Journal of Animal and Veterinary Advances 10 (2): 229-234, 2011
ISSN: 1680-5593

Serum Vitamin D Levels and Skeletal and General Development of Young Bearded Dragon Lizards (*Pogona vitticeps*), under Different Conditions of UV-B Radiation Exposure

[1,2,3]K.R. Kroenlein, [1]K.L. Zimmerman, [1]G. Saunders and [4]S.D. Holladay
[1]Department of Biomedical Sciences and Pathobiology, Virginia Maryland Regional College of Veterinary Medicine, Duckpond Drive, VA 24061, Blacksburg, Virginia
[2]Department of Anatomy and Radiology,
[3]Valley Animal Hospital, 24012, Roanoke, Virginia
[4]College of Veterinary Medicine, University of Georgia, GA 30602, Athens, Georgia, United States

Abstract: Vitamin D, synthesized in the skin secondary to UV-B radiation exposure is essential for bone formation and calcium metabolism in terrestrial vertebrates. Animals housed artificially indoors may be at risk for osteomalacia (older animals) or rickets (young animals) due to hyperparathyroidism from lack of natural sunlight, the usual source of UV-B radiation. The hypothesis of this study was that inland bearded dragon lizards, *Pogona vitticeps*, a commonly kept reptile pet, housed in enclosures with various artificial UV-B radiation sources would demonstrate increased bony changes and decreased overall health with decreasing amounts of UV-B radiation. About 25 captive-bred juvenile bearded dragons were housed in groups of 5 for 11 weeks with varying UV-B exposure levels provided by the following treatments: mercury vapor, full spectrum compact fluorescent, hard-quartz fluorescent tube, incandescent and natural sunlight. Contrary to the hypothesis, no significant differences were observed in weight, length or number of crickets consumed among the treatment groups. Serum vitamin D levels were significantly higher in the full spectrum compact fluorescent treatment which corresponded to the lowest UV-B radiation provided. All treatments demonstrated some degree of osteodystrophic changes upon bone histopathology, suggesting that none of the studied exposures may be adequate for optimal lizard health.

Key words: Hyperparathyroidism, inland bearded dragon, *Pogona vitticeps*, osteodystrophy, ultraviolet radiation, vitamin D

INTRODUCTION

Improper husbandry and inadequate nutrition are the two most common causes for illness in reptile pets (Ballard and Cheek, 2003). One of the most frequently overlooked requirements of some captive reptiles is UV-B radiation, a component of natural sunlight. UV-B radiation is essential for proper bone formation and calcium metabolism due to its involvement with the formation of physiologically active vitamin D (Holick *et al*., 1995). Vitamin D deficiency causes hypocalcemia due to inadequate calcium absorption across the intestines. The hypocalcemic state up-regulates the parathyroid gland to synthesize Parathyroid Hormone (PTH). Chronic hyperparathyroidism can cause potentially terminal osteomalaciain reptiles resulting in thin, brittle or misshapen bones (Frye, 1981).

Most captive reptiles are not exposed to direct sunlight, thus an artificial source is necessary to provide UV-B radiation. There are many UV-B lighting options available for purchase in pet shops and from distributors. Simple economy (price) most often drives the choice when all other known factors appear equal. However, different lights of differing cost supply different levels of UV-B radiation (Bagnall, 2004), the most important component for vitamin D synthesis in many reptiles (Ferguson *et al*., 2003).

Among large lizard species, UV-B requirement studies have almost exclusively focused on the green iguana (*Iguana iguana*). However, bearded dragons (*Pogona vitticeps*) recently replaced the green iguana as the most popular pet lizard (Bohme *et al*., 2009). Green iguanas are an arboreal, herbivorous species living in lush, green tropics ranging from northern Mexico, through, Central

Corresponding Author: S.D. Holladay, Department of Anatomy and Radiology, College of Veterinary Medicine, University of Georgia, GA 30602, Athens, Georgia, United States

America and into South America (Hatfield, 2002). Bearded dragons are a terrestrial, omnivorous species living in the arid regions of Australia (Ballard and Cheek, 2003). Due to these differences in natural habitats, diets and behaviors, the captive care of these two species differs. The purpose of this study was to investigate lighting differences in relation to skeletal and general development of young bearded dragons. Three commonly available UV-B light sources (mercury vapor, compact fluorescent and hard-quartz fluorescent), an incandescent lamp (complete absence of UV-B radiation) and natural sunlight exposure were utilized as the various lighting options. Growth rates, feeding rates, serum 25-OH-vitamin D levels and bone histopathology were recorded for these treatment groups. The hypothesis was that the bearded dragons housed in enclosures with the highest UV-B output would present with data most similar to bearded dragons maintained under natural sunlight exposure.

MATERIALS AND METHODS

Animals: Five treatment groups of 6 week old captive-bred bearded dragons, each containing 5 lizards were maintained for 11 weeks. Groups 1-4 were housed at the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM) and group 5 was housed in a protected outdoor environment offering a choice of shade or natural sunlight. Experiments described in this report were approved prior to their initiation by the Virginia Tech Institutional Animal Care and Use Committee (IACUC).

Housing: Laboratory enclosures consisted of 430-gallon, opaque plastic storage boxes (groups 1-4) and the outdoor enclosure was a 30 gallon 1/16 plastic mesh cage (group 5). The opaque enclosures were prepared by drilling ½ ventilation holes in the sides as well as holes cut in the enclosure lids as required for heating and lighting elements.

The mesh enclosure was a pre-fabricated reptile habitat constructed of plastic mesh and PVC pipe (Apogee Industries, 177 Telegraph Road, STE 584, Bellingham, Washington 98226; Reptarium). Interiors of the enclosures were matched as closely as possible. Each enclosure contained a wooden ramp and platform which would allow the bearded dragons to achieve a 10 basking distance from the sources of light and/or heat. Substrate consisted of ZooMed Laboratories, Inc. Vita-Sand®, a vitamin-fortified calcium carbonate substrate. Additionally, each enclosure contained an analog thermometer and plastic bowls for food and water.

Light/Heat sources: Laboratory enclosures were maintained at an ambient day/light temperature of 32.2°C (90°F) with a basking site temperature of 40.5-43.3°C (105-110°F) and night/dark ambient temperature of 26.6°C (80° F). Photoperiods were maintained at 14 h of light and 10 h of darkness. These conditions approximated the outdoor enclosure conditions. Light and heat were supplied to treatment group 1 with a ZooMed Laboratories, Inc. PowerSun® UV 100 W mercury vapor. Light was supplied to treatment group 2 with a 20 W full spectrum compact fluorescent bulb (Full Spectrum Solutions, Inc., P.O. Box 1087, Jackson, MI 49204, USA; Model PCF209155).

Light was supplied to treatment group 3 with a ZooMed Laboratories, Inc. Reptisun® (5.0) hard-quartz glass fluorescent tube light. Treatment groups 2 and 3 (fluorescents) were heat supplemented by 100 W ceramic heat elements. Light and heat were supplied to treatment group 4 with a standard 100 W incandescent bulb. The enclosure for group 5 was maintained outside in an area protected from wind and rain. The enclosure was relocated throughout the day to maintain a direct, bright basking site as well as a shaded area within the enclosure. UV-B radiation levels were measured using a digital ultraviolet radiometer (ZooMed Laboratories, 3650 Sacramento Drive, San Luis Obispo, CA 93401; Digital Ultraviolet Radiometer Item # ST-6).

A temperature rheostat with a 100 W ceramic heat element was added to group five to ensure a minimum ambient temperature of 32.2°C (90°F). Enclosures acclimated for 1 week prior to study onset.

Nutrition: The bearded dragons were fed twice daily for the first 4 weeks and then daily for the remainder of the study. The diet consisted of crickets and a vegetation mixture. The crickets were gut-loaded with a commercially available cricket diet (Fluker's Orange Cube Complete Cricket Diet, Fluker Laboratories, 1333 Plantation Avenue, Port Allen, LA 70767) for 2-3 days prior to feeding. The cricket diet contained brewers yeast, dried kelp and spirulina.

These diet constituents are dietary sources of vitamin D made available to the bearded dragons through these gut-loaded crickets. However, this does not present as a confounding factor for vitamin D3 analysis at the conclusion of the study as all the bearded dragons consumed this diet. Therefore, any variation in serum vitamin D3 levels between treatment groups can be attributed to the synthesis of vitamin D3 physiologically from UV-B exposure.

Cricket size did not exceed the average distance between the bearded dragon's eyes, a commonly used practice among bearded dragon breeders (Sommella, 2003). The vegetation mixture included: dandelion greens, mustard and collard greens, snap peas, green beans, acorn squash, pumpkin and small amounts of ripe fruit. Meal worms were offered once weekly. Rep-Cal® Juvenile Bearded Dragon Food (RepCal Research Lab, P.O. Box 727, Los Gatos, CA 95031) was also available daily but signs of consumption were not observed during the first 2 weeks of the study and thus offering the food was discontinued. This diet contained D-activated animal sterol as a source of vitamin D3 but as the diet was discontinued early in the course of the study, this source of vitamin D3 was considered inconsequential. Daily, the enclosures were misted with a fine-spray water bottle and the standing water was changed.

Weekly measurements: The following data collecting regimen occurred prior to any feeding of the bearded dragons. Dragons were sequentially removed, in arbitrary order, from each treatment group. Growth was recorded as body mass (g) and snout to vent length (mm). The individual bearded dragon was then placed in a five-gallon bucket with an as-needed continuous supply of crickets. The total number of crickets consumed by that lizard in 5 min was recorded. This value will be here after referred to as cricket consumption. Using the digital ultraviolet radiometer, UV radiation measurements were taken from the basking site of each enclosure approximately 40 cm from the light sources. In the case of the natural sunlight treatment group, the measurement was recorded between 11:00 am and 1:00 pm from the basking site.

Tissue collection: One lizard from each treatment group was arbitrarily selected and euthanized under the following protocol: the lizard was placed in an air tight container with an isoflurane (IsoFlo, 99.9% isoflurane, Abbott Animal Health) saturated cotton ball until unresponsive to handling; Ketamine hydrochloride (Ketaset, 100 mg mL^{-1}, Fort Dodge) was diluted to 1 mg 100 μL^{-1} and given IP at 100 μL 10 g^{-1} of body weight as an anesthetic overdose.

Blood was collected into sterile Eppendorf Micro Centrifuge tubes via jugular exsanguination and immediately placed in an ice bath at 0°C (32°F). Individuals were then eviscerated and placed in 4% buffered formalin. This protocol continued until all individuals had been euthanized. At this time, the blood samples were centrifuged. A micropipette was used to collect and transfer the serum supernatants to new sterile Eppendorf Micro Centrifuge tubes which were then refrigerated at 5°C (41°F).

Blood analysis: About 20 h post-collection, blood samples were analyzed using American Laboratory Products Company (ALPCO) Diagnostics 25-OH Vitamin D EPBA (Enzyme-based-Protein-Binding-Assay). This is a competitive protein binding assay test kit intended for the measurement of 25-hydroxy Vitamin D, the major circulating metabolite of vitamin D in the body. The assay is based on competition between 25-OH Vit D present in the sample with a 25-OH Vit D tracer in the assay, for the binding site of Vitamin D Binding Protein (VDBP, Gc-globulin). Varying levels of 25-OH Vit D result in degrees of yellow color within the wells of the test kit.

The intensity of the yellow color is indirectly proportional to the concentration of 25-OH Vit D in the sample. By plotting absorbance versus concentration of known standard concentrations and comparing the sample values to this curve, the concentration of 25-OH Vit D in the sample was quantified. Hereafter, 25-OH Vit D will be referred to simply as vitamin D.

Tissue analysis: Upon complete tissue fixation, the left femur was dissected from each individual and processed for histopathology. Each sample was interpreted by a board-certified veterinary pathologist and placed in one of four groupings based on abnormalities. Group 1 was defined as having normal bone structure. Group 2 was defined as having a thicker than normal hypertrophic zone of cartilage with decreased osteoidspicules. Group 3 was defined as similar to Group 2 in osteoid description but with a normal hypertrophic zone. Group 4 was defined as displaying severe osteopenia with a decreased amount of osteoidspicules and an extended and/or irregular zone of hypertrophy.

RESULTS AND DISCUSSION

UVB levels: Radiation levels remained constant throughout the study period, with the exception of the natural sunlight treatment group and are as follows: mercury vapor bulb provided 17 uW cm^{-2}; full spectrum compact fluorescent bulb, 2 uW cm^{-2}; hard-quartz fluorescent tube, 16 uW cm^{-2}; incandescent, 0 uW cm^{-2}; and natural sunlight, mean 19.1 uW cm^{-2}, range 2-35 uW cm^{-2}.

Weight, length and cricket consumption: A one-way Analysis of Variance (ANOVA) was utilized to test the equality of the means between each treatment group on specific sampling dates as well as within each treatment group over the duration of the study. First, variance analysis was performed for each sampling date to determine differences between treatment groups on specific dates. No significant differences in mean weight, mean length or mean cricket consumption were recorded between treatment groups on specific sampling dates.

Table 1: Variance analysis (one-way ANOVA) of weight and length within treatment groups over the study duration

Treatment	F	df	p-value
Mercury vapor			
Weight (g)	3.66	43	0.003
Length (mm)	2.66	43	0.019
Compact fluorescent			
Weight (g)	6.79	41	<0.001
Length (mm)	9.48	41	<0.001
Hard-quartz tube			
Weight (g)	1.62	46	0.146
Length (mm)	1.69	46	0.126
Incandescent			
Weight (g)	28.13	33	<0.001
Length (mm)	42.29	33	<0.001
Natural sunlight			
Weight (g)	2.08	46	0.057
Length (mm)	4.44	46	0.001

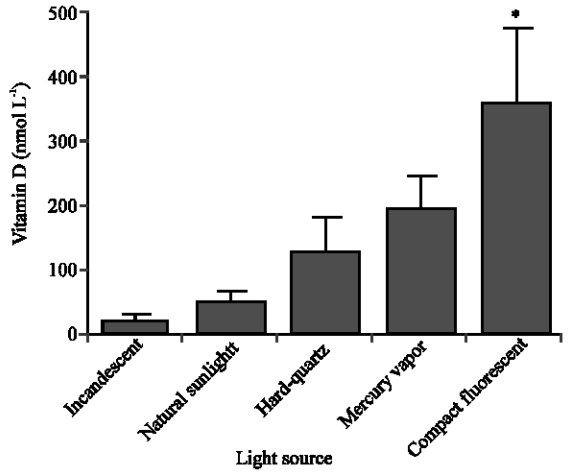

Fig. 1: Serum vitamin D concentration and type of light exposure

Second, variance analysis within each treatment group over time was performed to determine if mean differences existed in weight and length between specific sampling dates. This analysis represents a nonspecific demonstration of growth, length and weight, within each treatment group over the duration of the study. All treatment groups varied significantly in both mean weight and mean length over the duration of the study with the exceptions of weight in the natural sunlight group and both weight and length in the hard-quartz glass fluorescent tube light treatment group Table 1.

Vitamin D and bone histopathology: An analysis of variance showed a significant difference in mean vitamin D levels between the treatment groups on the sacrifice date (one-way ANOVA, F = 4.78, df = 18, P = 0.012; (Fig. 1). A χ^2-test using the histopathology grading scale of the femurs showed no significant differences between treatment groups. All groups showed some degree of osteodystrophy (Table 2).

Table 2: Mean bone histopathology scores

Treatment	Bone grade (1-4)	SD
Mercury vapor	2.50	1.00
Compact fluorescent	2.25	0.96
Hard quartz tube	2.00	0.82
Incandescent	2.67	0.58
Natural sunlight	3.25	0.96

The weight, length and cricket consumption data from the present bearded dragons do not indicate any significant differences between the lighting options. This outcome is similar to results of a 15 month study of chuckwalla lizards, *Sauromalu sobesus*, housed under two different light treatments (Aucone *et al*., 2003). Chuckwalla lizards are native to the south-western deserts of North America, a habitat similar to that of bearded dragon lizards in Australia. The light treatments consisted of one group exposed to an incandescent and fluorescent light source and a second group with mercury vapor lamp exposure. Although, no significant differences were seen in growth rates, differences in vitamin D levels were observed between the two chuckwalla groups.

The mercury vapor lamp treatment group expressed higher levels of vitamin D, similar to levels found in wild chuckwalla lizards which the study concurrently evaluated. The study on chuckwalla lizards, however, did not examine full spectrum compact fluorescent bulbs and it is this treatment that merits further discussion based on results from the present bearded dragon lizards.

The highest and only significantly different level of vitamin D we observed in lizards was in the full spectrum compact fluorescent treatment group. This bulb, however, provided the least amount of UV-B radiation (2 uW cm^{-2}), with the exception of the incandescent bulb that provided no UV-B radiation. Although, not statistically different, the trend of vitamin D levels for the treatment groups, from highest to lowest was as follows: compact fluorescent, mercury vapor, hard-quartz fluorescent tube, natural sunlight and incandescent. It might be expected that differences in bone histopathology may exist with varying levels of serum vitamin D. This, however was not the case, as all the bearded dragons demonstrated some degree of osteodystrophy. Metabolic bone disease can be divided into three general categories: osteoporosis, osteomalacia and osteopetrosis. Of these three disorders, reptiles are most prone to osteomalacia (therefore, it is to this category that the bearded dragon bone histopathology was compared.

Osteomalacia is divided into two basic subcategories in reptiles, rickets and fibrous osteodystrophy (Frye, 1981). Rickets typically results from a deficiency of vitamin D during early growth. This etiology seems appropriate for these bearded dragons which were in a high growth state throughout the study. However, with

rickets there is no deficiency in osteoid formation but rather an excess in osteoid formation. Despite the possibility of vitamin D deficiency in these growing bearded dragons, excess osteoid was not seen in these bone sections.

The second subcategory, fibrous osteodystrophy is commonly seen in reptiles. This condition is caused by prolonged and excessive Parathyroid Hormone (PTH) stimulation of the bone matrix. PTH affects the rate of transfer of calcium into and out of bone, resorption in the kidneys and indirectly increases absorption from the gastrointestinal tract. The mechanism by which it increases GI absorption is by regulating the synthesis of the active metabolite of vitamin D (vitamin D3). Excessive PTH can result from primary hyperparathyroidism, a parathyroid tumor causing increased PTH production or more commonly from secondary hyperparathyroidism. Secondary hyperparathyroidism occurs due to the physiological conditions of hypocalcemia, hyperphosphatemia or vitamin D deficiency (Liu, 2002).

PTH synthesis is regulated by a negative feedback mechanism primarily involving serum calcium levels. In the case of hypocalcemia, PTH synthesis is up-regulated to increase calcium mobilization from the bone, increase resorption in the kidneys and increase absorption from the GI tract. Hypophosphatemia has similar effects. Calcium and phosphorus ratios are tightly associated, therefore, an excess of phosphorus is interpreted by the body as an insufficiency of calcium and PTH synthesis is increased. Vitamin D deficiency can cause fibrous osteodystrophy via secondary hyperparathyroidism by decreasing the amount of calcium absorbed by the GI tract, thereby, decreasing serum calcium levels. The histopathology of fibrous osteodystrophy resulting from excessive PTH systhesis is as follows: increased osteoclasts, thinned cortical bone and increased osteoblast activity. The osteoblasts fill the marrow spaces of the affected areas with a fibrovascular tissue (Cotran *et al*., 1999). These signs were evaluated for but not observed in the bearded dragon bone sections.

The present lizards displayed a bone disorder throughout all of the treatment groups that does not fall squarely into previously described conditions. The fact that the compact fluorescent treatment group had the highest vitamin D level along with bone changes suggests that these changes may not be due to a deficiency in vitamin D but rather a nutritional deficiency or GI malfunction in calcium absorption; the most common in reptiles being hyerphosphatemia or an improper calcium/phosphorus ratio (Frye, 1981). However, if this were the case, one would expect to see the least amount of bony changes in the compact fluorescent group, as the higher vitamin D levels would enable them to absorb larger amounts of calcium from the GI tract.

Again, this was not the case. Another, unexpected result was the condition of the bearded dragons maintained under natural sunlight. We expected this group to provide normal values of vitamin D and bone morphology but such was not the case. However, the number of variables to which this group may have been exposed is numerous despite all efforts to maintain a controlled environment. Regardless of the cause, the results suggest that the artificial light sources we evaluated may be inadequate in preventing osteodystrophies. This is in agreement with a previous study on captive Fijian iguanas, *Brachylophus fasciatus* and *Brachylophus vitiensis* (Laing *et al*., 2001). Although, bearded dragons and iguanas are very different species, as described earlier, the possibility of some basic level of UV-B radiation requirements in both species cannot be ignored.

Future studies are required to determine the causes of observations made during this study. Possible extensions from this study could include: larger study populations, increased duration of study, serum vitamin D levels of healthy captive adults or multiple groups housed with compact fluorescent bulbs and varying calcium supplements to the diet. The size of studied lizards alsoshould be increased to ensure that adequate serum samples can be obtained for ionized calcium levels. Ionized calcium is the preferred analysis in reptiles as it represents a more clinically relevant and physiologically active form of calcium in the blood (Dennis *et al*., 2001). Data on calcium levels within the blood could be a missing key in better interpreting results of this study. Another less accurate option for calcium determinations would be collection and analysis of excreta throughout the study (Van der Wardt *et al*., 1999). These additional studies are neededto better support bearded dragon husbandry. In particular if current artificial light options are not adequate to maintain the health of captive bearded dragons then current husbandry techniques may need to be re-evaluated.

CONCLUSION

Exotic and reptile medicine and husbandry techniques historically have been a concern of zoological parks and enthusiasts alone. With the increased popularity of new exotic pets, more research needs to be dedicated to the health needs of these animals. UV-B radiation which is normally provided by the sun is of particular concern for species constantly maintained indoors. Although, the present study on bearded dragons does not point to a

best solution for the UV-B requirements of this species, this study strongly suggests that further research needs to be conducted on the currently available options. All study subjects demonstrated osteodystrophy to some degree, despite the fact that the pattern of bony changes did not fall into a previously described condition. Serum vitamin D levels varied for one of the treatment groups.

Nutritional sources would be the other route for increased vitamin D levels but all study groups were maintained on the same diet which was intended to contain adequate vitamin D. Perhaps some difference in light quality exists between the chosen light options beyond the UV-B radiation measured. The health and longevity of bearded dragons and other reptiles is dependent on the conditions of their care. Expansions on this study are necessary to ensure the best and most accurate information on these lizards.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Geraldine R. Dodge Foundation. We also thank ZooMed Laboratories, Inc. for providing supplies used within this study.

REFERENCES

Aucone, B.M., W.H. Gehrmann, G.W. Ferguson, T.C. Chen and M.F. Holick, 2003. Comparison of two artificial ultraviolet light sources used for Chuckwalla, *Sauromalusobesus*, husbandry. J. Herpetol. Med. Surg., 13: 14-17.

Bagnall, G., 2004. Making sense of reptile lighting. Reptiles, 12: 28-34.

Ballard, B. and R. Cheek, 2003. Exotic Animal Medicine for the Veterinary Technician. Iowa State Press, Iowa, pp: 31-43.

Bohme, H., A. Fruth, F. Rebmann, D. Sontheimer and W. Rabsch, 2009. Reptile-associated Salmonellosis in a breastfed infant. Klin Padiatr., 221: 74-75.

Cotran, R., S. Kumar, V. Collins and T. Robbins, 1999. Pathologic Basis of Disease. WB. Saunders Co., Philadelphia PA. USA., pp: 1228-1229.

Dennis P.M., R.A. Bennett, K.E. Harr and B.A. Lock, 2001. Plasma concentration of ionized calcium in healthy iguanas. J. Am. Vet. Med. Assoc., 219: 1093-1093.

Ferguson, G.W., W.H. Gehrmann, K.B. Karsten, S.H. Hammack and M. McRae *et al*., 2003. Do panther chameleons bask to regulate endogenous vitamin D3 production. Phyiol. Biochem. Zool., 76: 52-59.

Frye, F.L., 1981. Biomedical and Surgical Aspects of Captive Reptile Husbandry. Veterinary Medicine Publishing Company, Kansas, pp:19-40.

Hatfield, J.W., 2002. Green and sometimes mean. Reptiles, 10: 48-65.

Holick, M.F., X.Q. Tian and M. Allen, 1995. Evolutionary importance for the membrane enhancement of the production of vitamin D3 in the skin of poikilothermic animals. Proc. Natl. Acad. Sci, USA., 92: 3124-3126.

Laing, C.J., A. Trube, G.M. Shea and D.R. Fraser, 2001. The requirement for natural sunlight to prevent vitamin D deficiency in iguanian lizards. J. Zoo Wildlife Med., 32: 342-348.

Liu, S.K., 2002. Metabolic diseases in animals. Semin. Musculoskelet. Radiol., 6: 341-346.

Sommella, T., 2003. Beardie basics. Reptiles, 11: 34-43.

Van der Wardt, S.T., M.J. Kik, P.S. Klaver, M. Janse and A.C. Beynen, 1999. Calcium balance in Drakensberg crag lizards (*Pseudocordylusmelanotusmelanotus Cordylidae*). J. Zoo Wildlife Med., 30: 541-544.