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ORIGINAL ARTICLE

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A comparison of UVb compact lamps in enabling cutaneous vitamin D synthesis in growing bearded dragons

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Summary

The effect of exposure to different UVb compact lamps on the vitamin D status of growing bearded dragons (Pogona vitticeps) was studied. Forty-two newly hatched bearded dragons (<24 h old) were allocated to six treatment groups (n = 7 per group). Five groups were exposed to different UVb compact lamps for two hours per day, with a control group not exposed to UVb radiation. At 120 days of age, blood samples were obtained and concentrations of 25(OH)D_3, Ca, P and uric acid were determined. In addition, plasma 25(OH)D_3 concentration was determined in free-living adult bearded dragons to provide a reference level. Only one treatment resulted in elevated levels of 25(OH)D_3 compared to the control group (41.0 ± 12.85 vs. 2.0 ± 0.0 nmol/L). All UVb-exposed groups had low 25(OH)D_3 plasma levels compared to earlier studies on captive bearded dragons as well as in comparison with the free-living adult bearded dragons (409 ± 56 nmol/L). Spectral analysis indicated that all treatment lamps emitted UVb wavelengths effective for some cutaneous vitamin D synthesis. None of these lamps, under this regime, appeared to have provided a sufficient UVb dose to enable synthesis of plasma 25(OH)D_3 levels similar to those of free-living bearded dragons in their native habitat.

KEYWORDS

25(OH)D_3, lizard, *Pogona vitticeps*, reptile, ultraviolet light, vitamin D, vitamin D metabolites

1 | INTRODUCTION

The bearded dragon (*Pogona vitticeps*) is one of the most frequently kept reptile species in North America (Wright, 2008) and possibly in Europe. The most common disorders in captive *P. vitticeps* belong to the metabolic bone disease (MBD) complex (Wright, 2008). This complex of diseases includes rickets, osteoporosis and secondary hyperparathyroidism and is caused by an imbalance of calcium and/or phosphorus in the body (Divers & Mader, 2005). Vitamin D_3 regulates calcium metabolism by inducing intestinal absorption and renal reabsorption of Ca and P (Ajibade, Benn, & Christakos, 2010; Haxhiu et al., 2014). Furthermore, a misbalance in dietary vitamin D, Ca or P intake can cause renal disease in lizards (Miller, 1998), which leads to increased plasma levels of P and uric acid (Knotek, Hauptman, Knotkova, Hajkova, & Tichý, 2002).

Vitamin D_3 deficiency can result in hypocalcaemia, which in turn can lead to development of MBD (Holick, Tian, & Allen, 1995). Vitamin D_3 can either be provided exogenously or endogenously synthesized in the skin. The epidermal cells of the skin contain 7-dehydrocholesterol (7-DHC) which is transformed to pre-vitamin D_3 when exposed to ultraviolet b (UVb) radiation between 290 and 320 nm (Fraser, 1995). Pre-vitamin D_3 is isomerized to vitamin D_3 in a thermally dependent step (Holick et al., 1980). Vitamin D_3 in the skin binds to vitamin D-binding protein (VDBP) which transports it into the bloodstream.

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Vitamin D_3 is hydroxylated in the liver to 25(OH)D_3, which again binds to VDBP and re-enters the circulation. Circulating 25(OH)D_3 is regarded as the primary storage form of vitamin D_3 and is used to assess vitamin D_3 status (Gillespie, Frye, Stockham, & Fredeking, 2000; Holick, 1990). 25(OH)D_3 is metabolized by renal cells to the active endocrine hormone, 1,25$(OH)_2D_3$, which is a vital regulator of calcium and phosphorus homeostasis. In humans, and possibly other vertebrates, 25(OH)D_3 is also taken into the cells of many other organs possessing vitamin D_3 receptors, including the immune system, where intracellular conversion to 1,25$(OH)_2D_3$ takes place (Hossein-nezhad & Holick, 2013). Within the cells, 1,25$(OH)_2D_3$ has paracrine and autocrine functions (Björn, 2008).

Vitamin D_3 is utilized by almost all vertebrate species studied (Björn, 2008), and the formation of vitamin D_3 via 7-DHC photoconversion is an ancient process which has been conserved through evolution. Most vertebrates are able to utilize pre-formed vitamin D_3 from the diet, which has enabled many species of reptiles to be maintained in captivity without UVb lighting but with dietary supplementation of vitamin D_3. The first successful use of a fluorescent tube emitting UVb for reptile vitamin D_3 synthesis was described by Laszlo (1969).

Since then, cutaneous synthesis of vitamin D_3 under artificial UVb radiation has been demonstrated in several diurnal reptile species (Acierno, Mitchell, Roundtree, & Zachariah, 2006; Allen, Oftedal, & Horst, 1995; Bernard, Allen, & Ullrey, 1997; Ferguson et al., 2003, 2009; Gillespie et al., 2000; Hibma, 2004; Hoby et al., 2010; Kroenlein, Zimmerman, Saunders, & Holladay, 2011; Oonincx, Stevens, van den Borne, van Leeuwen, & Hendriks, 2010; Selleri & Di Girolamo, 2012), but also in crepuscular and nocturnal species (Acierno et al., 2008; Carman, Ferguson, Gehrmann, Chen, & Holick, 2000; Wangen, Kirchenbaum, & Mitchell, 2013). Most of these studies did not describe the UVb intensity and spectral distribution received by the subjects. The spectral power distribution of the light source determines the resulting photoproducts (MacLaughlin, Anderson, & Holick, 1982). The phosphor blend and transmission qualities of the outer glass tube from a UVb-emitting fluorescent lamp determine its spectral power distribution and hence its vitamin D_3 forming potential. Moreover, animal species are likely to have different optimal UVb exposure levels as a result of differing microhabitats and basking behaviours (Ferguson et al., 2005), and could also differ in their ability to utilize dietary vitamin D_3 (Allen et al., 1995). These differences and the lack of reference values for plasma 25(OH)D_3 concentrations in free-living specimens of most species make it difficult to determine "adequate" UVb exposure levels for captive reptiles. Reference values are necessary to determine the success of any supplementation regime, whether by dietary means or via provision of UVb. Vitamin D metabolite concentrations of free-living reptiles have been reported only for Komodo dragons (Gillespie et al., 2000), Chuckwalla, *Sauromalus obesus* (Aucone, Gehrmann, Ferguson, Chen, & Holick, 2003), Ricord's iguanas, *Cyclura ricordii* and rhinoceros iguanas, *Cyclura cornuta cornuta* (Ramer et al., 2005).

This study evaluates the effect of long-term exposure to five UVb compact lamps, marketed for use with reptiles, on 25(OH)D_3 plasma concentrations in bearded dragons. We hypothesize that the spectral power distribution of these lamps determines the vitamin D status of the exposed bearded dragons. Furthermore, to evaluate these plasma 25(OH)D_3 levels, blood samples were obtained from wild, free-living bearded dragons in Australia and analysed for 25(OH)D_3.

2 | MATERIALS AND METHODS

2.1 | Treatments and animals

This study was approved by the Committee for the Care and Use of Animals, Wageningen University, Wageningen, the Netherlands. The study consisted of six treatments in a parallel design: five types of commercial UVb compact fluorescent lamps and one control lamp which did not emit UVb radiation. A total of 42 animals (19 males and 23 females) from two clutches of newly hatched bearded dragons (*P. vitticeps*) originating from a single pair were obtained from a private breeder. Starting from the day of hatching, snout vent length (SVL) and total length (TL) were measured and body mass (BM) was determined using a precision balance (type HF-2000G, A&D Company Ltd, Tokyo, Japan) on a weekly basis. Bearded dragons were assigned to one of six terraria within 24 h of hatching. To reduce age differences, the 1st to the 7th animals hatched were allocated to the first terrarium, the 8th to the 14th to the second terrarium, and so on, until all terraria contained seven animals.

2.2 | Housing and measurements

Each terrarium measured 124 × 50 × 40 cm (L × W × H) and contained a 60-Watt reflector lamp (Philips lamp NR80; Philips Eindhoven, the Netherlands), which provided heat between 08:00 and 22:00 daily, and a compact fluorescent lamp. One control compact fluorescent lamp without UVb output and five compact fluorescent lamps sold for use with "desert" reptiles were used in this study (Table 1). Before the start of the study, the lamps were used for 100 h to stabilize UVb output. Once the first animal had been allocated to a terrarium, the compact fluorescent lamps were on between 12:00 and 14:00 daily using a timer.

TABLE 1 Treatment name, lamp type and power

Treatment	Lamp type	Power (Watt)
Control	Philips SoftoneT65 WW827	20
Trixie	Trixie Desert Pro Compact 10.0	23
JBL	JBL Reptile Desert UV 480 Desert Terrarium Lamp	23
Arcadia	Arcadia D_3 + 10%UVB Compact Reptile Lamp	23
ZooMed	ZooMed ReptiSun 10.0 UVB Desert Compact Fluorescent Lamp	26
ExoTerra	ExoTerra Repti Glo 10.0 UVB Desert Terrarium Lamp	26

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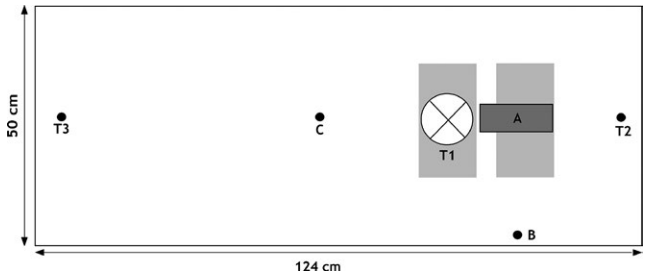

FIGURE 1 Terrarium set-up: A and B indicate where UV intensity and index were measured; T1, T2, T3 indicate where temperature was measured; ⊗ Reflector lamp; □ treatment lamp; ▮ brick

One house brick (21 × 10 × 5 cm) was placed underneath the reflector lamp, and one was placed directly below the compact fluorescent lamp to create 5 cm high basking sites (Figure 1). The reflector lamp was located 15 cm above the basking site, and the compact fluorescent lamp was mounted horizontally at a height of 25 cm above the second basking site. UVb radiation was measured twice a week at two locations in the terrarium; A) directly underneath the centre of the compact UVb lamp at a distance of 21.5 cm and B) at the front of the terrarium near the glass, 31 cm from the centre of the lamp (Figure 1). Total UVb output (μW/cm^2) and UV Index were determined with radiometers (Solartech Inc., Harrison Township, MI, USA; models 6.2 and 6.5).

Each week before the compact lamps were turned on, surface temperatures were measured directly underneath the spotlight (T1, Figure 1) and 3 cm from each side of the terrarium (T2 and T3, Figure 1), using a RayTemp 3 infrared thermometer (Electronic Temperature Instruments Ltd, West Sussex, UK).

At 120 days of age, a blood sample (1.5 ml) was taken from the ventral tail vein of each animal using a 2-ml heparinized syringe containing 80 IU electrolyte-balanced heparin (PICO50 syringe, Radiometer Medical ApS, Brønshøj, Denmark) and a 25-gauge needle (BD Microlance 3 25G 0.5 × 16 mm ref 300600). Directly after collection, the sample was divided into two equal parts with one part being centrifuged (750 × *g* for 10 min at 10°C) and stored at −80°C within 1 h after extraction until assayed for 25(OH)D_3. The other half was kept in the closed syringe and sent for analysis of calcium, phosphorus and uric acid concentrations.

2.3 | Diet and feeding

Bearded dragons were provided ad libitum with house crickets (*Acheta domesticus*), desert locusts (*Schistocerca gregaria*), migratory locusts (*Locusta migratoria*) and yellow mealworms (*Tenebrio molitor*) of appropriate size to their body mass throughout the study. All insects were purchased from a commercial supplier (Star Food BV, Barneveld, the Netherlands) and dusted with calcium carbonate powder prior to being offered. Feed intake per dietary constituent was determined daily per terrarium and calculated by subtracting the weight of the refusals from the weight offered the day before. At the end of the study, average total feed intake was calculated per dietary constituent.

2.4 | Blood samples from free-living bearded dragons

A permit for scientific research was acquired at the NSW Department of Environment & Conservation (DEC) National Parks and Wildlife Service under the National Parks and Wildlife Act 1974, Section 132C. Also a permit under the Animal Research Act was granted by the Animal Welfare Branch, NSW Department of Industry and Investment, Australia (licence number S13043). Bearded dragons were captured by hand in New South Wales, between Broken Hill (S 31.91021°, E 141.48497°) and Cobar (S 31.71187°, E 144.15192°) in the summer of 2010 (28 January–8 February) (Oonincx, van Leeuwen, Hendriks, & van der Poel, 2015). Gender was determined by visual inspection. Thirteen adults (four females and nine males) and one subadult male were sampled as described above. These blood samples were centrifuged in local hospitals within 12 hr and stored in a mobile freezer (Engel Fridge, Engel, Australia), before being shipped on dry ice to Wageningen University, Wageningen, the Netherlands. At the time and place of each capture, solar UVb readings were taken using the Solarmeter model 6.2 UVB meter.

2.5 | Laboratory analyses

Blood samples of the bearded dragons in the UVb lamp experiment were analysed for plasma 25(OH)D_3 concentrations at the Endocrine Laboratory of the VU University Medical Center (Amsterdam, the Netherlands) by ID-XLC-MS/MS as described by Heijboer, Blankenstein, Kema, and Buijs (2012) with modifications to optimize the method for bearded dragons as described in Oonincx et al. (2013). To conduct a statistical analysis, samples below the detection limit (4 nmol/L) were given an arbitrary value of 2 nmol/L. Calculations with 0, 2 or 4 nmol/L did not affect the outcome of the statistical analysis. Plasma concentrations of vitamin D metabolites (25(OH)D_3 and 1,25$(OH)_2D_3$) from the free-living bearded dragons were determined individually by radioimmunoassay as described in Oonincx et al. (2010).

Total Ca, P and uric acid (UA) concentrations were determined by means of a Unicel DXC-600 (Beckman Coulter, Woerden, the Netherlands). Ionized Ca concentration was determined by a blood gas analyser (Rapidlab 1265, Siemens Nederland B.V., The Hague, the Netherlands) at the Department of Clinical Sciences of Companion Animals of Utrecht University (Utrecht, the Netherlands).

2.6 | Lamp assessment

At the end of the trial, the fluorescent lamps used in the trial were mounted on a test bench, and after a standard 30-min warm-up time on line voltage regulated by a Variac transformer (Carroll and Meynell CMV2E-1) to 230V, metre readings were taken from each lamp with a Solarmeter 6.5 UV Index meter and a Solarmeter 6.2 broadband UVb irradiance meter. Recordings were made from the side of each lamp, with the sensor positioned perpendicular to the axis of the lamp, halfway down the coiled section of the bulb. Metre readings were taken at 5-cm increments from the lamp surface.

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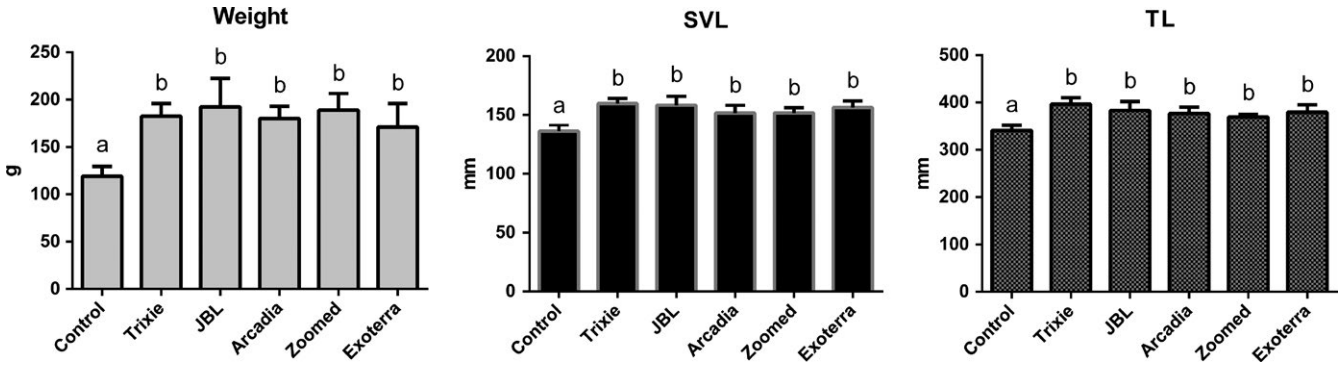

FIGURE 2 Bodyweight (BM), snout vent length (SVL) and total length (TL) of ~4-month-old bearded dragons (*Pogona vitticeps*) exposed to one control lamp and five UVb compact lamps. Different letters above bars represent significant differences ($p < .05$)

Spectrograms were obtained using an Ocean Optics USB2000 + spectral radiometer with a UVb-compatible fibre-optic probe with cosine adaptor (Ocean Optics Inc., Dunedin, Florida USA). Spectrometer recordings were made at a standard distance of 10 cm from the lamp surface. The measured spectral irradiance (μW/cm²/nm) from each lamp was weighted with the CIE previtamin D_3 action spectrum (Bouillon et al., 2006) to obtain a comparison of their vitamin D_3 effective (D-Eff) irradiance. A previous experiment (Oonincx et al., 2010) had used similar experimental parameters with a different compact lamp. A comparison was made with the spectrum and D-Eff from this lamp (ZooMed Reptisun 5.0 UVb Tropical 26-watt, ZooMed Laboratories Inc., San Luis Obispo, USA) and with reference solar spectra from Australia (Bernhard, Mayer, Seckmeyer, & Moise, 1997).

2.7 | Statistical analyses

Individual lizards were considered as experimental units; the interdependence of individual lizards within a terrarium was assumed to be negligible, but could not be evaluated due to the experimental design. To test whether animals came from a homogenous population, hatching BM, SVL and TL were subjected to an analysis of variance with treatment, gender and the interaction between treatment and gender as factors. An analysis of variance with treatment, gender and their interaction as factors was also conducted on the increase of BM, SVL and TL after ~4 months. Significance of differences ($p < .05$) in average daily feed intake, UV Index (UVI), UVb and temperature between treatments was tested by a one-way ANOVA followed by a LSD post hoc. Ca, P, UA and 25(OH)D_3 concentrations were analysed by means of a nonparametric analysis of variance (Kruskal–Wallis test) followed by a Mann–Whitney U test corrected for multiple comparisons. Statistical analyses were conducted using IBM SPSS Statistics 20 (IBM Corporation, Armonk, NY, USA).

3 | RESULTS

3.1 | Animals and Housing

Nine animals did not complete the study; in each of the UVb-exposed groups, one animal was removed because of size differences with its terrarium mates. Five of these were removed between twelve and fifteen weeks after the start of the experiment. One animal from the ZooMed group was removed at 4 weeks of age and euthanized because of severe injuries caused by terrarium mates. In the control group, three animals (one at 2 weeks, the other two at 21 weeks of age) were removed because of clinical signs of calcium deficiency (tetany). The remaining 33 bearded dragons remained visibly healthy. The data of the removed animals were excluded from the statistical analyses, except for feed intake, as this was determined per treatment group. Blood was obtained from all animals, except for one from the ZooMed group. Initial values for BM, SVL and TL of the bearded dragons were similar for the treatment groups. At the end of the study, BM ($p < .01$), SVL ($p < .02$) and TL ($p < .02$) were lower in the control group than in the other groups (Figure 2). Gender did not influence these parameters. Mean temperatures were similar over treatments during the study period; however, they differed between locations: directly under the heat lamp (T1) 52.2 ± 1.7°C, on the right side (T2) 33.5 ± 1.4°C and on the left side (T3) 29.8 ± 1.4°C.

3.2 | Ultraviolet b radiation

Intensity of UVb radiation for the five treatment lamps was measured at different distances (Table 2). The Arcadia lamp produced the highest UVb irradiance and UVI at all locations; the ExoTerra lamp had the lowest UVb irradiance. The UVb irradiance of all treatment lamps decreased during the experiment (Figure 3). The largest decrease was apparent for the Trixie lamp (27%), whereas the Arcadia lamp had the lowest decrease (10%).

The UVb intensity was also determined for the free-living bearded dragons. Readings were taken where the bearded dragons were found in the field as soon as the animal was caught (Figure 4). The presented data include measurements in both sun and shade. Animals were frequently found basking on sunny days, including during the heat of the day. The maximum solar UVb irradiance recorded at the location of one of the basking dragons was 506 μW/cm² at 14.00.

The UVb spectral irradiance of each of the five treatment lamps and the ZooMed lamp from the study by Oonincx et al. (2010),

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At a distance of (cm)	Total UVb (μW/cm²)				UV Index			
	20	25	30	35	20	25	30	35
JBL	31	21	15	12	0.9	0.6	0.4	0.3
ZooMed	42	27	19	15	1.4	0.9	0.7	0.5
Arcadia	86	57	42	31	2.9	1.9	1.4	1.0
ExoTerra	15	10	7	5	0.8	0.5	0.4	0.3
Trixie	38	26	19	14	1.1	0.7	0.5	0.4
Control	0	0	0	0	0	0	0	0

TABLE 2 UVb intensity and UV index of compact lamps at the end of the experiment, measured perpendicular to the lamp at different distances

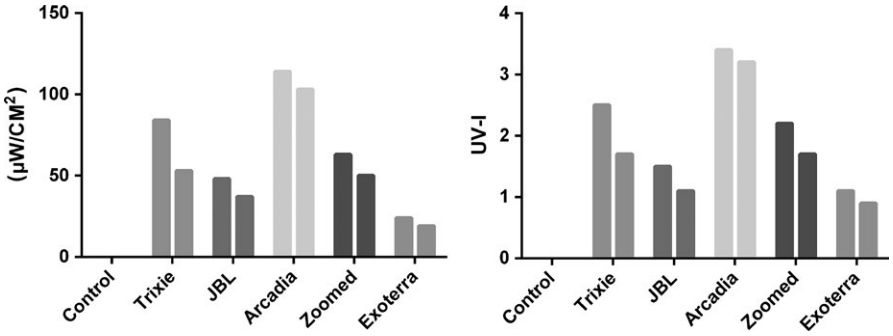

FIGURE 3 UVb intensity (left) and UV index (right) of treatment lamps at the start (100 h) and at the end of the experiment (340 h) at 25 cm distance

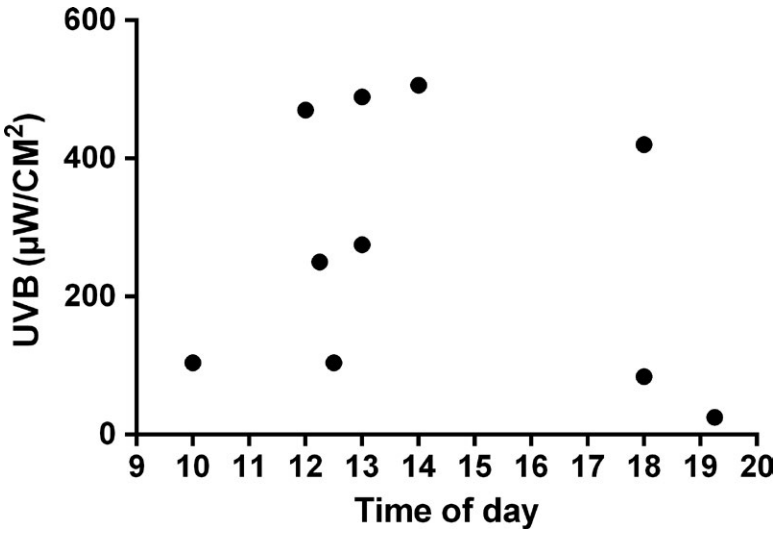

FIGURE 4 UVb intensity at locations where free-living adult bearded dragons were observed during the day

hereafter referred to as the ZooMed 2010 compact lamp, is shown in Figure 5. The spectral power distribution (SPD) of all five treatment lamps was similar, with the exception of a small peak around 297 nm from the Arcadia lamp. For this lamp, UVb irradiance was higher than for the other treatment lamps at all wavelengths. The ZooMed 2010 compact lamp had a different SPD, emitting wavelengths as short as 285 nm.

The D-Eff spectral irradiances for all lamps and for sunlight at a solar altitude of 60° (Bernhard et al., 1997) are shown in Figure 6. Total D-Eff irradiances at 10 cm distance, calculated from the spectral data, are as follows in order of their magnitude (μW/cm² D-Eff): Arcadia 28.3; ZooMed 2010 lamp 20.7; ZooMed 13.49; Trixie 10.05; JBL 9.35; ExoTerra 7.63. The sunlight had a much higher total D-Eff irradiance (49.5 μW/cm² D-Eff) than all of the lamps measured at 10 cm distance.

3.3 | Blood plasma concentrations

None of the animals in the control group had detectable levels of 25(OH)D_3, whereas in the Arcadia group, all animals had levels above the detection limit of 4 nmol/L (Table 3). This group also had a higher mean 25(OH)D_3 plasma concentration than the four other treatments, which were all close to the limit of detection. The free-living bearded dragons had ten times the plasma 25(OH)D_3

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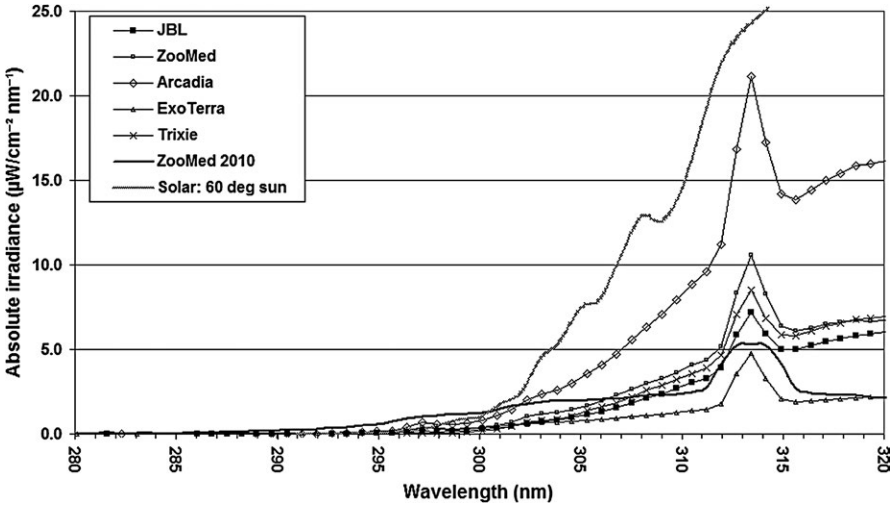

FIGURE 5 UVb intensity of six UVb compact lamps and a reference solar spectrum with solar altitude of 60° (mid-morning and mid-afternoon in summer), in North Queensland, Australia (Bernhard et al., 1997)

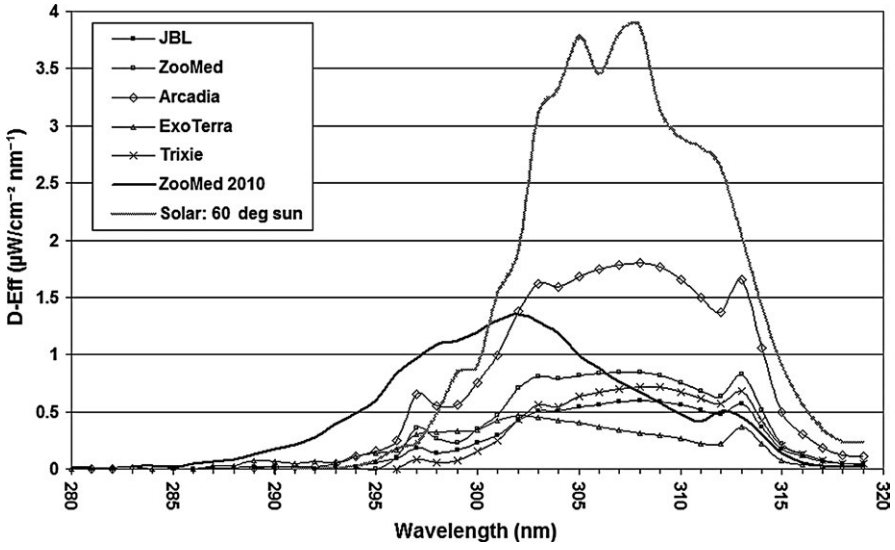

FIGURE 6 The vitamin D_3 effective (D-Eff) spectral irradiance of six UVb compact lamps and a reference solar spectrum with solar altitude of 60° (mid-morning and mid-afternoon in summer), in North Queensland, Australia (Bernhard et al., 1997). Calculation based on Bouillon et al. (2006)

concentration (409 ± 56 nmol/L) found for the Arcadia treatment (41.00 ± 12.85 nmol/L). The 1,25(OH)D_3 plasma concentration of the free-living bearded dragons was 770 ± 458 pmol/L.

Calcium concentrations were higher in the Arcadia treatment, compared to the control (p = .011), whereas plasma P levels in the Arcadia group were lower (p = .026) than in the control group, whereas the other treatments were intermediate. Uric acid concentrations were higher in the Arcadia treatment than in the ExoTerra treatment (p = .025).

4 | DISCUSSION

In this trial, only one lamp (the Arcadia lamp) elevated serum 25(OH)D_3 levels in the bearded dragons, compared to the control. This lamp was used for two hours per day and had a maximum irradiance of 57 μW/cm^2 and a UVI of 1.9 (at 25 cm distance). This total dose (irradiance × time) only resulted in serum 25(OH)D_3 levels of 41.00 ± 12.85 nmol/L, less than a quarter of the concentration found previously for growing bearded dragons exposed in a similar manner to the ZooMed 2010 compact lamp (178.4 ± 9.0 nmol/L; Oonincx et al., 2010), and only one-tenth of those found in free-living bearded dragons in their native habitat (409 ± 56 nmol/L). The maximum irradiance available to the dragons from the other lamps in the trial was lower (range 10–27 μW/cm^2 and UVI 0.5–0.9 at 25 cm distance), and this resulted in low serum 25(OH)D_3 concentrations. For each brand, only a single lamp was used during the experiment. Hence, this does not allow evaluation of the effectiveness of brands, but only for the actual lamp used and the light spectrum emitted by that lamp.

The high 25(OH)D_3 concentrations found in the free-living bearded dragons are likely explained by their exposure to a far greater dose of UVb from natural sunlight. The maximum solar UVb irradiance recorded at the location of one dragon was 506 μW/cm2 at 14.00 h (Figure 4), which is an order of magnitude higher than that received by the bearded dragons in the highest (Arcadia) treatment level in the trial. Every recording between 10:00 h and 15:30 h exceeded 100 μW/cm2. This indicates that high UVb exposures do occur, but we did not monitor exposure times and so their daily dose was unknown.

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TABLE 3 Concentration of 25(OH)D_3 in plasma, calcium (Ca), phosphorus (P) and uric acid (UA) in whole blood of bearded dragons (*Pogona vitticeps*) exposed to either a control or a UVb compact lamp during their first 120 days of age (mean ± standard deviation)

		UVb compact lamps					
Component	Control lamp (*n* = 4)	Trixie (*n* = 6)	JBL (*n* = 6)	Arcadia (*n* = 6)	ZooMed (*n* = 4)	ExoTerra (*n* = 6)	*p*-value
25(OH)D_3 (nmol/L)	2.00 ± 0.00[a]	4.33 ± 5.72[a]	4.50 ± 3.89[a]	41.00 ± 12.85[b]	5.50 ± 2.65[a]	10.33 ± 17.64[a]	.004
Ca (mmol/L)	2.20 ± 0.28[a]	3.45 ± 0.37[a,b]	3.36 ± 0.41[a,b]	4.73 ± 2.69[b]	3.30 ± 0.22[a,b]	3.20 ± 0.21[a,b]	.027
P (mmol/L)	4.05 ± 0.22[b]	3.36 ± 0.72[a,b]	2.96 ± 0.60 [a,b]	2.57 ± 0.61[a]	3.57 ± 0.72[a,b]	3.00 ± 0.34[a,b]	.029
UA (μmol/L)	228 ± 97[a,b]	336 ± 73[a,b]	307 ± 69[a,b]	517 ± 158[b]	226 ± 98[a,b]	236 ± 98[a]	.012

[a,b]Different superscript letters within rows represent significant differences between treatments.

Basking species typically do not remain fully exposed for long periods (Ferguson, Gehrmann, Brinker, & Kroh, 2014, 2015; Ferguson et al., 2010). Nevertheless, UVb was available to the free-living bearded dragons throughout their entire day.

The spectral power distribution of the UVb source is also a very important factor affecting cutaneous vitamin D_3 synthesis and resulting 25(OH)D_3 concentrations (MacLaughlin et al., 1982). Short-wavelength UVb has a higher vitamin D_3 synthesizing potential per microwatt compared to longer wavelengths, with an optimum calculated to be at 298 mm under natural circumstances. Longer wavelengths up to 320 nm also enable conversion from 7-DHC to pre-vitamin D_3 to take place, although the longer the wavelength, the higher the irradiance required to produce the same result (Bernhard et al., 1997).

The spectral power distribution (SPD) of natural sunlight, with a threshold between 290 nm and 295 nm, (Figure 5) is such that although 298 nm is the most *efficient* wavelength for vitamin D_3 synthesis, far greater irradiance is produced by longer wavelengths. As a result, the most *effective* wavelength for pre-vitamin D_3 formation from sunlight at solar altitude 60° is 308 nm (Figure 6).

The relative SPD of all the lamps in the current trial is similar to that of natural sunlight so the UVb dose would appear to be the main determining factor for the comparative differences in vitamin D status between the groups in this trial and sunlight. However, the SPD of the ZooMed 2010 compact lamp is different, resulting in a different D-Eff distribution (Figure 6). This lamp has a larger proportion of short-wavelength UVb than the other treatment lamps and the solar spectrum and includes highly energetic wavelengths <290 nm, not found in natural sunlight. This SPD produces its most effective wavelength for pre-vitamin D_3 formation at 302 nm. In the 2010 study, this compact lamp exposed bearded dragons to moderate UVb radiation (34 μW/cm^2 UVb at 19 cm, 20.7 μW/cm^2 D-Eff at 10 cm), but this resulted in much higher serum 25(OH)D_3 levels than produced under the Arcadia lamp despite the Arcadia lamp's higher total irradiance (57 μW/cm^2 at 25 cm) and vitD-Eff irradiance (28.3 μW/cm^2 D-Eff at 10 cm).

MacLaughlin et al. (1982) have demonstrated that the spectral character of UV radiation determines the percentage conversion of 7-DHC into the different photoproducts pre-vitamin D_3, lumisterol$_3$ and tachysterol$_3$, which provides a possible explanation for this anomaly, warranting further research. It is the quasi-equilibrium between these photoproducts and 7-DHC under UV radiation which is responsible for the self-regulation of vitamin D_3 synthesis, preventing overproduction. It is possible that the use of lamps with very different spectra from sunlight and in particular those emitting non-terrestrial short-wavelength UVb (<290 nm) may affect the self-regulation in some way. "Extraordinarily elevated" concentrations of serum 25(OH)D_3 were recorded in green iguanas given only low-level irradiation from an experimental lamp of this type (Bernard, 1995). More studies are needed to determine whether certain spectra can lead to overproduction of vitamin D_3. However, lamps emitting a high proportion of their UVb output in these shorter wavelengths have proven harmful to reptiles, causing eye and skin damage and death, and must be regarded as hazardous (Hibma, 2004; Gardiner, Baines, & Pandher, 2009; Baines 2010).

Different assay methods for 25(OH)D_3 were used for blood samples from dragons in the 2010 study, from the free-living dragons and from the dragons in the lamp trial. Commercially available assays for 25(OH)D_3 have been described as yielding differing results, not only between different methods of analyses, but also between different laboratories using the same assays (Binkley et al., 2004; Roth, Schmidt-Gayk, Weber, & Niederau, 2008). We conducted a small study (*n* = 20) to compare our two 25(OH)D_3 assay methods, using bearded dragon plasma from Oonincx et al. (2013). We found a significant correlation between the two methods (p = .006; Pearson R = .60), indicating that results from both methods can be qualitatively compared. However, more work is needed to quantify potential differences between samples analysed via the binding assay and the ID-XLC-MS/MS method.

Calcium levels were higher, and P levels were lower in the Arcadia group compared to the control group. Furthermore, the Ca:P ratio tended to be higher in the Arcadia treatment compared to the control (p = .067). In fact, the Ca concentration was only lower than the P concentration in the control group. In that group, three animals showed clinical symptoms of vitamin D deficiency, whereas these were not observed in the other treatments. This indicates that even minimal UVb exposure suffices to prevent clinical symptoms of vitamin D deficiency, despite a low level of 25(OH)D_3. From two animals, removed from the control group due to clinical vitamin D deficiency symptoms (tetany), blood samples were taken at day 91. Subsequently, these animals were housed together and exposed to a Zoomed Reptisun Desert 10.0 26W UVb lamp, as used in one of the treatments, for two hours daily during a period of 3 weeks. During the first 4 days, these two bearded dragons basked actively underneath the UVb lamp; they flattened their

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bodies and positioned themselves perpendicular to the lamp, thereby maximizing the exposed body surface. After 3 weeks of exposure, clinical symptoms had disappeared, and a second blood sample was obtained. The 25(OH)D_3 plasma levels had increased for one of the individuals (from <4 nmol/L to 26 nmol/L), whereas in the other individual, the 25(OH)D_3 plasma concentration remained under the detection limit. The bearded dragons that remained in the control group showed reduced growth compared to the UVB-exposed groups. Because vitamin D_3 and its metabolites facilitate bone growth, such effects can be expected (van Leeuwen, van den Bemd, van Driel, Buurman, & Pols, 2001). Similarly, Oonincx et al. (2010) described reduced growth in unexposed bearded dragons compared to exposed animals. However, in that study, this was only apparent in female bearded dragons.

Uric acid concentrations in the bearded dragons in the Arcadia group were higher than in the ExoTerra group (p = .012) and tended to be higher than the control and ZooMed group (p = .075, for both). Although high uric acid concentrations have been linked with renal insufficiency (Divers & Mader, 2005), the levels found in this study are within reference values (119–595 μmol/L (Diethelm, Stein, & Mader, 2006; Tamukai, Takami, Akabane, Kanazawa, & Une, 2011)). Future studies focusing on the effects of UVb radiation on vitamin D metabolite level should not only determine the spectral output of UV lamps, but also determine the actual exposure of individual animals. Solitary housing combined with behavioural analysis would facilitate this, while at the same time preventing interdependence of collected data. Furthermore, usage of lamps which have a broader spatial range would be preferred over compact lamps to standardize UVb exposure. Studies with the aim to gain insight in calcium metabolism would benefit from parathyroid hormone measurements, if assays for reptilian serum are developed.

In conclusion, none of the lamps used in this trial raised the 25(OH)D_3 plasma levels to those found in free-living dragons. Although the low dose of UVb from all lamps apparently sufficed to prevent clinical symptoms, subclinical vitamin D deficiency could have other effects on the health and welfare of bearded dragons. In this study, lamps with very little UVb radiation under 295 nm were used. With lamps having this spectral power distribution, UVb irradiance appeared to determine 25(OH)D_3 concentrations in growing bearded dragons. However, significant increases were only seen under the lamp with the highest output. Therefore, if lamps with this spectral power distribution are to be used to improve vitamin D status, a UVb irradiance at animal level at least this high may be required. The compact lamp used in a previous study, with low irradiance but emitting a higher proportion of UVb below 295 nm, resulted in strongly elevated 25(OH)D_3 plasma levels. This indicates that the spectral power distribution plays a key role in vitamin D synthesis, warranting further investigation.

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