

Effect of Vitamin D3/ Ascorbic Acid Supplementation on Growth and Tibia Characteristics of Green Water Dragons (*Physignathus cocincinus*, Cuvier 1829)

Sergio E. Oyarzun¹, Andrea L. Dufresne¹, Eduardo V. Valdes¹, Graham J. Crawshaw¹, and Steven Leeson²

¹*Metropolitan Toronto Zoo, Toronto, Ontario*

²*Department of Animal Science, University of Guelph, Guelph, Ontario*

A preliminary experiment was conducted to investigate the interactive effect of one level of a combined dose of vitamin D3/ascorbic acid (C) supplementation on growth performance and bone development in green water dragons (*Physignathus cocincinus*, Cuvier 1829). Ten forty-seven day old female green water dragons obtained from one clutch of eggs were selected for this study. From birth to age 46-days, the dragons were raised on the standard Metro Toronto Zoo (MTZ) diet of dusted crickets on an ad-lib basis.

At age 47-days, the lizards were weighed, measured and allocated randomly to 1 of 2 dietary treatments groups in a completely randomized design, replicated 5 times, one dragon per replicate. For the duration of the experiment, the animals were housed individually in glass tanks (30Wx60Lx35H cm) with sliding mesh-lids. Room fluorescent light fixtures provided a photoperiod of 14 hours of light and 10 hours of dark. Ambient temperature ranged from 22 to 32°C. A combination of ultraviolet (UV) lights (40W 122 cm Duro Test Vita-Lite and 40W 122 cm GE F-40-BL blacklight) suspended 15 cm above the perching branches provided 8 hours of UV-radiation during the 14-hour day light period.

Treatment Diet-A (control) was the normal MTZ diet for insectivorous reptiles consisting of live crickets dusted with a vitamin-mineral supplement and one live earthworm daily. Treatment Diet-B (experimental) consisted of Diet-A with the addition of vitamin D3 (0.12 IU/g BW) and ascorbic acid (0.10 mg/g BW) provided orally. The vitamin solution was offered via a 1 ml syringe from which they readily drank. The control animals received plain water via the same device. Feed and water was provided daily on an ad-lib basis. The crickets were fed a high calcium (9% Ca) mash-diet for a minimum of 3 days prior to being offered to the lizards.

The number and weight of crickets and worms consumed by each individual lizard were recorded on a daily basis to determine actual feed intake. Dragons body weights (BW), snout-vent body lengths (5-V) and snout-tip of tail length (5-T) were taken at the beginning and at 7-day intervals thereafter. Throughout the experimental period, the dragons were observed on a daily basis for signs of scoliosis (kinky-tail), a common problem in the MTZ green water dragon collection.

At the end of the 13-week experimental period, the lizards were sedated (isoflurane/oxygen) and euthanized (intracardiac T -61). Radiographs were taken to evaluate skeletal structures. Blood was obtained by cardiac puncture, collected into heparinized tubes, centrifuged, plasma separated and stored in liquid nitrogen until analysis. Plasma calcium and phosphorus was determined by reflectance spectrophotometry using a Kodak Ektachem DT60 analyzer (Kodak Canada Inc., Toronto, Ontario, Canada).

The tibia of both legs were removed, cleaned of flesh, weighed, measured with a caliper, and air-dried for bone breaking strength assay. Bone breaking strength (the force necessary to break each tibia) was measured using an Instron Universal Testing Machine Model 1122 (Instron Corporation, Canton, Ma., U.S.A.). Modifications were made to this apparatus to specifically examine these small bones. In this test, bones were supported on each end by stainless steel posts and force was exerted on the midspan of the bone by a crosshead moving at a constant speed of 50 mm/minute. A force deflection plot was created for each bone on chart paper moving at 200 mm/minute. The chart scale measured from 0 to 200 Newtons (N). Peak, or maximum force required to fracture each bone was recorded. Bones were then oven-dried, de-fatted by ether extraction method (AOAC, 1990), ashed at 600° C for 6 hours and subsequently assayed for calcium by atomic absorption spectrophotometry, and phosphorus by auto analyzer .

Data for all response variables between treatments were analyzed using the Student's t-test.

The objective of this preliminary trial was to evaluate the effects of one level of a combined dose of vitamin D3/ascorbic acid supplementation on growth performance and bone development in juvenile green water dragons. The results of this experiment are presented in Table 1. Diet treatment had no effect on body weight, body length, daily gain, and plasma calcium and phosphorus values. Although the values for all parameters were slightly higher in the dragons receiving the supplemental vitamin D3/C, the differences were not statistically significant.

There was a positive effect of diet on bone (tibia) breaking strength and length. Tibia strength values were greater ($P < 0.05$) for dragons fed diet treatment B (vitamin D3/C) as compared to those on the control diet (23.08 vs 17.37 N). Tibia length was also significantly greater ($P < 0.05$) for the dragons on treatment B (Table 1) .

Key words: green water dragons, vitamin D3, ascorbic acid, growth, tibia

Table 1. Plasma values, bone parameters and growth performance of juvenile female green water dragons (*Physignathus cocincinus*, Cuvier 1829) (mean \pm SEM).

	Diet-B (Vit D ₃ /C) n = 5	Diet-A (Control) n = 5
Plasma, mg/dl		
Calcium	11.86 ^a \pm 0.53	12.18 ^a \pm 0.37
Phosphorus	9.94 ^a \pm 0.66	9.44 ^a \pm 0.33
Left tibia		
Breaking strength, N	23.08 ^a \pm 1.04	17.37 ^b \pm 1.66
Weight, mg	69.22 ^a \pm 5.51	57.46 ^a \pm 4.64
Length, mm	194 ^a \pm 5.09	182 ^b \pm 3.74
Ash, %	35.17 ^a \pm 0.77	32.63 ^a \pm 1.22
Right Femur+Tibia		
Weight, mg	280.8 ^a \pm 24.22	235.7 ^a \pm 23.35
Calcium ¹ , %	16.82 ^a \pm 0.09	16.82 ^a \pm 0.42
Phosphorus ¹ , %	5.09 ^a \pm 0.2	5.05 ^a \pm 0.24
Body weight, g		
Age 47 days	6.60 ^a \pm 0.18	6.01 ^a \pm 0.29
Age 138 days	33.43 ^a \pm 1.93	27.97 ^a \pm 1.91
Overall daily gain, g	0.294 ^a \pm 0.02	0.241 ^a \pm 0.02
S-V length, cm		
Age 68	6.3 ^a \pm 0.37	5.8 ^a \pm 0.2
Age 138	8.72 ^a \pm 0.36	8.5 ^a \pm 0.27
S-T length, cm		
Age 68	21.7 ^a \pm 0.66	19.8 ^a \pm 1.19
Age 138	31.5 ^a \pm 1.19	29.6 ^a \pm 1.49

^{a-b}Means with different superscript within a row differ significantly (P < 0.05).

¹values expressed on a fat-free DM basis.