Hypovitaminosis A: Are captive-bred insect prey deficient in usable Vitamin A for *Anaxyrus* (Anura: Bufonidae)?

R. Andrew Odum, Toledo Zoological Society[1]
Kent Bekker, Toledo Zoological Society
Patrick Turski, Toledo Zoological Society
John Chastain, Toledo Zoological Society
Jennifer Pramuk, Woodland Park Zoo
Allan Pessier, San Diego Zoo
Ric Berlinski, Toledo Zoological Society
Chris Hanley, Toledo Zoological Society
Val Hornyak, Toledo Zoological Society
Timothy Herman, Toledo Zoological Society
Joseph R. Mendelson III, Zoo Atlanta

ABSTRACT:

Hypovitaminosis-A has been identified as a significant medical issue for the captive Wyoming toads (*Anaxyrus baxteri*) and other bufonid taxa (Pessier et al., 2002; Pessier et al., 2005). Low Vitamin A levels have been associated with lingual squamous metaplasia, first recognizable in Wyoming toads as "short tongue syndrome" because of an apparent difficulty that some toads had in catching insect prey. Hypovitaminosis-A has also been associated with other abnormalities in amphibians including poor vision, poor skin quality, and reproductive failure (Wright and Toddes, 2005). This study focused on testing three diets to treat hypovitaminosis A in a common species of toad.

We investigated the concentration of liver retinols in American toads (*Anaxyrus americanus*) raised on three different diets: (1) domestic crickets (*Acheta domesticus*) fed a 8–10 % calcium (Marion Zoological® Orthocal™ Insect Supplement) gut-loading diet as a control; (2) crickets maintained on the same high calcium diet and then gut-loaded with the complex enhanced diet (CED) described by Li et al. (2009) (see formulation below); and (3) crickets maintained on a high β-carotene diet consisting of equal parts by mass of kale, sweet potato, and carrots. Study animals were collected on 28 May 2010 as larvae from a single site in Lucas County, Ohio, and then were reared together in the laboratory through metamorphosis. On 15 June 2010, 15 randomly selected toadlets were transferred into each of six identical glass aquaria (61cm x 30.5cm x 30.5cm) for the three experimental groups with two replicates. On 7 August, an additional regime of dusting crickets with Tetrafauna Reptocal® vitamins (Tetraholdings [US], Inc.; Blacksburg, VA) was added to all feedings for all three groups to address what appeared to be calcium metabolic issues in the high β-carotene group. After approximately seven months, all animals were euthanized using MS-222 and their livers excised for retinol analysis.

Approximately two grams of each sample were homogenized with a 2% pyrogallol methanol solution and then alloquoted at one and a half grams each into three test tubes. Samples were saponified in a 60% v/v potassium hydroxide solution and a 70ºC water bath for 60 minutes. Following saponification, samples were extracted according to methods previously described (Stacewicz-Sapuntzakis et al., 1987) and analyzed for retinol, using a Waters™ 2695® separations module HPLC, with a Grace/Vydac 201TP54® column and a

[1] Toledo Zoological Society, 1 Hippo Way, Toledo, OH 43614 419-385-5721 (Andrew.Odum@toledozoo.org)

Waters™ 2487® dual wavelength absorbance detector. (Analysis performed at the Fort Worth Zoo Nutrition Department).

Results

The mean liver retinol concentration in the control animals fed the high-calcium gut-loaded crickets (Group 1) was less than 2.5 % the retinol concentration ($\overline{X}$ = 0.95413ug/g) found in the toads fed crickets gut-loaded with the CED (Group 2 - $\overline{X}$ = 40.08628ug/g) and the toads fed crickets maintained on the high β-carotene diet (Group 3 - $\overline{X}$ = 43.18979ug/g) (See Box and Whisker Graph, ANOVA analysis, and break-down table below). One of the replicates of toads fed the high β-carotene diet had a higher mortality rate and exhibited clinical indications of calcium deficiencies. These animals were not included in the analysis, leaving only one replicate for Group 3 in the results presented here.

Discussion and Recommendations

Both the high β-carotene and CED gut-loaded cricket diets produced significantly higher levels of Vitamin A in the livers of the studied American toads, while the control animals fed crickets gut-loaded with the commonly used high calcium diet had minimal, and in some case undetectable, levels of Vitamin A. These results indicate that either of the experimental diets could be used to address hypovitaminosis-A issues in captive bufonids when used in conjunction with a multi-vitamin and calcium dusting supplement. However, the CED gut-loading method had some distinct advantages in logistics and management over the high β-carotene crickets. The cricket colonies maintained on the high β-carotene had a tendency to become infested with fruit flies and required additional cleaning and maintenance. The crickets used for the CED gut-loaded diet were maintained on the high calcium cricket diet, then gut-loaded for 30 minutes prior to being fed to the toads. Li et al. (2009) demonstrated that Wyoming toads (*Anaxyrus baxteri*) fed the CED diet demonstrated improvements in their ability to catch prey, which may be an indirect indication of reduced incidence of squamous metaplasia or short-tongue syndrome, one symptom of hypovitaminosis-A, thereby suggesting improved Vitamin A levels. This study verifies these findings with liver retinol levels as a metric. Implementing the CED diet was a simple procedure that required minimal keeper time. The formula was mixed weekly and refrigerated for continuous availability. Because Vitamin A is oil soluble and stored for an extended time within the liver and fat reserves, it probably is not necessary to use this supplementation at every feeding. Currently at the Toledo Zoo we are feeding the CED diet approximately once a week and have seen no indications of hypovitaminosis-A in the amphibians under this diet.

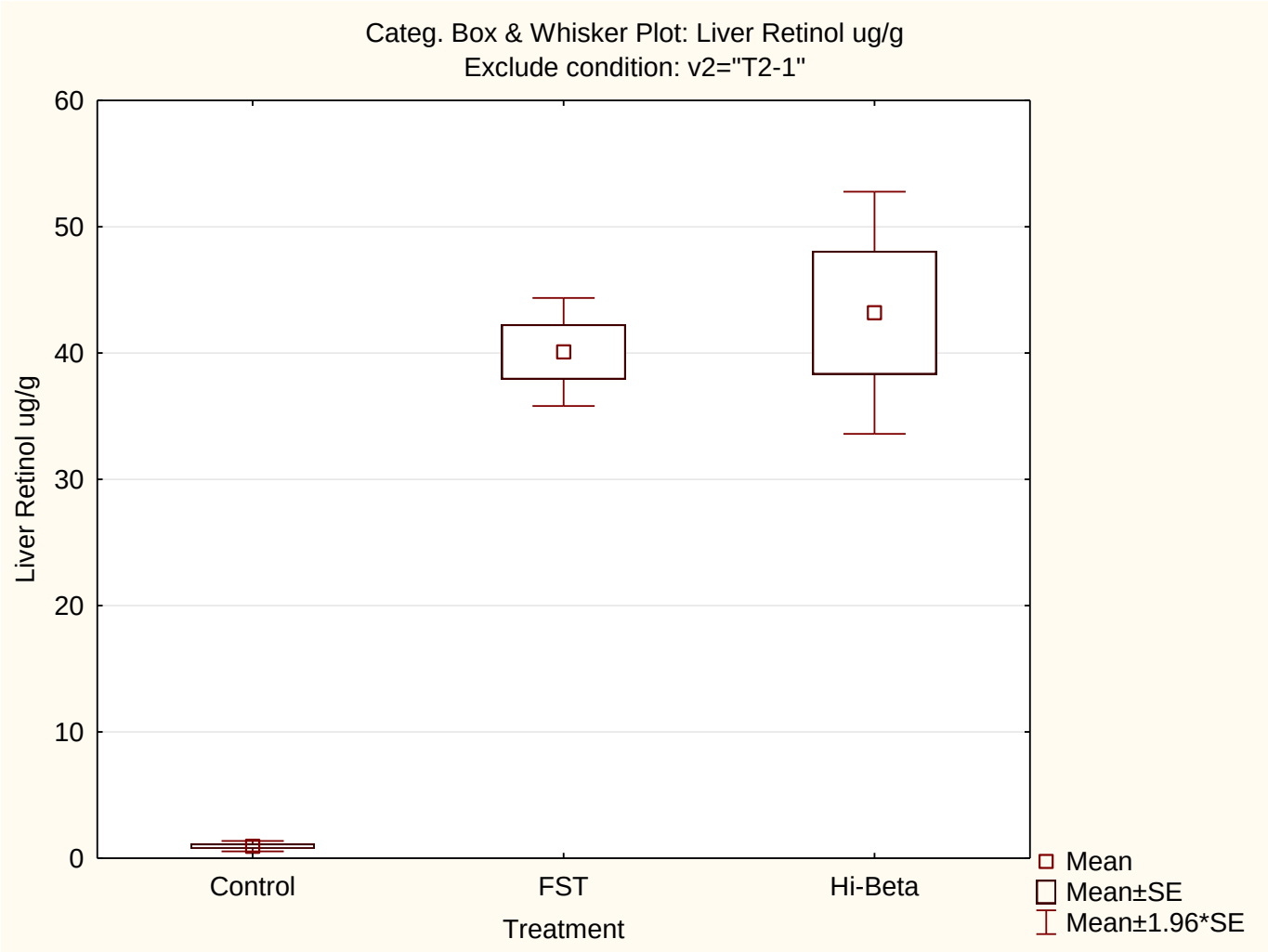
Colonies of crickets maintained on the Orthocal™ or other calcium gut-loaded diets for long periods appear to have decreased survivorship and reproduction (Bernard et al., 1997). To maintain healthy and productive cricket colonies, the Toledo Zoo has since switched to feeding crickets a commercial cricket breeding and rearing diet (i.e. Purina® Cricket Chow®, St. Louis, MO). This commercial cricket diet appears to have enhanced growth, reduced mortality, and increased fecundity in the crickets. Prior to feeding the crickets to amphibians, the insects are gut-loaded for 30 minutes with CED and then dusted with a powdered multiple vitamin/calcium supplement (Tetrafauna Reptocal®). Occasional calcium gut loading could also be added to the feeding regime if calcium deficiencies are encountered, although when this problem did appear in some of our study animals, the symptoms disappeared after dusting the crickets with the vitamin/calcium supplement was initiated. The toads consumed the crickets soon after they were introduced into the enclosure so that most of the vitamins were still adhering to the crickets when they were eaten.

For amphibian taxa that have known susceptibility to hypovitaminosis-A, we suggest the periodic use of the CED diet to provide Vitamin A in a form that is known to be absorbed by bufonids. The interval between treatments will depend upon whether the animal is already vitamin A deficient, which would require initial frequent treatments to increase vitamin A levels. There was no indication of hypervitaminosis-A in any of the animals during the seven months of this study including the group of toads offered the CED crickets at every feeding. Including a CED feeding in husbandry protocols once a week might provide adequate Vitamin A levels in bufonids. We have provided a simple recipe below to mix varius quantities of the CED diet.

GRAPH

BOX and WHISKER GRAPH OF LIVER RETINOL CONCENTRATIONS

The toads offered the CED gut-loading and high β-carotene cricket diets had more than 40 times the amount of retinols in their livers than did the animals offered the high-calcium diet. This analysis excludes the one replicate of the high β-carotene diet that had significant mortality during the experiment. Inclusion of these data did not significantly change the results; however the one replicate with the higher mortality was no longer comparable to the control and it was deemed appropriate to exclude it from these analyses. (In the graph below, FST = CED diet, Hi-Beta = high β-carotene diet)



ANOVA RESULTS

Variable	Analysis of Variance (Vitamin A analysis Totals July 2012.sta) Marked effects are significant at p < .05000 Exclude condition: v2="T2-1" (Animals in Group with high mortality)							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Liver Retinol ug/g	25117.95	2	12558.97	5753.749	61	94.32376	133.1475	0.000000

BREAKDOWN TABLE

Breakdown Table of Descriptive Statistics
(Vitamin A analysis Totals July 2012.sta)
N=64 (No missing data in dep. var. list)
Exclude condition: v2="T2-1"

Treatment	Liver Retinol ug/g Means	Liver Retinol ug/g N	Liver Retinol ug/g Std.Dev.
Control - (1)	0.95413	27	1.09488
CED - (2)	40.08628	26	11.12059
Hi-Beta - (3)	43.18979	11	16.22003
All Grps	24.11082	64	22.13655

ENHANCED MIXTURE (CED) USED IN THIS STUDY SIMILAR TO ENHANCED DIET (Li et al., 2009)

Item	Percent by Weight
Aquatic Turtle Food (ground) (Mazuri®, Brentwood, MO)	77%
Wild Alaskan Salmon Oil (Yummy Chummies®, Anchorage, AK)	12%
Spirulina Powder (Now Foods®, Bloomington, IL)	11%

CED RECIPE TO MAKE VARIUS AMOUNTS

Item	To Make 100g	To Make 50g	To make 25g
Aquatic Turtle Food (ground)	77g	38.5g	19g
Wild Alaskan Salmon Oil	12g	6g	3g
Spirulina Powder	11g	5.5g	3g
Total Amount	**100g**	**50g**	**25g**

LITERATURE CITED

Bernard, J., M. Allen and D. Ullrey. 1997. Feeding Captive Insectivorous Animals: Nutritional Aspects of Insects as Food. Nutrition Advisory Group Handbook, Fact Sheet 3: 1-7.

Li, H., M. Vaughan and R. Browne. 2009. A Complex Enrichment Diet Improves Growth and Health in the Endangered Wyoming Toad (*Bufo baxteri*). Zoo Biology 28(3): 197-213.

Pessier, A., M. Linn, M. Garner, J. Raymond, E. Dierenfeld, W. Graffam and C. Baer. 2005. Suspected Hypovitaminosis a in Captive Toads (*Bufo* Sp.). Proceedings of the American Association of Zoo Veterinarians, American Association of Wildlife Veterinarians and the American Zoo and Aquarium Association Nutrition Advisory Group.

Pessier, A., D. Roberts and M. Linn (2002). Short Tongue Syndrome, Lingual Squamous Metaplasia and Suspected Hypovitaminosis a in Captive Wyoming Toads, *Bufo baxteri*. 9th Annual Meeting of the Association of Reptilian and Amphibian Veterinarians, Reno, NV, Association of Reptilian and Amphibian Veterinarians.

Stacewicz-Sapuntzakis, M., P. E. Bowen, J. W. Kikendall and M. Burgess. 1987. Simultaneous Determination of Serum Retinol and Various Carotenoids: Their Distribution in Middle-Aged Men and Women. Journal of Micronutrient Analysis 1987(3): 27-45.

Wright, K. and B. Toddes. 2005. Amphibian Nutrition. **AZA Amphibian Biology and Conservation** K. Zippel and R. Odum. Silver Springs, MD, AZA.

ACKNOWLWEDGEMENTS

We would like to thank Ann Ward and Amy Hunt Coslik from the Fort Worth Zoo for providing input into the experiment design and performing the liver retinol analysis. We would also like to thank Kevin Wright for his input in the experimental design, and April Dieter and Erin Balfour for their processing of the samples and specimens at the Toledo Zoo. Finally we would like to thank Jan McKee and the members of the Wyoming Toad Recovery Team for their support in conducting this research.