

Komodo dragons J.Nijboer

**UV-B and vitamin D<sub>3</sub> metabolism in juvenile Komodo dragons (*Varanus Komodoensis*)**

J. Nijboer<sup>1</sup>, H. van Brug<sup>2</sup>, M.A. Tryfonidou<sup>3</sup> J.P.T.M. van Leeuwen<sup>4</sup>

<sup>1</sup> Veterinary Department, Rotterdam Zoo; <sup>2</sup>Optic Research Group, Technical University Delft,

<sup>3</sup>Department of Clinical Sciences of Companion Animal Medicine, Utrecht University.

<sup>4</sup>Department of Internal Medicine, Erasmus MC, Rotterdam,

**Running head:** UV-B and vitamin D<sub>3</sub> synthesis in Komodo dragons

**Corresponding author:**

Joeke Nijboer  
Rotterdam Zoo  
Veterinary Department  
Van Aersenlaan 49  
3039 KE Rotterdam  
The Netherlands

Tel.: +31 10 4431 441

Fax: +31 10 4431 414

Email: [J.Nijboer@Rotterdamzoo.nl](mailto:J.Nijboer@Rotterdamzoo.nl)

### Abstract

The aim of this research project was to assess the vitamin D status in juvenile Komodo dragons held in captivity in Rotterdam Zoo. In addition, the effect of interference with UV-B on the serum levels of vitamin D metabolites and on the serum calcium concentrations were investigated in three Komodo dragons. Supplying 450 IU vitamin D<sub>3</sub> /kg feed orally did not increase 25-hydroxyvitamin D<sub>3</sub> (25-(OH)D<sub>3</sub>), the 24-hydroxylated metabolite of vitamin D (24,25-(OH)<sub>2</sub>D<sub>3</sub>), 1,25-dihydroxyvitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>D<sub>3</sub>) and calcium levels.

In contrast, exposing the Komodo dragons to UV-B altered the levels of vitamin D metabolites. The amount of 25-(OH)D<sub>3</sub> increased in komodo dragon 1 (K1) (18 to 195 nmol/ml) and in komodo dragon no 2 (K2) (31 to 291 nmol/ml). The amount of 1,25-(OH)<sub>2</sub>D<sub>3</sub> did not change significantly in both komodo dragons (139.5.6 to 235.3 pmol/l).

Measurement of 24,25-(OH)<sub>2</sub>D<sub>3</sub> in K2 showed a dramatic improvement after exposing to UV-B; the amount of 24,25-(OH)<sub>2</sub>D<sub>3</sub> rose (7.5 to 448.1 ng/ml).

Komodo dragon 3 (K3) was sent to Gran Canaria where it received natural UV-B. The level of 25-(OH)D<sub>3</sub> improved from 18 to 272 nmol/l. The amount of 1,25-(OH)<sub>2</sub>D<sub>3</sub> did not increase either.

In all komodo dragons the calcium level remained stable and within the range 3.18 to 4.44 mmol/l.

The present study documents for the first time the levels of three vitamin D<sub>3</sub> metabolites and their regulation by UV-B in Komodo dragons. According to literature low levels of 25-(OH)D<sub>3</sub> have caused bone defects in juvenile Komodo dragons. The current data show a clear effect of UV-B on the 25-(OH)D<sub>3</sub> levels and a concomitant rise in serum 24,25-(OH)<sub>2</sub>D<sub>3</sub> levels while 1,25-(OH)<sub>2</sub>D<sub>3</sub> levels remained constant. Although we have no data on the bone metabolism in our 3 Komodo dragons it is tempting to speculate in view of the published improvements of bone after UV-B treatment, that 24,25-(OH)<sub>2</sub>D<sub>3</sub> is involved in bone metabolism in Komodo dragons. This would be in line with data obtained in chicken and human showing a positive effect on bone.

UV-B measurements of a UV-B radiating lamp shows that the amount of UV-B declines rapidly during time. Also the decay rate differs from lamp to lamp. If "UV-B" lamps are used for synthesising vitamin D<sub>3</sub> through the skin the UV-B radiation should be measured regularly and the lamp should be replaced in time before the UV-B radiation is too low for his synthesising purposes.

This study shows clearly that, although this is a preliminary study, there is a dramatic change in vitamin D metabolites when using feeds with vitamin D versus UV-B light in juvenile komodo dragons.

### Introduction

Komodo dragons (*Varanus komodoensis*) are rare animals, which only inhabit the islands of Komodo, Rintja, and the western half of Flores in Indonesia. Reports of animals on smaller islands nearby, including Padar and Gili Montang are probably based on observations of movement of transient animals by swimming to these islands. Komodo dragons live in the tropics on 8 Degrees Southern latitude which means that the intensity of sunlight is much higher there than in Western Europe for example. In nature Komodo dragons bask in the morning, from 15 minutes to more than 3 hours (Auffenberg 1981).

These animals are opportunistic carnivores, at the top of the food chain on these Indonesian islands. It has been suggested that Komodo dragons as alpha predators can survive on these islands because they are ectotherm, which means that they require less food than mammals in other parts of the world at the top of the food chain. As adaptation to survival during long periods of low prey density, a Komodo dragon in one meal can consume up to 80 % of its own body weight. They feed on live prey as well as on carrion. They are capable of taking

down deer, wild boars and water buffalos. When necessary they do not feed for months at a time. Young Komodo dragons feed on insects, small birds and mammals and on other reptiles which may be more readily available throughout the year (Walsh 1999).

Komodo dragons are listed on Cites Appendix 1 by IUCN. The wild population can be considered as several thousands of animals. The major threats include habitat alteration, poaching of prey species and perhaps tourism.

The total captive population as of November 1998 was 272 animals which consisted of 65 males, 50 females and 157 of unknown sexes in 49 institutions. In Indonesian zoos live 160 animals, in North America 82, in Europe 14 and in Asia (- Indonesia) and Australia both 8 animals. About a dozen successful breedings have been recorded world wide. Zoos in Europe which maintain Komodo dragons are Thoiry in France, Chester in United Kingdom, Lisbon in Portugal, Reptillad on the Canary Islands in Spain, Zoo Berlin in Germany, Pilzen in the Czech Republic and Rotterdam Zoo in the Netherlands.

In captivity an adult Komodo dragon eats 1.5 –3.0 kg of rats a week, depending upon the size of the lizard and the time of the year. In general no vitamins and minerals are supplemented. A diet of whole animals combined with access to hot spots up to 40° C and natural or artificial UV-B light are thought to be adequate to promote healthy growth and development for adult Komodo dragons.

Hatchlings are fed daily for the first eight months and then every third day throughout the next year. In captivity they live on a diet comprising 20 % of whole mice and 80 % of chopped beef or lamb to which a vitamin and mineral supplement is added (Walsh 1999). Bone problems in (juvenile) Komodo dragons

It was reported by Allen *et al.* (1994) that nine of the twelve hatched Komodo dragons in Washington Zoo had long bone fractures which were discovered at about two months of age. It was discovered that the 25-dihydroxivitamin D<sub>3</sub> (25-(OH)D<sub>3</sub>) level, which is one of the intermediaries metabolics in the Vitamin D<sub>3</sub> synthesis, was low. After exposing the animals during two months to UV-B the 25-(OH)D<sub>3</sub> level increased significantly. It was presumed that rapid growing animals have increased requirements for calcium (Ca), phosphorus (P), and vitamin D<sub>3</sub> and that non-reproductively active adults may be more tolerant to low levels of Ca, P and/or vitamin D<sub>3</sub>, or low exposure to UV-B.

In October 1995 Rotterdam Zoo obtained three juvenile Komodo dragons which were born in the National Zoo in Washington D.C. (USA). From Washington it was known that the juveniles received UV-B light in order to synthesize vitamin D<sub>3</sub> and to prevent bone problems. At first it was decided that the young Komodo dragons did not receive UV-B or extra vitamin D at all. At a later stage it was decided to add vitamin D<sub>3</sub> to their diet and months later to expose them to an UV-B emitting lamp. One of the juvenile dragons was sent to Gran Canaria in Spain in June 1999.

#### Vitamin D<sub>3</sub> metabolism

Rachitis is a deficiency disease of vitamin D, which appears to have been a problem recorded in ancient times; evidence shows that rickets occurred in the Neanderthal man about 50.000 BC. (Machlin, 1990). The major effects of vitamin D are to increase the active absorption of Calcium-ion from the proximal intestine and to increase the mineralisation of bones. Vitamin D represents a group of closely related compounds that possess anti rachitic activity. (Machlin 1990).

A diagram depicting the synthesis and initial step of metabolism via 24-hydroxylase activity is shown in Figure 1. There are two sources from which vitamin D<sub>3</sub> (cholecalciferol) is normally provided: it is produced in the skin and it is taken up via the diet.

In the skin 7-dehydrocholesterol is photo chemically converted by UV-B to provitamin D<sub>3</sub> that then isomerizes to vitamin D<sub>3</sub>. Vitamin D<sub>3</sub> from the intestines and from the skin vitamin D<sub>3</sub> is bound to vitamin D binding protein and moves to the liver where it is hydroxylated at the carbon 25 position by the enzyme 25-hydroxylase to form 25-hydroxyvitamin D<sub>3</sub> (25-(OH)D<sub>3</sub>). Finally, in the proximal tubules of the kidney the biologically most active vitamin D<sub>3</sub> metabolite, 1,25-dihydroxyvitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>D<sub>3</sub>), is formed. A second metabolite of vitamin D<sub>3</sub> is produced in the kidney, namely 24,25(OH)<sub>2</sub>D<sub>3</sub>. Generally, 24,25-(OH)<sub>2</sub>D<sub>3</sub> has been considered to be the first step in the degradation pathway of 1,25-(OH)<sub>2</sub>D<sub>3</sub> and 25-(OH)D<sub>3</sub>. However, several human and animal studies demonstrated a positive contribution of 24,25-(OH)<sub>2</sub>D<sub>3</sub> either alone or in combination with other hormones to bone metabolism (van Leeuwen *et al.*, 2001) Recent studies in chickens suggest that 24,25-(OH)<sub>2</sub>D<sub>3</sub> together with 1,25-(OH)<sub>2</sub>D<sub>3</sub> treatment improves fracture healing and that 24,25-(OH)<sub>2</sub>D<sub>3</sub> serum levels are correlated to fracture healing (Kato 1998, Seo 1997).

The synthesis of 1,25-(OH)<sub>2</sub>D<sub>3</sub> is tightly controlled in order to maintain the calcium homeostasis. The major stimulators of 1,25-(OH)<sub>2</sub>D<sub>3</sub> formation are low serum calcium, parathyroid hormone and low serum phosphate levels. Increased serum calcium levels (hypercalcemia) inhibits formation of 1,25-(OH)<sub>2</sub>D<sub>3</sub>. Most interestingly, 1,25-(OH)<sub>2</sub>D<sub>3</sub> itself inhibits its own formation but stimulates 24-hydroxylase activity and the formation of 24,25-(OH)<sub>2</sub>D<sub>3</sub> and 1,24,25-(OH)<sub>3</sub>D<sub>3</sub>. Thus the metabolic clearance of 1,25-(OH)<sub>2</sub>D<sub>3</sub> is enhanced. By these regulatory mechanisms toxic effects of hypercalcemia (too much calcium) in the blood is prevented.

Figure 1: Diagram of the vitamin D<sub>3</sub> synthesis. Details are described in the text above.

- insert figure 1 here-

#### UV and UV-B meter

The spectrum of irradiance of wavelengths that reach the earthly atmosphere from the sun is approximately from 100 to 3200 nanometer (nm). Molecules in the atmosphere absorb certain wavelengths, so that the solar spectrum is attenuated when the radiation reaches the surface of earth. Some of the solar radiation is partly absorbed by ozone, oxygen, carbon dioxide and water. It means that life on earth is in principal exposed to Ultra Violet (UV), Visible Light and Near Infra Red. The wave length of Near Infra Red is longer than 700 nm. The Visible Light has a wavelength from 400 to 700 nm. UV can be divided into UV-C with a range from 100-280 nm, UV-B with a range from 280-315 nm and UV-A with a length of 315-400 nm. As mentioned before UV-B plays a major role in converting 7-dehydrocholesterol into provitamin D<sub>3</sub> in the skin with a maximum conversion at 297 plus or minus 3 nanometer. (Bernard, 1995)

Depending on the degrees latitude and the time of the year in some places it is not possible for humans to produce provitamin D<sub>3</sub> by natural light. In locations on 52 degrees North latitude, for example Edmonton, in Canada no provitamin D<sub>3</sub> will be produced from October until the beginning of April (Holick 1997). Berlin, Warschau and Rotterdam in Europe are also situated on the same latitude. Tests in Boston (42 degrees North), have confirmed that no provitamin D<sub>3</sub> was produced from November until February. The European cities of Barcelona and Rome lie on 42 degrees North. Tests have demonstrated that at the latitude of Los Angeles (34 degrees North) enough provitamin D<sub>3</sub> is produced throughout the year. 34 degrees North is the same latitude as places in Morocco and Northern Syria in the old world! The data described tests related to humans but probably similar considerations can be made on the effect of provitamin D<sub>3</sub> synthesis in reptiles, for example Komodo dragons. Tests have also proven that through normal windows UV-B coming from outside is absorbed. If Komodo dragons rely on the availability of UV-B for their synthesis, it can be questionable if

especially juvenile Komodo dragons receive enough UV-B light. In order to measure the intensity of UV-B a special meter was designed.

The aim of the current study was to assess the vitamin D<sub>3</sub> status in Komodo dragons held in captivity in the Rotterdam Zoo (i.e. at 52 degrees North). In addition, the effect of interference with UV-B on the serum levels of vitamin D<sub>3</sub> metabolites and on the serum calcium concentration was investigated.

## Methods

### The Komodo dragons

In Rotterdam Zoo the three Komodo dragons were housed according to the suggestions made by the taxon manager (Walsh 1999). All three were housed separately in a cage with a surface area of 10 square m<sup>2</sup>. All the Komodo dragons were fed once a week. The diet consisted of whole rats and small rabbits. They were fed ad libitum. After 20 months K1 weighed 2.1 kg, K2 2.5 kg and K3 1.5 kg. The weight at 20 months for juveniles should be according figures of the National Zoo between 1.5 kg and 3.1 kg.

As mentioned above, in October 1995 the Komodo dragons arrived in Rotterdam Zoo.

Unfortunately it was not possible to take always blood samples from all the three Komodo dragons at the same time. Also it happened that not enough blood could be obtained for analyses.

In May 1996 (month 1) the first blood samples were taken from the tail vein using heparin tubes and immediately stored at minus 68 °C until analysing. In February 1997 (month 9) Carmix<sup>®</sup>, a vitamin and mineral supplement, was added to the diet which means 450 IU vitamin D<sub>3</sub> per kg food. Two months later blood samples were taken again.

Two Komodo dragons received UV-B by exposing them to the Osram Ultra-Vitalux<sup>®</sup>. The wattage of the lamp - according the manufacturer is 300 W and it has a service time of 1000 hours. The Osram Ultra-Vitalux<sup>®</sup> consists of a quartz burner and a tungsten filament which are blended in such a way that, in combination with the special glass bulb and its interior reflector, a certain radiation is emitted. The effect of this radiation is practically the same as the radiation of the natural sunlight occurring the Osram Ultra-Vitalux<sup>®</sup> manual (2001).

In month 13 K1 was exposed to UV-B and in month 21 K2 was exposed to UV-B. All lamps were hung between 60-80 cm above the ground surface in the cages in a way that the Komodo dragons had free access to the radiation of the lamps.

In month 25 K3 went to Gran Canaria, situated 28 degrees North, where it received an outdoor facility and was exposed to natural sunlight. In month 33 a blood sample of that dragon was obtained and analysed on the medical laboratory in Rotterdam.

Figure 2: Electric schedule of the UV intensity meter

- Insert Acrobat figure 2 here

### Vitamin D analyses

The 25-(OH)D<sub>3</sub> analyses were performed according the description of DiaSorin (Minnesota, USA). The assay consists of a two step procedure. The first procedure involves a rapid extraction of 25-(OH)D<sub>3</sub> and other hydroxylated metabolites from the serum. Following extraction, the treated sample is then assayed using an equilibrium radio immunoassay (RIA) procedure which is based on an antibody with specificity to 25-(OH)D<sub>3</sub>. The sensitivity of this assay has shown rates to be at or below 1.5 ng/ml.

The amount of  $1,25\text{-(OH)}_2\text{D}_3$  was analysed by the IDS Gamma-B kit by immunoextraction followed by quantitation by  $^{125}\text{I}$  radio immunoassay. The assay has a calculated sensitivity of 2.1 pg/ml.

Calcium analyses were performed using a colorimetric calcium assay (Sigma Diagnostics). All analyses were performed by the laboratories of the Department of Internal Medicine of the Erasmus MC in Rotterdam, except the analysis of  $24,25\text{-(OH)}_2\text{D}_3$  which was performed on the Department of Clinical Sciences of Companion Animal Medicine of the Utrecht University.  $24,25\text{-(OH)}_2\text{D}_3$  was quantitatively determined by a modified radio immunoassay (RIA) (DiaSorin, Stillwater, Minnesota, USA). Before processing, labeled standards  $24\text{R},25\text{-dihydroxy}[26,27\text{-methyl-}^3\text{H}]\text{cholecalciferol}$  (specific activity 15.4 GBq/mg, Amersham Pharmacia Biotech, UK) was added to plasma samples and to the standards of the RIA to determine individual sample recovery. Samples were extracted twice with ethylacetate:cyclohexane (1:1, v/v) and once with methanol:ethylacetate:cyclohexane (4:5:5, v/v) (Bosch 1983) and  $24,25\text{(OH)}_2\text{D}_3$  was separated by solid phase extraction using  $\text{NH}_2$  cartridges (Bakerbond spe Amino Disposable Extraction Columns, J.T. Baker, Phillipsburg, USA) according to the described method of McGraw and Hug (1990). The standard curve of the stable vitamin  $\text{D}_3$  metabolite showed good parallel dilution to the standard curve of the RIA. The intra- and inter-assay coefficient for  $24,25\text{-(OH)}_2\text{D}_3$  were 10.1% and 8.5%, respectively.

#### Design of the UV-B meter

The intensity of UV-B meter was designed and constructed by the Optic Research Group of the Technical University of Delft in the Netherlands. The UV-B meter is sensitive to a narrow wavelength band (10.17 nm at Full Width at Half Maximum) around 302.01 nm. The intensity meter consists of a photo diode, placed directly behind an interference filter to ensure that only the desired wavelengths impinge the sensor, and an LCD read out screen. For situations where it is not possible to read out this built in display, a connector is provided to connect a simple multi-meter to the UV-B meter. This enables the measurement of UV-B radiation to take place at larger distances and under otherwise impossible angles.

The photo detector from Centronic (code OSD 5.8-Q) was selected for its relatively high sensitivity for UV-B radiation. The interference filter was obtained from Oriel and was tested by the Optic Research Group on transmission besides the desired wavelength range. No leakage was observed, which means that no wavelength other than the desired ones could penetrate the filter. The peak transmission (17.25 %) of the filter was at 302.01 nm. The photons impinging on the photo detector result in a small voltage. This voltage is shown, after amplification, on the small liquid crystal display.

Up to now the UV-B meter is not calibrated absolutely and therefore it can only be used for relative measurements. It can be used to monitor UV-B radiation with time and the spatial distribution measurement of the UV-B radiation coming from a single lamp or a number of lamps. The UV-B meter can be used to check when lamps, have to be changed, and to get an impression where the UV-B rich spots are in certain areas. It can only be used to detect the light intensity differences between different animal facilities. The UV-B meter can easily be manufactured by properly educated technical staff. The costs of this meter is limited was approximately 700 Euro.

The readout in the display of the UV-B meter is in millivolts: which means with the used photo diode that every point read out is equal to about 5nW light falling on the surface of the sensor ( $1\text{ cm}^2$ ). The meter was calibrated in such a way that the read out value on a distance of 1 meter is 150. The numeric value of the UV-B meter is 0.218 V/microW. It means the amount of microW/cm<sup>2</sup> is the read out value divided by 218.

In cage no 1 (K2) the UV-B lamp was hung 80 cm above the ground and in cage 2 (K1) for practical reasons, 60 cm above the ground perpendicular under the lamp. The measurements are the average of two times measuring during the same day. The UV-B measurements were taken from the ground. Both Komodo dragons had free access to sunbath under the lamps. The lamps were connected to a timer which means that they were on for two hours in the morning and two hours in the afternoon, every day. Every six months the lamps were changed.

## Results

25-(OH)D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub> and 24,25-(OH)<sub>2</sub>D<sub>3</sub>

In table 1 and figures 3, 4 and 5 all data on changes of the amount of 25-(OH)D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub> and 24,25-(OH)<sub>2</sub>D<sub>3</sub> in the blood serum of K1, K2 and K3 can be found.

The Komodo dragons did not get any UV-B or orally vitamin D<sub>3</sub> supplement besides vitamin D from their normal diet (rabbits and rats) during their arrival from Washington in October 1995 until Mai 1997. Although they all got sufficient UV-B in Washington (Allen, personal communication) to maintain the normal level of 25-(OH)D<sub>3</sub> (150 –200 nmol/ml (Gillespie 2000) the amount of 25-(OH)D<sub>3</sub> dropped in 18 months after arrival in Rotterdam for K1, K2 and K3. In the tables and figures these data can be found as data in month 1. It was then decided to add vitamin D to the diet: In month 9 after arrival 450 IU vitamin D<sub>3</sub> was supplemented per kg food to the diet of each dragon. Analyses of the amounts of 25-(OH)D<sub>3</sub> in month 11 showed no significant changes.

In month 13 UV-B emitting lamps (Osram Ultra-Vitalux<sup>®</sup>) were installed in the cage of K1. The amount of 25-(OH)D<sub>3</sub> improved as can be seen in table 1 and figure 3. K2 was exposed to UV-B in month 21 which also resulted in an increase of 25-(OH)D<sub>3</sub> (table 1, figure 4). After K3 has been sent to Gran Canaria in month 25 blood analyses showed an increase of 25-(OH)D<sub>3</sub> (table 1; figure 5).

In the same samples used for 25-(OH)D<sub>3</sub> also the 1,25-(OH)<sub>2</sub>D<sub>3</sub> levels were determined. As can be seen in table 1 and the figures 3, 4, and 5, neither the 450 IU vitamin D supplemented food nor the UV-B treatment resulted in a clear change in 1,25-(OH)<sub>2</sub>D<sub>3</sub> levels.

Another important vitamin D<sub>3</sub> metabolite is 24,25-(OH)<sub>2</sub>D<sub>3</sub>. Because of the limited amount of heparin plasma available only the 24,25-(OH)<sub>2</sub>D<sub>3</sub> levels of K2 could be measured. Data were obtained before month 11 and after months 26 and 35. As shown in table 1 and figure 4, 24,25-(OH)<sub>2</sub>D<sub>3</sub> rose dramatically after exposure to adequate levels of UV-B.

Table 1: Levels of 25-(OH)D<sub>3</sub>, 1,25-(OH)<sub>2</sub>D<sub>3</sub> and 24,25-(OH)<sub>2</sub>D<sub>3</sub> in the blood of the Komodo dragons.

Month	Komodo dragon (K1)		Komodo dragon (K2)			Komodo dragon (K3)	
	25-(OH)D <sub>3</sub> nmol/l	1,25-(OH) <sub>2</sub> -D <sub>3</sub> pmol/l	25-(OH)D <sub>3</sub> nmol/l	1,25-(OH) <sub>2</sub> D <sub>3</sub> pmol/l	24,25-(OH) <sub>2</sub> D <sub>3</sub> ng/ml	25-(OH)D <sub>3</sub> nmol/l	1,25-(OH) <sub>2</sub> D <sub>3</sub> pmol/l
1	18	235,3	31	139,5		18	158,2
11	26	159,9	37	201,9	7,5	33	161,3
18	131	132,8	29	188		19	
20						17	121,6
26	195	177,9	201	143,8	294,6		
33						272	152,7
35			291	158,2	448,1		

- Insert figures 3, 4 and 5 here-

#### Calcium

Figure 6 shows, that for all 3 dragons throughout the UV-B treatment or the move to Gran Canaria; serum calcium levels remained stable. The absolute serum calcium values are shown in Table 2.

Table 2: Calcium levels in the blood of Komodo dragons (mmol/l)

Month	Komodo dragon 1 (K1)	Komodo dragon 2 (K1)	Komodo dragon 3 (K3)
11	4.16	3.84	4.48
18	3.18		
20			3.8
26	3.7	4.44	
33			3.98
35		3.86	

#### Lamp radiation

Table 3 shows the result of the declining of the amount UV-B of the used lamps in the komodo dragon facilities. The UV-B lamp from cage no 2 was after replacing used for other reptiles. Half a year later the amount of microwatts UVB/cm<sup>2</sup> was reduced to 0.16.

After replacing every six month similar values were found again (personal communication, van de Koore).

Table 3: Decline in lamp radiation (Watts UV-B/cm<sup>2</sup>) of two lamps hanging in two cages.

Month	Cage 1	Cage 2
19	0.72	
22	0.43	0.8
24	0.34	0.3
25	0.23	0.34



## Discussion

No systematic blood data could be obtained and therefore the analysed data represents a trend in the changes of the vitamin D metabolites under different circumstances.

Comparing to the first levels of 25-(OH)D<sub>3</sub> in month 1 to normal levels in Komodo dragons (150-200 nmol/L) when exposed to UV-B shows that these levels were very low and certainly would have caused bone problems. Similar levels were found in case of clinical lameness and poor density on radiographs, with fractures in several long bones (Gillespie 2000). Applying 450 IU vitamin D<sub>3</sub> extra per kg food for two month did not improve the 25-(OH)D<sub>3</sub> levels which suggests that juvenile Komodo dragons cannot rely on these amounts of vitamin D<sub>3</sub> when orally supplied to satisfy their vitamin D synthesis. Exposing Komodo dragons to artificial or natural light improves the amount of 25-(OH)D<sub>3</sub> within 5 months to their normal value which is 150-200 nmol/l.

No reference data were available on the amounts of 1,25-(OH)<sub>2</sub>D<sub>3</sub>. The observed data varied from 121.6 to 235.3 pmol/l. The average of the 13 blood samples from 3 komodo dragons is 163,9 pmol/l. No correlation is found after supplying extra vitamin D<sub>3</sub> or exposing the Komodo dragons to UV-B.

Calcium levels varied from 3.18 to 4.48 mmol/l with a mean of 3.93 mmol/l in 6 samples from 3 animals. In the article of Gillespie (2000) the mean values for 48 Komodo dragons were 3.62 mmol/l with an observed range for measured values of 2.94 to 4.30. It means that the levels found for calcium in Rotterdam fall within the mean range and variation.

Although normal values of 24,25-(OH)<sub>2</sub>D<sub>3</sub> are not known it is clear that when Komodo dragons exposed to UV-B the amount of 24,25-(OH)<sub>2</sub>D<sub>3</sub> improves significantly. Due to limited availability of serum the effect of supplying orally vitamin D<sub>3</sub> on 24,25-(OH)<sub>2</sub>D<sub>3</sub> levels could not be determined in this research project.

The limited data of the current study shows the effect of UV-B on the 25-(OH)D<sub>3</sub> levels in Komodo dragons. Although we have no data on the bone density of the dragons in our study, combining our observations with those in the Washington Zoo on increasing serum 25-(OH)D<sub>3</sub> and the reduction in bone problems and other clinical symptoms it is conceivable to suggest that adequate UV-B availability is important for the well being of the Komodo dragons. The current study reports for the first time 1,25-(OH)<sub>2</sub>D<sub>3</sub> levels in Komodo dragons. An interesting observation is that throughout the treatment period the levels of the biologically most active vitamin D compound, 1,25-(OH)<sub>2</sub>D<sub>3</sub>, and of serum calcium remained stable. This is logical from a physiological point of view as calcium is a very important ion whose level needs to be controlled very tightly, because both hypocalcaemia and hypercalcaemia can be life threatening. As 1,25-(OH)<sub>2</sub>D<sub>3</sub> is the most important regulator of serum calcium it is important that the level of this hormone is also strictly regulated. If the dramatic increase in 25-(OH)D<sub>3</sub> levels after UV-B treatment would have been followed by a comparable increase in 1,25-(OH)<sub>2</sub>D<sub>3</sub> then the animals would have become hypercalcemic. Putting Gillespie's data and the current data together it is intriguing that despite a similar 1,25-(OH)<sub>2</sub>D<sub>3</sub> level in case of low and high 25-(OH)D<sub>3</sub> level, there seem to be clinical problems for the Komodo dragons in case of low 25-(OH)D<sub>3</sub> (Gillespie et al. 2000).

Therefore, it is tempting to speculate that an additional vitamin D<sub>3</sub> metabolite might be important to restore the bone defects. A possible candidate is 24,25-(OH)<sub>2</sub>D<sub>3</sub> (van Leeuwen et al., 2001), which we show here to increase in parallel to 25-(OH)D<sub>3</sub>. This is not unique for Komodo dragons, as also observed in humans an increase in serum calcium or 1,25-(OH)<sub>2</sub>D<sub>3</sub> is followed by an increase in 24-hydroxylase activity in order to prevent further formation of 1,25-(OH)<sub>2</sub>D<sub>3</sub> and stimulated inactivation of 1,25-(OH)<sub>2</sub>D<sub>3</sub> by forming 1,24,25-(OH)<sub>3</sub>D<sub>3</sub>. A possible role for 24,25-(OH)<sub>2</sub>D<sub>3</sub> in this respect is supported by data in humans showing a beneficial effect on bone when 24,25-(OH)<sub>2</sub>D<sub>3</sub> was added to the treatment with 1 $\alpha$ -(OH)-