
DIETARY CAROTENOIDS ON FAT-SOLUBLE VITAMIN STATUS AND GROWTH IN TOMATO FROGS, *Dyscophus guineti*

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ABSTRACT: Some species in captivity develop some nutritional diseases such as hypovitaminosis A. There are some cases of this disease documented in amphibians. Adults and young of *Dyscophus guineti* were fed three diets over nine weeks to evaluate plasma vitamin A levels and dietary effects on animal growth. Treatment one (control) comprised feeder crickets injected with soy oil; treatment two was feeder crickets injected with betacarotene and treatment three was feeder crickets injected with mixed carotenoids.

Dry matter intake increased in all treatments, but the significant effect was the weekly intake throughout the period ($p=0.009$). Treatment three had a significant effect on growth of the animals ($p<0.0001$), and Pearson correlation coefficients showed that dry matter intake had an important effect on weight and length of the animals. Vitamin A from feeder crickets was higher in insects supplemented with carotenoids ($p=0.0001$) and frogs' plasma vitamin A levels were significantly higher in animals from treatment three ($p<0.02$).

Results suggests that both tomato frogs and feeder crickets could receive some provitamin A activity when they eat a diet supplemented with betacarotene, lutein and zeaxanthin, and that the carotenoids have an important effect on weight gain in this frog species.

KEY WORDS: frogs, vitamin A, carotenoids

INTRODUCTION

Tomato frogs belong to the family Mycrohylidae with three recognized species: *D. antongilli*, *D. guineti* and *D. insularis*. All are endemic to Madagascar, and are only found in the northern part of the island. These reddish-colored frogs earned their name due their appearance, being similar to tomatos, especially when they inflate. True colors, however, can vary a great deal, presenting with many shades of orange, red and yellow, even brown (Glaw and Vences, 2007). Because of these characteristics, tomato frogs are common in the pet trade; at least one species has a conservation status “near threatened.”

Pigmentation alterations and health issues, including vitamin A deficiency, have been documented for managed amphibian species, and are directly or indirectly linked with dietary

management. In captivity, insectivorous species can be prone to nutritional imbalances, due to dietary insufficiencies of both minerals and vitamins. Calcium and Vitamin A are known to be lacking in most feeder insects (Dierenfeld and Graffam, 1996). Skin pigmentation changes and health, specifically linked with dietary Vitamin A concentrations, and their relationship with reproduction, have been studied in some amphibian species in captivity, and all are related to nutrition. Critical aspects, including actual nutrient requirements, carotenoid metabolic pathways, and specific functions, remain totally unknown in most amphibian species.

Hypovitaminosis A was reported in Wyoming toads (*Bufo baxteri*). Deficiency symptoms were correlated with poor growth and feeding difficulties (Wright, 2006). This pathology is known as lingual squamous metaplasia and is caused by the transformation of the mucus producing glandular epithelium of the tongue to squamous keratinized epithelium (short tongue syndrome; Pessier et al., 2002).

Other authors (Valdes and Fleming, 2008) also reported hypovitaminosis A in giant tree frogs (*Litoria infrafroneta*), grey foam-nest tree frogs (*Chiromantis xerampelina*) and Puerto Rican crested toads (*Peltophrynus lemur*). Symptoms included loss weight, inability to catch prey, and skin problems.

The dynamics of carotenoid functions and conversion to Vitamin A activity have not been detailed in amphibians; further, biochemical pathways appear to vary distinctly among species that have been examined. In addition to meeting vitamin A nutritional needs, dietary carotenoids are directly linked to skin pigmentation in a variety of species, including amphibians. Alteration of pigmentation may affect potential recognition of breeding partners, perception of fitness, and could have a physiologic effect on health and reproductive output. This study provides a model for similar research with other colorful amphibian species that may be applied globally.

The purpose of the present study was to determine if carotenoids from the diet have an effect on vitamin A status and growth of tomato frog in captivity.

MATERIALS AND METHODS

Twenty-four false tomato frogs (*Dyscophus guineti*) were used for this study. Young (n=19) and adult (n=5) frogs were fed either small (108 mg) or adult (357 mg) crickets for a 9-week trial period. Food intake (*ad libitum* crickets) was determined over the first two weeks prior to beginning the study, and was adjusted depending on weight gain. Frogs were fed three times per week.

Animals were housed individually in glass terraria (40 cm X 20 cm X 25). Temperature and humidity conditions were 25.8°C and 24.5% respectively, controlled with a Zilla® thermometer-hygrometer. Photoperiod was 12 hours light and 12 hour darkness, light-day bulbs used were Philips® 40 Watts, and UV bulbs Sylvania 350® 40 Watts.

Feeder crickets *Acheta domestica* were injected between dorsal segments of their abdomen with known concentrations of beta carotene and/or mixed carotenoids treatments versus control. Animals were randomly assigned to three treatments (eight animals per treatment). Treatments used were:

- a) Treatment One: Control: Feeder crickets with no carotenoids, injected with soy oil.
- b) Treatment Two. Betacarotene: Feeder crickets injected with beta carotene dispersable solution (Betacol® 30%, CaroTech®) dissolved in soy oil.
- c) Treatment Three. Mixed Carotenoids: Feeder crickets injected with beta carotene (Betacol®) 30%, lutein (Lutecol® 20%), canthaxantin (Canthacol® 100g/kg) and xanthophylls (Xarocol® 20 g/kg) dissolved in soy oil. All carotenoids were from CaroTech®.

Blood samples were drawn by heart puncture at the beginning and end of the study for fat-soluble vitamin A as well as carotenoid analysis. All crickets and blood plasma samples were analyzed in the Biology Laboratory of the University of Arizona, by a high-performance liquid chromatography (HPLC) method. Individual frogs were weighed and measured weekly using a CQT-202 Adam Equipment Company 200g (± 0.01 g) scale and 180 mm Vernier calipers.

Statistical comparisons were expressed as the means and differences, and were considered significant at $p < 0.05$ using ANOVA and ANCOVA analysis. Duncan's multiple range test was used for multiple comparisons of means, and Pearson correlations were used for food intake analysis. All statistical analyses were performed using the SAS software package (SAS, 2003).

RESULTS

Food Intake and Growth

All the individuals increased food intake over the course of the study. Results are showed in Figure 1.

Food intake was not affected by the treatment ($p > 0.05$) but the increase in dry matter intake along the period was significant ($p = 0.009$). The animal's age had a significant effect on this parameter ($p < 0.0001$); adult frogs' intake was higher than young ones, which is directly related to their body size.

Concerning weight gain, Figure 2 show the responses obtained in all frogs over the study.

There was a constant weight increase of all animals throughout the trial period. Treatment had a highly significant effect ($p < 0.0001$); according to the Duncan's multiple range test, weight increase was significantly less in treatment 2 (beta carotene) animals; meanwhile, weights of animals in the mixed carotenoids treatment group were higher. Age of the animals had an important effect due to the adults having average weights higher than young ones (78.57 g and 9.30g respectively), showing growth rates of 1.07 g/week for young frogs compared to 2.07

g/week for adults. This effect is because adult frogs had higher average weights than young frogs from the beginning of the study.

Total weight gain data showed no significant effect by treatment ($p=0.5410$), but by age, treatment was significant ($p=0.05$). According to the Duncan's multiple range test, young frogs grew 189% and adult frogs grew 33% more than their starting body mass over 9 weeks. There was a significant effect in length between treatments two and three ($p=0.03$).

Pearson correlations showed that dry matter intake had an important effect on weight and length ($p<0.0001$). Pearson coefficients are shown in Table 1.

Carotenoid and Vitamin Analysis

Tables 2 and 3 show carotenoid and vitamin composition of feeder crickets used in the experiment.

Vitamin A levels showed a highly significant effect in crickets injected with carotenoids compared to not injected and control crickets ($p=0.0001$). Cricket's age and interaction had an important effect; according to Duncan's statistics, levels were different between treatment one and three, and between two and three.

Vitamin E was not added separately, but its amount was increased in all the treatments compared to non-supplemented crickets. However, the change was significant only in treatment 3 ($p=0.04$) compared to control. This could be due to the presence of vitamin E in soy oil.

Lutein levels increased in treatments two and three, but in treatment three levels were notably higher. Zeaxanthin level increased 5000 times its initial value and lutein increased 14000-fold.

Cricket's age had a significant effect on lutein and zeaxanthin levels ($p=0.0016$ and $p=0.0001$); young crickets showed higher levels. Interaction was important in both cases.

Betacarotene levels increased 600% in treatment 2, and 800% in treatment three compared to their initial level. Young crickets supplemented with betacarotene had the higher proportion of this pigment, but adult crickets did not show the same trend. Duncan's multiple test showed significant differences in treatment three crickets compared with control and treatment two. Cricket's age and interaction did not have a significant effect on betacarotene content.

Plasma Analysis

Frogs' plasma vitamin A levels did not differ among treatments before the experimental treatments were fed. Vitamin A levels increased numerically in all the treatments; however only the mixed carotenoids treatment effect was statistically significant ($p<0.02$). Figure 3 shows frog's plasma vitamin A levels obtained before and after the experiment.

Discussion

No effect of dietary carotenoids on food intake was found. Some authors obtained similar results with *Bufo baxteri* (Li, et al., 2009) offering enriched diets with vitamin A and carotenoids, and with finches (*Lonchura domestica*; McGraw et al., 2004). However, significant increases along the period could be due to inclusion of soy oil, which had a positive effect on the diet's palatability. In young frogs, increase in food intake possibly was due to their physiologic stage of growing.

Results in the animal's growth suggests that carotenoid pigments have an effect on weight gain patterns in this frog species. Results obtained in the present study are similar with another published study (Li et al., 2009), with a reported higher weight gain in young *Bufo baxteri* fed a diet with feeder crickets supplemented with fish oil, *Spirulina* and commercial turtle food, high in retinal and betacarotenes. However, the authors didn't specify if the effect was due to the caloric content of the diet. Likewise, another study (Cucco et al., 2006) reported important effects in growth of quail chicks fed with betacarotenes in the first 10 days.

Increase in weight and length was higher in young frogs than adults. This could be due to the fact that animals are more efficient during growth stage. However, until now no information was available concerning *D. guineti* growth curves. Increase in adult weights was possibly due to a higher caloric diet. Pearson correlation coefficients showed that dry matter intake affected the weight and length in tomato frogs, but more studies and a longer time are needed to confirm these data.

One study with *Schistocerca gregaria* (Heller et al., 2000), suggests that zeaxanthin can be synthesized from oxidation of betacarotene, because this pigment was not found in plants consumed by this worm. More studies are needed to confirm the ability to synthesize zeaxanthin in *Acheta domestica*. However, some authors have found zeaxanthin and lutein in some species of insects (Heller et al., 2000; Eeva et al., 2010).

From this experiment, we can conclude that carotenoids could be vitamin A precursors in frogs, and it may prove more effective to use a mixture of carotenoids instead of just beta-carotene. Some species of fish have the ability to form active vitamin A from carotenoids (Gross et al., 1966; Meyers, 1998) and others determined the presence of vitamin A in frogs species such as *Rana catesbeiana*, and *R. esculenta* and *R. temporaria* froglets (Collins et al., 1953).

It is suggested that crickets have an apparent capacity to synthesize vitamin A from carotenoids, so this may be something to explore further, by optimizing carotenoid nutrition of feeder crickets through dietary manipulation. Future investigations are suggested to study the transfer rate of carotenoid nutrient absorption/metabolism and mechanisms in frogs. It is necessary to develop more studies in vitamin A and carotenoids nutrition of amphibians, for longer periods of time (at least one year) to analyze growth rates and color changes, and to confirm these results.

This, and links with health and reproduction should be investigated further. More studies related to carotenoid and vitamin A are needed to better understand the biochemical pathways and functions of pigments in tomato frogs and other frog species.

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Table 1. Pearson (*r*) correlation coefficients obtained during the experiment.

Variable	Weight	Length	Dry Matter Intake
Weight	1.00	0.98	0.93
Length	0.98	1.00	0.96
DM Intake	0.93	0.96	1.00

Table 2. Average levels of lipid soluble vitamins and carotenoids in young and adult feeder crickets.

Nutrient	Young				Adult			
($\mu\text{g/g}$)	NS ^a	T1 ^b	T2 ^c	T3 ^d	NS	T1	T2	T3
Vitamin A (retinol)	0.06	0.08	0.20	1.00	0.05	0.10	0.17	0.33
Vitamin E (α -tocopherol)	1.96	81.19	101.93	263.24	2.40	121.43	121.09	105.05
Lutein	0.74	0.50	6.04	10164.55	1.53	1.02	4.02	4884.16
Zeaxanthin	0.22	0.11	2.71	1360.22	0.58	0.31	1.89	255.79
β -carotenes	0.34	0.00	471.73	268.53	0.46	0.14	306.39	363.55

^aNon supplemented,

^bTreatment 1: Control.

^cTreatment 2: Beta carotene.

^dTreatment 3: Mixed carotenoids.

Table 3. *P* values for vitamin and carotenoid levels ($\mu\text{g/g}$) for young and adult feeder crickets.

Effect	Vitamin A	Vitamin E	Lutein	Zeaxanthin	Betacarotene
Treatment	0.0001	0.047	0.0001	0.0001	0.0001
Age	0.0002	0.46	0.0016	0.0001	0.125
TreatmentXAge	0.0001	0.31	0.0005	0.0001	0.124

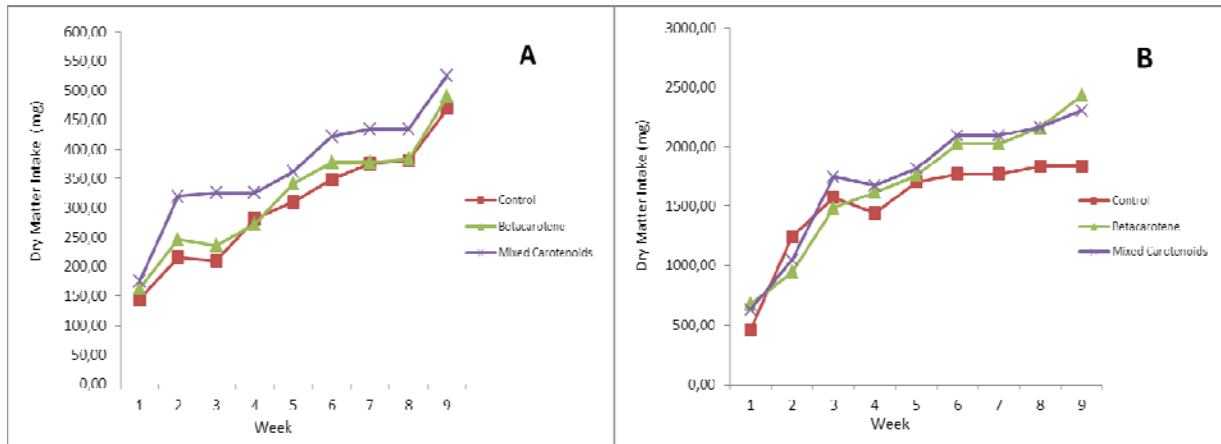


Figure 1. Dry matter food intake per week (insects) of young (A) and adult frogs (B) during the experiment.

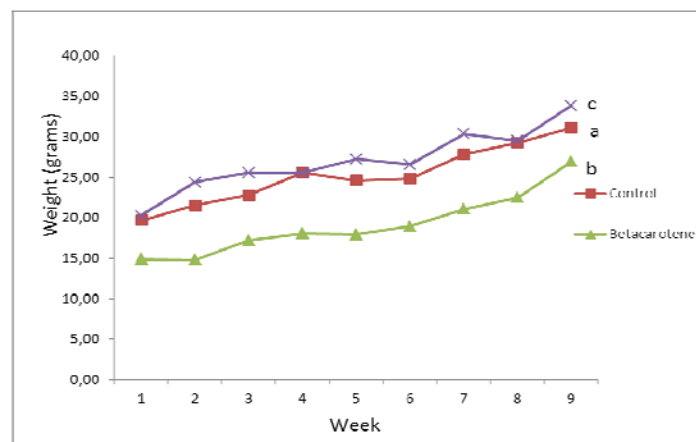


Figure 2. Average frogs' weights during the experiment (same letters don't differ statistically).

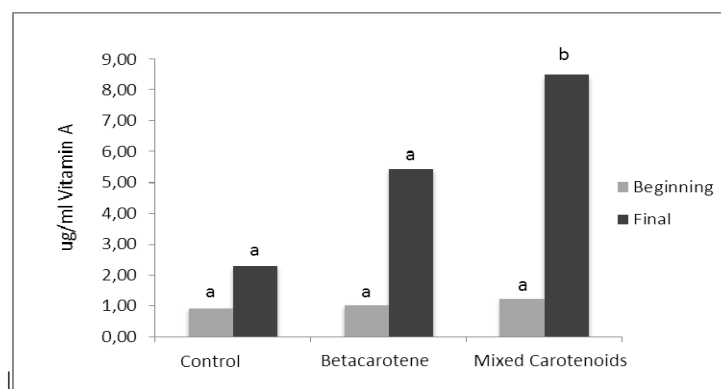


Figure 3. Average plasma vitamin A levels before and after the study (bars with the same letters do not differ statistically).