

Indoor Husbandry of the Panther Chameleon *Chamaeleo [Furcifer] pardalis*: Effects of Dietary Vitamins A and D and Ultraviolet Irradiation on Pathology and Life-History Traits

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To assess the importance of diet and light for indoor maintenance, hatchling panther chameleons were reared for 1 year on crickets fed diets that differed in vitamin concentrations and in different light environments. Dietary transfer of vitamins from the cricket diet to the lizards via the crickets was quantified, as was UV irradiance. There was a statistically significant dietary enhancement of growth by both vitamins on males. UV-A irradiation significantly suppressed growth of females. Low vitamin A shortened life span and resulted in a number of gross and histological pathologies. Hepatocellular lipidosis, indicating a possible toxicosis, occurred with all diets and light treatments. Higher vitamin A resulted in mild soft-tissue mineralization, and high vitamin D shortened the life span of females. Low vitamin A drastically reduced reproduction in both sexes. The intermediate levels of dietary vitamins resulted in the best production of viable eggs by females. However, without high UV-B irradiation, all viable eggs died at term and contained different vitamin levels than hatching eggs from wild-caught females. Baseline levels of egg calcium are given for hatching eggs from wild-caught

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females. Modifications in current husbandry procedures are recommended.

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INTRODUCTION

Chameleons as Captives

Old-world chameleons have become one of the most sought-after reptiles for captive maintenance in recent years. Thousands of wild-caught animals were exported from East Africa during the 1970s until the trade was curtailed [Dodd, 1982]. More recently the wild-caught chameleon exportation from West Africa and Madagascar has reached similar proportions [Anon., 1993; Glaw and Vences, 1994]. Most imported specimens have either died en route or shortly after arrival, due to their stress-prone nature and lack of proper husbandry. The large drain on the wild populations and the dismal prospects of survival after capturing have posed serious conservation and ethical concerns.

Despite these problems, justifiable reasons exist for the captive maintenance of chameleons and the development of proper husbandry techniques. Limited exportation of wild-caught specimens of some species based on sound knowledge of their population dynamics is justifiable and necessary. Some of the justifications for keeping chameleons follow.

Zoos and educational programs. Chameleons are specialized for climbing. Their special features (e.g., zygodactyl feet, slow deliberate movement, turret-shaped rotating eyes, rapid color change ability, and long projectile tongue used for insect capture) are bizarre and fascinating. Chameleons are one of the best animals to stimulate interest and fascination in children and the nonbiologically educated adult population. They are ideal for drawing attention to our increasingly critical global environmental crisis.

Biological research. While the unique features of chameleons have been studied (e.g., the biomechanics of their tongue: Altevogt and Altevogt, 1954; mechanisms of color change: Zoond and Eyre, 1934, 1935), many aspects of their behavior, physiology, and ecology remain unknown. Future research in both the laboratory and field on chameleons should advance our general biological understanding (i.e., they can become model organisms).

Conservation. Chameleons, adapted to tropical forests, are rapidly disappearing with the rapid deforestation of the tropics [Raxworthy, 1988; Glaw and Vences, 1994]. Only a few of the 130 or so described species [Klaver and Böhme, 1986] can survive the rampant habitat destruction. This justifies the establishment of captive breeding colonies. Such colonies can provide refuges for endangered species until suitable habitat can be restored and can reduce the demand for heavy harvesting of wild populations.

The pet trade. The keeping of pets dates back to the dawn of human civilization. While mammal, bird, and fish culture of selected species for the pet industry predates herpetoculture, the captive propagation of reptile and amphibian species is accelerating [Love, 1994]. Some chameleon species certainly will become part of this industry. Animal culture in its early stages of development can lead to serious conflict

with conservation, education, and research [e.g., see Swingland, 1994; Glaw and Vences, 1994]. But as culture technology matures, conflict should diminish, and culture techniques can significantly complement these other areas [Backner, 1994].

Chameleon Husbandry: The State of the Art

Despite the initially high mortality of wild-caught chameleons, a marketable number of individuals survive and, with proper care, adjust to captivity. Survivors of the first 4–6 weeks have a reasonably good prospect of at least a 1- or 2-year life span in captivity, especially males. Adults collected during the natural breeding season often will readily breed in captivity, but success of these breedings is rare.

Why long-term maintenance fails, especially for indoor captives, is unknown. Opinions and recommendations, based on little experimental study, vary. Problems and controversy include 1) the methods and amounts of nutrient supplementation, 2) light quality, quantity, and source, 3) proper temperature, thermal gradients, and seasonal thermal variation, 4) humidity and air circulation, 5) enclosure size, construction, and furniture, 6) social grouping and density for adults and juveniles, and 7) egg incubation environments. Current recommendations for “proper” care are published [Bustard, 1989; Obst et al., 1989; De Vosjoli, 1990a,b; Henkle and Heinicke, 1993; Schmidt et al., 1994; De Vosjoli and Ferguson, 1995; Le Berre, 1995].

Nutritional and Light Quality Needs of Chameleons Are Not Well Understood

While energy intake of chameleons has been measured or estimated for a few species [Burrage, 1973; Ferguson, 1991, 1994], nutritional quality requirements remain poorly understood. Senegal *Chamaeleo senegalensis* and panther chameleons (*C. [F.] pardalis*) prefer novel prey species [Eason, 1990; Ferguson, unpublished data] and may behaviorally balance their diet quality. However, no experimental data on chameleon nutrition and light requirements have been published.

Most experimental studies of nonhuman animal nutrition have utilized domesticated species, particularly rodents and commercially important livestock, such as cattle, rabbits, poultry, and fish. For good general explanations of the nutritional roles of calcium, vitamin A, vitamin D, and ultraviolet light in vertebrates, see Sebrell and Harris (1971) Fowler [1978] Norman [1979] Frye [1991] and Annis [1992]. For additional details of photoregulation of vitamin D, see Webb et al. [1989] and Holick [1989]. For discussion of symptoms of hypervitaminosis A, see Dorr and Balloun [1976], Stevens and Blair [1983], and Metz et al. [1985]. The roles of vitamin A and carotenoids are discussed by Pennino et al. [1991] and Dierenfeld et al. [1995]. Vitamin photodegradation is discussed by Berne et al. [1984, 1990], Drott et al. [1991], and Tang et al. [1994]. The interaction of fat soluble vitamins is discussed by Frigg and Broz [1984] and Metz et al. [1985].

Nutritional research on reptiles is at such a preliminary level that we do not know how well requirements and roles of nutrients for other vertebrates apply to reptiles. However, the scant information available suggests that reptiles are unique in their requirements and tolerances of vitamins A and D. These may differ between species and populations of the same species [Allen, 1989; Frye, 1991; Talent, unpublished data].

Our goal is to present the results of perhaps the first full-scale experimental studies of micronutrition in a chameleon. We tested the efficacy of gut-loading

TABLE 1. Design of Experiment 1*

Treatment		Dietary vitamin A	Dietary vitamin D	UVA
1	A	Low	Low	Low
	B	Low	Low	High
2	A	Low	High	Low
	B	Low	High	High
3	A	High	Low	Low
	B	High	Low	High
4	A	High	High	Low
	B	High	High	High
5	A	Medium	Medium	Low
	B	Medium	Medium	High

*See Table 3 and text for quantitative values of treatments and replications.

crickets with vitamins A and D to overcome deficiencies of these nutrients and the value of black lights and sunlamps as indoor sources of ultraviolet light. Based on the important new findings presented below, modifications of current husbandry procedures for panther chameleons are recommended.

METHODS

Subjects

Eggs were obtained from wild-caught imported panther chameleons *Chamaeleo [Furcifer] pardalis*. Methods of maintenance and egg collection are detailed elsewhere [Ferguson, 1994]. Two experiments were performed (Tables 1, 2). To minimize genetic variability, hatchlings for the first experiment were siblings from a single female. Due to limited availability of hatchlings, those for the second experiment were from three additional females. The geographic origin of all females was from the east coast of Madagascar.

Hypotheses and Experimental Designs

Our hypothesis for the first experiment (Table 1) was that dietary vitamins A and D delivered by gut-loading insect prey and UV-A irradiation provided by black light are factors important for normal growth, development, and reproduction of the panther chameleon in a captive indoor environment. It was derived from published recommendations [De Vosjoli, 1990a,b; Ferguson and Blades, 1991].

The second experiment (Table 2), performed after the results of the first experiment were known, tested the relative importance of dietary vitamin D and UV-B irradiance provided by sunlamps and natural sunlight on the same life-history traits.

We assumed that other physical and sensory needs were adequately provided by the laboratory procedures and that high and low experimental levels of the factors spanned the range of required levels. These assumptions were based on previous experience with lizard and chameleon keeping.

For the first experiment (Table 1) a four-way ($2 \times 2 \times 2 \times 2$) cross-classified factorial design, using high and low values of dietary vitamin A, dietary vitamin D, and UV-A for each sex, was used. Each of the 16 treatment groups was replicated twice ($n = 32$). An additional diet group with intermediate levels of both dietary vitamins A and D was cross-classified with the two UV-A levels for each sex. With

TABLE 2. Design for Experiment 2*

Treatment	Dietary vitamin D	UVB	Light source
1	Low	Low	Black light indoors
2	Low	High	Sunlamp indoors
3	High	Low	Black light indoors
4	High	High	Sunlamp indoors
5	Low	High	Sunlight outdoors
6	High	High	Sunlight outdoors

*See Table 3 and text for quantitative values of treatments and replications.

the two replicates of the four possible combinations, the total number of individuals in the experiment was 40. Females were bred to the experimental males.

For the second experiment (Table 2), the two levels of dietary vitamin D used in Experiment 1 were cross-classified with two levels of UV-B in an indoor environment identical to that of Experiment 1 for females only. The low UV-B level was similar to that in all treatments of Experiment 1. The high level approximated the total UV-B dose estimated to be available in outdoor environments from natural sunlight [see Gehrmann, 1987]. Additional females receiving the two dietary vitamin D levels were maintained in natural sunlight in outdoor environments that differed from the lab regimes in temperature and photoperiods but approximated the indoor UV-B dose of the high UV-B treatments. Dietary vitamin A levels were at the intermediate levels of Experiment 1 for all Experiment 2 females. Each of the six treatment groups was replicated three or four times (total $n = 21$). Experiment 2 females were bred to three males that were not part of the experiment but received crickets fed the medium vitamins A and D diet. The males were rotated evenly among all treatment groups for breeding. Each of the two experiments lasted 1 year.

Experimental Setup

Lizards in both experiments were maintained individually in plastic terraria. Initial terrarium sizes were $25 \times 17 \times 20$ cm. Sizes were adjusted at 2 months to $30 \times 19 \times 24$ cm and at 4 months (for males) to $38 \times 23 \times 28$ cm. Tops were covered with plastic mesh (mesh size at 2×7 mm) or 6 mm mesh hardware cloth lids; paper covered the sides of the terraria to visually isolate individuals in adjacent cages.

Cage furniture included a petri dish 8 cm in diameter for water, a petri dish 3.5 cm in diameter for cricket food, and one or more tree branches for climbing. Branches were suspended above the floor to allow lizards to escape from crickets, which mostly remained on the floor of the cage. Deionized water was added to the dish, and the sides of the cage were misted with deionized water daily. All cages were thoroughly cleaned once a week and as needed. Cricket food was changed weekly.

To minimize "position effects," mostly due to small temperature and light gradients, cage location was systematically rotated every second day. Outdoors (Experiment 2, treatment groups 5 and 6, Table 2), cages were moved among partially shaded outdoor light environments to avoid extreme photothermal conditions.

Indoors, a 24 h horizontal and vertical thermal gradient was established in each cage by placing one-half of the cage floor on an activated flex-watt heat pad. The mean thermal gradient within each cage was 41 to 28°C during the day and 41 to 24°C at night. There were no significant temperature differences in cage gradients. Most

important, there were no significant cloacal temperature differences among the chameleons in any of the indoor treatments in each experiment. Indoors, mean daytime cloacal temperature was 32°C; mean nighttime cloacal temperature was 24°C. Cloacal temperatures were not routinely monitored for Experiment 2 females maintained outdoors, but daytime values were often higher during the summer.

For Experiment 1, fluorescent lamps, suspended 34 cm above the cage floor, were the primary light source. Weak illumination from ceiling fluorescent fixtures, containing cool white lamps, supplemented all indoor cages about 8 h per day. Two 100 cm double 40 watt bulb luminaires, arranged parallel to a table top, serviced the cages on that edge. For the low UV-A treatment, one 40 watt Vita-Lite bulb (DuroTest Corp, Fairfield, NJ) was illuminated. For the high UV-A treatment, a 40 watt black light [General Electric BL-40, Light Bulb Solutions, Dallas, TX] was paired with a 40 watt Vita-Lite. Illumination for both treatments was 12 h daily. Visible light illuminance (measured with a General Electric Type 214 light meter) was similar for the two treatments (658.5 lux for the low, 697.2 lux for the high). However, UV-A (measured with a Spectroline UVA radiometer, model DM-365N, Spectronics Corp., Westbury, NY) irradiances were substantially different [$4.7 \mu\text{W}/\text{cm}^2$ for the low, $81.6 \mu\text{W}/\text{cm}^2$ for the high]. UV-B irradiances (measured with a Spectroline UVB radiometer, model DM-300N) were low (1.0 – $2.0 \mu\text{W}/\text{cm}^2$) for both treatments. Because Vita-Lites have a spectral power distribution (SPD) close to that of natural sunlight, the light quality of the low UV-A treatment group was close to natural. However, that of the high UV-A treatment group was strongly biased toward UV-A. These light measurements were made on the floor in the middle of each terrarium, with the mesh lid in place. The lid attenuated light transmission by about 25%.

For Experiment 2, indoor lizards in the low UV-B treatments were exposed to one black light (BL-40) 10 min per day five times per week. Those in the high UV-B treatments (indoors) received a sunlamp (FS-40) treatment for 1 h per day. For both treatments, the cages were partially shaded, and spatial UV-B gradient was established (from 0 – $2.1 \mu\text{W}/\text{cm}^2$ irradiance for the low treatment, from 0 – $89 \mu\text{W}/\text{cm}^2$ irradiance for the high treatment). Lizards were closely monitored during exposure to the gradients and voluntarily exposed themselves to 60–70% of the maximum dose possible during the treatments over the course of the experiment [Jones, 1995]. In addition, all indoor lizards in this experiment received illumination from a Vita-Lite fluorescent bulb ($1.0 \mu\text{W}/\text{cm}^2$ UV-B irradiance) 12 h per day.

The outdoor lizards in Experiment 2 received natural sunlight, often partially shaded by trees. Shading was necessary to prevent overheating throughout most of the first half of the experimental period (May through October, 1993). UV-B dose during this period approximated those for the high UV-B indoor treatment (about $25 \text{ J}/\text{cm}^2$ for the half year). During the last half of the experiment, animals were taken outdoors for as many hours per day as possible, but, on days when they could not be taken out due to cloud cover and cold temperatures, they were kept indoors under the same light conditions as the high UV-B indoor treatment. They were kept indoors less than 25% of the time during this half-year period.

Crickets (*Acheta domestica*) were obtained from Armstrong Cricket Farm (West Monroe, LA) as the sole food source for the experimental lizards. Crickets were maintained in $30 \times 19 \times 24$ cm terraria partially placed on heat tape to provide thermal gradients. Tap water was available on moistened paper towels. Diets specially manufactured by Zeigler Bros. (Gardners, PA) were available ad libitum for

TABLE 3. Vitamin A and vitamin D₃ concentrations in five cricket diets used in the study

Diet	Vitamin A (IU/g)	Vitamin D ₃ (IU/g)
Low A, no D	5.67 ^a	0
Low A, high D	5.67 ^a	100.2 ^b
High A, no D	102.97 ^c	0
High A, high D	102.97 ^c	100.2 ^b
Medium A, medium D	54.32 ^c	50.2 ^b

^aA baseline of 5.67 IU/g of vitamin A equivalent, due to carotenoids, was present in all five diets.

^bCholecalciferol.

^cAs added retinyl acetate in addition to baseline carotenoids; because of some degradation of vitamin A in freezer-stored diets, stores were replaced every six months.

2–7 days before crickets were harvested to assure maximum gut-loading (Allen and Oftedal, 1989).

The Zeigler diets (Table 3) were identical in composition to the standard Zeigler cricket diet (ingredients available upon request from Zeigler or the senior author), except for the levels of vitamins A and D. One ingredient of the standard Zeigler cricket diet of special note is the calcium level (8% by weight). Diets were maintained at -10°C until use.

Fifteen to 30 crickets (about 30% of the lizard body mass) were offered to each lizard three times per week. This amount matched that shown to be consumed ad libitum by this species [Ferguson, 1991, 1994]. Crickets remaining from the previous feeding were removed prior to the next feeding.

Traits Measured in Animals

A number of traits were analyzed for treatment effects. These included growth, survival, gross morbidity of general behavior and morphology, histopathology, male social behavior and fertility, female oviposition, egg viability, and hatching success. Animal tissues were processed and examined by author F.L.F. without prior knowledge of the diet and light treatments of each sample.

Vitamin, Mineral, and Toxin Assays

Cricket feed, crickets, and chameleon eggs were assayed using high performance liquid chromatography (HPLC) for vitamin A (by author E.S.D.) and vitamin D (by authors M.F.H., T.C.C., and Z.L.).

Calcium of eggs was assayed using atomic absorption spectrophotometry (by author T.S.G.). Bone minerals were analyzed using atomic absorption spectrophotometry for calcium and colorimetric analysis for phosphorus (by author J.J.V.).

Mycotoxins in cricket feeds were analyzed by the Animal Health Diagnostic Laboratory at Michigan State University, East Lansing.

RESULTS

Uptake of Vitamins From Feeds by Crickets

The vitamin transfer from feeds to crickets and the total concentration depended on cricket size (age). Larger crickets contained less vitamins per gram than smaller crickets.

Vitamin A. For larger crickets (0.25 g wet mass; two-thirds grown) fed the high vitamin A diet for 10 days, vitamin A concentrations were no higher than those of crickets sampled before feeding on the diet (1.8 IU/dry g in crickets, $n = 1$; $0.7 \pm 0.05 \text{ s.d., IU/g}$ $n = 2$, in crickets after feeding). The feed used at Armstrong's cricket farm contained low but measurable vitamin A concentration (about 5 IU/g). Smaller crickets (0.06 g wet mass) increased from $1.2 \pm 1.0 \text{ s.d. IU/g}$, $n = 3$ in prefed crickets to an average of 15.8 IU/g (range from $3\text{--}30 \pm 13.9 \text{ s.d., } n = 3$) in crickets feeding on the high vitamin A diet for 10 days, a severalfold increase of vitamin A. The difference between vitamin A concentration of gut-loaded small vs. large crickets was nearly statistically significant ($P = 0.083$, Mann Whitney-U Test).

Vitamin D. For larger crickets fed the high vitamin D diet, vitamin D concentrations increased from $1.1 \pm 0.06 \text{ s.d., } n = 2, \text{ IU/g}$ in the prefed samples to $4.3 \pm 1.1 \text{ s.d., } n = 2, \text{ IU/g}$ after 10 days of feeding, a fourfold increase. Smaller crickets increased from $0.9 \pm 0.2 \text{ s.d., } n = 2, \text{ IU/g}$ to $9.1 \pm 6.1 \text{ s.d., } n = 3, \text{ IU/g}$, a ninefold increase. However, the difference between vitamin D concentrations of gut-loaded small vs. large crickets was not statistically significant.

Because of few replicate samples (1–3), we cannot demonstrate statistically that uptake of vitamin D was more efficient than that of vitamin A, but it seems likely in the larger crickets. Also, final concentrations in larger crickets were consistently lower than in smaller crickets for both vitamins.

Oral Intake of Vitamins by Lizards

Ferguson [1991] reported the total wet mass of small and large crickets ingested ad libitum by panther chameleons. Ingestion was measured for each one month age class for the first nine months of life in a laboratory setup nearly identical to that in this study. Monthly intakes increased during the first six months and stabilized after that. Dry mass of crickets is one quarter of wet mass [Pennino et al., 1991]. Accordingly, because lizards were fed their ad libitum rate in this study, total dry mass ingested could be estimated for the first and second six months as a quarter of total wet mass for the first six months and a quarter of six times the average monthly ingestion for months 7–9. Only small crickets were fed to the lizards for the first three months. Only larger crickets were fed to them for the remaining months of the study. By assuming that mass of dietary intake matched closely the ad libitum feeding rate previously determined and measuring the vitamin concentration of gut-loaded crickets, we estimated the maximum intake of dietary vitamins A and D that could have been ingested during the experiment, using the high vitamin levels (Table 3). Accordingly, we calculated a maximum total dietary intake during the first six months of life of 100 IU of vitamin A and 150 IU of vitamin D. During the second six months of life, we estimated a maximum intake of 40 IU of vitamin A and 230 IU of vitamin D.

Due to reduced vitamin concentrations in large crickets, the weekly doses of vitamins A and D decreased. Vitamin A doses dropped precipitously from 3 IU/g/week for juveniles to 0.04 IU/g/week for adult females. Vitamin D doses dropped more gradually from 2 IU/g/week to 0.20 IU/g/week .

Growth

All Experiment 1 lizards surviving 4 months grew substantially and were approaching minimum mature size by the end of month 4. Females grew from $29.4 \pm 0.6 \text{ s.d., } n = 20$, to $114.5 \pm 12.8 \text{ s.d., } n = 20$, mm SVL, males from 29.5 ± 0.5

s.d., $n = 20$, to 130.8 ± 13.6 s.d., $n = 20$, mm SVL. However, there were some statistically significant treatment effects. For males, there was significant growth enhancement for high levels of both vitamins (high vitamin D plus high vitamin A males, group 4, Table 1, averaged 143.7 mm SVL, $n = 4$, $P < 0.01$ ANOVA). But the growth enhancement of vitamin A was stronger (vitamin A effect: $P < 0.001$, $R^2 = 0.63$; vitamin D effect: $P < 0.01$, $R^2 = 0.29$). Females did not show significant effects of diet on growth but showed a significant depression of growth with high UV-A (black light females, groups 1B–5B, Table 1, averaged 106.4 mm SVL, $n = 10$, $P < 0.05$, ANOVA).

Growth of Experiment 2 females showed no statistically significant diet or light effects. All animals exhibited rapid linear growth which sharply reached a plateau at sexual maturity.

Mortality

In Experiment 1 mortality was zero (0 of 40) during the first 3 months but increased as sexual maturity approached. By the end of the fourth month, 9 of 40 (24%) had died or were sacrificed just prior to death. By the end of the tenth month, 22 of 40 (55%) were dead. Of the dead, most were females (14 or 64%).

There were clear treatment effects. All eight of the lizards fed crickets eating diets low in both vitamins (group 1, Table 1) were dead by the end of the tenth month. Furthermore, the four (of eight) living animals on a low vitamin A plus high vitamin D diet (group 2, Table 1) were in poor condition, were unlikely to survive much longer, and were sacrificed. Low dietary vitamin A intake was therefore correlated with an earlier death.

In Experiment 2 there was no mortality before 6 months of age. After that, mortality was strongly associated with reproduction (82%, 9 of 11 deaths occurred in gravid or early postgravid females). Mortality was significantly higher for females on the high vitamin D diet (64%, 7 of 11 died, groups 3, 4, and 6, Table 2) than for those on the low vitamin D diet (40%, 4 of 10 died, groups 1, 2, and 5, Table 2, Fisher Exact Probability = 0.053).

Morbidity

Seven abnormalities of morphology and behavior were recorded, mostly in the Experiment 1 subjects. These are listed with little amplification:

1. Tail tip necrosis or skin lesions were the first symptom to appear (most cases appeared in month 4 after switching to the larger crickets); the condition was not debilitating but resulted in an attenuated tail in some individuals.
2. Swollen lips were mild, appeared about the same time as the first symptom, and had no permanently debilitating effect.
3. Gular or cervical edema (swelling) was seen only in adults. It was the subcutaneous accumulation of clear fluid.
4. Vertebral kinking or abnormally constant flexure at the pelvic region occurred in late adolescence or early maturity, was permanent, often debilitating, and associated with histologically documented metabolic bone disease, and was often accompanied or shortly followed by symptoms 5 and 6.
5. Eyelid closure and oozing occurred in early maturity, resulted in aphagia and

adypsia (stopped eating and drinking), and would have led to a rapid physical deterioration and death without treatment. All individuals with this symptom were sacrificed after eyes had been closed for a few days.

6. Loss of posture control or muscular grip occurred in early maturity. Its onset was closely correlated with that of symptom 4. Individuals would sit on the cage bottom with their bodies vertically upright, or sometimes they would be on their sides. Limbs often trembled during locomotion, and the tongue was often dysfunctional when attempting to feed.

7. Hemipenial impaction (males) occurred in newly matured males in Experiment 1. It resulted from failure of the normal regular expulsion of the shed epithelial lining of the hemipenis. The "plug" was easily removed from such males with forceps, but the impaction often recurred.

While at least one of these symptoms occurred in at least one individual in every Experiment 1 treatment group but one (high vitamin A, high vitamin D, high UV-A, group 4B, Table 1), there were clear treatment effects on their frequency and severity. Fifteen of the 16 animals (94%) receiving low dietary A (groups 1 and 2, Table 1) showed several or all of these symptoms. In contrast, only 6 of the 25 Experiment 1 animals (24%) receiving medium or high dietary A (groups 3, 4, and 5, Table 1) showed symptoms. Of the three most debilitating symptoms (4, 5, 6), only one animal, a male receiving medium vitamin A and high UV-A (group 5B, Table 1), showed mild kinking of the vertebral column. These symptoms seem, then, to be signs of vitamin A deficiency. There was no clear association of vitamin D manipulation with any of these symptoms in Experiment 1. All symptoms except 1 and 2 appeared only in adults.

Experiment 2 animals, all on medium levels of dietary vitamin A, were less symptomatic. Only one adult animal developed mild vertebral kinking (symptom 4). However, cervical edema, largely absent in Experiment 1, was significantly associated with high UV-B in adults (present in 50%, 7 of 14 animals, receiving high UV-B, groups 2, 4, 5, 6, Table 2, present in none of 7 animals receiving low UV-B, groups 1 and 3, Table 2, $P = 0.047$, Fisher Exact Probability).

Histopathology

Forty animals died or were euthanized and preserved for histopathological analysis (19 in Experiment 1, 21 in Experiment 2). All of the Experiment 1 treatment groups were represented except one (group 2B, Table 1). We investigated only dietary effects on internal tissues for the first experiment. Sample sizes for each of the five diet groups were three to five. The animals were preserved at 10 or 13 months of age. For Experiment 2, sample sizes for each of the six diet and light groups were three or four.

1. Hepatocellular vacuolar lipidoses or fatty liver was moderate to severe in 32 of the 40 individuals representing all treatments in Experiments 1 and 2. Because the liver is a robust organ, this is not necessarily a seriously debilitating condition and may indicate some type of dietary imbalance or toxicosis.

2. Nephritis or renal gout, a serious, ultimately fatal condition occurred in four of the 40 individuals. Two were in Experiment 1, group 4 (Table 1). One was in Experiment 1, group 5. One was in Experiment 2, group 3 (Table 2). Three of the

four were on high levels of vitamin D. This pathology is generally thought to result from toxicosis, dietary imbalance, or water deprivation [Frye, 1991].

3. Metabolic bone disease, a general category of disorders, has multiple manifestations and is given a variety of names, depending on what is wrong with the bones [see Fowler, 1978; Frye, 1991]. It occurred in all Experiment 1 animals in the low dietary vitamin A groups (1 and 2, Table 1), even those showing no vertebral kinking or abnormal flexure (symptom 4 above). One additional Experiment 1 female in the high dietary vitamins A and D group (group 4, Table 1) had this disorder along with severe renal gout. Manifestations included soft, easily cut bones, poorly formed, irregular cement lines, accompanied by (surprisingly) a statistically significantly higher mineral (ash) content in dry fat-free bone [$P < 0.05$ ANOVA], and a slightly but not statistically significantly higher percent calcium content than the harder, more normal looking bone found in the groups fed higher levels of vitamin A (groups 3, 4, and 5, Table 1). This symptom was absent in all Experiment 2 animals.

4. Soft tissue mineralization is an abnormality with multiple causes. In almost direct opposition to metabolic bone disease, all Experiment 1 animals receiving medium and high dietary vitamin A (groups 3, 4, and 5, Table 1) showed mild to moderate soft tissue mineralization, whereas none of the six receiving low dietary A (groups 1 and 2, Table 1) showed this symptom. This symptom was absent in all Experiment 2 animals.

Reproduction in Males

Males in all Experiment 1 diet groups showed courtship behavior or aggression, beginning at about 4 months of age. However, of the eight males on low dietary vitamin A (groups 1 and 2, Table 1), only two (25%), both on high dietary vitamin D, copulated. Of the 12 males on high or medium dietary vitamin A (groups 3, 4, and 5, Table 1), six (50%) copulated at least once (four of the six were also on high or medium dietary vitamin D, groups 4 and 5, Table 1). Most copulations of all females were by a single, particularly vigorous, male (on medium vitamins, low UV-A, group 5A, Table 1). We controlled this to reduce variation of male factors in assessing reproductive performance of females. Active spermatogenesis was histologically documented in at least one male from each of the groups on high or medium dietary vitamin A (3, 4, and 5, Table 1).

Six of the 20 males eventually showed strong male-male aggressive displays, including the suffusion of the bright orange or red color characteristic of males from east-coastal populations. All six of these males received medium or high dietary vitamin A, and five of the six also received medium or high vitamin D (groups 4 and 5, Table 1).

Reproduction in Females

In Experiment 1 some viable (surviving at least several weeks) eggs were produced by females on all five diet treatments, but there were clear experimental effects on the number and percent of viable eggs. In Experiment 1 only nine of 20 females (45%) mated and produced viable eggs. Of these, most (six) were receiving medium or high dietary vitamin A (groups 3, 4, and 5, Table 1). Viability of the five clutches produced by three females receiving low dietary vitamin A (groups 1 and 2, Table 1) was very low (0–31%, mean 17%), whereas viability of the 25 clutches produced by six females receiving medium or high vitamin A (groups 3, 4, and 5,

Table 1) was higher (range 0–75%, mean 50%). The best production of viable eggs was by the females on the medium vitamins (group 5, Table 1, three females produced 221 viable eggs); next was by females on the high vitamin A, low vitamin D diet (group 3, Table 1, one female produced 63 viable eggs); next was by females on the high vitamins diet (group 4, Table 1, two females produced 57 viable eggs); next was by the females on the low vitamin A, high vitamin D diet (group 2, Table 1, two females producing nine viable eggs); last was by the females on low dietary vitamins (group 1, Table 1, one female producing two viable eggs).

A critical result was that none of the viable eggs produced by any of the Experiment 1 females hatched. Most, however, went to full term before they died. Eggs contained fully formed embryos that survived and developed for 6–10 months.

In Experiment 2, reproduction was more successful. Twenty of 21 (95%) produced viable eggs (vs. 45% for Experiment 1 females). Overall egg viability for those reproducing was similar (54%, 362 of 675 eggs, in Experiment 1; 58%, 477 of 819 eggs, in Experiment 2). Significantly greater ($P < 0.0001$, chi-squared test) hatch success occurred with exposure to high vs. low UV-B in Experiment 2 (groups 2, 4, 5, and 6 vs. 1 and 3, Table 2). Of those four high UV-B treatment groups, the highest hatch success was with those constantly indoors receiving high dietary vitamin D plus sunlamp (group 4, Table 2); 38%, 52 of 173, viable eggs hatched. Next was the group constantly indoors receiving low dietary vitamin D plus sunlamp (group 2, Table 2); 22%, 23 of 105, hatched. The two outside groups were next. Fifteen percent, 19 of 128, hatched for the high dietary vitamin D group (group 6, Table 2). Thirteen percent, 20 of 156, hatched for the low dietary vitamin D group (group 5, Table 2). Only two eggs of 113 (2%) hatched in the high dietary vitamin D plus low UV-B group (group 3, Table 2), but they both died after a few days. No eggs of 144 hatched in the low dietary vitamin D plus low UV-B group (group 1, Table 2).

Vitamin and Calcium Content of Eggs

To obtain baseline values of egg vitamin and mineral content, samples of eggs from clutches of wild-caught parents laid within the first 2 months of captivity were deep-frozen [-70°C] within 6 h after the neonate slit the shell but had not yet emerged from the egg. More than 95% of the remaining sibling eggs hatched into robust juveniles, several of which have been successfully raised to adulthood. Similarly, live eggs approaching term from Experiment 1 females were deep-frozen after it was clear that siblings were unable to slit the egg shell and were dying at term.

Vitamin A. Egg contents, including neonate and all fluids, were analyzed. Five slit eggs, representing five clutches, from the wild-caught females averaged 20.6 IU of vitamin A/dry g (range 10.8–49.6 $\pm$ 16.3 s.d., $n = 5$). Nine term eggs from females captive-raised on the high vitamin A diet averaged 3.1 IU/g (range 2.9–3.7 $\pm$ 0.4 s.d., $n = 6$). Six term eggs from females captive-raised on the medium vitamin A diet averaged 1.5 IU/g (range 0.6–2.2 $\pm$ 0.5 s.d., $n = 9$). The difference in vitamin A content between eggs from the high vs. medium vitamin A diet mothers was statistically significant ($P < 0.001$ ANOVA). Vitamin A content of eggs from experimental mothers was somewhat higher (average 2.47 $\pm$ 0.9 s.d., IU/g $n = 6$) when the mothers had not been exposed to high UV-A than when they had (average 1.96 $\pm$ 0.9 s.d. IU/g, $n = 9$), but these differences were not statistically significant. Regardless, at the end of incubation, eggs from wild-caught females had 7–14 times

the vitamin A content of eggs from captive-raised females ($P < 0.001$, Mann-Whitney U Test).

Vitamin D. The contents of four slit eggs, siblings of those analyzed for vitamin A, from the wild-caught females averaged 0.32 IU vitamin D/dry g (range 0.16–0.44 $\pm$ 0.12 s.d.). A single term egg from a medium vitamin D experimental female contained 0.23 IU/g. Three term eggs from three high vitamin D experimental clutches contained an average of 2.16 IU/g with a very large range (0.28–5.60 $\pm$ 2 s.d.). While more results are needed for firm conclusions, it seems possible that the high dietary vitamin D levels may result in substantially higher vitamin D content of eggs than normally present in eggs from wild-caught females. However, conclusions based on comparison of hatched eggs with those that have not (and aren't going to) must be formed with caution (see Discussion).

Calcium. The shell and egg contents were analyzed separately. Calcium values are available only for slit eggs that are siblings with those from wild-caught females analyzed for vitamins. Twelve eggs averaged 6.27 $\pm$ 1.18 s.d. $n = 12$, mg of calcium per egg. Eighty-seven percent of the calcium was in the embryo and egg fluids. The rest was in the shell.

Mycotoxins

Analysis of the Ziegler cricket diets showed no levels of any mycotoxin judged to be clinically significant, although there were traces of vomitoxin (0.41 ppm).

DISCUSSION

The most important result of this study is that, despite normal appearing growth and reproduction, none of the indoor dietary and light regimes produced robust hatchlings without exposure to substantial UV-B irradiation. The dietary and light effects on growth and pathology are discussed separately from those on reproduction.

Growth

Obvious growth suppression and many of the pathologies reported here can be prevented with only the high and medium dietary levels of vitamins A and D supplemented into the insect feeds used in this study. The benefit of the intensive irradiation of chameleons with UV-A is at best marginal and had at least one possible negative effect (reduced female growth). The suppression of female growth may have been due to two factors. First, ultraviolet (sunlight) has a degradative effect on vitamin D stored in the skin of vertebrate animals [Webb et al., 1989]. UV-A also degrades vitamin A, both in vitro and in the skin [Berne et al., 1984, 1990; Drott et al., 1991], but this effect applies mainly to retinyl esters, not retinol [Tang et al., 1994]. Such degradation could reduce the strongly positive vitamin-induced growth effects. Second, growth in panther chameleons is strongly correlated with appetite measured by food consumption rate [Ferguson, 1994]. Strong UV irradiation could directly irritate the senses of the animals and cause appetite suppression independent of any specific effect of vitamin A deficiency. Visual sensitivity to UV irradiation has been demonstrated or strongly implicated in lizards [Alberts, 1989; Fleishman et al., 1993]. The slightly lower vitamin A content of eggs from females exposed to high UV-A irradiation suggests that the females could have been more deficient in vitamin A due to UV-A exposure.

That growth suppression occurred only in females is interesting. The UV-A degradation effect is stronger in female than in male humans [Roe, 1987]. Also, vitamin A requirements could be higher for females so that, being closer to deficiency with the same dietary levels, females were affected more strongly than males. No similar suppression of growth by UV-B occurred in Experiment 2 females.

Soft Tissue Mineralization

The low dietary vitamin A animals did not have mineralized soft tissues but, despite metabolic bone disease, did take up calcium and deposit it in their bones. So the pathology may be related to the combination of the dietary calcium, presence of substantial vitamin A, and at least some dietary vitamin D.

Curiously, soft tissue mineralization was absent from any of the Experiment 2 females, at least some of which should have shown it. The Experiment 2 animals were from different females (bloodlines). Those used for Experiment 1, all siblings, may have been more predisposed genetically to soft tissue mineralization under the dietary and light conditions in this study.

Reproduction in Males

The elevated levels of dietary vitamins, especially vitamin A, seemed to promote normal male maturation and the expression of appropriate social behavior. The generally poorer overall health and shorter life span due to vitamin A deficiency may explain why the appropriate social responses were not as apparent in the low dietary vitamin A males. Males do not require the high levels of UV-B needed by adult females.

Reproduction in Females

Elevated dietary vitamin A seemed to promote normal female maturation and reproduction up to a point. As in males, the poor performance of females on low dietary vitamin A was probably due to their overall poor health from vitamin A deficiency. That the medium levels in Experiment 1 resulted in the better production of viable eggs (and the most vigorously courting male) suggests that the high dietary levels of vitamins, while promoting faster growth, may have been detrimental. The increased female mortality associated with high dietary vitamin D in Experiment 2 and the association of three of the four cases of renal gout with high levels of vitamin D in both experiments suggest that the high levels of vitamin D may have been more of a problem than high levels of vitamin A.

Despite the absence of substantial mycotoxins in the Ziegler feed samples, the widespread hepatocellular lipidosis suggests that a toxicosis, unrelated to vitamins A and D, might have contributed to the poor hatch success. Perhaps mold germinated on the moist towels in the cricket containers and their toxins entered drinking crickets. Possibly another chemical such as thiram, a fungicide, contaminated the feed stock [Porter et al., 1994]. Alternatively, one or more of the other nutrient additives in the cricket diet could be the cause. Further toxicological research is needed.

Comparison of the vitamin levels of eggs that hatched with those that did not seems a good approach to gather clues regarding the appropriate dietary vitamin levels of the mothers. However, with no current knowledge of vitamin function and dynamics of reptile eggs, interpretation remains risky. The dramatically higher vitamin A content in hatching vs. nonhatching eggs suggests that dietary A levels of the

mothers may have been too low. Indeed, weekly doses dropped precipitously in adults. However, vitamin A metabolism in reptile eggs remains unstudied. Perhaps the storage of such high levels of a potentially toxic substance for 6–10 months is abnormal. Eggs may store less toxic carotenoids and rapidly convert them using the neonatal metabolism just before hatching and in preparation for rapid posthatching growth. Perhaps the levels of carotenoids in the Zeigler diet is a real deficiency. The low but measurable amount of vitamin A in the eggs from captive-raised females could represent the residual of a much higher dose, possibly toxic, present during most of the long egg incubation and which resulted in a defective neonate. Certainly the generally low vitamin A content of insects [Pennino et al., 1991; Dierenfeld et al., 1995] and the bright yellow-orange fat and yolk often observed in field-caught females suggest that dietary intake of carotenoids (vitamin A precursors) may be the more likely source of dietary vitamin A in nature for insectivores [see also Dierenfeld et al., 1995].

By similar reasoning, the possibly lower vitamin D₃ levels in hatched vs. nonhatched term eggs may simply reflect that the healthy neonates depleted a rich supply of vitamin D during the final stages of calcium mobilization from the shell. Shadrix et al. [1994] demonstrated mobilization of shell calcium at term in the skink *Eumeces*. We hypothesize that vitamin D deficiency in the eggs prevented hatchlings from mobilizing shell calcium for hardening their bones prior to hatching, thus causing the term deaths. The need for vitamin D to facilitate this process is currently undocumented. Whether or not the nonhatching eggs were calcium deficient, despite the rich calcium supply to their mothers, is not yet known. If not, then vitamin deficiency in the mother and egg becomes an important consideration.

Regardless of other causes of hatching failure, which occurred in all treatment groups of both experiments, Experiment 2 clearly demonstrated one cause to be insufficient UV-B irradiation. That UV-B partially relieved the hatching failure, while seemingly near-overdoses of dietary vitamin D did not, is inconsistent with claims that dietary vitamin D alone promotes successful propagation of chameleons [Henkle and Heinecke, 1993; Schmidt et al., 1994]. From the literature cited in the introduction, we know that vitamin D production, function, and regulation in some vertebrates involves a complex feedback loop related to dietary levels of calcium, vitamin D, other nutrients, UV-B irradiation, temperature, the production of intermediary metabolites of vitamin D, and the interaction of these with other hormones. Levels of vitamin D and calcium in wild invertebrates upon which chameleons feed are unknown. Perhaps the calcium levels used in this study were too high and somehow interfered with transfer of dietary vitamin D or calcium to eggs. The form of dietary vitamin D used in this study may be different than that produced endogenously with UV-B irradiation. The timing of high doses of dietary vitamins or calcium may be critically important, and improperly phased in these experiments. Clearly, more research is needed to understand the natural balance and ontogenetic timing of dietary and photosynthetic regulation of calcium and vitamin D in the panther chameleon.

Husbandry Recommendations

Based on the results and discussion presented here, several modifications of indoor maintenance practices described in the pet-care literature are recommended. These may be modified in the future as research progresses.

Ultraviolet B irradiation vs. dietary vitamin D. Because of the complexity of internal vitamin D metabolism, its known high toxicity as a dietary supplement, and the high levels needed by females for successful reproduction in this species, we recommend exposing animals 1 h daily for 5 days to a UV-B sunlamp (UVB lamp FS-40T12 (Ultraviolet Resources, Cleveland, OH) or equivalent light source) if they are kept mostly indoors. The weekly dose of UV-B should be about 1 joule/cm². Shade must be provided during this exposure so that they can photoregulate (they will do this [Jones, 1995]). Also, chameleons should not be allowed to get closer than 30 cm from the bulb. Sunlamps are potentially carcinogenic and can cause eye damage. However, following this regime, we had no symptoms of UV damage to any lizards. Most important, keepers must be aware of the potential danger of these bulbs to humans and protect themselves during their use.

To date the only other UV-B source commercially available in the US that might be beneficial for indoor propagation of panther chameleons is the Reptisun UVB310 or Reptiguana Light UVB310 from Zoo Med. (San Luis Obispo, CA). Two 40 watt fluorescent bulbs of either type in a two-bulb luminaire produce about 4–9 $\mu\text{W}/\text{cm}^2$ of UV-B at 30 cm (measured in our lab). This is about one-tenth to one-twentieth the average irradiance of a sunlamp but is several times higher than a double Vita-Lite or black light. So 50–80 h exposure a week (vs. 5) should provide a dose comparable to that of the recommended sunlamp treatment. Five to ten hours per week outdoor exposure to direct sunlight when temperatures are suitable should substitute appropriately [Gehrmann, 1987].

Dietary vitamin D₃ used in combination with sunlamps may enhance the benefit but should be given in doses no greater than the medium levels used in this study. Some herpetoculturists strongly recommend the use of dietary vitamin D₃ in lieu of UV light for chameleons but do not support their recommendation with quantitative data [Henkle and Heinecke, 1993; Schmidt et al., 1994]. One author [Le Berre, 1995] erroneously assumes that, because chameleons strongly reflect UV light, none can be simultaneously absorbed to stimulate photosynthesis of vitamin D₃ from provitamin D and that, therefore, chameleons probably rely mostly on dietary vitamin D in nature. We have preliminary evidence that oral administration of vitamin D₃, under conditions of no UV-B and lower calcium levels than used in this study, may result in enhanced reproductive success, including hatch success in captive-raised panther chameleons [Talent, unpublished data]. However, more research is needed to better define the conditions under which UV-B irradiation may be unnecessary. Also, chameleon species may differ in their need for UV-B exposure.

Vitamin A supplementation of insects. We have demonstrated the need for vitamin A during growth and reproduction. Vitamin A supplementation can result in reproductive success with captive panther chameleons. Nevertheless, some suggest that dietary vitamin A (retinol or its esters) supplementation may not be ideal. Supplementation of insect food with fresh carotene-rich vegetables such as carrots, yams, greens (collard, mustard, turnip) rather than with preformed vitamin A might work better. We have not yet tested this method for chameleons, so we cannot fully endorse this, based on our research. But preliminary evidence from the private sector suggests success [Frye, unpublished data], and insectivorous *Sceloporus* lizards can utilize β -carotene to overcome vitamin A deficiency [Talent, unpublished data]. For now, direct supplementation of retinol or retinyl-esters by mouth with periodic doses totaling 1–2 IU/g of chameleon per week or providing 50–100 IU/g of insect diet should

prevent vitamin A deficiency symptoms in growth and reproduction of panther chameleons. Supplementation with preformed vitamin A directly by mouth is especially recommended if animals show symptoms of vitamin A deficiency described in this article.

We did not test the efficiency of supplementation by "dusting" (agitating insects in a bag or cup containing powdered vitamin-mineral supplement). Some report success using this method [Annis, 1992]. If metabolic bone disease occurs, temporary dusting with calcium carbonate or di-calcium phosphate, once a week until the condition is cured, is a recommended treatment. But this won't help if the problem is vitamin A deficiency.

Fresh vegetables vs. water as a moisture source for insects. Because we suspect moist paper towels in the cricket cage as a potential source of toxin-generating molds, fresh vegetables supplied in small enough amounts to be consumed in 48 h should be a sufficient moisture source and reduce this possible source of toxins.

Feeding insects grain diets. The composition of commercial grain diets used to feed insects may not yet be optimal for maintaining chameleons. In addition, toxins have been detected in some of the grain diets [Porter et al., 1994]. A hepatic toxicosis was universally present in the animals raised on crickets fed grain diets in this experiment. In fairness, it could have been generated by our cricket watering techniques rather than by the diet itself. The basis for the ratios of the other nutrients added to these commercial cricket diets remains obscure.

Other Recommendations for Panther Chameleons

Temperature. Panther chameleons are facultative thermoregulators [Kingsbury, 1994]. When cool, they bask in the sun or heat lamp to raise their body temperature, but they have a broad thermoneutral zone (temperature range within which they don't actively try to modify their body temperature) and allow their body temperature to drift from 24–36°C during daytime activity. Readings of three temperatures—panther chameleon cloacal temperature, adjacent perch temperature, and shaded nearby air temperature ($n = 54$ sets of three)—taken in Madagascar in May, 1991, showed close correspondence between these three temperatures throughout the day within this range [Ferguson, unpublished data]. Low temperatures at night should be above 18°C, which approximates the average minimum temperature recorded in the range of this species. For best breeding results, adults should be maintained at daytime body temperatures of about 30–32°C.

Water. Panther chameleons are readily attracted to dripping water. They can learn to drink directly from a bowl of standing water by dripping water into it for a few weeks. While they are from a belt of high-humidity habitat, they do not seem to need high cage humidity in captivity as long as they can drink. Constantly moist perches can lead to foot infections.

Light intensity. While panther chameleons were not exposed to high light intensity in this study and appeared to adjust well to living at lower indoor light intensities, keeping them in well-lighted areas such as under skylights, near large windows, or in greenhouses seems to cause them to enhance their colors to brighter levels and is recommended when possible [see also Zoond and Eyre, 1934, 1935; Bustard, 1989; De Vosjoli, 1990a,b; Schmidt et al., 1994]. This effect may have nothing to do with ultraviolet-B exposure, since levels penetrating most glass or plexiglass are not particularly high.

Minimum enclosure size. While even adult males adjusted to the very small enclosures used in this study, when raised in them, we recommend larger enclosures for general maintenance, especially if the specimens are wild-caught. Panther chameleons can be more easily observed and more attractively displayed in larger cages. The ideal compromise between space economy and large enclosure size for a single full-sized adult male seems to be about 60 × 60 × 60 cm. Females can be kept in cages 45 × 45 × 45 cm or smaller. Visual obstruction of five of the six surfaces is recommended for such small enclosures, especially for males.

CONCLUSIONS

1. Raising panther chameleons on crickets gut-loaded with diets containing 50 IU/g performed vitamin A, 50 IU/g vitamin D₃, and 8% calcium by mass promoted the best combination of growth, survival, and production of viable eggs, but no viable eggs hatched without high UV-B irradiance. Chameleons fed crickets eating diets with the lowest concentrations of vitamin A showed multiple pathologies, poor reproduction, and early mortality.

2. High UV-A exposure (12 h black light/day) had no clear beneficial effect and slowed female growth.

3. High UV-B irradiation substantially improved hatching success when combined with medium dietary vitamin A levels, 8% calcium, and low or high dietary vitamin D. High UV-B in combination with high dietary vitamin D yielded the highest hatching success but increased mortality of the mothers. Black lights or Vita-Lites alone or in combination provide insufficient UV-B for successful reproduction of panther chameleons.

4. Three of four cases of renal gout were associated with high dietary vitamin D.

5. Hepatocellular lipidosis associated with all diets and light treatments suggested a general dietary toxicosis unrelated to vitamins A and D or light quality.

6. Panther chameleons eating crickets that in turn were receiving 100 IU/g of vitamin A and the same levels of vitamin D in their food ingested about 140 IU of vitamin A and 350 IU of vitamin D during their first year. Weekly doses to chameleons decreased ontogenetically, because, as crickets grow, they concentrate vitamins A and D less efficiently. Amounts of ingested vitamin A vs. vitamin D differed, because crickets concentrate the two vitamins differently.

7. Vitamin A content of eggs at term (hatched successfully) from wild-caught females averaged seven or more times that of eggs at term (failed to hatch) from captive-raised females. Eggs from the high vitamin A group in captivity averaged twice that of eggs from the medium vitamin A group.

8. Vitamin D content of eggs at term (hatched successfully) from wild-caught females averaged less than that of eggs at term (failed to hatch) from captive-raised females.

9. Calcium content of eggs at term (hatched successfully) from wild-caught females averaged 6.27 mg/egg. Eighty-seven percent was in the neonate and egg fluids; the rest was in the shell.

10. UV-B deficiency appears to have been a major, but not the only, cause of hatching failure in this study.

11. Significant amounts of dietary vitamin A (between 50 and 100 IU/g in

cricket food or 1–2 IU/g of chameleon, orally administered per week) and UV-B irradiation (50–60 $\mu\text{W}/\text{cm}^2$, 5 h per week) are recommended for successful captive maintenance and propagation of panther chameleons. The recommended use of dietary vitamin D in lieu of or in combination with UV-B irradiation remains controversial and needs further study.

12. Nutritional status is a function of interactions between diet and light; recommendations for one cannot be accurate without knowing the other.

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