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Effects of vitamin D₃ supplementation and UVb exposure on the growth and plasma concentration of vitamin D₃ metabolites in juvenile bearded dragons (*Pogona vitticeps*)

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ABSTRACT

The effectiveness of dietary vitamin D₃ 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 D₃ 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)D₃ and 1,25(OH)₂D₃. At 3 months of age, plasma concentrations of 25(OH)D₃ did not increase with increasing vitamin D₃ supplementation unlike the 1,25(OH)₂D₃. At 6 months of age, plasma concentrations of both 25(OH)D₃ and 1,25(OH)₂D₃ increased with increasing vitamin D₃ supplementation. Plasma concentrations in UVb-exposed animals were 18 times higher for 25(OH)D₃ (178.4 ± 9.0 vs. 9.9 ± 1.3 nmol/L) and 5.3 times higher for 1,25(OH)₂D₃ (1.205 ± 0.100 vs. 0.229 ± 0.025 nmol/L) than in vitamin D₃ 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 D₃ is ineffective in raising plasma concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ 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)D₃ in the liver, the storage form of vitamin D in the body, which is further converted to the biologically active 1,25(OH)₂D₃ 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)D₃. In humans, latitude is considered a factor in determining plasma 25(OH)D₃ 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)D₃ concentrations have been reported; African elephants (*Loxodonta africana*) in captivity were reported to have a 25(OH)D₃ 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)D₃ 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)D₃ 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)D₃ 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)D₃. 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)D₃ in iguanas held outdoors (105 ± 70 nmol/L) than in those held indoors (44 ± 25 nmol/L) or intermittently exposed to natural sunlight (78 ± 47 nmol/L). Plasma concentrations of 25(OH)D₃ 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)D₃ 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)D₃ 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 D₃ supplementation in reptiles. Allen and Oftedal (1994) suggested a daily dietary intake of 500–1000 IU vitamin D₃/kg of feed (1 IU = 0.025 µg) for captive carnivorous lizards, while Kik and Beynen (2003) recommended a concentration of 35 IU vitamin D₃/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 D₃/kg of feed where deficiencies have been reported to occur (Bernard and Allen, 1997). Nijboer et al. (2007) compared dietary supplementation of vitamin D₃ 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)D₃ were only 18 to 37 nmol/L for animals supplemented with 450 IU vitamin D₃/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 D₃ 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 D₃ and UVb exposure on plasma vitamin D metabolites in growing bearded dragons. The best estimates for the current minimum daily oral vitamin D₃ 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 D₃ supplemented ($n=6$ including a control group which was not supplemented with vitamin D₃) 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 D₃ 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 D₃ 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 D₃ 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 D₃ content of the penultimate and adult insects offered to the bearded dragons (*Pogona vitticeps*) throughout the study.

Insect	Organic matter	Crude protein	Crude fat	Crude fibre	Vitamin D ₃	Gross energy
	g/kg DM				IU/kg DM	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

2.2. Housing and treatments

Each group ($n = 7$) of bearded dragons was housed in a terrarium measuring $124 \times 50 \times 40$ cm (L \times W \times H) containing a 50 W halogen spot (E27, Namiba Terra, Kempen, Germany) in the centre at 27 cm above the ground. Five groups were offered dietary supplementation of vitamin D₃, six groups were exposed to various durations of UVb while one group was not supplemented with vitamin D₃ nor was exposed to UVb. Ultraviolet light was provided by means of a lamp (Reptisun 5.0 UVb, Zoomed Laboratories, Inc., San Luis Obispo, CA, USA) controlled by a timer turning on the light for either 2, 4, 6, 8, 10 or 12 h each day. The lamps were placed horizontally in the cages at a distance of 29 cm from the bottom in the top corner of the terrarium. Ultraviolet output of the lamps was measured inside the cages by means of a model 6.2 UV meter (Solartech Inc., Harrison Township, MI, USA) at a distance of 19 cm, directly underneath the middle part of the lamp.

Supplementation of the vitamin D₃ treated group was achieved by dissolving a vitamin D₃ preparation (Davitamon D, 0.029 mg vitamin D₃/mL, Chefaro Nederland BD, Rotterdam, The Netherlands) in peanut oil containing added vitamin E (0.5 g/L) and weekly oral administration to the animal via a syringe fitted with a metal probe. Kik and Beynen (2003) suggested a daily vitamin D requirement for reptiles between 10–70 IU/MJ. Based on this range a concentration of 35 IU/MJ was assumed as the requirement level. The vitamin D₃ supplemented groups were assigned to 25, 50, 100, 200 and 400% of this concentration. The gross energy intake, and thus vitamin D requirement, was estimated based on the weekly recorded body mass for each individual animal (Mader 1996). In addition, the non-supplemented animals (6 UVb exposure groups and 1 control group) also received a weekly supplementation of peanut oil containing vitamin E, but without vitamin D₃. The amount of peanut oil administered to a 100 g bearded dragon was 0.188 mL. Temperature measurements were conducted three times a week for each terrarium by means of a RayTemp 3 infrared thermometer (Electronic Temperature Instruments Ltd, West Sussex, UK) on the left, right and in the middle underneath the spotlight. During the study the body mass (BM), snout to vent length (SVL) and total length (TL) of the animals were recorded on a weekly basis. Tail length was calculated by subtracting SVL from TL. At an average age of three and six months of the animals in each terrarium, blood samples were obtained from the tail vein of each animal using a 1 mL, heparin rinsed syringe fitted with a 1.2 by 40 mm needle. Plasma was separated after centrifugation (2500 g for 10 min at 10 °C) and stored at –80 °C within 1 h after collection pending analysis for 25(OH)D₃ and 1,25(OH)₂D₃.

2.3. Chemical analysis

Vitamin D₃ and the gross energy content of the insects were analysed prior to the start of the experiment by means of HPLC (AOAC 2002.05; AOAC 982.29) and bomb calorimetry (IKA calorimeter C7000, Staufen, Germany), respectively. Samples of the insects were taken at regular intervals throughout the trial and frozen in closed containers at –20 °C. At the end of the study insects were freeze-dried, ground to pass a 1 mm sieve (Retsch mill, ZM100, Retsch BV, Ochten, The Netherlands) and analysed for nitrogen, crude fat, crude fibre and ash. Nitrogen content was determined using the Kjeldahl-procedure (ISO 5983:2005). Crude fat determinations were conducted by means of the Soxhlet-method (ISO 6492:1999) with crude fibre fraction determined according to the ISO 6865:2000 guidelines. The ash fraction was determined by incineration of samples for four hours at 550 °C (ISO 5984:2002).

Analyses of 25(OH)D₃ in plasma were performed using a commercially available kit (DiaSorin, Minnesota, USA) whereby 25(OH)D₃ was extracted from the plasma and analysed by radio immunoassay (RIA) using a specific 25(OH)D₃ antibody (Hypponen

et al. 2007). Concentrations of 1,25(OH)₂D₃ were determined using the IDS Gamma-B kit (Immunodiagnostic Systems, Tyne and Wear, UK) by immuno-extraction followed by a ¹²⁵I RIA for quantification.

2.4. Statistical analysis

Individual lizards were considered as experimental units. The inter-independence of individual lizards within a cage could not be evaluated due to the experimental design and was assumed to be negligible.

The BM, SVL, tail length and TL data at the start and the end of the study were subjected to analysis of variance with treatment (vitamin D₃ supplementation and UVb exposure), gender and the interaction between treatment and gender as variables using the General Linear Model procedure in SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). Main effects of treatment (vitamin D₃ supplementation vs. UVb exposure) on plasma parameters were tested by analysis of variance with gender as a co-variable. When a significant effect of treatment was observed, dose–response effects of vitamin D₃ supplementation and UVb exposure were separately analysed by linear regression with gender as a co-variable. For dietary vitamin D₃, a log₂ transformation was then applied because the independent variable (i.e. dose) was not normally distributed. Within treatment, a contrast comparison was conducted between the control group (no vitamin D₃ supplementation, no UVb exposure) and the average of the treatment groups. The effect of dosage was assessed by linear regression, either including (if contrast was not significant) or excluding (if contrast was significant) the control group. Values were considered significant when $P < 0.05$ and are presented as (non-transformed) means \pm SEM.

3. Results

3.1. Animals and housing

The temperature in the cage on the left side was 31.7 ± 1.6 °C, on the right side 32.6 ± 2.0 °C, and 25 cm directly under the heat lamp 51.2 ± 4.3 °C. The output of the UVb lamps (recorded at the end of the study) was 34 ± 3.0 μ W/cm². Table 2 shows the distribution of male and female bearded dragons over the treatments. Six animals did not survive the 180-day study; three animals (control, 25 and 100% vitamin D₃ group) died of unknown causes, two animals (control and 8 h UVb group) were killed by terrarium mates and one animal (6 h UVb group) was euthanized because of a severe tail infection after blood sampling at 3 months of age. In addition, during the study two animals were removed, one animal (12 h UVb group) because it was much smaller than its terrarium mates and one animal (100% vitamin D₃ group) because of signs of calcium deficiency (tetany and convulsions). The remaining 76 bearded dragons remained visibly healthy and increased body mass throughout the study. The data of the animals removed from the study were not included in the statistical analyses with exception of the feed intake, as this was determined per treatment group.

Table 2

Distribution of male and female bearded dragons (*Pogona vitticeps*) over the treatment groups.

	Vitamin D ₃ supplementation ^a (%)						UVb exposure (h)					Total	
	0	25	50	100	200	400	2	4	6	8	10		12
Male	3 (1)	3	3	1 (2)	5	2	2	2	3	4 (1)	4	2 (1)	34 (5)
Female	2 (1)	3 (1)	4	4	2	5	5	5	3 (1)	2	3	4	42 (3)
Total	5 (2)	6 (1)	7	5 (2)	7	7	7	7	6 (1)	6 (1)	7	6 (1)	76 (8)

Value between brackets indicates the number of animals that did not complete the study.

^a As percentage of the estimated daily requirement of 35 IU/MJ (Kik and Beynen 2003).

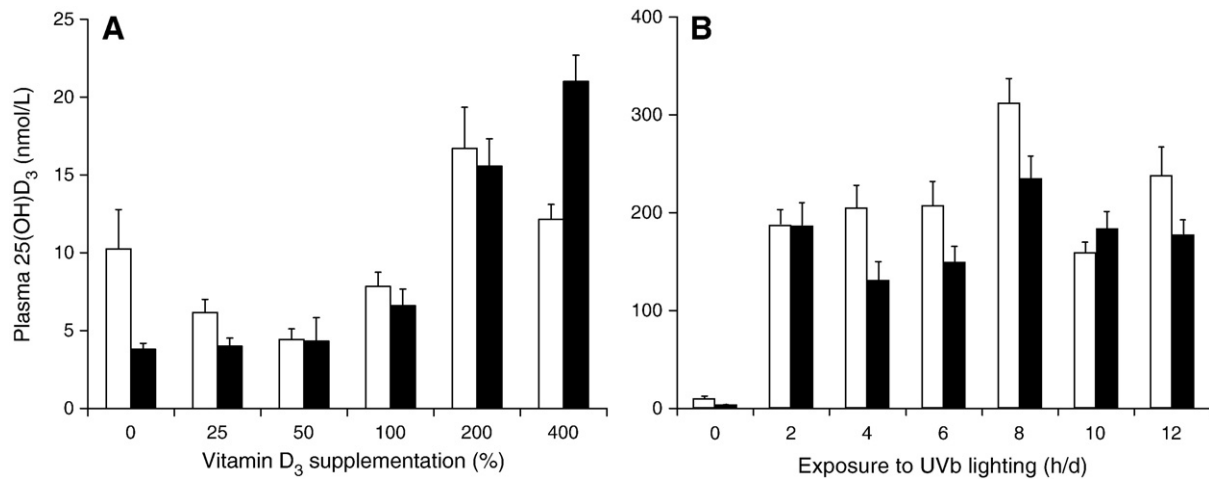


Fig. 1. Plasma concentrations of 25(OH)D₃ at 3 (□) and 6 (■) months of age in bearded dragons (*P. vitticeps*) supplemented with various dosages of vitamin D₃ relative to the estimated daily requirement of 35 IU/MJ (Kik and Beynen 2003) (panel A) or exposed to various lengths of time to UVb (panel B). Values are means ± SEM.

3.2. Feed intake and feed conversion

The average fresh feed intake for all animals was 5.10 ± 0.59 g per day, corresponding with 1.70 ± 0.20 g of dry matter per day during the 180-day trial. No significant effects of treatment on feed intake were found between the vitamin D supplemented animals and the UVb-exposed animals.

The feed conversion efficiency for all animals was 3.68 ± 0.06 kg of fresh feed per kg of body mass gain (1.25 ± 0.02 kg of dry matter per kg of body mass gain). No significant differences regarding feed conversion efficiency were found between treatments. Vitamin D supplemented animals selected feed items with a higher ($P=0.004$) vitamin D concentration than the UVb-exposed animals did (13.59 vs. 11.86 vitamin D/MJ). Within the vitamin D supplemented animals a lower supplementation level was associated with a higher ($P=0.024$) intake of dietary vitamin D (Vitamin D/MJ). Within the UVb-exposed animals a tendency to select feed with lower vitamin D concentrations was present ($P=0.057$), when exposure time was increased. Also, a tendency to consume more

($P=0.095$) vegetables was found with increasing exposure times to UVb.

3.3. Metabolite concentrations

With increasing age, plasma concentrations of 25(OH)D₃ decreased ($P=0.016$) from 217.1 ± 12.2 nmol/L at 3 months to 178.4 ± 9.0 nmol/L at 6 months of age in the UVb-exposed groups but not in the vitamin D supplemented groups (13.3 ± 3.7 and 18.6 ± 6.4 nmol/L, respectively). In both treatment groups, plasma concentrations of 1,25(OH)₂D₃ were lower ($P<0.05$) at 3 than at 6 months of age (0.17 ± 0.01 vs. 0.22 ± 0.03 nmol/L in the UVb-exposed group and 0.83 ± 0.06 vs. 1.21 ± 0.10 nmol/L in the vitamin D₃ supplemented group). As a consequence, the ratio between 1,25(OH)₂D₃ and 25(OH)D₃ was higher ($P<0.01$) at 6 than at 3 months of age in both treatment groups. Therefore, dosage effects of vitamin D₃ supplementation as well as UVb exposure on plasma concentrations were analysed by linear regression for the two ages separately.

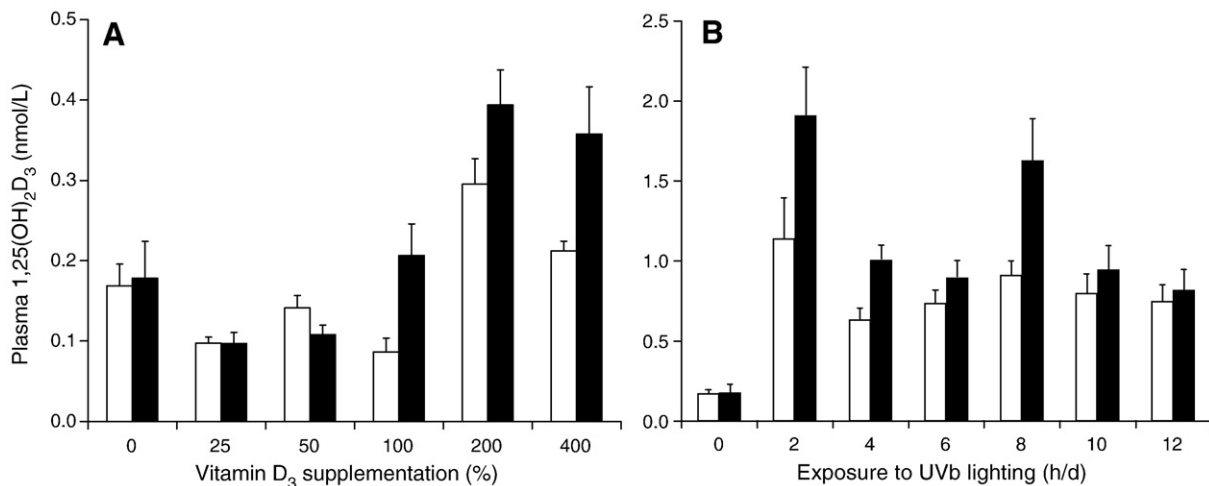


Fig. 2. Plasma concentrations of 1,25(OH)₂D₃ at 3 (□) and 6 (■) months of age in bearded dragons (*P. vitticeps*) supplemented with various dosages of vitamin D₃ relative to the estimated daily requirement of 35 IU/MJ (Kik and Beynen 2003) (panel A) and exposed to various lengths of time to UVb (panel B). Values are means ± SEM.

Table 3
Effects of exposure to UVb and gender on growth performance in bearded dragons (*Pogona vitticeps*).

	Vitamin D ₃ supplementation		UVb exposed		P-value		
	Male	Female	Male	Female	T ¹	G ²	T×G
<i>Day 0</i>							
Number of animals	20	22	19	23	–	–	–
Body mass, g	2.52	2.51	2.49	2.53	0.939	0.736	0.715
Snout vent length (SVL), mm	38.6	38.4	38.0	38.4	0.343	0.849	0.372
Tail length, mm	53.8	52.8	52.4	53.9	0.902	0.883	0.343
Total body length (TL), mm	92.5	91.2	90.4	92.2	0.735	0.857	0.282
SVL:TL	0.42	0.42	0.42	0.42	0.866	0.963	0.383
<i>Day 180</i>							
Number of animals	17	20	17	22	–	–	–
Body mass, g	271 ^a	214 ^b	278 ^a	279 ^a	0.015	0.055	0.040
SVL, mm	176 ^a	160 ^b	182 ^a	179 ^a	<0.001	0.007	0.060
Tail length, mm	417 ^a	370 ^b	415 ^a	412 ^a	0.075	0.027	0.050
TL, mm	241 ^a	210 ^b	233 ^a	233 ^a	0.395	0.077	0.075
SVL:TL	0.42	0.43	0.45	0.44	0.140	0.898	0.327

¹T is the effect of exposure to UVb (no exposure vs. 2 or more h/day).

²G is the effect of gender (male vs. female).

^{a,b}Within a row, means with different superscripts differ ($P < 0.05$).

The amount of vitamin D₃ supplemented did not affect plasma levels of 25(OH)D₃ at 3 months of age, although the 200 and 400% groups showed numerically higher concentrations (Fig. 1). The plasma concentrations of 1,25(OH)₂D₃ increased ($P = 0.002$) with increasing vitamin D₃ intake, but the ratio between the two vitamin D₃ metabolites was not affected. At 6 months of age, plasma concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ increased ($P < 0.001$) with increasing vitamin D₃ supplementation, whereas the ratio between 1,25(OH)₂D₃ and 25(OH)D₃ decreased ($P = 0.008$) simultaneously.

The duration of UVb exposure (2 to 12 h/day) did not affect plasma levels of 25(OH)D₃ and 1,25(OH)₂D₃ or their ratio at 3 months of age (Fig. 2). At 6 months of age, 1,25(OH)₂D₃ concentrations decreased ($P = 0.011$) with increasing duration of UVb exposure, whereas 25(OH)D₃ concentrations were unaffected. Hence, the ratio between 1,25(OH)₂D₃ and 25(OH)D₃ concentrations decreased ($P = 0.002$) with increasing duration of UVb exposure.

3.4. Growth and development

Initial values for BM, SVL, tail length, and TL of the bearded dragons were similar for the treatment groups (Table 3). However, at the end of the study BM and SVL were higher ($P < 0.05$) and tails tended to be longer ($P = 0.075$) in the UVb-exposed animals than in the vitamin D₃ supplemented animals. In addition, females had lower ($P < 0.05$) SVL and tail length and tended to have lower BM and TL ($P < 0.10$) than males. This was mainly caused by the females in the supplemented vitamin D₃ groups (treatment×gender, $P < 0.05$ or $P < 0.10$) which were lighter at the end of the study compared to their counterparts in the UVb group (214 vs. 279 g) as well as shorter in terms of SVL, tail length and TL. The contribution of SVL to TL, which ranged from 42% in the vitamin D₃ supplemented males to 45% in the UVb-exposed males, was not affected by treatment or gender.

3.5. Main results

On average, UVb-exposed animals had 12.5 times higher ($P < 0.001$) concentrations of 25(OH)D₃ (198.5 ± 7.6 vs. 15.9 ± 3.6 nmol/L) and 5.1 times higher ($P < 0.001$) concentrations of 1,25(OH)₂D₃ (1.020 ± 0.061 vs. 0.198 ± 0.023 nmol/L) in plasma than the vitamin D₃ supplemented animals. The ratio between 1,25(OH)₂D₃ and 25(OH)D₃ was, however, higher ($P < 0.001$) in the vitamin D₃ supplemented group (25.8 ± 1.8) than in the UVb-exposed group (5.4 ± 0.3). Gender

did not affect plasma concentrations of vitamin D₃ metabolites nor their ratio.

4. Discussion

The current study shows that oral supplementation of vitamin D₃ to growing bearded dragons is far less effective in raising plasma 25(OH)D₃ and 1,25(OH)₂D₃ to normal physiological concentrations compared to exposure to UVb (295–300 nm). Vitamin D₃ is required for normal development and clinical deficiency symptoms include tetany, or rickets and osteomalacia, osteoporosis or nutritional secondary hyperparathyroidism after a prolonged period (Donoghue and Langenberg, 1996). In the present study, tetany was observed in one animal in the 100% supplemented group. Vitamin D metabolite levels of this animal at the age of 3 months were higher than the treatment group average (11.0 vs. 7.3 nmol/L for 25(OH)D₃ and 126.9 vs. 86.4 pmol/L for 1,25(OH)₂D₃). None of the other animals, including the non-supplemented group, showed any clinical signs of vitamin D deficiency indicating that the diet contained sufficient vitamin D to prevent clinical deficiency symptoms within the first six months. Although vitamin D deficiencies were not observed in the unsupplemented group, low plasma concentrations of vitamin D metabolites may have affected the body size and mass of female bearded dragons. Supplemented female bearded dragons in this study had a lower body mass and length at the end of the study compared to the supplemented males and to both the males and females in the UVb groups (Table 3). Whether this reduced growth was caused by the supplementation remains to be determined as there was no dose-dependent effect within the supplemented females and concentrations of the measured plasma vitamin D metabolites were not different from the supplemented males. The reduced development of the supplemented females may have been caused by a lower feed intake as this was only measured for each terrarium and not for individual animals. However, vitamin D metabolites, combined with hormones are reported to influence bone metabolism and could therefore have influenced growth rates (Van Leeuwen et al. 2001). Other long term effects of low plasma vitamin D concentrations, might have become apparent if a longer timeframe was chosen.

The bearded dragons in this study appeared to select for vitamin D intake levels through the feed. Animals not exposed to UVb light selected feed items richer in vitamin D. Also, animals with a lower

vitamin D supplementation had a stronger preference for feed items higher in vitamin D content (Table 1); more crickets were consumed than locusts or mealworms. Dietary selection for specific nutrients has been described for many taxonomically distinct animal species; for instance insects (Waldbauer 1991), fish (Rubio et al. 2009), birds (Cerrate et al. 2008) and mammals (McCaughey et al. 2005).

The concentrations of vitamin D₃ metabolites in the animals exposed to UVb (122–234 nmol/L 25(OH)D₃) are in line with concentrations reported in other reptile species exposed to natural or artificial UVb. Gillespie et al. (2000) reported that adult komodo dragons (*V. komodoensis*) with daily UVb exposure have typical plasma concentrations of 25(OH)D₃ of 150–250 nmol/L. Nijboer et al. (2007) found concentrations between 195 and 291 nmol/L for 25(OH)D₃ in wild komodo dragons. In adult iguanians (including bearded dragons) exposed to natural light, Laing et al. (2001) reported a mean concentration of 105 nmol/L for the group. Higher plasma concentrations of 582 nmol/L were reported by Oftedal et al. (1997) for adult green iguanas (*I. iguana*) exposed to UVb while unexposed animals had concentrations below the detection limits of the assay (25 nmol/L). Normal physiological plasma concentrations for vitamin D metabolites in wild bearded dragons have not been described in the literature. However, based on the concentrations for different lizard species provided above, it seems plausible to assume that the normal physiological 25(OH)D₃ concentration should be between 150 and 250 nmol/L. To the authors' knowledge there are no studies reporting plasma concentrations of 1,25(OH)₂D₃, the biological active metabolite of vitamin D, in other reptile species exposed to UVb. The average concentration for growing bearded dragons exposed to UVb was 1.020 nmol/L. Duration of UVb exposure did not affect plasma concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ and exposure for 2 h appeared sufficient to reach a plateau. Plasma concentrations of these metabolites are closely regulated by the activities of a number of enzymes including hepatic 25-hydroxylase and renal 1- α -hydroxylase (Holick 2003). In addition, 24- α -hydroxylase is present in the proximal convoluted tubule cells of the kidney and in virtually all target cells of 1,25(OH)₂D₃ and produces 24R,25(OH)₂D₃, a metabolite reported to circulate in the blood at concentrations about 1,000 times higher than 1,25(OH)₂D₃. 24-Hydroxylation has been considered the first step in the degradation pathway of 1,25(OH)₂D₃ and 25-(OH)D₃ yielding 24R,25(OH)₂D₃ which appears to have physiological effects on chicken egg hatchability and resting zone cells in cartilage (Van Leeuwen et al. 2001). Whether the degradation product 24R,25(OH)₂D₃ is formed in bearded dragons and whether it has physiological activity as shown in other animals remains to be determined.

For attaining concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ similar to those in UVb-exposed bearded dragons, much higher oral concentrations of vitamin D₃ would be required than used in the current study. Further elevation of the oral vitamin D₃ dosage will increase the risk of hypervitaminosis and subsequent clinical symptoms of toxicity. Allen and Oftedal (1994) recommend a daily dietary intake of 500–1000 IU vitamin D₃/kg diet for captive carnivorous lizards while Kik and Beynen (2003) recommended a concentration of 35 IU vitamin D₃/MJ gross energy consumed (approximately 260–1,800 IU/kg diet) in order to prevent deficiencies. Bernard and Allen (1997) noted however that deficiency symptoms have been observed at 2000–3000 IU vitamin D₃/kg diet, indicating that the recommendations by Kik and Beynen (2003) and Allen and Oftedal (1994) may be too low to prevent deficiencies in all individuals. In the current study, the unsupplemented group exhibited no clinical vitamin D deficiency symptoms although their dietary vitamin D intake was 14.3 IU/MJ gross energy consumed, 358 IU/kg dry matter or 1.075 IU/kg diet. Species and individual differences in vitamin D requirements may be the reason for the differences between studies. UVb exposure for 2 or more hours resulted in significantly higher concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ in the blood of the bearded dragons than in non-exposed animals, including the vitamin D supplemented group. Further studies should investigate the minimum UVb exposure time required to maintain normal physiological concen-

trations of 25(OH)D₃ and 1,25(OH)₂D₃ and should include measurement of 24R,25(OH)₂D₃. Since de novo endogenous synthesis of vitamin D₃ appears to be highly species-specific in the case of anole lizards (Ferguson et al. 2005), comparative studies should investigate the effectiveness of ultraviolet radiation between reptile species (Ferguson et al. 1996; Carman et al. 2000).

In conclusion, the present study shows that exposure of bearded dragons for 2 or more hours to UVb ensures normal physiological concentrations of plasma 25(OH)D₃ and 1,25(OH)₂D₃ to be maintained. For female bearded dragons UVb exposure also resulted in higher growth rates. In addition, oral supplementation of vitamin D₃ at 4 times the average dietary recommendation of 35 IU/MJ was ineffective in raising plasma concentration of 25(OH)D₃ and 1,25(OH)₂D₃ to levels observed in animals exposed to UVb.

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