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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-

mones resulting in reduced growth rate (stunting).

This review of a decade of research into freshwater turtles presents non-nutritive and dietary factors that affect turtle Ca homeostasis. Non-nutritive, essential factors in aquatic-turtle nutrition include ontogeny, environmental temperature and humidity, and photoreception and ultraviolet light. Dietary factors that affect Ca homeostasis are the metabolism of Ca, vitamin A and vitamin D.

ESSENTIAL, NON-NUTRITIVE FACTORS IN AQUATIC TURTLE Ca HOMEOSTASIS

Ontogeny Nutritional needs to support bone and shell growth and maintain Ca homeostasis differ for aquatic turtles depending on their developmental stage. The growth rate for Red-eared sliders *Trachemys scripta elegans,* for example, is most rapid between 12 and 35 weeks of age, during which time the turtles require 3·4 times the food [dry matter (DM) on comparative diets], an 80% animal-protein diet and a faster gut transit time (GTT) than adult turtles (Avery *et al.,* 1993; Barten, 1996; Stancel *et al.,* 1998; McCauley & Bjorndal, 1999).

Environmental temperature and humidity Environmental temperature affects vitamin D synthesis, which is essential to maintain Ca homeostasis in aquatic turtles. Appropriate environmental temperatures also increase metabolic rate, digestion rate and digestibility, all factors that indirectly contribute to the maintenance of Ca homeostasis. For example, aquatic turtles need an environmental temperature of between 25 and 30˚C to obtain an average body temperature of 34˚C (Meek, 1995; Manning & Grigg, 1997; Bury *et al.,* 2000; Steyermark & Spotila, 2000). Metabolic rate affects energy needs and expenditure, and will influence both interspecies and intraspecies comparisons for dietary formulations.

The physiological need to bask in order to thermoregulate and synthesize vitamin D which, in turn, promote and maintain Ca homeostasis, may be less pronounced in soft-shelled turtle species than in hard-shelled species. Vitamin D synthesis in turtles depends on basking for exposure to heat and UV light. The synthesis of provitamin D to previtamin D is UV-light dependent photolysis and the isomerization of previtamin D to vitamin D_3 is also temperature dependent. For example, the in-vitro conversion of previtamin D to vitamin D_3 occurred after 1200 hours at 5˚C but the conversion only took 91 hours at 25˚C (Holick *et al.,* 1995).

Basking was the least observed activity in the eastern Spiny softshell turtle *Apalone spinifera* (Galois *et al.,* 2002). The population observed in this study, however, was highly mobile in an extensive home range indicating that a large percentage of time was spent in transit. This population also spent all of their time in shallow water *(c.* 1·5 m) when not overwintering, suggesting exposure to the higher air temperature and UV light without formal basking behaviour. Snapping turtles *Chelydra serpentina,* another aquatic species, also do not tend to bask out of water, yet they thermoregulate and bask by floating at the water surface (Bury *et al.,* 2000). The molecular properties of water and the fact that in coastal regions more suspended particulate matter exists, mean that UV light penetration is <5 m or is measured at 10^{14}–10^{15} photons $m^{-2}\,s^{-1}$ (Losey *et al.,* 1999).

The digestive efficiency, digestibility and GTTs of aquatic turtles are affected by environmental temperature and food particle size (Melenberger *et al.,* 1993; Boyer & Boyer, 1996; Kollias & Gentz, 1996; Spencer *et al.,* 1998). The lack of, or existence of, digestive efficiency, digestibility and GTT can inhibit or promote metabolic use of dietary factors essential for Ca homeostasis. For example, only 39% of juvenile Red-eared slider turtles kept at an environmental temperature of 15˚C could digest their food but juveniles

kept at 28 and 34°C consumed more and were able to digest their food (Avery *et al.,* 1993). Large particles of food limit the rate of microbial fermentation even with increased body temperature (Spencer *et al.,* 1998). For example, 1 mm^2 plastic markers were excreted in turtle faeces before larger pieces. The GTT for turtles maintained at 15°C was 6 days but it was <2 days for turtles maintained at 34°C. Feed efficiency for the Macquarie or Murray River turtle *Emydura macquarrii* was 49% at 30°C and 45% at 20°C (Spencer *et al.,* 1998). Factors promoting desirable GTT in turtles appear to be gut volume (negative correlation), higher environmental temperatures and humidity, food recognition, frequent feeding and dietary fibre (Melenberger *et al.,* 1993; Boyer & Boyer, 1996; Kollias & Gentz, 1996; Spencer *et al.,* 1998). Stress, social isolation and disease all impede GTT.

Environmental humidity contributes to normal bone and shell development by producing larger embryos and hatchlings when eggs are incubated in wet substrates or smaller embryos and hatchlings when eggs are incubated in dry substrates (Miller & Packard, 1992; Packard, 1999; Packard & Packard, 2002). Larger hatchlings have longer survival times and the survival time of juvenile snapping turtles increased exponentially with increasing humidity (Finkler, 2001).

Photoreception and UV light Many reptiles are sight feeders and vision is an important aspect of nutrition. Photoreception in reptiles is essential for feeding, social and reproductive behaviours, circadian rhythms and hormonal secretion. Photoreceptors in reptiles include sensory cells (cones and rods with photopsins and scotopsins, respectively), the integument, the pineal gland [including the pineal organ, the parapineal organ (parietal eye) and paraphysis] and endogenous pigments (Tosini, 1997). Endogenous pigments include melatonin, melanin, haemoglobin, carotenes and keratin, but there is some evidence that ingested pigments (e.g. melanin) can enhance cellular light absorption in some species (Coohill, 1995). Melatonin has a role in maintaining normal thermoregulatory processes essential to Ca homeostasis and it is also a neuromodulator in reptiles.

The absorption of light by biological cells and tissues is wavelength and pigment dependent. UVA (320–380 nm) and UVB (290–320 nm) are essential for the functioning of chelonian physiological processes. UVA can cause cellular death, cellular mutation and DNA damage but, in reptiles, UVA also stimulates feeding, sexual and territorial behaviours (Coohill, 1995; Boyer, 1996). UVB light is necessary for vitamin D biosynthesis and photoreception in reptiles (Bernard & Ullrey, 1995; Bidmon & Stumpf, 1995; Gascon *et al.,* 1995; Holick *et al.,* 1995; Kohen *et al.,* 1995; Dickinson & Fa, 1997). However, the roles of UVA and UVB light in reptile physiology are still not fully understood and more research is needed on UV light and vitamin D biosynthesis (Bernard & Ullrey, 1995; Liesegang *et al.,* 2001).

It is difficult to replicate UV light successfully in captivity and several limiting factors, such as accurate duplication of flux, spectral output and heat, may account for the prevalence of hypovitaminosis D in reptile collections that are provided with UV light. The flux, spectral output and wavelength of UV radiation in natural light are not constant but in UV lamps these factors are both constant and specific (Coohill, 1995). Full-spectrum lamps are not comparable to natural light and many do not emit UVB wavelengths (Gehrmann, 1992, 1996; Ullrey & Bernard, 1999). UV light and heat coexist in nature but not in most UV lamps and many reptiles show a preference for basking under heat lamps rather than under cooler UV lights (Dickinson & Fa, 1997).

A study by Kollias *et al.* (1997) appears to contradict much of the existing

research indicating the physiological need for UV light in turtles. In this study, Blanding's turtle *Emydoidea blandingii* hatchlings were raised without full-spectrum lighting and they did not appear to develop shell and long-bone deformities. However, the duration of the study was limited and the turtles were released at 9–10 months of age.

The colour rendering index (CRI) and the colour temperature index (CTI) are two other areas where accurate replication cannot be achieved using lamps. CRI is the ability of the light source to represent the colours of an object as they would appear in natural light. The CTI is a measure of the distribution of colour in wavelength of the light emitted by the lamp. Both these factors may be important for aquatic turtle species because they are sight feeders, yet most research using UV-light emitting lamps does not report on CRI and CTI. The action spectrum is a measure of the biological effect as a function of wavelength and it is rarely reported. In the future, research should be carried out into the effects of the action spectrum on aquatic turtles. The effectiveness of lamps depends on the species, the length of exposure to artificial light, the distance between the lamp and the animals, dietary factors and thermal factors (Coohill, 1995; Gehrmann, 1996).

CALCIUM METABOLISM

The dietary Ca requirements of chelonians and most other reptiles are unknown (Dierenfeld & Barker, 1995; Liesegang *et al.,* 2001). Dietary Ca deficits or excesses are caused by an inadequate dietary source of Ca, excess dietary phosphorus (P) relative to Ca (Ca:P ratio), inadequate dietary vitamin D_3, a surplus of dietary vitamin D_3 or ingestion of a form of vitamin D incompatible with the physiology of the organism.

Dietary Ca requirements may also differ between species. For example, based on shell-ash percentage, the Spiny softshell *A. spinifera* and the Western painted turtle *Chrysemys picta bellii* have the same percentage of shell relative to body mass but the Ca and P in the shell of the Spiny softshell species is only 20–25% of mass compared to *c.* 45% of mass in the hard-shelled species (Jackson *et al.,* 2000). Based on spectrophotometric analysis and automated lactate analysis, Spiny softshell turtles also appear to have a smaller compensatory release of Ca into plasma compared to the Western painted turtle and this may be related to the lesser degree of shell mineralization (Jackson *et al.,* 2000).

Ca is often added to the diets of turtles in captivity through bone meal, ground cuttlefish bones, Ca tablets and/or powders. Tablets and powders can contain Ca in the form of Ca citrate, Ca carbonate, Ca chloride, Ca gluconate, Ca phosphate, Ca hydroxyapatite or Ca lactate. Vitamin D (D_2 or D_3) and magnesium may also be included in tablets and powders. These supplements are variable in available Ca and many are used even though there has not been enough research to support their use to prevent or cure MBD in chelonians (Stancel *et al.,* 1998).

Hypercalcaemia, as a primary disease, is caused by hypervitaminosis D and can impede growth and cause soft-tissue mineralization and shell pyramiding. The growth of juvenile Red-eared slider turtles was stunted when on a diet supplemented with Ca at 2·24% DM compared to the growth of turtles on a control diet (no supplementation) that contained 1·38% DM Ca (Stancel *et al.,* 1998). The carapace length increase during the study was smaller in the supplemented group (an average increase of 5·19 mm compared to 9·87 mm in the control group) and the turtles on the higher Ca diet gained less mass during the study (an average mass gain of 5·82 g compared to 10·83 g in the control group). Although shell pyramiding, which is found in both captive and wild populations, is thought to indicate an excess of dietary Ca, some

research indicates excess dietary Ca or P does not cause shell pyramiding (Stancel *et al.,* 1998). Hard water appears to have health benefits that are attributed to bicarbonate and sulfate salts of Ca and magnesium that may increase the skeletal health of aquatic turtles housed in it (Donoghue & Langenberg, 1996).

Hypocalcaemia is usually a secondary disease process of MBD. A deficit in serum Ca causes the osteocytes to remove Ca from bone and, as the disease progresses, bone thinning results in a plasma Ca deficit. Clinical symptoms of hypocalcaemia may include tetany, reduced neurological functioning and heart failure (Barten, 1996). Despite an apparently appropriate dietary Ca content, some foods can inhibit Ca absorption; for example, phytates (soy beans and cereal grains), oxalates (spinach and other vegetables, fruits and greens), high-fat diets and high dietary acid (such as in commercial cat foods) (Donoghue, 1995). If high concentrations of dietary Ca are combined with a high-fat diet, fat–calcium soaps form in the digestive tract and interfere with the digestion and absorption of Ca (Donoghue & Langenberg, 1996).

Plasma indices of Ca metabolism may be of limited use in clinical nutrition. The plasma biochemistry of reptiles can vary with season, age, reproductive status and sex (Divers *et al.,* 1996). Most ♀ reptiles, including chelonians, will have an increased serum Ca level during oogenesis (Raphael *et al.,* 1999). This may indicate a need to question plasma biochemistry as a nutritional status indicator (Divers *et al.,* 1996).

The investigation of some foods with dietary associative effects may contribute to the understanding of Ca metabolism in aquatic turtles. A dietary associative effect occurs when one item in the diet affects the digestion of another item either positively or negatively (Bjorndal, 1991). Associative effects are more common in the diets of herbivores or omnivores (many aquatic chelonian species) than in carnivores because of the nutritional differences in the diet items offered. Herbivores and omnivores often utilize microbial fermentation to digest their food and this may increase the occurrence of associative effects.

VITAMIN A METABOLISM

The dietary vitamin A requirements for reptiles have not been determined and may be less for animals in captivity than species in the wild (Dierenfeld & Barker, 1995). MBD is most likely concurrent with a vitamin A deficit (hypovitaminosis A) (Boyer, 1996; Williams, 1996; Slifka, 1997). Hypervitaminosis in aquatic turtles is usually iatrogenic and clinical symptoms include epidermal petechia, erythema that progresses to skin sloughing, gout, blepharedema (unilateral and bilateral), immune function deficits, fatty liver, lethargy, anorexia, mass loss, nasal discharge, multifocal squamous metaplasia, limb oedema secondary to kidney failure, respiratory-tract infections and hyperkeratosis in the respiratory, ocular, endocrine, gastro-intestinal and genito-urinary systems (Barten, 1996; Boyer, 1996; Williams, 1996). The ocular swelling associated with blepharedema can cause blindness and prevent the sight-feeding chelonians from eating.

VITAMIN D METABOLISM

The term 'vitamin D' is used to refer to vitamin D_2 (ergocalciferol) or vitamin D_3 (25-OH-cholecalciferol). It is considered a hormone because it is produced in the kidney and is distributed by the vascular system to act on specific targets, such as the intestine and bone cells (Oftedal *et al.,* 1997). Vitamin D_3 is essential for the functioning of the immune system and the stimulation of the active-transport process of Ca from the small intestine into the plasma (Lemire, 1992; Bernard & Ullrey, 1995; Dierenfeld & Barker, 1995; Boyer, 1996). Cutaneous synthesis of vitamin D_3 occurs when the most appropriate air temperature and UVB light are available

for a species (Bernard & Ullrey, 1995; Bidmon & Stumpf, 1995; Gascon *et al.,* 1995; Holick *et al.,* 1995; Kohen *et al.,* 1995; Dickinson & Fa, 1997).

Red-eared sliders (Family Emydidae) have a plasma-binding protein with high-affinity binding sites for both vitamin D_3 and thyroxine (T_4). In many species, vitamin D_3 and T_4 bind to separate proteins; for example, Snapping turtles have a binding site for vitamin D_3 separate from T_4 (Licht, 1994; Horowitz & Licht, 1996). The dual, high-affinity, binding protein found in Red-eared sliders is called thyroxine-binding protein/vitamin D_3 binding protein (TBP/DBP) or thyroxine vitamin D_3 binding protein (TDBP). The Emydidae appears to be the one turtle family that has evolved a single protein to transport two different hormones.

Hypovitaminosis D is a problem in turtles in captivity because providing sufficient UV light of the appropriate wavelength and intensity is difficult. Vitamin A deficiency can also be caused by providing insufficient dietary vitamin D or dietary vitamin D in a form that a species cannot metabolize (Bernard & Ullrey, 1995).

Hypervitaminosis D can cause hypercalcaemia and hypercalciuria. Clinical symptoms may include fatigue, weakness, anorexia and abdominal pain with osteoporosis and calcification of synovial bursae, tendon sheaths and periarticular structures (Barten, 1996; Boyer, 1996; Williams, 1996).

There is some potential for research into the possibility of using calcinogenic plants as a minimal and inexpensive source of vitamin D_3. Rats *Rattus rattus* and domestic chicks *Gallus gallus* fed *Cestrum diurnum* leaf powder at 2% DM on a vitamin D-deficient diet show biological activity similar to a response to the presence of vitamin D_3 (Prema & Raghuramulu, 1993, 1994). The advantages of using a vegetative source for vitamin D include maintaining GTT and the ability to provide minimal supplementation of the vitamin without using potent drugs.

DISCUSSION

Kollias & Gentz (1996) stated that '... reptile feeding is an art, not a science' and, 8 years later, this statement is still fairly accurate owing to a lack of research. Despite some important and relevant research on Ca homeostasis in aquatic turtles in the past decade, it is difficult to create suitable environments and formulate appropriate diets for aquatic turtles in captivity to ensure their health and well-being because of the dearth of research-based quantifiable data. For example, MBD is still the primary nutritional pathology in reptiles in zoological institutes (McWilliams, unpubl.).

Research has been carried out for the hatchling to 1 year-old life stages of Red-eared sliders and Snapping turtles but it is too limited to enable confident recommendations for suitable environments or husbandry guidelines. Recommendations in this article will be limited to research areas that appear to be vital for the survival of aquatic turtles in captivity, both now and in the future, and associated conservation programmes.

A major factor impeding research into chelonian Ca homeostasis and nutrition may be the slow growth rate of turtle species. Long-term research programmes require extensive funding and even short-term research programmes lack suitable monies. However, long-term research programmes need to be developed for aquatic turtles and this is the major recommendation in this paper. Other recommendations are listed below.

1. *Photoreception, UV light and biosynthesis.* This area of health and nutrition is of singular importance in aquatic turtles. Feeding and reproductive behaviours, circadian rhythms, metabolism, hormonal functioning and Ca homeostasis are all dependent on photoreception, especially in relation to aspects of UV light (Tosini, 1997). There are few controlled studies on

UV light and biosynthesis (Bernard & Ullrey, 1995; Liesegang *et al.,* 2001) and these have been limited to Snapping turtles and Red-eared sliders. Future studies must include data on CRI, CTI, action spectrum and spectral composition (Coohill, 1995; Gehrmann, 1996).

2. *Ca homeostasis and vitamin and mineral supplementation.* The frequent manifestation of MBD in reptiles in captivity appears to be related to excess vitamin and mineral supplementation, and/or environmental deficits. Despite the relative plethora of research on Ca homeostasis in aquatic turtles, the vitamin A and vitamin D requirements of most reptiles are still not known (Bernard & Ullrey, 1995; Dierenfeld & Barker, 1995; Barten, 1996; Boyer, 1996; Williams, 1996). Future research on Ca supplementation for aquatic turtles must include the amounts of Ca relative to dietary P, vitamin A and vitamin D, and non-nutritive factors (e.g. temperature, UV light) essential to chelonian metabolism, with an emphasis on minimal supplementation (Dierenfeld & Barker, 1995).

3. *Developmental indices, GTT and energy requirements.* The energy needs of aquatic turtle species and their normal developmental indices relative to energy needs are not known. Healthy turtles appear to have lower metabolisms, lower feed intakes and longer GTTs than many other mammalian and avian species (Bjorndal & Bolten, 1990a,b). These factors may explain the tendency to underfeed turtles in captivity and they can lose 35% of their body mass and decrease metabolic rate by up to 50%. Clinical signs of malnutrition in chelonia include sunken eyes, inactivity and increased susceptibility to infection and parasites (Donoghue, 1995; Barten, 1996). Long-term research is needed to establish developmental indices, GTT, energy requirements relevant to ontogeny and immune-system functioning.

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Analysis of the maintenance diet offered to lories and lorikeets
(Psittaciformes; Loriinae)
at Loro Parque Fundación, Tenerife

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Field observations and anatomical adaptations of wild Loriinae show a high degree of diet specialization. Although these birds are commonly maintained in captivity, there are limited data about adequate diet and nutritional requirements. The aim of this study is to make qualitative and quantitative reviews of the diet and nutrition of these birds at Loro Parque, Tenerife, and identify possible nutrient excesses or deficiencies, in order to provide a basis for future investigations and improved feeding and nutrition of lories and lorikeets in captivity. The daily maintenance diet of 35 pairs of four genera *(Chalcopsitta, Eos, Lorius* and *Trichoglossus)* was analysed over 10 days and the amounts of various food items ingested were recorded in order to determine the nutritional content of the diet consumed. Daily intake values for each food were calculated from the difference between the mass of each item offered and the mass left over, corrected for evaporative wet-mass loss. The concentrations of 36 essential nutrients of diets offered and ingested were calculated and compared with the requirements for psittacines recommended in the literature. Each pair ingested a daily average of: $26{\cdot}3 \pm 3{\cdot}1$ g fruit and vegetables (*c.* 53% of the amount offered), $18{\cdot}3 \pm 5{\cdot}2$ g pellet (*c.* 80% of the amount offered) and $153{\cdot}4 \pm 7{\cdot}3$ ml nectar (*c.* 99% of the amount offered). The analyses show important differences in the nutritional content between the diet offered and the diet

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