



## Effects of vitamin D<sub>3</sub> supplementation and UVb exposure on the growth and plasma concentration of vitamin D<sub>3</sub> metabolites in juvenile bearded dragons (*Pogona vitticeps*)

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### ABSTRACT

The effectiveness of dietary vitamin D<sub>3</sub> and UVb exposure on plasma vitamin D metabolites in growing bearded dragons (*Pogona vitticeps*) was studied. A total of 84 (40 males and 44 females) newly hatched bearded dragons were allocated to six levels of oral vitamin D<sub>3</sub> supplementation (0 to 400%) or six UVb exposure times (2 to 12 h). At 3 and 6 months of age, blood samples were obtained from each animal and analysed for 25(OH)D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub>. At 3 months of age, plasma concentrations of 25(OH)D<sub>3</sub> did not increase with increasing vitamin D<sub>3</sub> supplementation unlike the 1,25(OH)<sub>2</sub>D<sub>3</sub>. At 6 months of age, plasma concentrations of both 25(OH)D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> increased with increasing vitamin D<sub>3</sub> supplementation. Plasma concentrations in UVb-exposed animals were 18 times higher for 25(OH)D<sub>3</sub> (178.4 ± 9.0 vs. 9.9 ± 1.3 nmol/L) and 5.3 times higher for 1,25(OH)<sub>2</sub>D<sub>3</sub> (1.205 ± 0.100 vs. 0.229 ± 0.025 nmol/L) than in vitamin D<sub>3</sub> supplemented animals at 6 months of age. This study shows that 2 h of UVb exposure enables adequate physiological concentrations of plasma vitamin D metabolites to be maintained in growing bearded dragons. Oral supplementation of vitamin D<sub>3</sub> is ineffective in raising plasma concentrations of 25(OH)D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> to concentrations observed in UVb-exposed animals.

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### 1. Introduction

In reptiles, low plasma vitamin D concentrations lead to a complex of diseases, collectively called Metabolic Bone Disease (MBD). MBD is considered the most prevalent complex of diseases of reptiles in captivity (Mader 1996; Laing and Fraser, 1999; Laing et al. 2001; Kik and Beynen 2003). Vitamin D status of animals capable of dermal cholecalciferol synthesis is determined mainly by exposure to UVb (natural or artificial). Oral vitamin D supplementation may be relatively inefficient in maintaining vitamin D status compared to exposure to UVb (Fraser 1983). Vitamin D can be hydrolyzed to 25(OH)D<sub>3</sub> in the liver, the storage form of vitamin D in the body, which is further converted to the biologically active 1,25(OH)<sub>2</sub>D<sub>3</sub> in the kidneys (Laing and Fraser, 1999). Vitamin D can also be inactivated in the liver and excreted via bile. Vitamin D absorbed by the intestine is rapidly taken up by the liver, while vitamin D formed in the skin slowly diffuses into the blood stream. Therefore absorbed vitamin D might reach higher concentrations in the liver leading to inactivation and thereby a lower utilization

efficiency (Fraser 1983). Reptiles held in captivity may be limited in their exposure to direct sunlight and therefore artificial UVb lighting might be required to ensure adequate plasma vitamin D metabolite concentrations (De Lang 1970; Bernard and Allen, 1997; Carman et al. 2000; Schmidt and Barbiers, 2003).

Across species, it is difficult to determine adequate or normal plasma concentrations of 25(OH)D<sub>3</sub>. In humans, latitude is considered a factor in determining plasma 25(OH)D<sub>3</sub> concentrations. Recently, Schoenmakers et al. (2008) recommended to change the lower threshold from 25 to 50–100 nmol/L for people in the UK. For sun-exposed, pregnant African women, average concentrations of 103–111 nmol/L were found and a lower threshold of 50–80 nmol/L was indicated (Prentice et al. 2009). For several animal species 25(OH)D<sub>3</sub> concentrations have been reported; African elephants (*Loxodonta africana*) in captivity were reported to have a 25(OH)D<sub>3</sub> plasma concentration of 41 nmol/L (Miller et al. 2009) and for Felidae species held in Zoological Institutions average concentrations of 70–95 nmol/L were found and deemed adequate (Crissey et al. 2003). Little data on animals in the wild are available, however wild black rhinos (*Diceros bicornis*) are reported to have a plasma 25(OH)D<sub>3</sub> concentration of 145 nmol/L (Clauss et al. 2002). Alpacas (*Lama pacos*) in Australia were sampled throughout the year to determine seasonal differences of 25(OH)D<sub>3</sub> concentrations. Plasma concentrations of 264 nmol/L in summer and 35 nmol/L in early spring were reported, with early spring concentrations possibly being insufficient. Concentrations of less than 15 nmol/L have been observed

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in camelids with clinical rickets (Van Saun et al. 1996). From such comparative data it seems that plasma 25(OH)D<sub>3</sub> concentrations below 80 nmol/L are to be considered low and concentrations between 100 and 250 nmol/L are considered normal.

In reptiles housed indoors, artificial or natural UVb positively affects plasma 25(OH)D<sub>3</sub>. Corn snakes (*Pantherophis guttatus*) without UVb exposure were reported to have a plasma concentration of 57 nmol/L; exposure to UVb light increased concentrations to 196 nmol/L (Acierno et al. 2008). Laing et al. (2001) reported higher plasma concentrations of 25(OH)D<sub>3</sub> in iguanas held outdoors (105 ± 70 nmol/L) than in those housed indoors (44 ± 25 nmol/L) or intermittently exposed to natural sunlight (78 ± 47 nmol/L). Plasma concentrations of 25(OH)D<sub>3</sub> in komodo dragons (*Varanus komodoensis*) increased 8–60 times when animals housed indoors (without UVb exposure) were exposed to sunlight (Gillespie et al. 2000). Typical 25(OH)D<sub>3</sub> blood values for komodo dragons exposed to UVb light are between 150 and 250 nmol/L (Gillespie et al. 2000). Oftedal et al. (1997) reported plasma 25(OH)D<sub>3</sub> concentrations of 559 nmol/L for green iguanas (*Iguana iguana*) exposed to UVb and <25 nmol/L for unexposed animals. Ferguson et al. (2002) found a parabolic relation between the exposure to UVb lighting and the reproductive success in female panther chameleons, indicating that excessive ultraviolet lighting could be detrimental. Certain species of lizards will actively regulate their UVb exposure in order to compensate for vitamin D insufficient diets (Ferguson et al. 2003). The latter research indicates that exposure to either artificial UVb or sunlight is effective in a number of lizard species in raising plasma concentrations of vitamin D metabolites.

Dietary supplementation of vitamin D can also be used to prevent deficiencies in animals. However, little research exists to assess the effectiveness of dietary vitamin D<sub>3</sub> supplementation in reptiles. Allen and Oftedal (1994) suggested a daily dietary intake of 500–1000 IU vitamin D<sub>3</sub>/kg of feed (1 IU = 0.025 µg) for captive carnivorous lizards, while Kik and Beynen (2003) recommended a concentration of 35 IU vitamin D<sub>3</sub>/MJ gross energy consumed for reptiles. The latter corresponds to approximately 260–1,800 IU/kg of feed and is lower than the 2,000–3,000 IU vitamin D<sub>3</sub>/kg of feed where deficiencies have been reported to occur (Bernard and Allen, 1997). Nijboer et al. (2007) compared dietary supplementation of vitamin D<sub>3</sub> to UVb exposure in komodo dragons (*V. komodoensis*) and concluded that the effect of artificial lighting on plasma vitamin D metabolite concentrations was much larger than that of dietary supplementation. Plasma levels of 25(OH)D<sub>3</sub> were only 18 to 37 nmol/L for animals supplemented with 450 IU vitamin D<sub>3</sub>/kg feed while for animals exposed to UVb, concentrations of 195 to 291 nmol/L were recorded (Nijboer et al. 2007). The latter research indicates that oral supplementation of vitamin D<sub>3</sub> may be far less effective in maintaining normal physiological concentrations of vitamin D metabolites in the plasma of reptile and lizard species. The objectives of this study were to compare the effectiveness of dietary supplementation of vitamin D<sub>3</sub> and UVb exposure on plasma vitamin D metabolites in growing bearded dragons. The best estimates for the current minimum daily oral vitamin D<sub>3</sub> requirements of reptile species were used to supplement growing

bearded dragons and exposure time to artificial UVb lighting was varied from 2 to 12 h per day.

## 2. Material and methods

### 2.1. Animals and diet

The study reported here was approved by the Committee for the Care and Use of Animals of Wageningen University, Wageningen, The Netherlands. A total of 84 (40 males and 44 females) newly hatched bearded dragons (*P. vitticeps*) with a mean ± SEM body mass of 2.57 ± 0.03 g (range 2.11–3.04 g) originating from a private breeder were used in this experiment. The animals originated from four females which had been mated to one male bearded dragon. The animals from two nests which were co-located were indistinguishable and as a result parentage could not be established. All animals were equally distributed over two dietary treatments: vitamin D<sub>3</sub> supplemented ( $n=6$  including a control group which was not supplemented with vitamin D<sub>3</sub>) and UVb-exposed groups ( $n=6$ ). Each treatment group consisted of 7 animals and was assigned to a single terrarium. In order to minimise body mass differences between and within treatments the following allocation procedure was used. The 1st, 3rd, 5th, 7th, 9th, 11th and 13th animals to hatch were allocated to terrarium 1 (vitamin D<sub>3</sub> supplementation) while the 2nd, 4th, 6th, 8th, 10th 12th and 14th animals to hatch were allocated to terrarium 2 (UVb exposure). This procedure was repeated another 5 times so that the uneven numbered terraria contained all the animals for the vitamin D<sub>3</sub> treatment and the even numbered terraria the animals for the UVb treatments. Treatments started as soon as the first animal was housed in a terrarium.

All animals were provided with fresh water on a daily basis and feed was provided *ad libitum* throughout the 180-day study. A selection of insects was offered throughout the study including crickets (*Acheta domesticus*), grasshoppers (*Locusta migratoria* and *Schistocerca gregaria*) and mealworms (*Tenebrio molitor*). The insects were purchased from a commercial supplier (Starfood, Barneveld, The Netherlands). The gross chemical composition including vitamin D<sub>3</sub> content of the insects is provided in Table 1. All insects were dusted with calcium carbonate before they were offered to the bearded dragons to ensure sufficient calcium intake. In addition to insects, endive (*Cichorium endivia*), Chinese cabbage (*Brassica pekinensis*) and red bell pepper (*Capsicum annuum*) were offered as vegetable feed sources but were not accepted during the first months of life and only sparsely during the later months of the study. The average intake of feed vegetables over the entire study period across the 12 terraria was 0.44% of the total dry matter intake (range 0.36–0.56%). Feed intake per terrarium was measured by weighing the feed provided to each terrarium and subtracting refusals. Feed refusals were bulked and analysed at the end of the study for dry matter to calculate actual dry matter intake for each terrarium.

**Table 1**

Analysed nutrient composition and vitamin D<sub>3</sub> content of the penultimate and adult insects offered to the bearded dragons (*Pogona vitticeps*) throughout the study.

Insect	Organic matter g/kg DM	Crude protein	Crude fat	Crude fibre	Vitamin D <sub>3</sub> IU/kg DM	Gross energy MJ/kg DM
<i>Penultimate</i>						
Desert locusts ( <i>Schistocerca gregaria</i> )	956	655	176	94	204	26.2
Migratory locusts ( <i>Locusta migratoria</i> )	968	652	162	108	109	24.0
Mealworms ( <i>Tenebrio molitor</i> )	962	585	266	70	150	24.7
<i>Adult</i>						
Desert locusts ( <i>S. gregaria</i> )	975	611	274	131	317	29.3
Migratory locusts ( <i>L. migratoria</i> )	974	631	226	140	213	30.1
House crickets ( <i>Acheta domesticus</i> )	944	697	165	90	934	25.7

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