COMPARISON OF TWO GUT-LOADING REGIMENS ON NUTRIENT CONTENT OF FEEDER CRICKETS (*GRYLLODES SIGILLATUS*)

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Abstract

Captively-managed amphibian species are commonly fed diets consisting primarily of captive-raised crickets (*Acheta domestica, Gryllodes sigillatus*) due to the multi-faceted challenges of providing diverse diets on a consistent basis in managed settings (Livingston *et al*., 2014). Since crickets are known to be poor sources of key nutrients to amphibian health (Ferrie *et al*., 2014), feeder crickets typically are supplemented through the practices of gut-loading and dusting with vitamin/mineral supplements to enhance the nutrient content of these items to prevent dietary deficiencies that can produce health problems such as metabolic bone disease and hypovitaminosis A (Ferrie *et al*., 2014; Pessier, 2013). Gut-loading regimens for feeder crickets vary widely across institutions, and many studies have evaluated particular regimens for their effects on resulting nutrient content (Livingston *et al*., 2014).

Based on Iske's (2018) findings suggesting specific gut-loading diets can result in significant levels of calcium, vitamin A, and carotenoids, the Fort Worth Zoo decided to test Iske's successful diets in a new regimen. The new regimen will ultimately be assessed by review of reproductive, health, and pathology records for Puerto Rican crested toads (*Peltophyrne lemur*) and Chiricahua leopard frogs (*Lithobates chiricahuensis*).

Banded crickets (*Gryllodes sigillatus*), ½-inch size, were obtained from Phat Jack's Farms (Grand Prairie, TX). Crickets were maintained on one of two diets: Fort Worth Zoo standard gut-loading diet of Zeigler Hi-Cal Cricket Monster Diet (Zeigler Bros, Inc., Gardners, PA 17324), available *ad lib* daily along with fresh water (WaterGel crystals, The Don Carr Company, San Antonio, TX), and an experimental alternating diet of Repashy Superfoods SuperLoad insect gut-load formula (Repashy Ventures, Inc., Oceanside, CA 92056; fed 4 days a week) and a diced produce mix (33% each raw carrot, sweet potato, and kale, diced to ½" by ½" size; fed 3 days a week). Crickets were gut-loaded for two days after arrival before starting to feed out. Samples for nutrient content analysis were taken after 2-3 days on each diet and again at 6-8 days, representing the earliest and latest days they would typically be fed out. Additionally, samples were taken at each time point and dusted with a 50/50 mix of Nekton-Rep (Nekton GmbH, Keltern, Germany) and Spectrum Ca-130 calcium carbonate (Spectrum Chemical Mfg Corp., New Brunswick, NJ 08901).

Gut-loading diets (Zeigler, Repashy Superload, and produce mixture) and Nekton-Rep/CaCO3 dust were each sampled for analysis. Samples were sent to Midwest Laboratories (Omaha, NE) to determine gross energy, proximate (crude protein, crude fat, crude fiber, ash, moisture), and mineral (calcium, phosphorus, magnesium, sodium, potassium, iron, zinc, copper, manganese, and selenium) concentrations according to AOAC (proximates and minerals) and ASTM (gross energy) methods (AOAC, 1996; ASTM, 2013). Samples also were extracted according to methods previously described (Stacewicz-Sapuntzakis *et al*., 1987; Coslik *et al*., 2009) and analyzed for

retinol and alpha-tocopherol using a Waters™ 2695® separations module HPLC, with a Grace/Vydac 201TP54® column and a Waters™ 2487® dual wavelength absorbance detector.

The results of nutrient analysis (Table 1) suggest neither gut-loading regimen consistently produced superior levels of any nutrient analyzed, and values for specific nutrients varied based on duration of total gut-loading. Dusted samples (Table 2) also did not show consistent trends for all nutrients between the diets. In general, except for the Ca:P ratio of most of the undusted crickets, all values fell within suggested nutrient targets for amphibians (Ferrie *et al*., 2014). Analysis of the gut-loading diets and dust are provided in Table 3. The samples from this study most comparable to those reported by Iske (2018) show much lower Ca:P ratios, but much higher vitamin A content. In comparison to a larger review (Livingston *et al*., 2014), the Ca:P ratios from samples in this study fall within reported ranges, while vitamin A content falls within or above reported values. These differences could be due to differences in gut-loading regimens, gut loading and dusting products, length of time gut loading, vitamin A analysis methodology, and dust adherence.

While neither gut-loading regimen appears clearly superior based on the resulting nutrient content of crickets, the crickets fed the rotating diet were perceived to live longer than those fed only the Zeigler diet. Carotenoids were not measured in this study but are expected to be different between regimens due to their presence in the produce diet and may have possible health benefits.

Literature Cited

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Table 1. Micronutrient, mineral and vitamin A and E content (dry-matter basis) of un-dusted ½" banded crickets (*G. sigillatus*) before and after gut-loading with Zeigler Monster Diet Hi-Cal and alternation of Repashy SuperLoad and mixed produce diets at Fort Worth Zoo.

Nutrient	**Unit**	**B**	**Z2[3]**	**Z8**	**R/P2[4,5]**	**R/P3**	**R/P6**	**R/P8**
Moisture	%	73.8	74.4	73.6	74.5	75.3	74.5	76.6
Crude Protein	%	67.3	67.6	69.7	72.2	74.5	75.4	71.8
Crude Fiber	%	7.2	7.8	7.2	7.3	8.4	9.1	9.3
Crude Fat	%	19.8	18.8	15.3	18.2	16.8	13.6	12.3
Ash	%	4.3	5.8	6.6	5.6	5.5	6.4	5.8
Ca:P	ratio	0.16	0.85	1.25	0.63	0.41	0.96	0.64
Calcium	%	0.15	0.9	1.33	0.67	0.45	1.07	0.68
Phosphorus	%	0.97	1.05	1.06	1.06	1.09	1.11	1.07
Magnesium	%	0.09	0.12	0.11	0.12	0.08	0.12	0.13
Potassium	%	1.23	1.25	1.29	1.29	1.3	1.39	1.41
Sodium	%	0.38	0.43	0.42	0.43	0.45	0.45	0.43
Iron	ppm	71.8	134.8	168.9	84.3	64.0	74.6	72.2
Zinc	ppm	165.9	192.6	185.6	188.2	172.9	181.1	180.8
Copper	ppm	38.4	49.2	51.1	44.7	42.9	51.2	46.6
Manganese	ppm	34.2	53.9	71.6	34.9	124.7	34.8	39.7
Selenium	ppm	0.66	0.59	0.57	0.78	0.53	0.66	0.56
Vitamin A[1]	IU/kg	4214	15103	2146	12025	20376	60376	20938
Vitamin E[2]	ppm	84.2	344.9	271.1	279.6	382.8	197.4	278.5

B=baseline sample of crickets collected on arrival (sub-sampled over 5 weeks); Z2=crickets gut-loaded with Zeigler diet for 2 days; Z8=crickets gut-loaded with Zeigler diet for 8 days; R/P2=crickets gut-loaded for total of 2 days, last fed Repashy SuperLoad diet; R/P3=crickets gut-loaded for total of 3 days, last fed produce mix diet; R/P6=crickets gut-loaded for total of 6 days, last fed Repashy SuperLoad diet; R/P8=crickets gut-loaded for total of 8 days, last fed produce mix diet.

[1]Vitamin A as retinol

[2]Vitamin E as alpha-tocopherol

[3]Zeigler Monster Diet Hi-Cal Cricket (Zeigler Bros, Inc., Gardners, PA 17324)

[4]Repashy SuperLoad (Repashy Ventures, Inc., Oceanside, CA 92056)

[5]Produce mix: 33% each diced raw carrot, sweet potato, and kale

Table 2. Micronutrient, mineral, and vitamin A and E content (dry-matter basis) of dusted[1] ½" banded crickets (*G. sigillatus*) before and after gut-loading with Zeigler Monster Diet Hi-Cal and alternation of Repashy SuperLoad and mixed produce diets at Fort Worth Zoo.

Nutrient	Unit	B	Z2[4]	Z8	R/P2[5,6]	R/P3	R/P6	R/P8
Moisture	%	73.8	69.7	73.2	71.5	73.7	71.4	75.3
Crude Protein	%	67.3	64.7	69.4	66.0	70.6	73.8	70.0
Crude Fiber	%	7.2	7.4	5.7	8.0	7.9	7.5	8.7
Crude Fat	%	19.8	16.6	15.9	16.6	19.0	13.9	12.6
Ash	%	4.3	10.7	9.9	9.6	9.1	92	9.2
Ca:P	ratio	0.16	4.43	3.18	2.96	2.66	2.93	2.79
Calcium	%	0.15	4.09	3.32	2.91	2.69	3.00	2.71
Phosphorus	%	0.97	0.92	1.04	0.98	1.01	1.03	0.97
Magnesium	%	0.09	0.13	0.15	0.11	0.10	0.11	0.12
Potassium	%	1.23	1.16	1.27	1.19	1.22	1.33	1.30
Sodium	%	0.38	0.36	0.41	0.39	0.42	0.42	0.45
Iron	ppm	71.8	188.1	208.6	110.2	105.9	108.7	101.6
Zinc	ppm	165.9	196.0	198.1	184.2	180.8	187.5	172.9
Copper	ppm	38.4	45.5	53.7	42.8	41.3	48.7	42.1
Manganese	ppm	34.2	100.3	98.5	62.1	61.5	69.2	59.1
Selenium	ppm	0.66	nm[7]	nm	0.81	0.59	0.61	0.53
Vitamin A[2]	IU/kg	4214	134200	123619	127636	149868	77774	75903
Vitamin E[3]	ppm	84.2	322.4	336.1	330.3	344.4	239.5	231.8

B=baseline sample of crickets collected on arrival (sub-sampled over 5 weeks); Z2=crickets gut-loaded with Zeigler diet for 2 days; Z8=crickets gut-loaded with Zeigler diet for 8 days; R/P2=[c]rickets gut-loaded for total of 2 days, last fed Repashy SuperLoad diet; R/P3=crickets gut-loaded for total of 3 days, last fed produce mix diet; R/P6=crickets gut-loaded for total of 6 days, last fed Repashy SuperLoad diet; R/P8=crickets gut-loaded for total of 8 days, last fed produce mix diet.

[1]Composed of 50% NektonRep (Nekton GmbH, Keltern, Germany) and 50% Spectrum CA-130 calcium carbonate (Spectrum Chemical Mfg Corp., New Brunswick, NJ 08901)

[2]Vitamin A as retinol

[3]Vitamin E as alpha-tocopherol

[4]Zeigler Monster Diet Hi-Cal Cricket (Zeigler Bros, Inc., Gardners, PA 17324)

[5]Repashy SuperLoad (Repashy Ventures, Inc., Oceanside, CA 92056)

[6]Produce mix: 33% each diced raw carrot, sweet potato, and kale

[7]nm, not measured due to insufficient sample

Table 3. Micronutrient, mineral and vitamin A and E content (dry-matter basis) of gut-loading diets and supplement mix used in treatment groups.

Nutrient	Unit	Zeigler Monster Diet[3]	Repashy SuperLoad[4]	Produce Mix[5]	50/50 NektonRep/CaCO3 dust[6]
Moisture	%	9.0	6.54	85.71	5.72
Crude Protein	%	23.2	19.9	15.9	nm[8]
Crude Fiber	%	5.2	4.9	4.9	nm[8]
Crude Fat	%	5.7	5.5	2.1	nm[8]
Ash	%	22.6	24.6	9.0	nm[8]
Ca:P ratio		8.8	14.8	3.8	352.9
Calcium	%	9.56	9.44	1.05	24.7
Phosphorus	%	1.09	0.64	0.28	0.07
Magnesium	%	0.43	0.31	0.28	0.17
Potassium	%	1.16	1.17	2.73	nd[7]
Sodium	%	0.24	0.26	0.49	0.14
Iron	ppm	864.0	111.0	86.1	1660
Zinc	ppm	238.0	38.8	21.0	670
Copper	ppm	37.2	6.1	10.5	145
Manganese	ppm	348.0	70.4	53.9	825
Selenium	ppm	0.32	nd[7]	nd[7]	nd[7]
Vitamin A[1]	IU/kg	41533.5	27887.9	6997.4	4326784
Vitamin E[2]	ppm	326.0	335.1	32.2	7332.7

[1]Vitamin A as retinol

[2]Vitamin E as alpha-tocopherol

[3]Zeigler Monster Diet Hi-Cal Cricket (Zeigler Bros, Inc., Gardners, PA 17324)

[4]Repashy SuperLoad (Repashy Ventures, Inc., Oceanside, CA 92056)

[5]Produce mix: 33% each diced raw carrot, sweet potato, and kale

[6]Composed of 50% NektonRep (Nekton GmbH, Keltern, Germany) and 50% Spectrum CA-130 calcium carbonate (Spectrum Chemical Mfg Corp., New Brunswick, NJ 08901)

[7]nd, not detected by analysis

[8]nm, not measured due to insufficient sample