001	0.027	<0.001
		Female	61.5	<0.001	0.027	<0.001
	Cerreta	Adult	63.2	<0.001	0.042	<0.001
		Male	64.2	<0.001	0.344	<0.001
		Female	62.2	<0.001	0.027	<0.001
	Oonincx	HPHF	67.3	<0.001	0.027	<0.001
		HPLF	66.3	<0.001	0.027	<0.001
		LPHF	61.5	<0.001	0.027	<0.001
		LPLF	72.4	0.016	0.027	0.023
		Control	68.4	<0.001	0.027	<0.001
	Yi	Adult	67.4	<0.001	0.027	<0.001
Dry matter (%)	Barras	—	—	28.8	35.5[a]	28.3
	Pei Yee	Male	37.3	<0.001	0.027	<0.001
		Female	38.5	<0.001	0.027	<0.001
	Cerreta	Adult	36.8	<0.001	0.042	<0.001
		Male	35.8	<0.001	0.344	<0.001
		Female	37.8	<0.001	0.027	<0.001
	Oonincx	HPHF	32.7	<0.001	0.027	<0.001
		HPLF	33.7	<0.001	0.027	<0.001
		LPHF	38.5	<0.001	0.027	<0.001
		LPLF	27.6	0.016	0.027	0.023
		Control	31.6	<0.001	0.027	<0.001
	Yi	Adult	32.6	<0.001	0.027	<0.001
Crude protein (%)	Barras	—	—	72.9[a]	59.6	73.6
	Pei Yee	Male	54.3	0.027	0.003	<0.001
		Female	52.9	0.027	0.001	<0.001
	Oonincx	HPHF	60.7	0.027	0.314	<0.001
		HPLF	72.5	0.462	<0.001	0.403
		LPHF	37.5	0.027	<0.001	<0.001
		LPLF	53.9	0.027	0.002	<0.001
		Control	69.8	0.027	<0.001	0.024
	Yi	Adult	19.3	0.027	<0.001	<0.001
Crude fat (%)	Barras	—	—	15.6	15.2	15.3
	Pei Yee	Male	35.5	<0.001	<0.001	<0.001
		Female	35.5	<0.001	<0.001	<0.001
	Cerreta	Adult	13.3	0.007	0.032	0.039
		Male	14.6	0.115	0.388	0.364
		Female	12.1	0.001	0.005	0.007
	Yi	Adult	7.7	<0.001	<0.001	<0.001
Ash (%)	Barras	—	—	7.2	12.2	6.5
	Pei Yee	Male	4.2	<0.001	<0.001	<0.001
		Female	3.7	<0.001	<0.001	<0.001
	Cerreta	Adult	6.4	0.059	<0.001	0.599
		Male	6.3	0.04	<0.001	0.289
		Female	6.5	0.088	<0.001	0.953
	Yi	Adult	5.6	0.004	<0.001	0.003
Calcium (%)	Barras	—	—	1.16	3.75	0.92
	Cerreta	Adult	0.15	0.002	<0.001	<0.001
		Male	0.11	0.001	<0.001	<0.001
		Female	0.18	0.002	<0.001	<0.001
Phosphorus (%)	Barras	—	—	0.72	0.74	0.69*
	Cerreta	Adult	0.57	<0.001	<0.001	0.026
		Male	0.59	<0.001	<0.001	0.026
		Female	0.54	<0.001	<0.001	0.026
	Oonincx	HPHF	1.83	<0.001	<0.001	0.026
		HPLF	1.72	<0.001	<0.001	0.026
		LPHF	1.22	<0.001	<0.001	0.026
		LPLF	2.14	<0.001	<0.001	0.026
		Control	1.96	<0.001	<0.001	0.026
Ca:P Ratio	Barras	—	—	1.6	5.1	1.3
	Cerreta	Adult	0.3	0.002	<0.001	<0.001
		Male	0.2	0.002	<0.001	<0.001
		Female	0.3	0.002	<0.001	<0.001

HC, high calcium; LC, low calcium; HPHF, high protein high fat; HPLF, high protein low fat; LPHF, low protein high fat; LPLF, low protein low fat.

[a] Non-normal data, single sample Wilcoxon signed rank test used instead of a single sample *t*-test.

acids and secretes digestive enzymes similar to mammals (Banks, 1963; Tinker and Ottesen, 2016, 2021).

A final hypothesis is based on layman reports. The dubia roach has an especially long digestive tract and can hold digesta in their foregut for up to 3 days before it is excreted through the rest of the alimentary system (Dubia Roach Depot, 2023). If true, it is possible that the long length of the gut and the foregut's holding ability allowed for the roaches to maintain a stable Ca:P ratio despite being fasted for so long.

When comparing the Ca:P ratio of dubia roaches with previous studies, the results closely mimic the results found in percent calcium. This finding is unsurprising because the percentage of calcium changed much more than the percentage of phosphorus when dubia roaches were fed different gut-loading diets.

All calcium content dropped in adult roaches when they were gut-loaded for 7 days (high calcium 8.7 g/kg; low calcium 1.4 g/kg). Yet again, it is critical to note that fasted dubia roaches may have been previously exposed to the high calcium diet, and thus the 24 h fasting period was not able to accurately reflect true baseline in adult roaches. Previous research indicates that adult dubia roaches have a calcium content ≤0.7 g/kg (Kulma *et al.*, 2016; Cerreta *et al.*, 2021).

According to the Nutritional Research Council, the amount of calcium (g/kg) required in the diet of rats for growth and mice for maintenance is 5 g/kg (Benevenga *et al.*, 1995). Although these recommendations are for mammals, they are generally used as a guide for reptiles and amphibians because of the limited evidence available for these species (Finke, 2003). However, a qualitative reference (not tied to

Table 12. Nutritional contents of dubia roaches compared to the nutritional recommendations from the Nutritional Research Council in the diets of rodents on a dry matter basis (Benevenga *et al.*, 1995). Data presented as mean in g/kg or ppm.

	Dry matter basis (g/kg)												
	Crude protein	Crude fat	Calcium	Phosphorus	Ca:P ratio	Magnesium	Potassium	Sodium	Iron ppm	Zinc ppm	Copper ppm	Manganese ppm	Molybdenum ppm
Phase one: diet comparison of nymphs													
Baseline (24 h fast)	196.4	45.4	0.3	2.0	0.2	0.47	3.9	1.7	61	243	9	11	1.6
Control (48 h fast)	194.6	46.2	0.3	2.3	0.1	0.47	4.2	1.8	63	260	13	10	1.6
HC	192.5	47.4	3.5	2.3	1.5	0.58	4.1	1.7	88	233	10	19	1.7
LC	187.1	44.9	0.4	2.0	0.2	0.44	3.8	1.6	59	238	10	9	1.7
Phase two: adults left on the gut-loading diet													
Fast	209.6	44.8	3.3	2.1	1.6	0.46	3.9	2.0	75	231	15	13	1.2
HC 24H	211.2	53.9	13.3	2.6	5.1	0.79	4.7	1.9	140	177	16	37	1.4
LC 24H	178.2	43.3	2.6	1.9	1.3	0.42	4.0	2.0	70	229	15	10	1.0
HC 7D	243.4	59.9	8.7	2.5	3.4	0.64	4.5	2.0	100	210	16	22	1.1
LC 7D	194.9	46.8	1.4	1.7	0.8	0.37	3.9	1.9	65	208	14	9	0.8
Nutritional Research Council guidelines for rodents													
Rat maintenance	50.0	50.0	~	~	~	~	~	~	~	~	~	~	~
Rat growth	150.0	50.0	5.0	3.0	1.7	0.45	3.6	0.50	35	12	5	10	0.15
Mouse maintenance	180.0	50.0	5.0	3.0	1.7	0.45	2.0	0.50	35	10	6	10	0.15

ppm, parts per million; HC, high calcium; LC, low calcium; HC 24H, high calcium diet for 24 h; LC 24H, high calcium diet for 24 h; HC 7D, high calcium diet for 7 days; LC 7D, low calcium diet for 7 days.

a peer-reviewed, evidence-based reference) in the Merck Veterinary Manual suggests that reptiles should be provided 0.8–1.1% calcium (DM basis) for carnivorous reptiles, 1–1.5% for omnivorous reptiles, and 1.4–2% for herbivorous reptiles (Nijboer, 2020).

The results from the current study suggest that all nymphal dubia roaches and adult dubia roaches fed the low calcium diet do not satisfy the needs of mice or rats, whereas adult dubia roaches fed the high calcium diet do (1 day, 13.3 g/kg; 7 days, 8.7 g/kg). Nymphal dubia roaches fed the high calcium diet (1.2% Ca on DM basis) satisfy the needs of carnivorous and omnivorous reptiles (Nijboer, 2020). Additionally, adult dubia roaches fed the high calcium diet for 1 day (3.8% Ca) or 7 days (2.4% Ca) satisfy and exceed the reported needs of carnivorous, omnivorous, and herbivorous reptiles, whereas adult dubia roaches fed the low calcium diet for 1 day (0.9% Ca) and 7 days (0.5% Ca) do not (Nijboer, 2020). However, this would suggest

Table 13. Nutritional contents of dubia roaches compared with the nutritional recommendations for the diets of reptiles in the Merck Veterinary Manual (Nijboer, 2020). Values for dubia roaches are presented as the mean on a percent dry matter basis.

	Percent dry matter basis											
	Crude protein	Crude fat	Calcium	Phosphorus	Ca:P ratio	Magnesium	Potassium	Sodium	Iron ppm	Zinc ppm	Copper ppm	Manganese ppm
Phase one: diet comparison of nymphs												
Baseline (24 h fast)	76.9	17.8	0.1	0.8	0.2	0.2	1.5	0.7	61	243	9	11
Control (48 h fast)	76.7	18.2	0.1	0.9	0.1	0.2	1.6	0.7	63	260	13	10
HC	69.6	17.1	1.2	0.8	1.5	0.2	1.5	0.6	88	233	10	19
LC	74.1	17.8	0.2	0.8	0.2	0.2	1.5	0.6	59	238	10	9
Phase two: adults left on the gut-loading diet												
Fast	72.9	15.6	1.2	0.7	1.6	0.2	1.4	0.7	75	231	15	13
HC 24H	59.6	15.2	3.8	0.7	5.1	0.2	1.3	0.5	140	177	16	37
LC 24H	62.9	15.3	0.9	0.7	1.3	0.1	1.4	0.7	70	229	15	10
HC 7D	65.9	16.2	2.4	0.7	3.4	0.2	1.2	0.5	100	210	16	22
LC 7D	70.4	16.9	0.5	0.6	0.8	0.1	1.4	0.7	65	208	14	9
Merck Veterinary Manual												
Carnivorous reptiles	30 to 50	~	0.8 to 1.1	0.5 to 0.9	1.2 to 1.6	0.04	0.4 to 0.6	0.2	60 to 80	50	5 to 8	5
Omnivorous reptiles	20 to 25	3 to 6	1 to 1.5	0.6 to 0.9	1.6 to 1.7	0.2	0.4 to 0.6	0.2	200	130	15	150
Herbivorous reptiles	18 to 22	3 to 6	1.4 to 2	0.8 to 1	1.7 to 2	~	~	~	~	~	~	~

HC, high calcium; LC, low calcium; HC 24H, high calcium diet for 24 h; LC 24H, high calcium diet for 24 h; HC 7D, high calcium diet for 7 days; LC 7D, low calcium diet for 7 days.

that the digestibility of nutrients is static. In a recent study by Boykin *et al.* (2020a), corn snakes (*Pantherophis guttatus*) in a complete cross-over study were found to digest calcium differently based on the concentration of the mineral in the diet. The calcium content in the black soldier larvae offered to the snakes was 17.7 g/kg, and the neonatal and juvenile mice offered over the study were 15.1 g/kg, and 19.5 g/kg, respectively. During the first half of the study, calcium digestibility was higher in the snakes fed the lower calcium content neonatal mice (85.1%) compared with the black soldier fly larvae (63.2%), but calcium digestibility reversed in the second half of the study when the snakes were offered the lower calcium content black soldier fly larvae (71.3%) compared with the higher calcium juvenile mice (62.5%; Boykin *et al.*, 2020a). These results suggest that the reptile can alter the digestibility of the nutrients based on nutrient concentration; thus, we might expect a higher rate of digestibility for reptiles or amphibians fed diets with lower calcium content (i.e., gut-loaded dubia roaches). The authors are currently testing this theory in blue-tongued skinks (*Tiliqua scincoides*) in a complete cross-over study evaluating the digestibility of dubia roaches fed the low and high calcium diets outlined in the current study, and the results are similar (Barras *et al.*, 2024a).

Phosphorus is a vital nutrient because of its roles in the formation of bones, DNA, cell membranes, and energy production and storage (Morris and Mohiuddin, 2023). Although statistical significance was found in the phosphorus concentrations of dubia roaches based on diet fed or how long the diet was offered, little clinical significance was found. All concentrations were lower than the recommendations for mice and rats (3 mg/kg; Benevenga *et al.*, 1995), but they all (0.6–0.8% phosphorus on DM basis) were able to satisfy the recommendations for reptiles (0.5–1.0% phosphorus; Nijboer, 2020). Caution should be taken with the recommendations for mammals because the needs for mammals and reptiles are different (Finke, 2003). Additionally, many insectivorous diets have calcium concentrations that are lower than the phosphorus concentrations; the nymphal dubia roaches fed the low calcium diet are a good example. Reptiles and amphibians fed these types of diets are more susceptible to nutritional secondary hyperparathyroidism (Mans and Braun, 2014). The authors believe it is more valuable to feed these animals a diet with a positive Ca:P ratio than the previously noted phosphorus concentrations. This is further evident in the recommendations for mice, rats, and reptiles for diets to have a positive Ca:P ratio (Benevenga *et al.*, 1995; Nijboer, 2020).

In general, iron concentrations of roaches were significantly higher in roaches fed the high calcium diet. All concentrations of iron exceed the current recommendations for mice and growth in rats (35 ppm), as well as carnivorous reptiles (60–80 ppm); however, these concentrations were considered inadequate for omnivorous reptiles (200 ppm; Benevenga *et al.*, 1995; Nijboer, 2020). Because the recommended values for reptiles are subjective, it is currently not possible to confirm whether these recommendations are appropriate. It is interesting to note that the recommendation for carnivorous reptiles is lower than that for omnivores because carnivores would be more likely to encounter this mineral in prey species. Iron is a key nutrient because it allows hemoglobin to function as an oxygen carrier and is essential for collagen production and vitamin D metabolism (Underwood, 1977; Balogh *et al.*, 2018). Iron deficiency in reptiles can lead to ferropenic anemia (Saggese, 2009); in rats, iron deficiency can impair running ability (Finch *et al.*, 1976). Additionally, low concentrations of iron are a risk factor in humans for the development of osteoporosis (Zheng *et al.*, 2014).

Manganese concentrations were also higher in both the nymphal and adult roaches fed the high calcium diet compared with the low calcium diet and dropped significantly over time in adult dubia roaches. Similar to iron, the recommendations for manganese in the diets of mice and rats (10 ppm), as well as carnivorous reptiles (5 ppm), are much lower than for omnivorous reptiles (150 ppm; Benevenga *et al.*, 1995; Nijboer, 2020). Likewise, the dubia roaches fed the high and low calcium diets had manganese concentrations that were similar to the recommendations for the former but not the latter. Again, the wide range between these recommendations makes it difficult to draw any conclusions or make any recommendations. Manganese plays valuable roles in immune function, reproduction, digestion, and bone growth (Aschner and Erikson, 2017). Animals with manganese deficiency may present with skeletal abnormalities, impaired reproductive performance, and improper lipid, carbohydrate, and protein metabolism (Keen *et al.*, 2000).

The amount of copper measured in dubia roaches (9–16 ppm) was considered high for mice (6 ppm) and growing rats (5 ppm) regardless of age, diet fed, or time fed (Benevenga *et al.*, 1995). The amount of copper in nymph and adult dubia roaches was considered high for carnivorous reptiles (5–8 ppm) regardless of diet fed or time fed. Only adult dubia roaches that were fasted or fed the low calcium diet for 24 h had a copper content considered appropriate for omnivorous reptiles (15 ppm; Nijboer, 2020). Copper is essential for hemoglobin synthesis, collagen synthesis, and cross linking of organic bone matrix (Watts, 1989). In human infants, copper deficiency can lead to pancytopenia and osteopenia; in adult humans, copper deficiency can result in anemia, changes in ossification, and, in rare cases, seizures (Zatta and Frank, 2007). Copper deficiency is also associated with bone fragility in animals (Jonas *et al.*, 1993).

Magnesium concentration of roaches fed the high calcium diet, regardless of age or time gut-loaded (0.6–0.8 g/kg), exceeded the recommendation for mice and rats (0.5 g/kg), whereas all roaches fed the low calcium diet (all = 0.04 g/kg) fell short of this recommendation for mice and rats (Benevenga *et al.*, 1995). All roaches (Mg = 0.1 to 0.2% on DM basis), regardless of age, diet fed, or time fed, exceeded the recommendations for carnivorous reptiles (0.04%) but were sufficient for omnivorous reptiles (0.2%), with the exception of adult dubia roaches gut-loaded on the low calcium diet (Mg = 0.1%; Nijboer, 2020). Magnesium plays a key role in the metabolism of calcium (Donoghue and McKeown, 1999) because it stimulates calcitonin to inhibit osteoclastic activity and increase renal clearance of calcium and phosphorus (Austin and Heath, 1981). Additionally, magnesium is essential in vitamin D activation, which in turn is vital for calcium absorption from the intestine (Dai *et al.*, 2018; Uwitonze and Razzaque, 2018). Because of the integral role of magnesium in calcium metabolism, low magnesium concentrations in the diet can lead to hypocalcemia and nutritional secondary hyperparathyroidism (Rude and Singer, 1981). Future studies should be conducted to evaluate the role of magnesium in gut-loading diets and to determine best practices for this mineral.

No significant differences were found in zinc concentrations of all roaches based on diet fed or time (177–238 ppm). All roaches exceeded the zinc recommendations for mice (10 ppm), growth in rats (12 ppm), and carnivorous and omnivorous reptiles (50 and 130 ppm, respectively) (Benevenga *et al.*, 1995; Nijboer, 2020). Zinc is a heavy metal that plays valuable roles in the structure of proteins and enzymes, as well as bone formation and mineralization (Yamaguchi, 1998; Morris and Mohiuddin, 2023). Zinc toxicosis is a potential concern in reptiles because it is a heavy metal that has been associated with intravascular hemolysis and anemia (Fitzgerald and Newquist, 2008). Zinc toxicosis is generally attributed to high dietary concentrations or ingestion of galvanized metal (Fitzgerald and Newquist, 2008). Future research should focus on determining appropriate dietary concentrations of this critical, but potentially deadly, metal.

No significant differences were found in molybdenum concentration of all roaches based on age, diet fed, or time fed (0.8–1.7 ppm). All roaches had molybdenum concentration that were higher than the recommendation for rodents (0.15 ppm; Benevenga *et al.*, 1995). There are no published molybdenum recommendations for the diet of reptiles. The role of molybdenum in animal nutrition is poorly understood, although it is known to influence copper metabolism (Marston, 1952). In laboratory animals, excretion of molybdenum is rapid and primarily via urine. Excessive molybdenum can inhibit intestinal absorption of iron and copper. The lethal dose for repeated oral administration of molybdenum in rodents is 60–333 ppm/day, which is far above the concentrations measured in the roaches (Vyskočil and Viau, 1999). Because of the interaction of this nutrient with other minerals, it is essential for future research to determine appropriate recommendations for molybdenum in reptile diets.

Potassium (K) concentrations were significantly different only in adult dubia roaches based on diet fed not on time and did not differ significantly for nymph roaches. All roaches exceeded the recommendations for mice (2.0 g/kg), growth in rats (3.6 g/kg), and reptiles (0.4–0.6% K on DM basis; all dubia roaches: 1.2–1.6% K; Benevenga *et al.*, 1995; Nijboer, 2020). Potassium plays key roles in maintaining cell membrane potential and transmission of action potentials in nerve cells, regulating blood pH, and water balance (Mitchell, 2009; Morris and Mohiuddin, 2023). Likewise, sodium concentrations for nymphs (high calcium: 1.7 g/kg; low calcium: 1.5 g/kg) and adults (all diets and times between 1.9–2.0 g/kg) were higher than recommendations for mice, rats (0.5 g/kg), and reptiles (0.2% Na on DM basis; all dubia roaches: 0.5–0.7% K; Benevenga *et al.*, 1995; Nijboer, 2020). Sodium is the primary electrolyte associated with extracellular fluid osmolality in vertebrates; it plays key roles in nerve innervation and muscle contraction (Morris and Mohiuddin, 2023; Shrimanker and Bhattarai, 2023). Potassium and sodium concentrations are regulated by the kidneys and the salt glands (Minnich, 1972; Palmer, 2015). Salt glands are typically associated with the nares of reptiles, if present (Shoemaker and Nagy, 1977). Excess concentrations of sodium or potassium can be excreted by these glands when plasma osmotic concentrations are too high (Barten, 2006). Because of the roles of these electrolytes in cellular function, it is essential that we develop a better understanding of the sodium and potassium dietary requirements for reptiles.

The amount of sulfur measured in the dubia roaches cannot be compared with the dietary needs of mice, rats, or reptiles because no references for these species are available. No significant differences were found in all roaches based on diet fed, but time did have a significant effect on sulfur concentration in adult dubia roaches. Sulfur is key as a substrate for amino acids, proteins, enzymes, and vitamins; thus, it is critical to determine appropriate recommendations for dietary sulfur for reptiles (Komarnisky *et al.*, 2003).

Conclusions

Overall, the high and low calcium diets offered to the dubia roaches in these studies did not alter the overall nutritional value of these insects, except for calcium and iron in both nymphs and adults and CP in adults. Additionally, low mortality in all roaches regardless of the diet fed or time spent on the diet demonstrate that both diets can be used in dubia roaches without causing overt mortality. The results of this study also found that roaches really “are what they eat,” and comparisons to historic data confirmed that the diet fed to dubia roaches can significantly alter the nutritional composition of these insects. This suggests that it may be possible to tailor the nutrition of a reptile by what is gut-loaded into a dubia roach. Gut-loading diets for invertebrates fed to reptiles and amphibians are primarily focused on increasing the calcium content and thus the Ca:P ratio of the prey items. Based on the results from this study, this appears to have been successful for the dubia roaches fed the high calcium diet; the low calcium diet did not correct the natural inverse Ca:P ratio for this species. It is worthwhile to validate the claims of commercial gut-loading diets because they are not strictly regulated.

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