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Dietary Vitamin D_3 and UV-B Exposure Effects on Green Iguana Growth Rate: Is Full-spectrum Lighting Necessary?

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Abstract

The effects of dietary vitamin D_3 and exposure to ultraviolet light on growth of juvenile green iguanas (*Iguana iguana*) was investigated. Thirty-two iguanas (2–3 weeks old) were randomly assigned to one of four groups corresponding to four experimental testing protocols. The findings give a current perspective on the best vitamin D_3 acquisition method by green iguanas in a husbandry situation.

Green iguanas (*Iguana iguana*) are large, herbivorous, arboreal lizards indigenous to Central and South American tropical rainforests. Because of their unique, "exotic" nature and ubiquitous availability, iguanas are one of the most popular reptilian pets. Most iguana owners acquire their new pet when it is small, usually no more than 0.3 m (1 ft) in total length with most of that being tail. Unfortunately, most new iguana owners do not realize that their new exotic pet can reach lengths exceeding 1.8 m (6 ft). It is for this reason that green iguanas are now being called Giant Green Iguanas, a trend that began on the internet. Sadly, many iguana owners decide against keeping the iguana when it grows to adulthood. Animal shelters are full of iguanas; in fact, iguanas are the most common reptile up for adoption in programs such as that of the Chicago Herpetological Society. There are entire organizations dedicated to saving these reptiles such as the *Iguana iguana* Sanctuary, Inc., of Pennsylvania and the Northwest Green Iguana Rehabilitation Center in Oregon.

Numerous books and publications are dedicated to the care of green iguanas in captivity. Most of them discuss the role of ultraviolet (UV) or "full-spectrum" lighting in the health of iguanas. The term "full-spectrum" was coined by a light bulb manufacturer and implies a simulation of the spectral output of the sun. Within this full-spectrum light, it is the UV bandwidth that is necessary for the synthesis of vitamin D_3 in the skin of reptiles. Like mammals, reptiles manufacture vitamin D_3 in their skin (Holick, 1989). The UV stimulates a cutaneous vitamin D_3 precursor that goes through a series of biochemical reactions resulting in activated cholecalciferol or vitamin D_3. Vitamin D_3 is an essential part of calcium absorption. Therefore, without a source of vitamin D_3 calcium is not absorbed from the intestinal tract no matter how much calcium is present in the diet. This UV – vitamin D_3 link is the reason why vitamin D_3 is often called the "sunshine vitamin".

When an iguana is calcium or vitamin D_3 deficient, a condition known as Metabolic Bone Disease (MBD) develops (Boyer, 1996). MBD is actually a general term for a number of conditions relating to atrophy of bone tissue caused by either a deficiency in calcium itself or a deficiency in vitamin D_3 thus preventing calcium from being absorbed. The basis of MBD is the mobilization to the bloodstream of calcium from the bones, the largest reservoir of calcium in an animal's body (Boyer, 1996). Common manifestations of MBD in iguanas include nutritional secondary hyperparathyroidism (dietary-induced MBD), fibrous osteodystrophy (fibrous connective tissue replaces atrophied bone), and osteomalacia/rickets (failure of bone calcification and decrease in bone density) (Fowler, 1978; Boyer, 1996). MBD is one of the most commonly diagnosed health problems in iguanas (Wissman and Parsons, 1994; Allen et al., 1995; Mader, 1997).

Most iguana "care in captivity" publications discuss the requirement of "full-spectrum" lighting for the reptile's health. Although direct sunlight is always the best, in a husbandry situation this is not always possible, so iguana owners turn to the so-called full-spectrum fluorescent light bulbs available on the pet market. These bulbs are said to produce the UV that iguanas need for vitamin D_3 synthesis. Unfortunately, their efficacy of supporting vitamin D_3 synthesis has yet to be determined by scientifically-controlled animal studies. In addition, Gehrmann (1996) has examined a number of bulbs common on the pet market and has found that while they do produce UV, they have a peak output outside the known peak wavelength for vitamin D_3 synthesis.

While UV-induced vitamin D_3 synthesis is the natural source of vitamin D_3 for iguanas, diet-supplemented vitamin D_3 is absorbed into the bloodstream and thought to be assimilated and utilized in the same matter (Holick, 1989), although the metabolism of dietary forms of vitamin D_3 in reptiles is not fully understood. If exposure to natural sunlight is not an option for captive iguanas and artificial light sources produce only very small quantities of D_3-synthesizing UV at best, dietary sources of vitamin D_3 are the only other alternative.

Most of the aforementioned iguana "care in captivity" publications rarely if ever mention dietary forms of vitamin D_3 as a potentially important alternative to UV-induced vitamin D_3 synthesis. A two-part iguana study at the National Zoo in Washington, D.C. (Allen, 1988; Allen et al., 1989; Allen et al., 1995; Bernard et al., 1991; Bernard, 1995), concluded that either iguanas need extremely high doses of dietary vitamin D_3 or they cannot utilize it at all. This study is cited quite commonly in the iguana literature as evidence that iguanas cannot use dietary vitamin D_3 and therefore must have a source of UV

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for vitamin D_3 synthesis.

With over 4.8 million live green iguanas imported to the U.S. from 1991 to 2002 (no data for 1997) (USFWS, 1997, 2003) and with MBD cited as one of the largest threats to iguana health, understanding the metabolism of dietary vitamin D_3 and its role in green iguana health is crucial. The main objective of this study was to delineate the best vitamin D_3 acquisition method (dietary or UV-induced synthesis) in green iguanas by looking at rates of growth in juveniles. If direct sunlight is not possible in a husbandry situation, is the use of these expensive, scientifically unsubstantiated full-spectrum lights the only other option iguana owners have?

Materials and Methods

A sample of 32 juvenile (3–4 weeks old) green iguanas was obtained from Fluker Laboratories, Baton Rouge, Louisiana. The captive iguanas hatch under the sun in a multi-acre outside enclosure at Fluker's iguana farm in El Salvador. After about 10 days in El Salvador, the iguanas are shipped to Fluker's facilities in Louisiana where they are housed in an indoor enclosure for 2–3 weeks before shipment to customers. The iguanas are fed Fluker's Iguana Diet® supplemented with Fluker's Repta Vitamin® supplement during these first few weeks of their lives. Fluker's Repta-Sun® artificial lights are provided for the iguanas as well as a spotlight for heat while they are in the indoor enclosure at the Louisiana facilities.

Iguanas were randomly assigned to one of four treatment groups using a 2 × 2 factorial research design. The treatment groups were as follows:

Group 1: UV light, dietary vitamin D_3
Group 2: UV light, no dietary vitamin D_3
Group 3: Fluorescent light, no dietary vitamin D_3
Group 4: Fluorescent light, dietary vitamin D_3

Each animal was assigned an individual number, and claws were so-marked with nail polish. Philips F40UVB® (TL40W/12/RS) bulbs (Philips Lighting Co., Somerset, New Jersey) provided the UV light and Philips Econ-o-watt Cool-White® F40CW/RS/EW-II bulbs provided the fluorescent (FL) light.

Four modular housing units were built to accommodate 8 separately-housed iguanas each (Figure 1). Each individual iguana cage measured 60.96 cm wide × 60.96 cm high × 60.96 cm long. The 121.9 cm (4 ft) UV or FL bulb (depending on group) extended down the middle of each unit giving each iguana cage access to the light via the inside corner. Window glass covered this inside corner in the FL groups and 1.27 cm (½ in) galvanized hardware cloth covered the inside corner in the UV groups. Window glass was used in the FL groups because glass would filter out any tiny amounts of UV that might irradiate from the FL bulbs, and the hardware cloth was used in the UV groups to allow for maximum flux of the UV light into the cages while preventing the escape of the iguanas. Each cage was carpeted with green indoor/outdoor carpeting and was equipped with a carpeted ramp that extended from midway up the back wall, across the inside corner, and ended on the floor.

The UV or FL light was turned on for 15 minutes per day

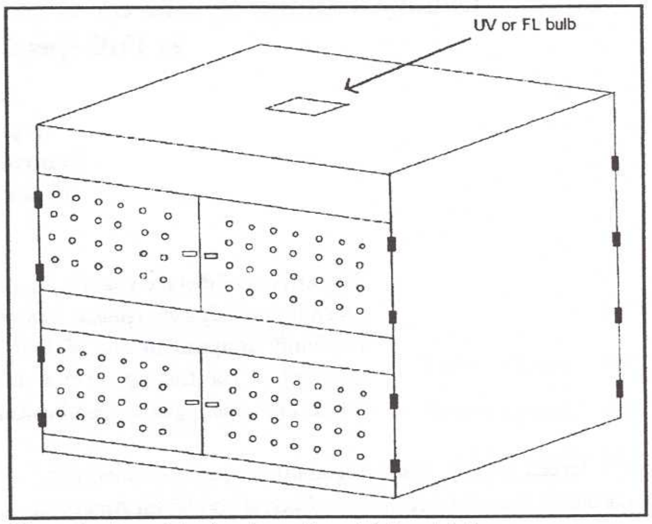

Figure 1. A modular housing unit containing eight iguana cages. Only four cages are showing; the other four face the back. A hole extending down the center accommodates either the UV or FL bulb.

(2 min at noon, 3 min at 1 P.M., 2 min at 2 P.M. etc. for six periods), 5 days a week. Each cage was heated by a 100-watt Pearlco® ceramic heating element (RAM Network, Encino, California) and was kept at ~30°C during the day and ~24°C at night using thermostats. A 12 hr light/12 hr dark photoperiod was set up using 40-watt G.E. SoftWhite® 40A/W (General Electric Co., Cleveland, Ohio) incandescent bulbs.

The 6-month study was conducted from June through December 1996. Iguanas were housed in a laboratory on Central Michigan University's Biological Station (CMUBS) located on Beaver Island, Michigan, from June through August. At the conclusion of the summer, the iguanas were moved to the main Central Michigan University (CMU) campus and housed in a biology laboratory for the remainder of the study period. At no time, from the arrival of the iguanas from Louisiana in June until the conclusion of the study in December, were the iguanas exposed to sunlight. Overhead (room) lighting in the CMUBS lab came from 150-watt G.E.® incandescent bulbs, while overhead lighting in the CMU biology lab came from Philips Econ-o-watt Cool-White® F40CW/RS/EW-II fluorescent bulbs.

Ultraviolet output of the Philips UV bulbs was measured at the beginning of the study in June and at the conclusion of the study in December using a Spectroline® DM-300X digital radiometer (Spectronics Corp., Westbury, New York) sensitive to a 280 – 320 nm bandwidth with peak sensitivity at 300 nm. The irradiance of the Philips bulb was found to be 50 microwatts per cm^2 ($\mu W/cm^2$) at 30 cm below the bulb and 20 $\mu W/cm^2$ at 61 cm below the bulb. As a comparison, the Sylvania 2096 UV bulb used in Allen and Bernard's work was found to have an irradiance of 50.9 $\mu W/cm^2$ at 61 cm below the bulb (Bernard, 1995) and all of the full-spectrum bulbs Gehrmann (1996) analyzed had an irradiance of less than 10 $\mu W/cm^2$ at 30 cm below the bulb. It was also confirmed that the window glass-filtered fluorescent lights (of the FL light groups) and overhead G.E. incandescent bulbs emitted no detectable amounts of UV.

Fresh water was available ad libitum in a large bowl, and all iguanas were misted with water regularly to maintain a humid environment. Diet was an essential part of this study and consisted of a "salad-type" diet using nutritionally balanced fruits and vegetables. Iguanas were fed daily for most of the study period but occasionally time restraints required the skipping of a day. The diet for any particular day was picked based on protein content and calcium phosphorus (Ca:P) ratios. A calcium phosphorus ratio of 2:1 is the ideal ratio for the nutritional requirements of green iguanas (Burgmann et al., 1993; Boyer, 1996; Mader, 1997). Calcium-rich leafy greens (e.g., collard greens, mustard greens, kale, turnip greens) made up at least 50% of each daily diet. At every feeding time, each food item was weighed out separately on an electronic balance to ensure that each iguana was given the same amount of each food item. The mean total amount of food (as fed basis) offered to the iguanas each day ranged from 8 g in June to 25 g in December in accordance to their growth. The cages were cleaned and vacuumed as needed.

The dietary vitamin D_3 was provided by Rep-Cal™ multivitamin supplements (Rep-Cal Research Labs, Los Gatos, California). This vitamin supplement is a two-part supplement consisting of Herptivite™ containing numerous vitamins, amino acids and minerals, and Calcium with Vitamin D_3™ containing only calcium and vitamin D_3. These two parts are mixed in equal proportions when used. A special lot of Calcium with Vitamin D_3™ was ordered that was free of vitamin D_3. The vitamin supplement was also weighed out daily on an electronic balance for each iguana and lightly dusted on top of the food. A mean of 1245 I.U. vitamin D_3 per kg of diet (as fed basis) was given over the entire study period. Because plants do not naturally contain vitamin D_3 (Norman, 1979), the only vitamin D_3 the iguanas received was from the supplement.

Both versions of the Rep-Cal Calcium with Vitamin D_3™ supplement were analyzed by Dr. James Ball of Ford Motor Company, Dearborn, Michigan, using gas chromatograph/mass spectrograph (GC/MS) and high performance liquid chromatography (HPLC) techniques. The vitamin D_3 supplement was found to contain 671 I.U. vitamin D_3 per gram of supplement and the vitamin D_3-free supplement was found to be free of any vitamin D_3.

Initially and every two weeks throughout the study, five growth parameters were measured and general health and behavior were noted. The five growth parameters measured were mass, snout-to-vent length, vent-to-tail tip length, head width, and base-of-tail width. Mass was determined by placing each iguana into a large plastic container on an electronic balance. Snout-to-vent and vent-to-tail tip lengths were both determined by carefully placing each iguana adjacent to a meter stick taped to a countertop. Head width was determined by carefully measuring the width of the head between the eye sockets (the widest part of the head) using vernier calipers. Base-of-tail width was determined by measuring the width of the tail just posterior to the pelvic girdle using vernier calipers. At select times throughout the study, all iguanas were photographed to illustrate general morphological changes.

Although five growth parameters were measured during the study, only mass and snout-to-vent length were analyzed because these two factors give the best indication of overall growth. Mass and snout-to-vent length were analyzed by linear regression analysis with the regression coefficients (slope of the regression line = rate of growth) being analyzed with a one-way ANOVA with Tukey's multiple comparison.

Results

The Philips F40UVB® bulb used in this study was chosen because of its high UV output. To my knowledge, this bulb has never been used in a study involving green iguanas, although a very similar bulb has been used in a panther chameleon (*Chamaeleo pardalis*) study (Ferguson et al., 1996). Because of the Philips F40UVB bulb's lack of use in reptilian husbandry and because little is known about the quantity of ultraviolet light needed by reptiles, UV exposure times and distances could only be inferred. Using the Ferguson et al. (1996) study as a base, the cages were designed so that the iguanas could be as close as 10.16 cm to the UV/FL bulb or as far away as 71.12 cm. An exposure time of 1 hour per day (10 min at noon, 10 min at 1 P.M., etc. for six periods) was initially used.

After about a week into the study, a darkening of the iguanas' skin pigmentation in groups 1 and 2 (UV groups) was observed. Consequently, these two groups were watched closely for signs of overexposure to ultraviolet radiation. Two weeks after the beginning of the study, a very lethargic iguana from group 1 died. Although no further signs of overexposure to UV were evident (other than dark skin pigmentation), the UV/FL exposure times were decreased from 1 hr to ½ hr per day (5 min at noon, 5 min at 1 P.M., etc.). Unfortunately, over the next week-and-a-half, two additional iguanas from group 1 and one iguana from group 2 had died, all very lethargic before death.

During the first week of July, the iguanas from groups 1 and 2 were observed to sit motionless with their eyes closed during the day indicating possible eye irritation caused by the UV. On 10 July, one additional iguana from group 1 had died with the same symptoms as the other four. After consultation with an authority on UV lighting (Gehrmann, pers. com.), the UV/FL exposure times were decreased to that listed previously in the Materials and Methods section of this manuscript (from 30 to 15 min per day). The UV/FL exposure times remained at this level throughout the remainder of the study.

The death of these five iguanas was most likely due to overexposure to ultraviolet light. No signs of MBD were observed in any of these iguanas at any time. As to why four iguanas from group 1 died but only one iguana from group 2 can only be speculated, but one possible explanation is that group 1 iguanas also received dietary vitamin D_3 supplementation suggesting possible vitamin D_3 toxicity in this group brought about by high amounts of UV light in addition to the vitamin D_3 supplement. Necropsies of these iguanas were not performed.

The results reported here are based on the remaining sample sizes for the four groups: Group 1: n = 4, Group 2: n = 7, Group 3: n = 8, and Group 4: n = 8.

As mentioned previously, only mass and snout-to-vent length (SVL) were analyzed because these two factors give the best indication of overall growth. Figure 2 shows a growth curve for mass of the four groups over the entire study period. Each time growth data were taken (every two weeks), mass values for each iguana were averaged to give one value per group. Figure 3 shows a growth curve for SVL of the four groups over the entire study period. Again, each time growth data were taken, SVL values for each iguana were averaged to give one value per group.

Linear regression analysis was performed on the mass and SVL growth curves for each iguana and the results averaged per group. Regression coefficients (slopes of the regression line, equal to the rate of growth) for both mass and SVL were analyzed with a one-way ANOVA with Tukey's multiple comparison. Results of the analysis of mass regression coefficients (Table 1) indicated a significant difference between group 1 (mean = .3946, SEM ± 0.043) and group 3 (mean = .1470, SEM ± 0.025) and between group 3 and group 4 (mean = .3796, SEM ± 0.046). Results of the analysis of SVL regression coefficients (Table 2) indicated a significant difference between group 1 (mean = .0295, SEM ± 0.003) and group 3 (mean = .0102, SEM ± 0.002) and between group 3 and group 4 (mean = .0269, SEM ± 0.003).

Discussion

Despite the death of five iguanas from groups 1 and 2 early on in the study, the most important comparison is between groups 3 (no UV, no D_3) and 4 (no UV, D_3) because this comparison directly addresses the question of the efficacy of dietary vitamin D_3 supplements in supporting the vitamin D_3 needs of green iguanas.

The growth curves for both mass and SVL (Figures 2 and 3 respectively), indicate groups 1, 2 and 4 grew throughout the study while group 3 did not. Interestingly, groups 1 and 4 (the groups receiving dietary vitamin D_3) grew the most. It appears that dietary vitamin D_3 elicited a growth advantage to groups 1 and 4 compared to that of groups 2 and 3. The significant differences between regression coefficients for groups 3 and 4 clearly indicates dietary vitamin D_3 utilization by group 4.

Unfortunately, scientifically-controlled research on the UV and vitamin D_3 requirements of the green iguana is greatly lacking. Thus far, the only extensive work in this area has been that of Allen and Bernard (Allen, 1988; Allen et al., 1989; Allen et al., 1995; Bernard, 1995; Bernard et al., 1991) who concluded that either green iguanas need extremely high levels of dietary vitamin D_3 (>3000 I.U./kg diet) or that the iguanas cannot utilize dietary vitamin D_3 at all and therefore must rely on a source of UV light for synthesis. This conclusion would have a significant impact on iguana husbandry: The use of so-called full-spectrum light bulbs could dramatically increase with devastating consequences. First, it is commonly thought that if iguanas cannot use dietary vitamin D_3 from vitamin supplements, then there is no need to give the vitamin supplement to the iguanas. This is dangerous because the other vitamins and minerals found in the supplement (e.g., vitamin A, vitamin B, calcium) are needed by the iguana. Second, and

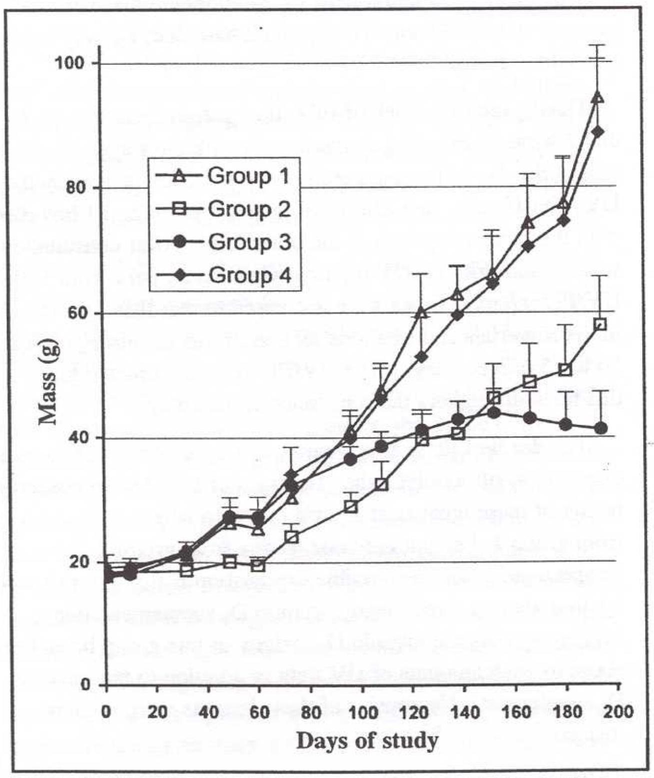

Figure 2. Growth curves for mean mass per group throughout the study. Sample sizes were: Group 1, n = 4; Group 2, n = 7; Group 3, n = 8; Group 4, n = 8. Error bars depict +1 SE.

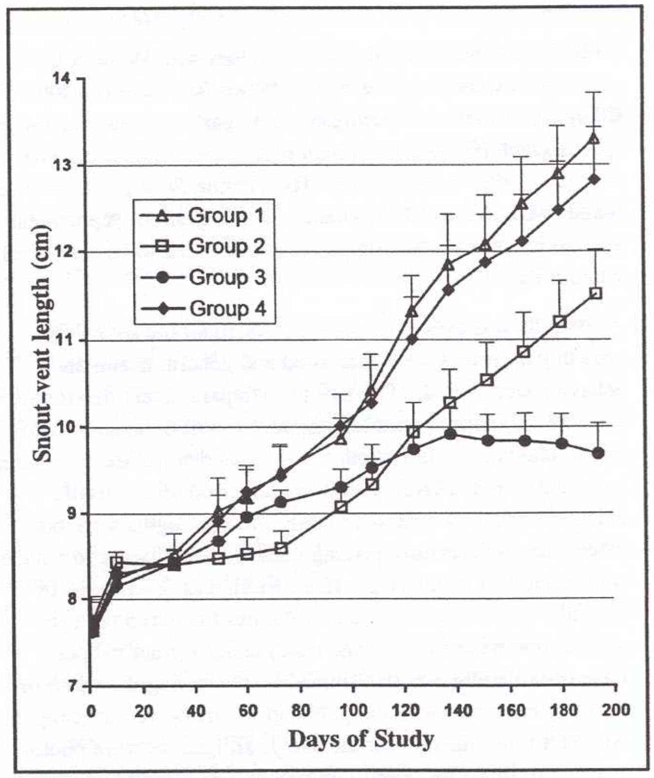

Figure 3. Growth curves for mean snout-vent length per group throughout the study. Sample sizes were: Group 1, n = 4; Group 2, n = 7; Group 3, n = 8; Group 4, n = 8. Error bars depict +1 SE.

Table 1. Linear regression lines and slopes (= rates of growth) for mass of the four groups. Note groups with same letters indicate significant differences in slope values (one-way ANOVA, $F(3,23) = 3.129$, $p < 0.001$, with Tukey's multiple comparison).

	Regression line	Slope ± SEM (g/day)
Group 1[a]	mass = 8.41 + .3946 × days	.3946 ± 0.043
Group 2[c]	mass = 11.24 + .2210 × days	.2210 ± 0.041
Group 3[ab]	mass = 19.34 + .1470 × days	.1470 ± 0.025
Group 4[b]	mass = 10.65 + .3796 × days	.3796 ± 0.046

Table 1. Linear regression lines and slopes (= rates of growth) for SVL of the four groups. Note groups with same letters indicate significant differences in slope values (one-way ANOVA, $F(3,23) = 10.990$, $p < 0.001$, with Tukey's multiple comparison).

	Regression line	Slope ± SEM (g/day)
Group 1[a]	SVL = 7.53 + .0295 × days	.0295 ± 0.003
Group 2[c]	SVL = 7.63 + .0188 × days	.0188 ± 0.003
Group 3[ab]	SVL = 8.20 + .0102 × days	.0102 ± 0.002
Group 4[b]	SVL = 7.63 + .0269 × days	.0269 ± 0.003

most important to this study, it is thought that if vitamin D_3 can not be supplied via dietary supplements, then a "full spectrum" light bulb must be used to stimulate the synthesis of vitamin D_3. As previously stated, the problem with these "full spectrum" bulbs is that their output is questionable with regards to their support of vitamin D_3 synthesis.

The results of this study clearly indicate dietary vitamin D_3 utilization by green iguanas. Consistently throughout the study, groups 1 and 4 were found to be the healthiest groups. Although group 1 received both UV light and dietary vitamin D_3, group 4 received only dietary vitamin D_3 indicating clearly dietary vitamin D_3 utilization by group 4. As mentioned earlier, the most important group comparison is that between groups 3 (no UV, no D_3) and group 4 (no UV, dietary D_3). The significant differences between groups 3 and 4 clearly indicate that dietary vitamin D_3 supplementation is an effective option for vitamin D_3 acquisition in green iguanas, at least for the first six months of their life.

While an argument could be made about the short time period of this study (six months), this is the best time frame to do this kind of work because young, rapidly growing juvenile iguanas have one of the greatest demands for calcium in their lifetime during this period, hence the high potential for calcium deficiency problems (i.e., MBD) (Mader, 1997).

Is full spectrum lighting absolutely necessary for the husbandry of green iguanas? Since green iguanas are obligate herbivores and therefore do not receive dietary vitamin D_3 in the wild, UV-induced vitamin D_3 synthesis is the "natural" source of vitamin D_3 for iguanas and therefore the preferred acquisition method. Because of the potential for dietary vitamin D_3 toxicity, the use of a safe, proven effective light source would be the best acquisition method of vitamin D_3 in captivity. Unfortunately, an effective light source is not currently available. The present research indicates that until there is such a light source available to the general public that has been thoroughly tested in controlled scientific studies, dietary vitamin D_3 can and should be used as an effective vitamin D_3 source. Full-spectrum lighting is not a necessity.

Research results looking at other growth and development factors such as general morphology and radiographic anatomy, serum vitamin D_3 and calcium levels, and bone ash analyses will be published at a later date. Further research is greatly needed to test the effectiveness of the various so-called "full spectrum" light bulbs at vitamin D_3 synthesis. Research is also needed to delineate just how much vitamin D_3 is needed by green iguanas in captivity.

Acknowledgments

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