

Nutritional Metabolic Bone Disease in Juvenile Veiled Chameleons (*Chamaeleo calytratus*) and Its Prevention^{1–3}

Stefan Hoby,^{4,5} Christian Wenker,⁵ Nadia Robert,⁴ Thomas Jeremann,⁵ Sonja Hartnack,⁶ Helmut Segner,⁴ Claude-P. Aebischer,⁸ and Annette Liesegang^{7*}

⁴Center for Fish and Wildlife Health, Institute of Animal Pathology, Vetsuisse Faculty, University of Berne, Berne 3012, Switzerland; ⁵Zoo Basel, Basel 4054, Switzerland; ⁶Section of Epidemiology and ⁷Institute of Animal Nutrition, Vetsuisse Faculty, University of Zurich, Zurich 8057, Switzerland; and ⁸DSM Nutritional Products Ltd, Basel 4052, Switzerland

Abstract

Nutritional metabolic bone disease (NMBD) is one of the most frequently observed pathological conditions in herpetoculture. To develop guidelines for NMBD prevention in growing veiled chameleons (*Chamaeleo calytratus*), 56 hatchlings were divided into 6 groups [group UV, with UVB exposure; group No: no supplements; group CaAUV: with calcium (Ca), vitamin A, UVB; group CaA: with Ca, vitamin A; group CaADUV: with Ca, vitamin A, cholecalciferol, UVB; and group CaAD, with Ca, vitamin A, cholecalciferol] and reared for 6 mo on locust-based diets. The nutrient composition of the locusts' diet and the locust-based diet for the chameleons was determined. The diagnosis included the detailed description of clinical findings, histopathology, measurements of serum Ca, 25-hydroxycholecalciferol (25-OHD₃), liver 25-OHD₃, vitamin A, bone mineral density, and bone mineral concentration. Chameleons that received no dietary supplementation of Ca, vitamin A, and cholecalciferol developed NMBD. When Ca and vitamin A were supplemented, the chameleons did not develop NMBD, independently of additional UVB and dietary cholecalciferol. The best prevention for NMBD was achieved by chameleons that received locusts gut-loaded with 12% Ca and dusted with 250,000 IU/kg (75 mg/kg) vitamin A and 25,000 IU/kg (0.625 mg/kg) cholecalciferol plus provision of long (10 h/d), low irradiation exposure (3–120 μW/cm²) to UVB. Chameleons that were fed diets low in vitamin A, cholecalciferol, and Ca were diagnosed with fibrous osteodystrophy. We noticed an interaction of vitamin A and cholecalciferol supplementation in the storage of vitamin A in the liver and formation of colon calcifications. From these findings, recommendations for the rearing of juvenile chameleons were derived. J. Nutr. 140: 1923–1931, 2010.

Introduction

Whereas the global trade of chameleons is still dominated by animals caught in the wild, increasing knowledge of chameleon husbandry has resulted in successful captive breeding of several chameleon species, including veiled chameleons (*Chamaeleo calytratus*), in recent years (1). As a consequence, this progress in breeding may reduce the capture of wild chameleons. However, captive chameleons require specific care and they should not be considered domesticated animals.

Diseases in captive chameleons are most often caused by inadequate husbandry and chronic stress (2,3). Nutritional

metabolic bone disease (NMBD)⁹ is one of the most frequently observed pathological conditions (4). The disease is characterized by functional and morphological bone changes that result from dietary calcium (Ca) and phosphorus (P) imbalance, a deficiency of Ca and vitamin D in the diet, or a lack of UVB light exposure followed by the effects of secondary hyperparathyroidism. Individuals in life stages experiencing intensive growth and enhanced demands on Ca and P metabolism, such as juveniles and reproducing females, are most affected.

Specific mineral and vitamin requirements have rarely been investigated for most reptiles. However, it has been recognized

¹ Supported by the Swiss Association of Wildlife, Zoo Animal and Exotic Pet Medicine, and by the Zebra Foundation.

² Author disclosures: S. Hoby, C. Wenker, N. Robert, T. Jeremann, S. Hartnack, H. Segner, C-P. Weber, and A. Liesegang, no conflicts of interest.

³ Supplemental Figures 1–3 and Supplemental Table 1 are available with the online posting of this paper at jn.nutrition.org.

* To whom correspondence should be addressed. E-mail: aliese@vetphys.uzh.ch.

⁹ Abbreviations used: BMD, bone mineral density; DM, dry matter; group CaA, with Ca and vitamin A; group CaAD, with Ca, vitamin A, and cholecalciferol; CaADUV, with Ca, vitamin A, cholecalciferol, and UVB; group CaAUV, with Ca, vitamin A, and UVB; group No, no supplements; group UV, with UVB exposure; HE, hematoxylin and eosin; NMBD, nutritional metabolic bone disease; 25-OHD₃, 25-hydroxycholecalciferol; pOCT, peripheral quantitative computer tomography; SVL, snout-to-vent length.

that a variety of commercially raised insects, the main food source of captive veiled chameleons, have low Ca concentrations and imbalanced Ca:P ratios that result in nutritional imbalances and disease in reptiles reared in captivity (5,6). Hence, methods to increase the Ca content in insects have been established, including the dusting of the insects before offering them as food or increasing their internal Ca content by feeding the insects Ca, a process known as gut-loading (7–9).

The question of whether reptiles depend on dietary vitamin D or are capable of UVB-mediated endogenous vitamin D synthesis is controversially discussed (10). If chameleons depend on endogenous vitamin D synthesis, exposure to UVB light could be a limiting factor, because many captive chameleons are kept in temperate climates or indoors. It has been previously shown that artificial UVB exposure of panther chameleons (*Furcifer pardalis*) enhanced hatching success, vitamin D status of adult females, and epidermal vitamin D production (11–13). In contrast, it has been stated that adequate nutritional provision of cholecalciferol without added UVB light was sufficient to keep and breed veiled chameleons successfully (14).

The role of vitamin A, in addition to its function in reproduction, vision, growth, and the integrity of the immune system, as an additional etiological factor for NMBD in chameleons is unclear. In growing panther chameleons, a connection between NMBD and low dietary vitamin A content, but not between NMBD and variations of vitamin D contents in the diet, was demonstrated (11). In veiled chameleons, symptoms of hypovitaminosis A were similar to those in other reptiles (skin, eye, respiratory, and neurological lesions) but also included bone lesions (15). Previous studies have shown that commercially raised insects are a poor source of preformed vitamin A (5,6). Hence, dietary supplementation may be crucial. On the other hand, it has been noted that excess supplementation with vitamin A may interfere with vitamin D absorption, resulting in a clinical condition similar to NMBD (15).

Overall, the knowledge of nutritional and environmental requirements for chameleons in human care to prevent NMBD is too fragmentary to derive recommendations on optimal feeding and light conditions. This situation has direct implications for the welfare of the species. For instance, under the current animal welfare regulations, an official authorization for keeping chameleons is necessary (16), but recommendations on diets and light conditions are not specified.

Based on the literature, NMBD appears not to be caused by a specific dietary or environmental factor but has a multi-factorial etiology. The aim of this study was to provide a comprehensive evaluation, considering the known etiological factors of dietary and light conditions for the prevention of NMBD in growing veiled chameleons. In addition, this study aimed at providing tools that support a reliable diagnosis of the disease at early stages of its manifestation.

Materials and Methods

Animals and experimental setup. Fifty-six veiled chameleon hatchlings (29 males, 27 females) of 2 clutches were reared for 6 mo. They were randomly divided into 6 groups consisting of 9–10 individuals each (group UV: with UVB exposure; group No: no supplements; group CaAUV: with Ca, vitamin A, and UVB; group CaA: with Ca and vitamin A; group CaADUV: with Ca, vitamin A, cholecalciferol, and UVB; group CaAD: with Ca, vitamin A, and cholecalciferol) (Supplemental Table 1). A clinical trial with 2×3 factorial design (2 levels UV light; 3 levels of supplementation) with predefined criteria for euthanasia was conducted. This study was performed in accordance with the Swiss animal welfare regulations and approved by the cantonal veterinary office (permit no.

2172). The chameleons were kept solitary in cylindrical plastic terraria (diameter 31 cm, height 42 cm) with ventilation slots (4×15 cm) at the sides covered by a cloth mesh (mesh size at 2×2 mm). The bottom consisted of paper that was changed weekly. Two or more branches of blackthorn and branches of bamboo were provided for climbing and hiding. Independent of the light source, temperature varied from 22 to 30°C (vertical gradient and day-night gradient) and humidity ranged from 40 to 65% (Hygrolog D –1.0, Rotronic). The terraria were misted using temperate tap water in the morning and in the evening. To minimize position effects caused by small temperature and light gradients, the terraria were systematically rotated every week. The general condition and behavior of the chameleons were evaluated on a daily basis. Criteria were defined to interrupt the experiment for the euthanasia of poorly performing chameleons. At the end of the study, the chameleons were anesthetized using isoflurane (induction chamber with 5 vol% isoflurane in oxygen 0.6 L/min, anesthesia maintenance with mask) and blood was collected by cardiocentesis. Serum was stored in tubes. The chameleons were euthanized intracardially using sodium pentobarbital (324 mg/kg body weight, Vetanarcol, Veterinaria).

Diet regimen. In the first 2 wk, all hatchlings were fed locust nymphs (*Locusta migratoria*), cricket nymphs (*Acheta domestica*), fruit flies (*Drosophila* sp.), and flour moths (*Ephesia kuehniella*) dusted with Korvimin ZVT + Reptil [WDT; 15% Ca, 8.3% P, 500,000 IU (150 mg) vitamin A and 50,000 IU (1.25 mg) cholecalciferol/kg mineral and vitamin mix] to support initial acclimation and stimulate feeding. Thereafter, the chameleons were offered locust nymphs that differed in supplementation with Ca, vitamin A, and cholecalciferol (Supplemental Table 1; Table 1). To increase the Ca content of the offered insects, they were gut-loaded for at least 48 h with a diet containing 12% Ca/kg wet weight (10 g Ca citrate and 33 g Ca carbonate, Hänseler) mixed with 57 g wheat seedlings produced at the Basel Zoo and a cricket diet (Grillen 3600, Provimi Kliba), respectively. To supplement the insects with vitamin A [250,000 IU/kg powder (75 mg/kg)] and cholecalciferol [25,000 IU/kg powder (0.625 mg/kg), Vital AG], the insects were dusted prior to feeding to the chameleons. The insects were consumed daily by each individual on a controlled ad libitum basis according to body weight and appetite. The remaining insects were removed on the same day they were fed. Three different sizes of locust nymphs (size 1 = 0.015 g, size 2 = 0.05 g, size 3 = 0.5 g body weight) were fed depending on the body weight of the growing chameleons. The body weight was determined every 3 wk.

Proximate analysis was performed to determine dry matter (DM), crude protein, crude fiber, and crude fat of the locusts' diet and the locusts themselves. Levels of vitamin A, cholecalciferol, and vitamin E were measured using HPLC (17–19). Ca, P, and Mg concentrations were measured with an autoanalyzer (Cobas Mira, Roche).

Light regimen. Groups UV, CaAUV, and CaADUV were exposed to light containing UVB (Replux UV-plus 23 W, Namiba Terra) and groups No, CaA, and CaAD to light without UVB (fluorescent tubes, Master TLD 36 W/840, Philips) (Supplemental Table 1). These primary light sources were provided for 10 h/d and were placed individually above the terraria and additional weak illumination from fluorescent tubes in the ceiling supplemented all indoor terraria 12 h/d. Irradiance was recorded repeatedly during the experiment with the cloth mesh in place at various distances in the terraria below the midpoint of the lamps using a solarmeter meter (Model 6.2 UVB, Solartech). Boron-silicate ampules containing provitamin D₃ (7-dehydrocholesterol) were placed at the same positions in the terraria as the solarmeter and were exposed for 2 h to maximize conversion to cholecalciferol and other photoproducts. The contents were then analyzed by HPLC and the percentage of synthesized photoproducts was calculated from the original substance (20,21). These measurements were performed after 3 mo.

Determination of serum Ca and 25-hydroxycholecalciferol and liver vitamin A and 25-hydroxycholecalciferol. Blood samples were centrifuged immediately at $1900 \times g$ for 10 min at 20°C and serum was stored at –80°C until analysis. The samples of each group were pooled using equal amounts (10%) according to weight of the samples. Total Ca concentrations were determined using an autoanalyzer (Cobas Mira,

TABLE 1 Selected minerals, crude nutrients, and fat-soluble vitamins in diets for locust nymphs (*Locusta migratoria*) and in locust nymphs that were or were not supplemented with Ca, vitamin A, and cholecalciferol¹

Feed	Minerals				Proximate nutrient components				Vitamins		
	Ca	P	Ca:P ratio	Mg	DM total	Crude protein	Crude fat	Crude fiber	Vitamin A	Cholecalciferol	Vitamin E
	% DM	% DM		% DM	% DM	% DM	% DM	% DM	mg/kg DM	μg/kg DM	mg/kg DM
Wheat seedlings	0.12	0.69	0.17	0.13	16.8	31.4	n.d. ³	n.d.	0	0	23.7
Cricket diet ²	1.25	0.69	1.80	0.26	89.6	22.5	n.d.	n.d.	3.65	147.8	166
Locust nymphs size 1 ⁴	0.32	1.16	0.28	0.20	23.2	67.7	n.d.	n.d.	0	0	27.6
Locust nymphs size 2 ⁵	0.04	0.90	0.04	0.16	27.1	53.5	8.5	7.9	0.21	0	22.3
Locust nymphs size 3 ⁶	0.09	0.87	0.10	0.15	24.7	57.6	12.8	7.8	0.42	0	20.5
Locust nymphs size 1 ⁴ with Ca, vitamin A, and cholecalciferol	3.01	0.88	3.82	0.29	34.1	67.2	n.d.	n.d.	1.95	65.2	21.2
Locust nymphs size 2 ⁵ with Ca, vitamin A, and cholecalciferol	0.47	1.05	0.44	0.37	43.6	66.5	5.1	12.6	2.19	41.9	20.1
Locust nymphs size 3 ⁶ with Ca, vitamin A, and cholecalciferol	0.84	0.67	1.64	0.12	30.7	57.7	12.0	8.9	2.47	23.8	18.6

¹ Values are means; samples were measured twice, sample size 200 g of insects, cricket diet, or wheat seedlings.

² Cricket diet Grillen 3600, Provimi Kliba, Kaiseraugst, Switzerland: 89% DM; 22.3% crude protein; 4.3% crude fiber; 4.8% crude fat; 6.7% crude ash.

³ n.d., not determined.

⁴ Body weight size 1 = 0.015 g.

⁵ Body weight size 2 = 0.05 g.

⁶ Body weight size 3 = 0.5 g.

Roche). Serum 25-hydroxycholecalciferol (25-OHD₃) measurements were performed using a kit from Chromsystems Instruments and Chemicals, which allows the chromatographic determination of 25-OHD₃ using a simple isocratic HPLC system employing a UV detector (HP-1100, Agilent Technologies). At necropsy, liver samples of ~0.5 g were collected and stored at -20°C. Samples of each group were pooled and vitamin A and 25-OHD₃ contents per gram wet weight were analyzed following HPLC (22).

Pathological investigations. Snout-to-vent length (SVL) and body weight were determined and complete necropsy was performed. Tissue samples of the internal organs (heart, lung, liver, kidney, gonads, stomach, small and large intestine) and the skeleton (right foreleg, left hind leg, dorsal vertebra, and head) were collected. Parathyroid glands were available only in rare cases due to their small size and their alterations in the course of euthanasia. The samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin (HE), and examined microscopically. Selected slides were also stained with Periodic Acid Schiff, Ziehl-Neelsen, Grocott, and Kossa stains. The mineralized samples (legs, head) of groups CaAUV, CaA, CaADUV, and CaAD were decalcified (Decalcifier rapid, Mallinckrodt Baker) for 3–6 h after fixation in formalin and most of the skin and skeletal muscles were removed. The histological evaluation of the skeleton focused on the orbital and parietal crest of the head, the dorsal vertebrae, the humerus, radius and ulna of the forelegs, and the femur and tibia of the hind legs.

Bone mineral density and mineral content in bone. Total bone mineral density (BMD) was measured in the vertebral column at the cranial end of the dorsal crest, and trabecular BMD was determined in the left humerus at the distal metaphysis by using peripheral quantitative computer tomography (pQCT) (Stratec XCT 960A bone scanner, Stratec Medizintechnik). To define the metaphyseal point for measuring, the length of the humerus was recorded and the point was set at nine-tenths of that distance from the distal bone end. Total and trabecular BMD were calculated by automated computation with a threshold > 71 mg/cm³.

For the determination of the ash in the right femur, the bone was dried for 96 h at 105°C and used for the determination of the dry weight. The samples were then put in the muffle oven at 600°C for 96 h and the ash was analyzed. Ca, P, and Mg concentrations were determined with an autoanalyzer (Cobas Mira, Roche).

Statistical analysis. The 6 groups were tested for potential significant differences of SVL and body weight at the beginning of the experiment

with a 1-way ANOVA. Values in the text are means (95% CI). For statistical analyses, BMD levels < 71 mg/cm³ were arbitrarily set at 70 mg/cm³. The influence of dietary supplementation and light on body weight, SVL, BMD, and femoral mineral content were studied using 2-way ANOVA with Tukey-Kramer multiple comparison tests. The logrank test was applied to assess the survival of group UV and No chameleons. Statistical procedures were performed with the NCSS 2007 software (Number Cruncher Statistical Systems) and Microsoft Excel. Differences of *P* < 0.05 were considered significant.

Results

Ca content and Ca:P ratios of supplemented locusts were several times higher than the unsupplemented locusts (Table 1). With the exception of DM, which was notably higher in the Ca-supplemented locusts, Ca-supplemented and -unsupplemented locusts had comparable levels of proximate nutrients (crude protein, crude fat, and crude fiber). Vitamin A and cholecalciferol contents were considerably higher in dusted locusts [6493–8234 IU/kg (1.95–2.47 mg/kg) vitamin A and 951–2608 IU/kg (23.8–65.2 μg/kg) cholecalciferol] compared with undusted locusts [0–1406 IU/kg (0–0.42 mg/kg) vitamin A and 0 IU/kg cholecalciferol] (Table 1).

The measurements of both the irradiance with a solarmeter and the percentage of photoproducts synthesized in ampules containing provitamin D₃ at different positions in the terraria showed that the UVB output of the fluorescent tube was negligible compared with the UVB light source (Table 2). UVB irradiance was proportional to the formation of photoproducts.

At the beginning of the clinical trial, the chameleon hatchlings were active, in good general condition, weighed 1.5 g (1.4–1.6 g), and had a SVL of 38.8 mm (38.0–39.7 mm), with no differences among groups. At the end of the trial, dietary supplementation had a positive effect on body weight (*P* < 0.0001) and SVL (*P* < 0.0001), whereas the influence of UVB exposure on body weight (*P* = 0.56, *P*-interaction = 0.20) and SVL (*P* = 0.06, *P*-interaction = 0.055) was not significant (Table 3). Owing to clinical symptoms consistent with NMBD, all 10 chameleons of group No and 6 of 10 chameleons of group UV had to be euthanized prior to the scheduled end of the

TABLE 2 Characteristics of a UVB light source (Reflux UV-plus 23 W) and a fluorescent tube (Master TLD 36W)

Light source position terrarium	Distance	Temperature ¹	Mean irradiance ²	Irradiance range	Dose ³	Photoproduct formation ⁴
	cm	°C	μW/cm ²	μW/cm ²	mJ/cm ²	%
Reflux UV-plus 23W						
Bottom	46	25.1	3	5–2	21.6	0.07
Middle	25	25.7	7	9–6	50.4	0.14
Top	4	28.3	120	131–114	864	1.05
Master TLD 36W						
Bottom	52	25.3	0	0	0	0
Middle	31	26.0	1	1–0	7.2	0
Top	10	28.1	5	5–4	36	0.09

¹ Temperature was determined at the beginning of the measurements.

² Irradiance was determined with a solarmeter model 6.2 UVB meter.

³ Dose (mJ/cm²) = [irradiance (μW/cm²) × time (s)] ÷ 1000.

⁴ Ampules containing provitamin D₃ (7-dehydrocholesterol) were used to assess the photoproduct formation ability during an exposition of 2 h. Analysis according to (20,21).

experiment. With the exception of 2 group UV chameleons that had moderate to emaciated body condition (narrowed tail base, decreased fat bodies), all others were in good body condition. The onset of clinical symptoms occurred later in group UV chameleons than in those of group No ($P = 0.0005$) (Table 4). In general, clinical symptoms were similar in both affected groups (Table 4). However, casque deformation was almost exclusively present in group No. The other 4 groups showed no clinical signs. The bending of forearms and lower legs was very frequent in both groups. The proximal metaphysis of the radius and ulna and the distal metaphysis of the tibia and fibula were most often affected (Supplemental Fig. 1). Molting problems were not seen in any chameleons of groups UV or No.

The chameleons of groups CaAUV, CaA, CaADUV, and CaAD developed normally without showing clinical signs of NMBD. They were all in good body condition with the exception of 2 individuals from groups CaADUV and CaAD that showed a moderate body condition. Independently of sex, diet, and light regimen, marked individual differences in growth rates were noted.

The pooled total serum Ca concentrations were lower in groups UV and No than in groups CaAUV, CaA, CaADUV, and CaAD (Table 3). Due to serum shortage, 25-OHD₃ concentrations could not be measured in groups No and CaAUV. The concentration was markedly higher in group CaADUV than in

groups UV, CaA, and CaAD (Table 3). Pooled 25-OHD₃ liver concentrations were distinctly higher in groups that were exposed to UVB radiation (UV, CaAUV, CaADUV) than in those that were not (No, CaA, CaAD) (Table 3). Liver concentrations of vitamin A ranged from 0.06 μmol/g (group No) to 0.12 μmol/g (group CaA) (Table 3).

Histopathological lesions consistent with NMBD were seen only in chameleons of groups UV and No. In the parietal and orbital crests of the casques, the changes were characterized by thinned laminae and wide medullary cavities filled with fibrovascular tissue (Fig. 1). Endosteal surfaces were scalloped due to the resorptive activity of numerous large multinucleated osteoclasts in prominent Howship's lacunae and surrounded by wide unmineralized osteoid seams lined by numerous osteoblasts. The histological findings in the dorsal vertebrae of groups UV and No chameleons were comparable with the lesions in the casques. No pathological lesions were seen in chameleons of groups CaAUV, CaA, CaADUV, and CaAD with the exception of 2 group CaA chameleons that showed increased osteoclastic resorption of otherwise well-formed and mineralized parietal crest laminae in the casques. In the long bones of the extremities, chameleons of groups UV and No showed thinned cortices with layers of osteoblasts and unmineralized osteoid on the periosteal surface (Fig. 2). The basic structure and thickness of the physis was preserved in all the chameleons. However, the chondrocytes of

TABLE 3 Baseline body weight and SVL and serum and liver Ca, 25-OHD₃, vitamin A concentrations, and ash characteristics of femora in veiled chameleons after being fed locust-based diets differing in the concentrations of these nutrients and being exposed to UVB light

Group	n	Body dimensions		Serum ¹		Liver (wet weight) ¹		Ash characteristics femur		
		Weight	SVL	Total Ca	25-OHD ₃	25-OHD ₃	Vitamin A	DM	Ca	P
		g	mm	mmol/L	nmol/L	pmol/g	μmol/g	% DM		
UV	10	26.7 (19.2–34.2)	100.1 (90.4–109.8)	2.2	142	51	0.06	57.4 (52.2–62.6)	4.6 (3.9–5.2)	2.5 (2.2–2.8)
No	10	13.5 (11.3–15.7)	74.4 (69.1–79.7)	2.0	n.d. ²	<LLQ ³	0.06	59.4 (54.6–64.1)	3.9 (3.4–4.3)	2.4 (2.2–2.7)
CaAUV	9	58.2 (47.3–69.2)	144.2 (133.8–154.6)	2.8	n.d.	62	0.08	59.1 (56.6–61.5)	11.1 (10.3–11.9)	5.0 (4.7–5.3)
CaA	9	60.5 (52.1–68.9)	144.2 (133.4–155.1)	3.1	160	26	0.12	63.4 (59.6–67.2)	10.7 (9.7–11.6)	4.8 (4.4–5.1)
CaADUV	9	54.3 (38.1–70.5)	138.2 (117.6–158.8)	3.1	> 250	71	0.09	62.2 (59.8–64.6)	9.5 (7.0–12.0)	4.8 (4.2–5.1)
CaAD	9	57.9 (38.2–77.6)	136.8 (117.8–155.7)	4.6	102	25	0.08	60.9 (58.2–63.6)	10.8 (8.5–13.1)	4.4 (3.8–5.1)

¹ Values are means (95% CI), n = 9–10 for serum and liver. It is a pool of n = 9–10 and then the pool mixture was measured twice.

² n.d., not determined.

³ <LLQ, below the lower limit of quantification of 10 ng/g.

TABLE 4 Clinical symptoms of NMBD in juvenile veiled chameleons fed unsupplemented diets that were or were not exposed to UVB light¹

Clinical signs	Group UV, <i>n</i> = 10	Group No, <i>n</i> = 10
Onset of clinical symptoms (<i>d</i> , <i>range</i>)	169 (152–186) ²	128 (110–146)*
Diseased animals	8	10
Inappetence	5	9
Apathy	5	4
Weakened stance	5	6
Tremor, ataxia	6	8
Discoloration	3	2
Casque deformation	1	8
Bending of forearms	4	8
Bending of lower legs	7	10

¹ Values are *n* of chameleons showing clinical signs if not stated specifically.

*Different from group UV ($P < 0.05$) (logrank test for survival analysis).

² *n* = 8.

the hypertrophic zone of group UV and No chameleons were arranged in irregular clumps rather than in longitudinal columns as found in groups CaAUV, CaA, CaADUV, and CaAD, and there was a markedly decreased production of metaphyseal trabecular bone (Fig. 3). The trabeculae in the metaphysis were shortened and plump and the primary spongiosa was replaced by disorganized spicules of woven bone without a cartilage core (Fig. 3). In several specimens of group No, primary spongiosa was virtually absent and the flattened physal cartilage was covered by woven bone, preventing vascular invasion from the metaphyseal side. Fibrous connective tissue formed a loose layer surrounding the irregular trabeculae in group UV and filled the metaphyseal cavities in group No (Fig. 3). Large multinucleated osteoclasts were abundant along metaphyseal and endosteal surfaces in chameleons of both affected groups and intense osteoblastic activity was also present. The long bones of CaAUV, CaA, CaADUV, and CaAD chameleons were characterized by broad, completely mineralized cortices and longitudinally aligned trabeculae containing a distinct cartilage core (Fig. 2). Moderate numbers of osteoclasts were located along subphysal surfaces.

Histological examination of the liver revealed mild, chronic, focal to multifocal pyogranulomas with centrally located remnants of fungal hyphae and spores consistent with *Candida* sp. in several chameleons of all groups. In segmental areas of the colon in chameleons of group CaAUV (7 of 9), CaA (8 of 9), and CaADUV and CaAD (4 of 9), multifocal calcifications of small blood vessels were present in the lamina propria (Supplemental Fig. 2). Kidney lesions were limited to group No chameleons (8 of 10) and were characterized by multifocal tubular degeneration and calcification (Supplemental Fig. 3). No important lesions or group-specific differences were detected in sections of other internal organs.

The BMD levels of the groups with dietary supplementation (CaAUV, CaA, CaADUV, and CaAD) differed from those without (UV and No) at both points measured in the vertebral column and the distal humerus ($P < 0.0001$) (Fig. 4). UVB exposure had no effect, although a trend could be noticed for the distal humerus ($P = 0.05$) but not for the vertebral column ($P = 0.31$) mineral density. Interactions between UVB exposure and dietary supplementation were significant for both the distal humerus ($P = 0.005$) and the vertebral column ($P = 0.01$) mineral

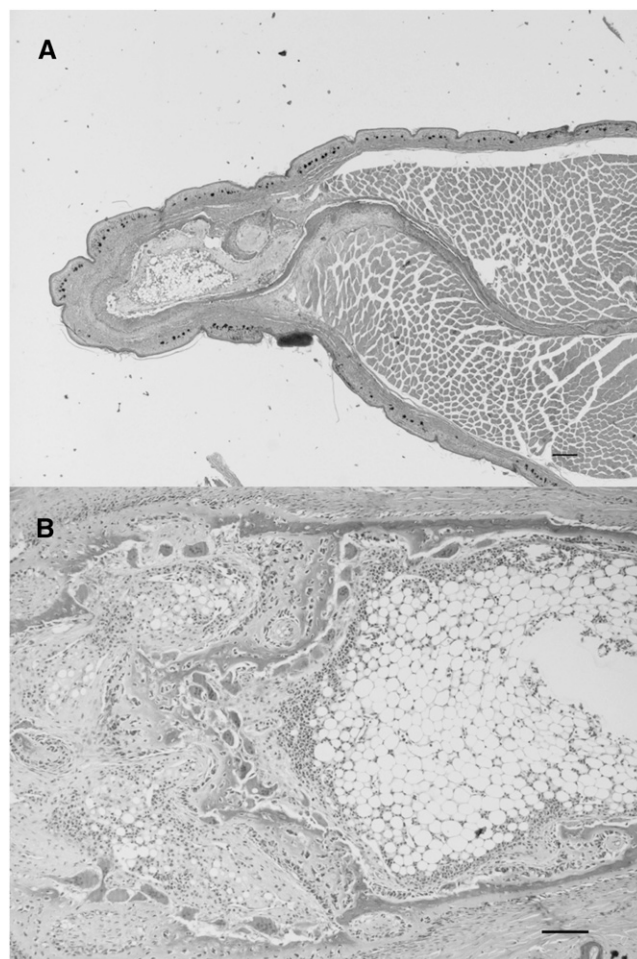


FIGURE 1 NMBD-associated histopathological casque lesions in veiled chameleons fed diets not supplemented with in Ca, 25-OHD₃, and vitamin A and that were or were not exposed to UVB light. (A) Cross section of a deformed casque (rostral end of the parietal crest) in a group No chameleon. HE, $\times 25$, bar = 200 μm . (B) Thinned laminae with pale eosinophilic unmineralized osteoid seams and wide medullary cavities partly filled with fibrovascular tissue in a casque of a group UV chameleon. Note the scalloped endosteal surfaces due to the resorptive activity of numerous large multinucleated osteoclasts and the marked osteoblastic activity. HE, $\times 100$, bar = 100 μm .

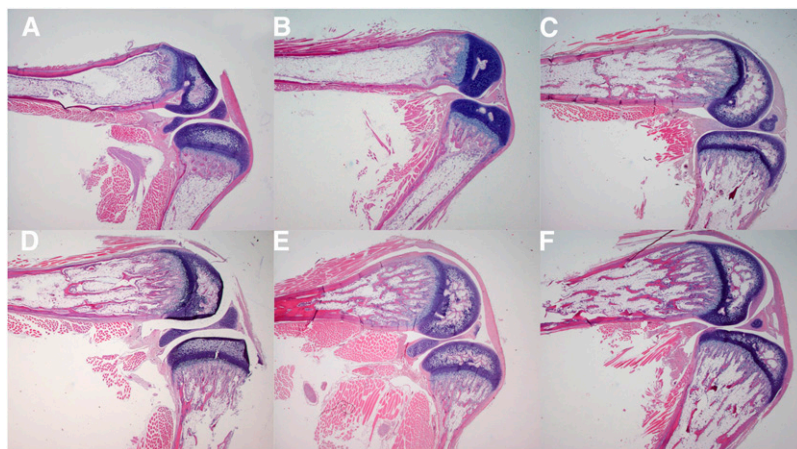
density. The humerus BMD of 5 individuals of group No and 1 specimen of group UV were below the threshold of 71 mg/cm³.

Dietary supplementation increased both the Ca and P contents of the femora ($P < 0.0001$) (Table 3). In contrast, the UVB exposure did not affect Ca ($P = 0.90$) or P ($P = 0.14$) content, and interactions between UVB exposure and supplementation did not influence Ca ($P = 0.24$) or P ($P = 0.78$) content.

Discussion

The most important result of this study was that juvenile veiled chameleons that received no dietary supplementation of Ca, vitamin A, or cholecalciferol developed NMBD, although the beneficial effects of UVB exposure delayed the onset of NMBD symptoms. When Ca and vitamin A were provided, the chameleons did not develop NMBD, independently of additional UVB and/or dietary vitamin D. The best results were in chameleons with all dietary supplements and exposure to UVB.

FIGURE 2 Cross section of the knee with the physes of the left distal femur and proximal tibia of group UV (A), No (B), CaAUV (C), CaA (D), CaADUV (E), and CaAD (F) veiled chameleons. Thinned cortices with layers of unmineralized osteoid on the periosteal surface and absence of mineralized trabeculae in the metaphysis in A and B. HE, $\times 12.5$.



Locusts were shown to be a valuable diet for veiled chameleons. The palatability for the chameleons was not influenced by the different treatments of the locusts, but the mortality rate of the insects increased when they were fed Ca-supplemented diets. The gut-loading with Ca and the dusting with vitamins A and D resulted in levels recommended for reptiles (23,24). Data on the diet composition of free-ranging veiled chameleons in their natural habitat are not available for comparison.

Clinical symptoms of NMBD were observed in groups UV and No. Remarkably, UVB light alone (group UV) led to a delayed development of the clinical symptoms but did not prevent them. It is assumed that the chameleons of group UV were able to compensate for the nutritional Ca deficiency by endogenous production of vitamin D and hence increased intestinal Ca absorption, but when a certain body weight was reached, the demand of Ca exceeded these compensatory efforts. The NMBD symptoms in the juvenile veiled chameleons of this study were similar to the observations from previous studies (3,15). Casque deformation was a frequently observed sign of NMBD and may be an important clinical feature for early diagnosis of NMBD in veiled chameleons. The bending of bones was limited to the limbs, presumably related to the forces acting on the skeleton of these climbing arboreal lizards. The clinical symptoms did not correspond with previous descriptions of vitamin A deficiency in panther and veiled chameleons that included vertebral kinking, neurological dysfunction, and dysecydysis (11,15). It is therefore suggested that they were predominantly related to Ca and vitamin D deficiency.

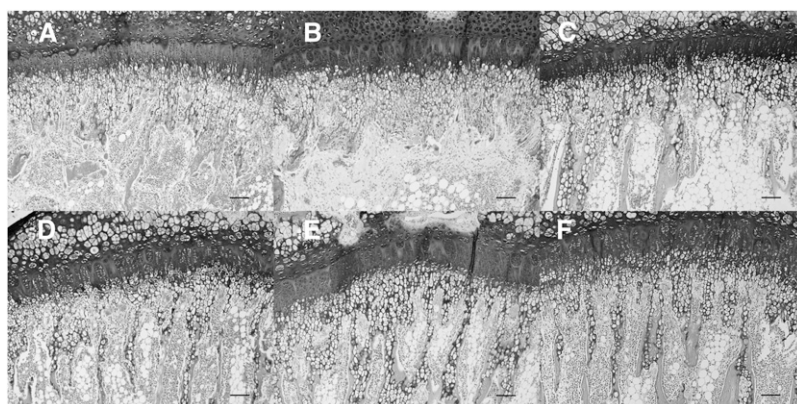
The amount of photoproducts formed in this study was in the range of the recommended amounts for female panther chameleons (25) and was suitable for the indoor rearing of veiled

chameleons when dietary supplementation with Ca and vitamin A was provided. The corresponding irradiance determined by the broadband UVB radiometer of this study was clearly different from the irradiance measured by other broadband UVB radiometers and UVB sources (21,26). This highlights the different spectral sensitivity of commercially available UVB radiometers and the need to determine the cholecalciferol photosynthesizing ability of a light source as an independent measure of UVB irradiance.

The serum Ca concentrations of groups UV (2.2 mmol/L) and No (2.0 mmol/L) were below the reference range and that of group CaAD (4.6 mmol/L) was above the reference range of 2.3–3.5 mmol/L for lizards (27). Still, to the authors' knowledge, specific reference ranges for veiled chameleons are lacking. Because total Ca levels are different per lizard species, age, and sex (28), the presented results must be interpreted with caution. However, low concentrations of total serum Ca in growing veiled chameleons may be indicative for NMBD. The high Ca concentration in group CaAD may have been linked to egg formation (29), which was noted in 2 females of this group at necropsy.

The serum 25-OHD₃ concentration was markedly higher in group CaADUV than in groups UV, CaA, and CaAD. This indicates that group CaADUV chameleons used both the dietary and UVB light-dependent sources to meet their vitamin D needs. 25-OHD₃ serum concentration is considered to be the most useful measure to assess vitamin D status in lizards (30). The concentration reflects the total vitamin D obtained from the diet and photobiogenesis over a period of several weeks (31). Baseline 25-OHD₃ concentrations have been reported for wild and captive Komodo dragons (*Varanus komodoensis*), which

FIGURE 3 Proximal left tibial physes of group UV (A), No (B), CaAUV (C), CaA (D), CaADUV (E), and CaAD (F) veiled chameleons. Irregular arrangement of chondrocytes in the hypertrophic zone in A and B. Short and plump trabeculae in the metaphysis and spicules of woven bone without cartilage core (A), or rather absence of primary spongiosa, replaced by fibrous connective tissue (B). Increased osteoblastic and osteoclastic activity in A and B. HE, $\times 100$, bar = 100 μm .



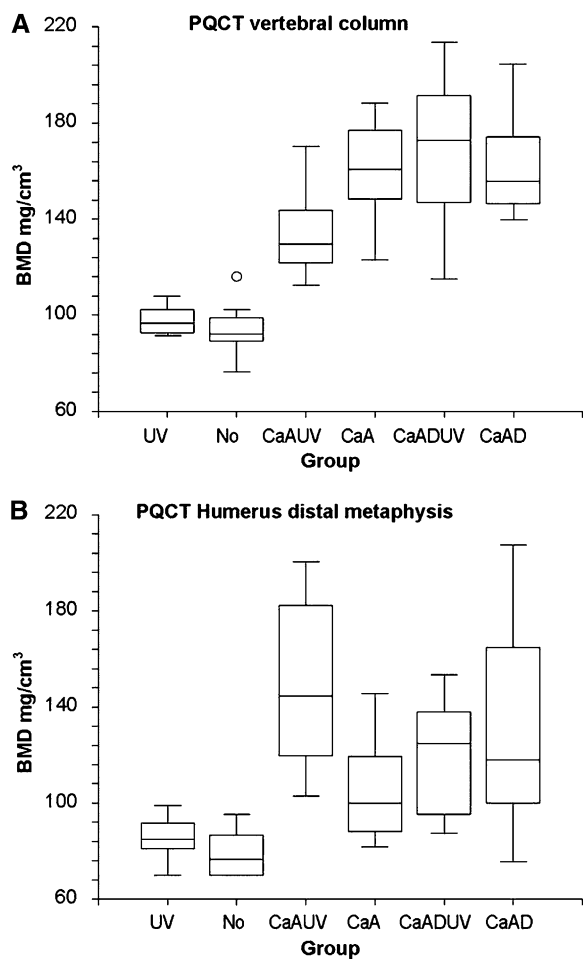


FIGURE 4 BMD as assessed by pQCT of the vertebral column (A) and the distal metaphysis of the left humerus (B) in veiled chameleons that were or were not exposed to UVB light and were fed diets varying in Ca, 25-OHD₃, and vitamin A. Box plots represent medians \pm quartiles, $n = 9$ –10. There were interactions between diet and UVB light exposure for both variables ($P \leq 0.01$). Dietary supplemented groups and unsupplemented groups differed ($P < 0.0001$), whereas UVB-exposed and -unexposed groups did not differ in A and B.

were exposed to the sun daily (32), as well as different agamid and iguanid species under artificial light or free-ranging conditions (33), captive green iguanas (*Iguana iguana*) housed outdoors (31), healthy wild Ricord's iguanas (*Cyclura ricordii*), and wild and outdoor-housed captive rhinoceros iguanas (*Cyclura cornuta cornuta*) (34). The observed variations may represent genetic, behavioral, physiological, dietary, or ecological differences between species. Comparative data for chameleons are lacking, but the results of this study indicate that 25-OHD₃ serum concentrations above 100 $\mu\text{g/L}$ (>250 nmol/L) could be ideal for growing veiled chameleons, although values as low as 41 $\mu\text{g/L}$ (102 nmol/L) were sufficient to prevent NMBD when the diet regimen was optimized.

To the authors' knowledge, this is the first report of 25-OHD₃ and vitamin A measurements in livers of chameleons. Pooled 25-OHD₃ concentrations were markedly higher in groups that received UVB compared with those that did not. This is in accordance with the fact that cholecalciferol, formed in the epidermis after UVB photolysis of provitamin cholecalciferol and subsequent thermal isomerization, enters the circulation and is transported to the liver where conversion to 25-OHD₃ takes place (35). The fact that the 25-OHD₃ liver concentrations of

chameleons not exposed to UVB radiation were comparably low, independently of dietary supplementation with vitamin D, indicates that the intestinal absorption of vitamin D was less important than exposure to UVB radiation. Another possible explanation could be that the form of vitamin D provided in this study could not be absorbed appropriately.

The quantification of vitamin A in the liver is considered to be very useful for assessing the vitamin A status in chameleons (2). However, it is difficult to interpret whether the concentrations reported here represent normal levels, because the data of baseline concentrations of vitamin A in reptile livers are fragmentary. The few available investigations revealed considerable species differences among Hermann's tortoises (*Testudo hermanni*) (36), free-ranging box turtles (*Terapene carolina*) (37), Komodo dragon, and 3 common European vipers (*Vipera berus*) (38). The vitamin A concentrations in this study were similar to those found in the studies on tortoises and turtles mentioned above and no pathologies were observed in chameleons with levels above 0.07 $\mu\text{mol/g}$ (21 $\mu\text{g/g}$). It is assumed that the concentrations in groups UV and No originated from low levels of vitamin A in untreated locust nymphs and potentially from the conversion of β -carotenes (not determined in this study) from the gut contents of these locusts. It has been suggested that insectivores may benefit from carotenoids that can be converted when required (39). Hence, a combination of preformed vitamin A and carotenoids may be optimal for veiled chameleons. The vitamin A concentration was higher in group CaA than in groups CaAUV, CaADUV, and CaAD, although supplementation with vitamin A was equal in all 4 groups. In contrast to the group CaAUV, CaADUV, and CaAD specimens, those of group CaA did not receive either cholecalciferol or UVB. Consequently, the elevated vitamin A level in group CaA might be related to either an antagonistic interaction in the intestinal absorption of these vitamins, as hypothesized for turkey poult (40), or interactions in the liver storage, as seen in broiler chickens (41).

In this study, the first, to our knowledge, detailed histological descriptions of NMBD in growing veiled chameleons were provided. The lesions were consistent with descriptions of fibrous osteodystrophy due to concurrent vitamin D and Ca deficiency in domestic mammals and reptiles (42). The lesions were different from rickets, which is characterized by disruption of the normal architecture of the physis. Moreover, lesions of vitamin A deficiency, such as inadequate resorption of endosteal bone associated with secondary changes in the nervous system and squamous metaplasia of various epithelia in domestic mammals (42) or metaplasia of the respiratory epithelium, changes in the epiphyseal cartilage zone of the femur and in the periosteum of the vertebrae seen in chicks (43), were absent. However, the skeletal lesions in this study were similar to the rudimentary descriptions in panther chameleons with hypovitaminosis A (11), and a conclusive differentiation of the effects of Ca, vitamin D, and/or vitamin A deficiency on bone pathology was, therefore, not possible. The increased osteoclastic resorption of the casques of 2 group CaA chameleons may be related to a clinically unnoticed lack of vitamin D even though pooled serum 25-OHD₃ concentrations in group CaA were higher than in group CaAD. Segmental calcifications in the colon were seen only in the groups that were fed locusts supplemented with dietary Ca and vitamin A. Only a few reports of soft-tissue calcification have been reported in reptiles. Severe cases of metastatic calcifications in green iguanas (44) and dystrophic calcifications in *Uromastyx* spp. (45) were found to be related to low plasma or serum 25-OHD₃ concentrations (12.5–107.3 nmol/L). In chameleons, mild to moderate soft-tissue mineral-

ization without specified localization has been correlated with supplementation of dietary vitamin A (11) and hypervitaminosis A (2). This is consistent with the suggestion that insectivores may have low dietary requirements of vitamin A and may be prone to hypervitaminosis A (46). A strictly controlled, slightly decreased supplementation with vitamin A and Ca may be adequate for growing veiled chameleons. Tubular degeneration and calcification of the kidneys was only seen in group No chameleons. This was probably related to chronically high levels of parathyroid hormone (not determined in this study) paired with hypovitaminosis D. It was suggested that this combination may result in a breakdown of inhibitors of soft-tissue mineralization over time (47).

The baseline results of BMD provided in this study may be used as a reference to monitor the bone quality in growing lizards in vivo by means of pQCT. This noninvasive technique has been shown to provide satisfactory results with regard to precision and accuracy, even in small animal species such as budgerigars (*Melopsittacus undulatus*) (48) or mustached bats (*Pteronotus parnellii rubiginosus*) (49). The results of the present study emphasize the beneficial effects of UVB radiation on bone metabolism.

In this study, unexpected higher mineral contents in bone, as described in panther chameleons suffering from NMBD associated with low dietary vitamin A (11), were not recorded. To the authors' knowledge, reports on the mineral composition of bones are not available for other reptile species.

It can be concluded that the indoor rearing of veiled chameleons fed with locusts gut-loaded with 12% Ca, dusted with vitamin A (250,000 IU/kg) (75 mg/kg) and cholecalciferol (25,000 IU/kg) (0.625 mg/kg) immediately before feeding, and provision of long (10 h/d), low irradiation exposure (3–120 $\mu\text{W}/\text{cm}^2$ UVB) provided the best combination for the prevention of NMBD. A total serum Ca concentration below 2.3 mmol/l was a useful clinical parameter for NMBD diagnosis in growing veiled chameleons. A mean serum concentration of 25-OHD₃ above 100 $\mu\text{g}/\text{L}$ (>250 nmol/L) was ideal for growing veiled chameleons. A decreased amount of Ca (8–10% Ca) and vitamin A [4000–6000 IU/kg DM (1.2–1.4 mg/kg)] of the diet or 150,000–200,000 IU/kg powder (45–60 mg/kg), in addition to providing carotenoids, may be ideal for growing veiled chameleons. BMD below 100 mg/cm³ (vertebral column, distal humerus) was consistent with NMBD.

Acknowledgments

Special thanks go to Andrea Aebischer, Thomas Aerni, Aline Borer, Jasmin Frei, Wolfgang Geuss, Isabel Keller, Björn Lehner, Joana Meier, Veronika Preite, and Jürg Völlm for their participation in this study. Furthermore, we thank Manuela Bozzo, Erika Garchi, Ines Mittner, Evelyne Rohrer, and Barbara Schneider for excellent technical assistance. Dr. Michael F. Holick and Dr. Tai C. Chen are acknowledged for the provision and analysis of cholecalciferol ampules, and Dr. Gilbert Weber and Dr. Peter Hofmann for the analysis of the dietary vitamins. S.H., C.W., N.R., T.J., and A.L. designed research; S.H. and C.W. conducted research; S.H., N.R., S.H., C.P.A., and A.L. analyzed the data; S.H. and A.L. wrote the paper; and S.H., N.R., H.S., and A.L. had primary responsibility for the final content. All authors read and approved the final manuscript.

Literature Cited

1. Carpenter AI, Rowcliffe JM, Watkinson AR. The dynamics of the global trade in chameleons. *Biol Conserv.* 2004;120:291–301.

2. Abate AL, Coke R, Ferguson GW, Reavill D. Chameleons and vitamin A. *J Herpetol Med Surg.* 2003;13:23–31.
3. Donoghue S. It's all about ontogeny. *Exotic DVM.* 2002;3:38–9.
4. Mader DR. Metabolic bone diseases. In: Mader DR, editor. *Reptile medicine and surgery.* 2nd ed. St. Louis: Saunders Elsevier; 2006. p. 841–51.
5. Barker D, Fitzpatrick MP, Dierenfeld ES. Nutrient composition of selected whole invertebrates. *Zoo Biol.* 1998;17:123–34.
6. Finke MD. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biol.* 2002;21:269–85.
7. Allen ME, Oftedal OT. Dietary manipulation of the calcium content of feed crickets. *J Zoo Wildl Med.* 1989;20:26–33.
8. Anderson SJ. Increasing calcium levels in cultured insects. *Zoo Biol.* 2000;19:1–9.
9. Coke R. Old world chameleons: captive care and breeding. *Bull Assoc Rept Amph Vet.* 1998;8:4–10.
10. McWilliams DA. Nutrition research on calcium homeostasis. I. Lizards (with recommendations). *Int Zoo Yearb.* 2005;39:69–77.
11. Ferguson GW, Jones JR, Gehrman WH, Hammack SH, Talent LG, Hudson RD, Dierenfeld ES, Fitzpatrick MP, Frye FL, et al. Indoor husbandry of the panther chameleon (*Furcifer pardalis*): effects of dietary vitamins A and D and ultraviolet irradiation on pathology and life-history traits. *Zoo Biol.* 1996;15:279–99.
12. Ferguson GW, Gehrman WH, Karsten KB, Hammack SH, McRae M, Chen TC, Lung NP, Holick MF. Do panther chameleons bask to regulate endogenous vitamin D₃ production? *Physiol Biochem Zool.* 2003;76:52–9.
13. Ferguson GW, Gehrman WH, Chen TC, Holick MF. Vitamin D-content of the eggs of the panther chameleon *Furcifer pardalis*: its relationship to UVB exposure/vitamin D-conditions of mother, incubation and hatching success. *J Herp Med Surg.* 2005;15:9–13.
14. Schmidt W. *Chamaeleo calytratus*: Das Jemenchamäleon. 5th ed. Münster (Germany): Natur und Tier, Verlag; 2006.
15. Stahl SJ. Captive management, breeding, and common medical problems of the veiled chameleon (*Chamaeleo calytratus*). Proceedings of the Association of Reptilian and Amphibian Veterinarians. Houston (TX): ARAV; 1997. p. 29–40.
16. Anonymous. Swiss federal ordinance for animal welfare (TschV), 455.1, Appendix II. Bern: Eidgenössische Druck- und Materialzentrale; 2008.
17. Anonymous. Foodstuffs: determination of vitamin A by high performance liquid chromatography. Part 1. Measurement of all-trans retinol and 13-cis-retinol. Brussels: European Committee for Standardization: EN 12823-1; 2000.
18. Anonymous. Foodstuffs: determination of vitamin D by high performance liquid chromatography. Measurement of cholecalciferol (D3) and ergocalciferol (D2). Brussels: European Committee for Standardization: EN 12821; 2000.
19. Anonymous. Foodstuffs: determination of vitamin E by high performance liquid chromatography. Measurement of alpha-, beta-, gamma-, and delta-tocopherols. Brussels: European Committee for Standardization: EN 12822; 2000.
20. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D₃: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D₃ synthesis in human skin. *J Clin Endocrinol Metab.* 1988;67:373–8.
21. Gehrman WH, Jamieson D, Ferguson GW, Horner JD, Chen TC, Holick MF. A comparison of vitamin D-synthesizing ability of different light sources to irradiances measured with a solarmeter model 6.2 UVB meter. *Herpetol Rev.* 2004;35:361–4.
22. Rettenmaier R, Schüep W. Determination of vitamins A and E in liver tissue. *Int J Vitam Nutr Res.* 1992;62:312–7.
23. Zwart P, Langerwerf BWA, Claessen H, Leunisse JJC, Mennes J, van Riel C, Lambrechts L, Kik MJL. Health aspects in breeding and rearing insectivorous lizards from moderate climatic zones. *Zool Garten.* 1992;62:46–52.
24. Donoghue S. Nutrition. In: Mader DR, editor. *Reptile medicine and surgery.* 2nd ed. St. Louis: Saunders Elsevier; 2006. p. 251–98.
25. Ferguson GW, Gehrman WH, Chen TC, Dierenfeld ES, Holick MF. Effects of artificial ultraviolet light exposure on reproductive success of the female panther chameleon (*Furcifer pardalis*) in captivity. *Zoo Biol.* 2002;21:525–37.
26. Gehrman WH, Horner JD, Ferguson GW, Chen TC, Holick MF. A comparison of responses by three broadband radiometers to different ultraviolet-B sources. *Zoo Biol.* 2004;23:355–63.

27. Kölle P. Labordiagnostik bei Reptilien. In: Kraft W, Dürr U, editors. *Klinische Labordiagnostik in der Tiermedizin*. 6th ed. Stuttgart (Germany): Schattauer; 2005. p. 549.
28. Carpenter JW. *Exotic animal formulary*. 3rd ed. St. Louis: Saunders Elsevier; 2005.
29. Harr KE, Alleman AR, Dennis PM, Maxwell LK, Lock BA, Bennett RA, Jacobson ER. Morphologic and cytochemical characteristics of blood cells and hematologic and plasma biochemical reference ranges in green iguanas. *J Am Vet Med Assoc*. 2001;218:915–21.
30. Laing CJ, Fraser DR. The vitamin D system in iguanian lizards. *Comp Biochem Physiol B*. 1999;123:373–9.
31. Ullrey DE, Bernard JB. Vitamin D: metabolism, sources, unique problems in zoo animals, meeting needs. In: Fowler ME, Miller RE, editors. *Zoo and wild animal medicine, current therapy 4*. Philadelphia: Saunders; 1999. p. 63–78.
32. Gillespie D, Frye FL, Stockham SL, Fredeking T. Blood values in wild and captive Komodo dragons (*Varanus komodoensis*). *Zoo Biol*. 2000;19:495–509.
33. Laing CJ, Trube A, Shea GM, Fraser DR. The requirement for natural sunlight to prevent vitamin D deficiency in iguanian lizards. *J Zoo Wildl Med*. 2001;32:342–8.
34. Ramer JC, Maria R, Reichard T, Tolson PJ, Chen TC, Holick MF. Vitamin D status of wild Ricord's iguanas (*Cyclura ricordii*) and captive and wild rhinoceros iguanas (*Cyclura cornuta cornuta*) in the Dominican Republic. *J Zoo Wildl Med*. 2005;36:188–91.
35. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr*. 2004;79:362–71.
36. Palmer DG, Rübél A, Mettler F, Volker L. Experimentally induced skin changes in land tortoises by giving high doses of vitamin A parenterally. *Zbl Vet Med A*. 1984;31:625–33.
37. Holladay SD, Wolf JC, Smith SA, Jones DE, Robertson JL. Aural abscesses in wild-caught box turtles (*Terapene carolina*): possible role of organochlorine-induced hypovitaminosis A. *Ecotoxicol Environ Saf*. 2001;48:99–106.
38. Jensen HB, With TK. Vitamin A and carotenoids in the liver of mammals, birds, reptiles and man, with particular regard to the intensity of the ultraviolet absorption and the Carr-Price reaction of vitamin A. *Biochem J*. 1939;33:1771–86.
39. Dierenfeld ES, Norkus EB, Carroll K, Ferguson GW. Carotenoids, vitamin A, and vitamin E concentrations during egg development in panther chameleons. *Zoo Biol*. 2002;21:295–303.
40. Metz AL, Walser MM, Olson WG. The interaction of dietary vitamin A and vitamin D related to skeletal development in the turkey poult. *J Nutr*. 1985;115:929–35.
41. Aburto A, Edwards HM Jr, Britton WM. The influence of vitamin A on the utilization and amelioration of toxicity of cholecalciferol, 25-hydroxycholecalciferol, and 1,25 dihydroxycholecalciferol in young broiler chickens. *Poult Sci*. 1998;77:585–93.
42. Thompson K. Diseases of bones. In: Grant Maxie G, editor. *Jubb, Kennedy and Palmer's pathology of domestic animals*. 5th ed. Edinburgh: Saunders Elsevier; 2007. p. 1–129.
43. Howell MC, Thompson JN. Lesions associated with the developing of ataxia in vitamin A-deficient chicks. *Br J Nutr*. 1967;21:741–50.
44. Richman LK, Montali RJ, Allen ME, Oftedal OT. Paradoxical pathologic changes in vitamin D deficient green iguanas (*Iguana iguana*). Proceedings American Association of Zoo Veterinarians (AAZV)/Wildlife Disease Association (WDA)/American Association of Wildlife Veterinarians (AAWV) Joint Conference Denver, CO, AAZV. 1995:203–4.
45. Raphael BL, James SB, Cook RA. Evaluation of vitamin D concentrations in *Uromastyx* spp. with and without radiographic evidence of dystrophic mineralization. Proceedings American Association of Zoo Veterinarians (AAZV). Puerto Vallarta, MX, AAZV. 1999:20–2.
46. Dierenfeld ES, Barker D, McNamara TS, Walberg JA, Furr HC. Vitamin A and insectivore nutrition. *Verh ber Erkr Zootiere*. 1995;37:245–9.
47. Gibbons PM. Comparative vertebrate calcium metabolism and regulation. Proceedings Association of Reptilian and Amphibian Veterinarians (ARAV). Orlando, FL (ARAV). 2001:267–79.
48. Liesegang A, Fischer I, Hatt J-M. Quantitative computed tomography for the assessment of bone mineral content and density in female and male budgerigars (*Melopsittacus undulatus*). *Verh ber Erkr Zootiere*. 2005;42:61–3.
49. Liesegang A, Firzlaff U, Kiefer B, Streich WJ, Clauss M. Different supplementation of minerals in bats and the consequences on bone mineral density. In: Fidgett AL, Clauss M, Eulenberger K, Hatt J-M, Hume I, Janssens G, Nijboer J, editors. *Zoo animal nutrition*. Fürth: Filander; 2006. p. 295–300.