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Nutrition research on calcium homeostasis. I. Lizards (with recommendations)

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This review presents information from a decade of nutrition research into calcium homeostasis in lizards, including non-nutritive factors essential to lizard nutrition; for example, ontogenetic dietary shift, microbial fermentation, environmental temperature, ultraviolet light and photoreception. Recommendations are made for possible nutrition research to be carried out in the future. These recommendations may be essential for the survival of lizards in captivity and include minimal vitamin and mineral supplementation, standardized ultraviolet light research, species requirements for spectral radiance, normative and pathological plasma indices, the relationship of gut transit time to diet, normative indices for growth and nutritional requirements, and development of an accurate method for identifying animals with, or at risk from, metabolic bone disease.

Key-words: calcium homeostasis, clinical nutrition, lizard, metabolic bone disease, nutrition, vitamin A, vitamin D

Calcium (Ca) metabolism and metabolic bone disease (MBD) have been studied in reptiles in captivity for at least four decades (Truitt, 1962; Reichenbach-Klinke & Elkan, 1965), however, crucial information about Ca metabolism in reptilian species is still lacking (McWilliams & Leeson, 2001). The pathogenesis of MBD in reptiles begins with nutritional and environmental factors that create a plasma Ca deficit causing parathyroid-gland stimulation of osteoclasts. Osteoclasts absorb bone tissue to increase plasma Ca concentrations and prolonged osteoclast activity reduces bone density and causes secondary disease.

The disruption in Ca homeostasis (MBD) negatively affects the skeletal health of lizards by preventing the formation of bone density in juveniles or by impairing bone maintenance with loss of bone density in adults (Raisz, 1999). Clinical symptoms of MBD are similar in most species and include weakness, gastrointestinal tract (GIT) dysfunction, stunted growth, neurological dysfunction (tetany, tremors and/or convulsions) and paralysis from fractured vertebrae and skeletal deformities (Burgmann *et al.,* 1993; Barten, 1996; Bennett, 1996; Boyer, 1996). Stunted growth and retention of the rounded infantile skull shape of hatchlings result from a decrease in anterior pituitary hormones [e.g. thyroid stimulating hormone (growth hormone), TSH] because of chronic plasma Ca deficits.

Non-nutritive factors essential to lizard nutrition are also discussed in this review, including ontogenetic dietary shift, microbial fermentation, environmental temperature, ultraviolet (UV) light and photoreception.

NON-NUTRITIVE FACTORS IN LIZARD NUTRITION

Ontogenetic dietary shift Lizard species are primarily either carnivorous or herbivorous but many are classed as omnivores either because their dietary needs change at a life stage or because their diets include both plants and animals. For example, most adult herbivorous lizard species have an ontogenetic dietary shift from juvenile carnivory in their second year (Auffenberg, 1995). A physiological change affecting diet that correlates with an ontogenetic shift is observed in the teeth. Lizards may have either homodont or heterodont dentition. A juvenile Gray's monitor *Varanus olivaceus* eats an invertebrate diet with homodontic teeth (all teeth are the same shape but may vary in size) that are sharp and suited to cutting through exoskeleton. When these lizards are approaching adulthood they begin to include plants in their diet and, at that time, they develop heterodontic teeth (a variety of teeth differentiated by function).

Microbial fermentation Some iguanid and agamid species utilize microbial fermentation to digest plant materials (Mackie *et al.,* 1999). Microbial fermentation, for these animals, also produces volatile fatty acids (VFAs) that are needed as a cellular energy source. Hatchling lizards of such species may need to consume the faeces of adult conspecifics to establish gut microbial colonies (Brice, 1995).

Microbial fermentation in herbivorous iguanas takes *c.* 5 days and this implies that a balanced diet must be consumed over a period of days (Brice, 1995). This process also suggests that interruption of food consumption by a few days may result in an additional 5 day delay before the nutrients are available for absorption.

Digestion of food for lizard species that utilize microbial fermentation is most efficient at higher environmental temperatures (Brice, 1995; Divers, 1995). For example, efficient GIT function in Green iguanas *Iguana iguana* occurs when environmental temperatures are 29–39·5°C and the optimum temperature range for maintaining microbial fermentation is 35–38°C. Ambient temperatures below 29°C disrupt both the composition and the activity of microbial populations.

Environmental temperature In addition to microbial fermentation, lizards are dependent on the ambient temperature for optimal functioning of other physiological processes, such as thermoregulation, digestion and vitamin D metabolism. Many lizard species are shuttling heliotherms and thermoregulate by moving from the sun to shade/shade to sun. Green anoles *Anolis carolinensis,* for example, use extensive sun basking and may spend as much as 92% of their time basking in cooler seasons (Cope *et al.,* 2001). To facilitate digestion, Green iguanas were observed to bask first, in order to increase metabolic function, then eat (at *c.* 1100–1600 hours) and then bask again (Divers, 1995). The efficient conversion of previtamin D_3 to vitamin D_3 is an environmental temperature-dependent process because the isomerization of previtamin D to vitamin D_3 is a thermal process. Previtamin D_3 conversion to vitamin D_3 in the skin of Green iguanas during *in vitro* incubation required 72 hours at 5°C compared to 8 hours at 25°C (Holick *et al.,* 1995).

Ultraviolet light The spectral composition of light is important for lizards for processes that include thermoregulation and GIT function (Tosini & Avery, 1996). Ultraviolet light includes UVA wavelengths (320–380 nm) and UVB wavelengths (290–320 nm). Light absorption by biological cells and tissues is wavelength dependent and direct absorption is by endogenous pigments (melanin, haemoglobin, carotene, keratin). Some exogenous pigments (e.g. ingested melanin) may also increase UV light absorption. Green iguanas ingesting an exogenous hormone (melatonin) increased metabolic rate (Tosini & Menaker, 1998).

UVA (black light) stimulates not only the appetite of reptiles but also agonistic, reproductive, social and territorial behaviours (Boyer, 1996; Gehrmann, 1996). A requirement for UVA for reptiles in captivity has not been proven and the growth of ♀ Panther chameleons *Chamaeleo pardalis* was stunted when they were exposed to UVA (Gehrmann, 1996).

UVB is important for Ca homeostasis in lizards and for ultraviolet vision (Bidmon & Stumpf, 1995). The integument produces vitamin D_3 (cholecalciferol) when UVB light irradiates 7-dehydrocholesterol (provitamin D_3) (Bernard & Ullrey, 1995; Bidmon & Stumpf, 1995; Dierenfeld & Barker, 1995; Gascon *et al.,* 1995; Holick *et al.,* 1995; Frye, 1997; Laing & Fraser, 1999; Ullrey & Bernard, 1999). Green iguanas housed in appropriate environmental temperatures could not absorb dietary Ca without UVB exposure and developed hypovitaminosis D despite receiving 3000 IU/kg (international units per kilogram) vitamin D_3 (Allen *et al.,* 1993; Oftedal & Allen, 1996). A study comparing plasma vitamin D_3 of Komodo dragons *Varanus komodoensis* with and without daily exposure to UVB found that the plasma vitamin D_3 values in 75% of wild and captive Komodo dragons with daily exposure to UVB were 150–250 nmol/litre (Gillespie *et al.,* 2000). However, Komodo dragons in captivity that did not have daily exposure to UVB had plasma vitamin D_3 below 100 nmol/litre. Komodo dragons and Green iguanas without access to UVB were unable to use dietary sources of vitamin D_3 (Bernard & Ullrey, 1995). After repeated exposure to UVB light, vitamin D_3 in the plasma of these animals increased and skeletal fractures healed.

There may be species differences to UVB-associated metabolism. Mediterranean geckos *Hemidactylus turcicus,* a nocturnal lizard species, appear to have an increased integument ability to absorb UVB as an adaptive mechanism for limited exposure to UVB (Carman *et al.,* 2000). Skin samples of *H. turcicus* produced vitamin D_3 in higher quantities than a diurnal species, the Texas spiny lizard *Sceloporus olivaceus,* despite similar concentrations of provitamin D_3 (Carman *et al.,* 2000).

In a 5 month-long study, Green iguanas injected with equivalents of 2000 IU/kg dietary cholecalciferol had lower plasma 25-hydroxyvitamin D_3 than iguanas exposed to UV light (35% Sylvania 2096 phosphor experimental lamp or 100% Sylvania phosphor experimental lamp) at distances of 5–70 cm (Bernard *et al.,* 1996). Lack of shade provision in this study may be the source of some pathologic 25-hydroxyvitamin D_3 plasma levels (as much as 1200 ng/ml) that may be attributed to a constant 12 hour UV light exposure under a 100% phosphor. Heliothermic lizard species, in general, need exposure to UVB light for 10–14 hours daily (Divers, 1996; Korber, 1997). The GE Chroma 50 (General Electric Lighting) appears to emit sufficient UVB to promote vitamin D_3 biosynthesis in healthy Green iguanas if the lamp is within 48 cm and the animals are exposed for 12 hours per day (Bernard, 1997).

Full-spectrum lamps are not comparable to natural light and usually do not have UVB wavelengths (Gehrmann, 1992). Full-spectrum lamps, even when they emit UV wavelengths, will not attract lizards in captivity if the lamp is not paired with radiant heat. Spiny-tailed iguanas *Oplurus cuvieri* preferred light sources that were also thermal sources independent of the substrate and spectral qualities of the light (Dickinson & Fa, 1997).

There is some research that does not support the necessity of UVB exposure for

Ca homeostasis in lizards. Eyed skinks *Chalcides ocellatus* and Leopard geckos *Eublepharis macularius* developed normally with a dietary source of vitamin D_3 but without exposure to UVB light (Allen *et al.,* 1994).

Exposure to UV light also controls integumentary disease and parasites, usually by causing cellular death, cellular mutation and/or deoxyribonucleic acid (DNA) damage (Coohill, 1995; Gascon *et al.,* 1995). For example, *Pseudomonas aeruginosa* is a soil and aquatic microorganism that is killed by exposure to UVB (Degiorigi *et al.,* 1996). UVB-induced bacterial killing depends on other environmental conditions including growth media and UVA.

Lizards from temperate climates are exposed to daily variation in environmental temperatures and infrared irradiances and they behaviourally thermoregulate by moving from the sun to shade/shade to sun (Tosini *et al.,* 1995). However, in captivity enclosures are usually furnished with permanent stationary heat and light sources that limit choice and encourage inactivity, contributing to the development of MBD.

Photoreception In lizards appetitive behaviours and physiological processes are dependent on photoreception. Many lizards are sight feeders and photoreception is important for finding prey, plants and other sources of food. The pineal gland (also some non-sensory cells, cytoplasm and the integument) is a photoreceptor in lizards and entrains endogenous rhythms with exogenous cycles to establish circadian rhythms (Tosini, 1997; Tosini & Menaker, 1998).

CALCIUM METABOLISM

Dietary factors, such as oxalates, protein and fat, can interfere with dietary Ca absorption in lizards. Oxalates are a salt of oxalic acid and are present in spinach, rhubarb, cabbage, peas, potatoes and beet greens (Brice, 1995; Donoghue & Langenberg, 1996). In Green iguanas oxalates inhibit Ca absorption by binding to it, although oxalates in spinach may only limit Ca absorption if Ca intake is marginal (Donoghue, 1995). Deficiencies in dietary protein (hypoproteinaemia) can interfere with Ca absorption because Ca is protein-bound (Boyer, 1996). For example, in the Bearded dragon *Pogona barbata,* vitamin D-binding protein (DBP) influenced the biosynthesis of vitamin D_3 (Laing & Fraser, 1999). DBP binds with 25-hydroxylated metabolites of vitamin D (biologically available D_3) and facilitates blood circulation of the metabolites. Ca supplements and feeding a high-fat diet can allow fat–calcium soaps to form in the digestive tract and these interfere with the digestion and absorption of Ca (Donoghue & Langenberg, 1996).

Ca dietary supplementing (finely ground oyster shell) for Green iguanas with chronic vitamin D deficiencies did not improve their nutritional state (Oftedal *et al.,* 1997) and Green iguanas with vitamin D deficiencies did not ingest Ca if it was offered separately from the food ration. Drakensberg Crag lizards *Pseudocordylus melanotus melanotus* appeared to maintain Ca homeostasis with 1·4–5·8% Ca dry matter (DM) of oral administration of Ca carbonate (Van der Wardt *et al.,* 1999).

Symptoms of hypocalcaemia in lizard species include weakness, hypotension, GIT and cardiac dysfunction, tetany and seizures (Barten, 1996; Bennett, 1996; Boyer, 1996). Hypocalcaemia is a factor in dystocia and retained eggs can cause death (DeNardo, 1996). Non-dietary factors in dystocia include lack of a proper nesting site, improper environmental temperature, dehydration and poor muscle tone as a result of inactivity (DeNardo, 1996; Korber, 1997).

Hypercalcaemia can be either a primary disease in reptiles caused by iatrogenic hypervitaminosis D or a secondary disease caused by renal failure, osteolytic lesions and immobilization. Clinical symptoms of hypercalcaemia include fatigue, weakness, anorexia, abdominal pain, osteoporosis and soft-tissue calcification. Hypercalcaemia can also result from egg resorption. In iguanas egg resorption is an adaptive mechanism for recycling the protein, fat and Ca of an egg. The Ca in two to three eggs can represent 40–100% of a ♀'s skeletal stores. Resorption of the eggs elevates plasma Ca and the lizard may test hypercalcaemic (Allen *et al.,* 1993; Frye, 1997).

In some lizard species endolymphatic sacs may also be important in Ca homeostasis because they contain Ca deposits. For example, the endolymphatic sacs of Fox geckos *Hemidactylus garnotii* reduce in size after egg formation or egg laying and this reduction may indicate Ca mobilization for egg-shell formation (Allen *et al.,* 1993).

VITAMIN A METABOLISM

Vitamin A (retinol) requirements of most reptiles are unknown and lack of information often results in hypervitaminosis A as an iatrogenic disease in lizards in captivity (Dierenfeld & Barker, 1995; Boyer, 1996). Clinical symptoms of hypervitaminosis A include erythema, skin sloughing, depression, anorexia, vomiting, soft-tissue calcification and bone lesions (Donoghue, 1995; Donoghue & Langenberg, 1996). Iatrogenic hypervitaminosis A may be most common in insectivorous lizard species which may not require vitamin A supplementation because they can convert insect carotenoids (e.g. betacarotene or provitamin A) to a biologically active form of vitamin A (Dierenfeld & Barker, 1995).

VITAMIN D METABOLISM

As in avian and mammalian species, the Green anole has high-affinity receptor sites for 1,25-dihydroxy-vitamin D_3 (1,25-D_3 or cholecalciferol) in chondrocytes (Bidmon & Stumpf, 1995). Vitamin D_3 is important for the growth and differentiation of cartilage, the elevation of plasma Ca and P (phosphorus), and it stimulates the active transport process of Ca and P from the small intestine to plasma (Holick *et al.,* 1995). Research in vitamin D_3 (1,25-D_3) metabolism in mammals also indicates it may be an immunomodulator (Lemire, 1992; Bidmon & Stumpf, 1995). Vitamin D may have a role in embryogenesis in some lizard species. The yolks of Green iguana and Bearded dragon eggs contained vitamin D_3 concentrations similar to those detected in the eggs of domestic chickens *Gallus gallus* (Laing & Fraser, 1999).

Hypervitaminosis D is usually iatrogenic, brought about by incorrect supplementation, and can cause renal failure and soft-tissue mineralization (Richman *et al.,* 1995; Boyer, 1996; Frye, 1997). Calcinogenic plants may be more appropriate for herbivorous lizard species than vitamin D supplementation. For example, rats *Rattus rattus* and chicks fed a vitamin D-deficient diet had calciferol-like activity when fed *Cestrum diurnum* leaf powder at 2% DM. In addition, intestinal Ca transport was increased, plasma Ca reached normal values and bone density increased (Prema & Raghuramulu, 1993, 1994).

DISCUSSION

The past decade of research in lizard nutrition related to calcium homeostasis has a greater emphasis on quantified inquiry. However, peer criticism within this decade indicates that reptile nutrition '... is an art, not a science' (Kollias & Gentz, 1996) and most of the information about reptile nutrition is based on observation, not quantification (Carman *et al.,* 2000).

Two caveats in utilizing existing, quantified lizard nutrition research on Ca homeostasis are (1) the limited range of the studies and (2) the limited variety of species that have been studied. The limited range focuses on those dietary and environmental factors that can be manipulated to reduce clinical disease: that is, those observed symptoms that have been identified as disease indicators. In other words, nutrition research in lizard species has been reactive, not investigative, resulting in an incomplete knowledge base about the nutritional pathology of these species. The incomplete knowledge base is further reduced by sparse normative data: it may be possible to diagnose confidently when a lizard is unwell but identifying a healthy animal creates some ambivalence. Research has been limited to studies on a few common lizard species, such as Green iguanas and Green anoles, resulting in a lack of comparative studies and creating a disproportionate reliance on extrapolation. In the past decade research has been carried out for other lizard species (*Sceloporus* spp, Eyed skinks, Komodo dragons and Spiny-tailed iguanas, to name a few), and in order to augment the expertise with lizards more species must be investigated.

A barrier to progressive lizard nutrition research is the limited funds available for zoological research. Judicious decisions about the use of these limited funds must include factors that eliminate many lizard species as timely and important research subjects (McWilliams, 2001). Smaller lizard species may not engender as much conservation interest nor be as attractive to visitors as mammal species. As a result, the research history on nutrition in lizard species is erratic and, at times, dwindling. This review of nutrition research relative to calcium homeostasis in lizard species must be read and understood within these limits.

Research reported in this paper offers valuable insight into lizard nutrition relevant to Ca homeostasis and outlines the need for future research into lizard nutrition.

1. *Vitamin and mineral supplementation.* Many known health problems in lizard species in captivity result from excessive vitamin and mineral supplementation of Ca, vitamin A and vitamin D. More research is needed to determine the most appropriate products to use and minimal supplementation levels, in order to avoid common nutritional pathologies, such as soft-tissue mineralization (Dierenfeld & Barker, 1995).

2. *Ultraviolet light.* There is an apparent need for lizard species to have a biosynthesized source of vitamin D_3 from UVB light. Research is needed to (a) establish effective, standardized UVB light replication (Ball, 1995; Bernard & Ullrey, 1995), (b) investigate the role of UV light in gustatory behaviours, biosynthesis and thermoregulation (Tosini & Avery, 1996) and (c) study and report on the correlated colour temperature (CCT) and the colour rendering index (CRI).

3. *Spectral radiance.* Spectral radiance is a measure of light forming the background against which objects are seen and it can be specific for some species. For example, Gundlach's anole *Anolis gundlachi,* Crested anole *Anolis cristatellus,* Krug's anole *Anolis krugi* and Puerto Rican anole *Anolis pulchellus* all showed a preference for a spectral radiance of 350–700 nm (Fleishman *et al.,* 1997). Research is needed on species differences and preferences in relation to light perception.

4. *Plasma indices.* Nutrition research in lizards often uses plasma indices to verify the effects of dietary levels of nutrients on physiology without correlating these measures to other factors. For example, plasma biochemistry and lymphocyte counts in reptiles can vary with health status, ambient temperatures, season, age and sex (Boyer, 1996; Divers *et al.,* 1996; Klingenberg, 1996). Research is needed on indices in specific conditions and life stages with an emphasis on normative indices.

5. *Gut transit time.* Green iguanas do not chew their food and larger particle sizes may mean longer gut transit time (GTT) (Frye, 1997). Large amounts of fresh food eaten by Green iguanas had faster GTTs than smaller amounts (Marken-Lichtenbelt, 1992). Iguanas fed between 1100 and 1400 hours after basking had faster GTT and more efficient digestion than when they were fed at other times (Divers, 1995; Barten, 1996; Frye, 1997). More research is needed on GTT in relation to nutrient absorption, clinical nutrition and microbial fermentation.

6. *Ontogeny.* There are not sufficient normative data on growth indices and nutritional requirements for life stages and reproductive stages for many lizard species. More research is needed in these areas, especially research into species with ontogenetic shifts.

7. *Metabolic bone disease.* Radiographs did not identify osteodystrophic animals despite bone density losses of 5·22% of the body mass composition (Allen *et al.,* 1993). Research is needed to identify a method to accurately and definitely diagnose those animals at risk from, or those which have developed, MBD.

PRODUCT MENTIONED IN THE TEXT

GE Chroma 50: light bulb, manufactured by General Electric Lighting, Cleveland, OH 44112, USA.

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Nutrition research on calcium homeostasis. II. Freshwater turtles (with recommendations)

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This review reports on a decade of nutrition research into calcium (Ca) homeostasis in freshwater turtles, including research on non-nutritive factors that are essential for Ca homeostasis [i.e. ontogeny, environmental temperature and humidity, and ultraviolet (UV) light and photoreception]. Recommendations for future research include long-term research programmes in three specific areas: (1) photoreception, UV light and biosynthesis, (2) Ca homeostasis and vitamin and mineral supplementation, and (3) developmental indices, gut transit time (GTT) and energy requirements.

Key-words: calcium homeostasis, clinical nutrition, freshwater turtles, metabolic bone disease, nutrition, vitamin A, vitamin D

Despite at least four decades of research (Truitt, 1962; Reichenbach-Klinke & Elkan, 1965) metabolic bone disease (MBD) is the prevalent nutritional pathology in reptilian populations in captivity (McWilliams, unpubl.). MBD results from either nutritional deficiencies or excesses of calcium (Ca), vitamin A and vitamin D that disrupt Ca homeostasis. The shell of a turtle is skeletal (mineralized components) fused with connective tissue. Shell and bone abnormalities are the most obvious clinical symptoms of MBD in chelonians, although symptoms are also age-dependent (Boyer, 1996; Jackson *et al.,* 2000). Hatchlings usually develop a firm shell by 1 year of age but the shell will not harden if the animal has MBD. In addition, the carapace may appear small relative to the animal (diet is sufficient for soft-tissue development but not for carapace growth), scute deformities develop and the beak may curve downward. Clinical symptoms of MBD displayed by adult turtles are progressive mineral loss of the shell, shell and endoskeleton fractures, pyramidal shell growth, egg retention and concurrent hypovitaminosis A (Barten, 1996).

The pathogenesis of MBD starts with a plasma Ca deficit that signals the parathyroid gland to stimulate osteoclast activity to increase plasma Ca concentrations by removing Ca from bone. If the Ca deficiency is long term, hyperplasia of the parathyroid develops (nutritional secondary hyperparathyroidism or NSH). In juveniles, NSH also causes a decrease in secretion of the anterior pituitary hor-