**University of Veterinary Medicine, Doctoral School**

# The most important nutritional disorders of tortoises (*Testudo* sp.) and bearded dragon *(Pogona vitticeps)*

Theses of PhD dissertation

Nikoletta Hetényi

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Supervisor and members of the scientific committee:

……………………………..
Dr. István Hullár
supervisor
associate professor
University of Veterinary Medicine,
Department of Animal Breeding, Nutrition and Laboratory Animal Science

Tamás Sátorhelyi, DVM
Exo-Pet Veterinary Clinic,
member of the scientific committee

………………………
Nikoletta Hetényi, DVM
University of Veterinary Medicine,
Department of Animal Breeding, Nutrition and Laboratory Animal Science

**Tartalom**

# 1. List of abbreviations

ALP = alcalic phosphatase,

ALT = alanin-aminotransferase,

AST = aspartate-aminotransferase

BL = body length,

BW = body weight,

Ca = calcium,

$Ca^{2+}$ = ionised calcium,

CK = creatin cinase,

SCL = straight carapace length,

LDH = lactate-dehydrogenase

MBD = metabolic bone disease,

p.o = per oral,

DM = dry matter,

PL = plastron length,

PH = plastron height,

SW = shell width,

PTH = parathyroid hormone,

## 2. Summary

Diseases of reptiles in captivity are frequently in connection to inadequate housing and feeding conditions. The most common health problem is the metabolic bone disease (MBD) which is predominantly caused by Ca and vitamin $D_3$ deficiency. In reptiles feeding and housing conditions have an impact on the blood biochemical parameters which are important diagnostic tools. I chose two commonly kept species, the Hermann’s tortoise *(Testudo hermanni)* and the bearded dragon *(Pogona vitticeps)* to the subject of the investigations in order to have useful data for the praxis as well. In my dissertation I summarized 7 experiments, in these I investigated the long term effects of different dietary supplements on selected blood parameters, health and growth rate of Hermann’s tortoises and bearded dragons (additionally, life spans of UVB bulbs were alto tested), nutritional composition of reptile foods and the dry matter intake, gut passage time and digestibility in Hermann’s tortoises.

The main statements are the following. The investigations regarding the vitamin and mineral supply of reptiles need to be long term (>10-12 months). For growing tortoises a dietary supplement containing 50 000 IU/kg vitamin $D_3$ and 150 g/kg Ca is adequate in the dosage of 1.5 g/100 g fresh food/day. For growing bearded dragons either a dietary supplement containing 50 000 IU/kg vitamin $D_3$ and 150 g/kg Ca or Ca supplementation + UVB bulb dedicated to desert species can be used. For adult bearded dragons either a dietary supplement containing 32 000 IU/kg vitamin $D_3$ and 350 g/kg Ca or Ca (350 g/kg) + UVB exposure =(33,5±5,5 $\mu W/cm^2$) can be used. None of these treatments are adequate for adult Hermann’s tortoises, accordingly they require supplement with high vitamin $D_3$ content (50 000 IU/kg) or stronger UVB irradiation.

In omnivore, insectivore and carnivore reptiles it is advised to have 24 hours long fasting prior blood sampling in order to have standardized uric acid concentrations. In bearded dragons the concentration of total protein increases with the age.

According to their nutritional value, cockroach species can be alternatives of crickets, but hissing cockroach can only be given occasionally because of its high Cu content (176.2 mg/kg). The dry matter intake of the tortoises in connection with the food varies between 0.4-1,2 % of the body weight. Tortoises have significantly higher dry matter intake if lettuce is fed.

## 3. Introduction

The number of reptile species in captivity is continuously increasing. In many cases the nutritional requirements and composition of foods in the nature are not known. That is why the diseases of reptiles in captivity are mainly in connection with inadequate housing and feeding. Among these the metabolic bone disease (MBD) is the most important disorder. It has a complex aetiology but the nutritional and renal secondary (lack of Ca/vitamin $D_3$) MBD is considered the most prevalent (Girling és Raiti, 2004; McArthur, 2004; Mader, 2006; Wright, 2008).

Blood biochemistry may help the early diagnosis, but for many parameters data in the literature are contradictory or missing. In reptiles the housing conditions (outdoor or indoor), as well as age, sex and feeding have great impact on these. Sources of the data are often from animals from the wild which cannot be easily interpreted in the veterinary praxis. According to the available data, huge differences can be found between herbivore, omnivore or carnivore reptiles. Because of this it would be important to set up reference values for these categories.

I chose two reptile species the Hermann's tortoises *(Testudo hermanni)* and the bearded dragon *(Pogona vitticeps)* which are commonly kept as pets. Through these results can be useful for the praxis as well.

## 4. Aims of the study

According to my former data collection, more than half of the reptiles had clinical symptoms of MBD despite the fact that they received dietary supplement. That is why I chose to test commercial dietary supplements. By knowing the long term effects of these on the health and blood parameters of the animals, useful recommendations can be made for pet owners.

My aim was to test whether it is possible to reach higher $25(OH)D_3$ concentration by using dietary vitamin $D_3$ or UVB exposure. In connection with this I also tested the level of UVB irradiation – and its changes with distance and time – of popular product of three companies.

As blood biochemistry is an important tool of the early diagnosis, I also aimed to set up blood reference values for healthy animals in both species. Here emphasis was put on the long term examinations and repeated sample takings.

Many of the diseases can be prevented with the adequate feeding, that is why I examined the nutritional composition of some well known and new invertebrate preys as well as turtle foods. Effects of age and diet were also tested in case of cricket species. Comparisons of mono- and dicotyledonous plants as well as alternatives of these in winter for tortoises were also made.

Information on voluntary dry matter (DM) intake, gut passage time and digestibility of nutrients may support the adequate feeding of tortoises and may also help to prevent fast growth rate due to overfeeding of the animals. The aim of the study was to determine voluntary DM intake and gut transit time in Hermann's tortoises and also to investigate whether it is possible to determine digestibility with the method of total collection. Here dandelion *(Taraxacum officinale)* leaves were tested as it is part of the diet in the nature and can be collected even in parks of cities. The two other plants are less recommended (cucumber and lettuce) but according to my previous data collection, these are widely used by pet owners. By knowing the level of consumption and digestibility the most suitable among these can be chosen.

## 5. Review of the literature

The Hermann's tortoises are herbivore tortoises native to southern part of Europe, while the bearded dragons are omnivores from Australia.

MBD in connection with inadequate feeding is frequently seen in both species, which is not a single disease but collection of clinical symptoms affecting the bone integrity (Jacobson, 1994; Fodor et al., 2004a,b; Girling és Raiti, 2004; McArthur, 2004; Mader, 2006; Gál, 2014). It can have both nutritional (**secondary alimentary hyperparathyroidism**) and renal origin (**secondary renal hyperparathyroidism**). The **primary** form of **hyperparathyroidism** – in connection with tumours in the parathyroid gland and increase parathyroid hormone (PTH) – is rarely described (Gál et al., 2006). It has a complex aetiology, the lack of Ca and/or vitamin $D_3$ (cholecalciferol) in the diet, inadequate Ca:P ratio or lack of UVB irradiation is frequently seen (McArthur, 2004; Mader, 2006, Gál 2014). The disease is common in young animals with accelerated growth rate because of overfeeding. Renal origin of MBD can be caused by lack of drinking water, drugs damaging the kidneys (e.g.: gentamicin) or feeding of proteins of animal origin in herbivores. Because of the tubulonephrosis Ca excretion via the kidneys is increased causing lower blood Ca concentration and the elevation of PTH. The lower number of tubular epithet cell leads to the lower activity of 1-α-hydroxilase enzyme and decreased level of calcitriol.

Among the animal origin foods vitamin $D_3$ (calcitriol) can be found in the liver, fat tissue and in smaller concentration in egg yolk and dairy products. The other source can be the endogenous synthesis in the skin which requires UVB radiation. The utilisation rate of plant origin vitamin $D_2$ (ergocalciferol) in reptiles is not known (Mader, 2006), but it seems to be less effective than cholecalciferol (Houghton and Vieth., 2006). The UVB irradiation can be classified into three categories. The UVA (316-380 nm) has a role in the pigmentation and also increases the appetite. However, overexposure may decrease growth rate and vitamin A content of the egg (Ferguson et al., 1996). The UVB (281-315 nm) plays a role in the endogenous synthesis of cholecalciferol. The UVC (100-280 nm) is filtered by the ozone layer (Mader, 2006).

Blood test may help the early diagnosis of MBD, lower than 1:1 Ca:P ratio is one of these. Elevated alcalic phosphatise (ALP) can be a sign of MBD as well. Several data of the literature are coming from single sampling of animals living in the wild (Marks and Citino, 1990; Cranfield et al., 1996; Eliman, 1997; Christopher et al., 1999; Mathes et al., 2006; Rangel-Mendoza et al., 2009), only few studies used repeated samplings (Erler, 2003; Holz, 2007; Eatwell, 2009; Oonincx et al., 2010, 2013; Tamuaki et al., 2011). In reptiles housing, feeding, age, as well as sex have a great impact on the biochemical parameters (Dennis et al., 2001, Kölle et al., 2001, Eatwell, 2009; Tamukai et al., 2011; Szőke et al., 2012; Scope et al., 2013; Andreani et al., 2014). That is why it would be important for the praxis to set up reference values according to sex and age, with special focus on the indoor or outdoor housing.

The invertebrate preys of reptiles in captivity are limited into few species – such as house cricket *(Acheta domesticus)*, mealworm *(Tenebrio molitor)* and superworm *(Zophobas morio)*. In carnivore turtles dried foods (e.g.: fish, shrimp) and commercial pellets are widely used by pet owners which may lead to vitamin A deficiency and MBD. Instead of these whole frozen fish – which contains the inner organs as well – and mollusk are recommended (Mader, 2006). Mediterranean tortoises have a Ca rich diet in the nature (Ca:P=4:1; Highfield, 1994). For them dicotyledonous plants can be offered, which should be at least 70-80% of the diet.

## 6. Experiments

- Animals were housed in the animal house of the Department of Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine.
- The work has been approved by the ethics review of the Committee of Animal Welfare (registration number: 22.1/380/003/2010) and also the Committee of Animal Welfare at the University of Veterinary Medicine.
- Lighting: 60W spot bulb with or without UVB exposure; 12 h/day.
- Ambient temperature: 23-28°C daytime, 18-22°C at night; 35-60% air humidity.

- Statistics: R 2.9.2. (R Development Core Team 2009); normality was tested with qqplot; level of significance $p<0,05$. UVB irradiation was measured with Solartech Solarmeter 6.2.
- Body weights were measured with Sartorius Scaltec SBC61; X-ray: Mediroll-2 type X-ray machine, 52 kV, 5.4 mAs, 0.18 sec.
- Blood taking: samples (0,5-1 ml/animal) were collected from the ventral (tortoise) or dorsal (bearded dragons) coccygeal vein with 22G needle into heparin collection tubes. Plasma was used for the analysis. In chapters 6.2 and 6.3 Radiometer ABL 500 blood gas analyser and Randox Rx Daytona analyzer, in chapter 6.4. Roche Cobas 411 (calcitriol) and Olympus AU400 were used.

## 6.1. EFFECTS OF TWO DIETARY VITAMIN AND MINERAL SUPPLEMENTS ON THE GROWTH AND HEALTH OF HERMANN'S TORTOISE

### 6.1.1. Materials and methods

- Housing: two 1.2 x 1.6 m wooden terrariums, using natural soil as substrate. Above each terrarium 1.2 m UVB lamp (4-6 μW/cm$^2$). One month-old male Hermann's tortoises, n=6/group.
- Feeding: two different commercial dietary supplements were given (product "A" and "B"; **table 1.**). The recommended dosage for products "A" and "B" per 100 g fresh food was 1.5 g and 2 g, respectively. The most important ingredients of the commercial supplements ("A" and "B") used in this study are shown in **table 2**.

**Table 1.** The Ca, P, and vitamin $D_3$ contents of diets made for groups A, and B

| | Diet of group A and B without supplement | Diet of group A with supplement | Diet of group B with supplement |
|---|---|---|---|
| Ca (g/100 g DM) | 1.5 | 3.1 | 3.5 |
| P (g/100 g DM)) | 0.2 | 1.1 | 0.4 |
| Ca : P | 7.5 : 1 | 2.8 : 1 | 8.8 : 1 |
| Vitamin $D_3$ (IU/100 g DM)) | - | 591.7 | 30.3 |

**Table 2.** The most important ingredients of the commercial supplements

| Components | product "A" | product "B" |
|---|---|---|
| Calcium (g/kg) | 150 | 148 |
| Phosphorus (g/kg) | 83 | 17 |
| Vitamin $D_3$ (IU/kg) | 50 000 | 2000 |
| Vitamin $B_1$ (mg/kg) | 160 | 3500 |
| Vitamin $B_2$ (mg/kg) | 500 | 5000 |
| Vitamin $B_6$ (mg/kg) | 300 | 1800 |
| Vitamin $B_{12}$ (µg/kg) | 1800 | 5000 |
| Biotin (µg/kg) | 10 000 | 29 000 |
| Vitamin E (mg/kg) | 1500 | 50 000 |
| Vitamin K (mg/kg) | 30 | 7500 |

Tortoises were fed once every day, in the morning. Diets were based on garden weeds from which two mixtures (mixture 1 and 2) were prepared (**table 3.**). Within mixture 1, two subgroups were used (mixture 1a from spring to autumn, and mixture 1b in winter). Animals had ad libitum access to mixture 1. The main components of mixture 1a were dandelion leaf (2/3 part), chickweed, grass and plantain, cloverleaf (together 1/3 part). In winter (approximately 2.5 month), due to weather conditions, weeds were not available, so tortoises were fed with mixture 1b containing rucola (2/3 part), turnip-tops and lettuce (together 1/3 part). Mixture 2 was given independently of the season, throughout the experiment and contained grated vegetables and fruit (2/3 part cucumber, 1/3 part apple and carrot).

**Table 3.** The nutritional values of the feeds used in the study on dry matter basis

| Feed | Ca % | P % | Crude fat % | Crude fibre % | Crude protein % |
|---|---|---|---|---|---|
| Mixture 1a | 1.8 | 0.2 | 3.5 | 10.3 | 20.9 |
| Mixture 1b | 1.7 | 0.5 | 4.2 | 15.9 | 34.8 |
| Mixture 2 | 0.2 | 0.3 | 0.6 | 5.6 | 9.6 |

- Body weight (in grams), straight carapace length, straight plastron length, shell width and shell height were measured (with a slide-gauge, in mm) weekly for 12 months. The possible effect of supplements on tortoise activity levels was assessed twice a day (in the morning before feeding and in the afternoon at 3 pm) by recording if the tortoises were active (moving or eating) or inactive (sleeping or basking).
- Additionally, X-ray examinations were made at ages 6 and 12 months to check the shell status.

- Data analysis: Welch's two sample t-test was used to calculate the difference in weight gain (and between the initial data). Non-normally distributed data were analysed with a Brunner-Munzel test. The growth curve was cut into two parts and these two parts were described by two mixed models separately. The linear part of the growth curve included the data of the first 17 days of the experimental period. On the data, which were collected from day 18 till the end of the experiment, an exponential curve was fitted.

### 6.1.2. Main results and conclusions

- At the beginning of the study, no significant differences were found between the groups in any of the parameters (**table. 4**). Animals in group B gained significantly more weight (p = 0.044) than in group A, so the final weight and shell parameters were significantly higher.

**Table 4.** Descriptive statistic for weight and shell parameters in group A and B

| Parameters | Group A | | | Group B | | | |
|---|---|---|---|---|---|---|---|
| | mean | SD | SE | mean | SD | SE | P |
| Initial weight (g) | 16.5 | 1.77 | 0.72 | 15.9 | 0.91 | 0.37 | 0.4822 |
| Final weight (g) | 131.6 | 28.70 | 11.71 | 186.7 | 50.14 | 20.48 | 0.0291* |
| Initial scl (mm) | 36.3 | 2.00 | 0.80 | 36.0 | 0.90 | 0.36 | 0.7167 |
| Final scl (mm) | 80.3 | 5.90 | 2.41 | 92.1 | 9.90 | 4.04 | 0.0035* |
| Initial pl (mm) | 30.5 | 2.30 | 0.95 | 31.5 | 2.30 | 0.95 | 0.4772 |
| Final pl (mm) | 68.8 | 5.08 | 2.07 | 81.4 | 9.30 | 3.79 | 0.0001* |
| Initial sh (mm) | 18.8 | 1.20 | 0.47 | 19.7 | 1.50 | 0.61 | 0.3109 |
| Final sh (mm) | 43.7 | 3.71 | 1.51 | 47.9 | 3.67 | 1.49 | 0.0273* |
| Initial sw (mm) | 31.0 | 2.30 | 0.93 | 31.7 | 1.80 | 0.71 | 0.5834 |
| Final sw (mm) | 69.0 | 4.74 | 1.93 | 77.3 | 6.11 | 2.49 | 0.0296* |

scl = straight carapace length, pl = plastron length, sh = shell high, sw = shell width, SD = standard deviation, SE = standard error. *Significant difference between the groups

- According to the results of mixed models, the type of supplement had no effect on the linear part of the growth curve (p=0.644; 0-17 days; **figure 1.**), while a significant effect (p=0.026) on growth was observed in the second, exponential part.

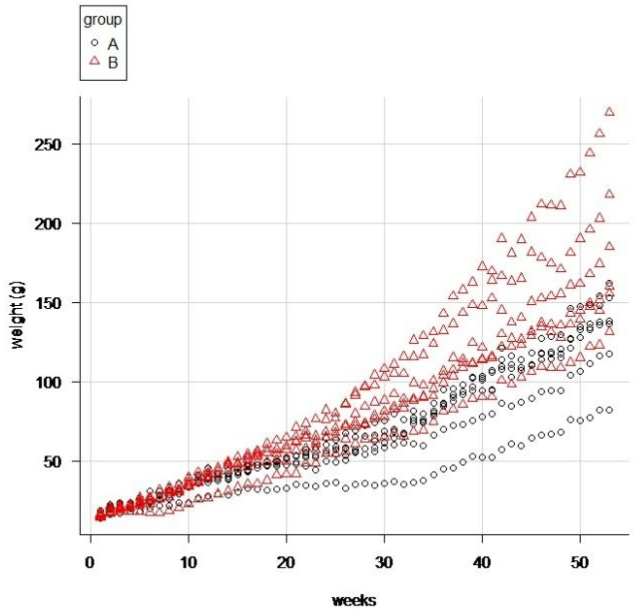


**Figure 1:** The growth curves of tortoises in group A and B

- The shell of the animals in group A was firm and healthy, while in group B all of the tortoises had weakened shells, as one of the symptoms of developing MBD. Both the carapace and the plastron were affected in 3 animals (B/1,4,5) and only the plastron of 3 (B/2,3,6) other tortoises. X-ray examinations did not show differences between the groups. Symptoms were not serious and were detected at 9-10 months of age.
- Animals were significantly more active ($p=0.038$) in the afternoons, but the difference between the two groups was not significant.

The main conclusions are the following

- For testing dietary supplements studies of longer duration (10-12 month) are recommended.
- According to this study daily application of a product containing high amount of vitamin $D_3$ and Ca is recommended. The product used in this study with 50 000 IU $D_3$/kg and 150 g/kg Ca – with the dosage of 1,5g powder/100 g fresh food − is adequate to avoid MBD in tortoises.

## 6.2. EFFECTS OF DIFFERENT DIETARY MINERAL AND VITAMIN SUPPLEMENTS ON SELECTED BLOOD PARAMETERS AND GROWTH OF BEARDED DRAGONS

### 6.2.1. Materials and methods

- Housing: one month old bearded dragons (n=36, 31 male, 5 females); housed individually in 790x70x420 mm size transparent plastic terrariums with paper bedding material. UVB bulbs were placed individually (26 W, 30-42 μW/cm$^2$).
- Two different commercial dietary supplements with or without UVB exposure (**table 5.**).

**Table 5.** The different treatments in the study

| | Calcium (g/100 g DM of diet) | Vitamin $D_3$ (IU/100 g DM of diet) | UVB exposure |
|---|---|---|---|
| $LCa_1HD_3$+noUV | 2.7 | 897.5 | − |
| $LCa_1HD_3$+UV | 2.7 | 897.5 | 12h/day |
| $LCa_1LD_3$+noUV | 2.7 | 35.9 | − |
| $LCa_1LD_3$+UV | 2.7 | 35.9 | 12h/day |
| $HCa_1$+UV | 5.4 | − | 12h/day |
| $HCa_2$+UV | 5.4 | − | 12h/day |

$LCa_1HD_3$+noUV − low Ca (Ca-carbonate/phosphate) + high dosage po vitamin $D_3$ without UVB light; $LCa_1HD_3$+UV − low Ca (Ca-carbonate/phosphate) + high dosage po vitamin $D_3$ + UVB light; $LCa_1LD_3$+noUV− low Ca (Ca-carbonate/phosphate) + low dosage po vitamin $D_3$ without UVB light; $LCa_1LD_3$+UV− low Ca (Ca-carbonate/phosphate) + low dosage po vitamin $D_3$ + UVB light; $HCa_1$+UV− high Ca (Ca-carbonate/phosphate) + UVB light; $HCa_2$+UV − high Ca (Ca-gluconate) + UVB light

- Feeding: five times a week, in the morning with foods of plant origin (1/3 part iceberg lettuce, 1/3 part cucumber + 1/3 part apple and carrot), and in the early afternoon with foods of animal origin. The latter was provided according to the age of lizards. Until the age of 5 month lizards received foods of animal origin contained lesser mealworm larvae *(Alphitobius diaperinus)*, after that mealworms *(Tenebrio molitor)*. House crickets *(Acheta domesticus)* were also offered until the age of 10 month, after that lizards consumed black crickets *(Gryllus bimaculatus)*.
- BW and body length (BL) were measured weekly for 16 months. Blood samples were taken from 3 animals in each group (n=18) seven times, once at 7, 9, 11, 13, 15 and twice at 16 months of age (with 2 weeks difference). In order to examine, whether the feeding before the blood sample taking affects the blood parameters (especially uric acid), before the second (9$^{th}$ month), fourth (13$^{th}$ month) and fifth (15$^{th}$ month) sampling animals were fasted for 24 hours.

- Data analysis. BW and BL of the groups were compared with ANOVA test. Reference Value Advisor v2 was used to calculate reference values of the measured blood parameters for the clinical practice. Effects of treatments were analysed with nonparametric methods.

### 6.2.2. Main results and conclusions

- None of the lizards showed symptoms of MBD. The BWs and BLs of the animals did not differ significantly neither at the beginning nor at the end of the study (**table 6**).

**Table 6**. The effects of treatments on the initial and final BW (g) and BL (mm) of the animals

| | $LCa_1HD_3$ noUV | $LCa_1HD_3$ +UV | $LCa_1LD_3$+ noUV | $LCa_1HD_3$+ UV | $HCa_1$+ UV | $HCa_2$+ UV | p value |
|---|---|---|---|---|---|---|---|
| I BW | 6.8±1.6 | 7.0±1.3 | 6.5±1.6 | 7.4±0.9 | 6.3±1.9 | 6.5±1.5 | 0.833 |
| F BW | 293.2±59.4 | 234.2±34.0 | 303.4±46.8 | 294.6±47.9 | 316.7±75.9 | 303.2±56.8 | 0.357 |
| I BL | 52.5±5.2 | 53.8±4.9 | 52.8±7.2 | 55.8±3.8 | 52.8±7.2 | 55.8±3.8 | 0.914 |
| F BL | 183.0±8.1 | 169,3±10.0 | 182.7±12.0 | 179.6±16.0 | 187.6±16.4 | 182.7±11.6 | 0.409 |

I=initial, F=final,
$LCa_1HD_3$+noUV − low Ca (Ca-carbonate/phosphate) + high dosage po vitamin $D_3$ without UVB light; $LCa_1HD_3$+UV − low Ca (Ca-carbonate/phosphate) + high dosage po vitamin $D_3$ + UVB light; $LCa_1LD_3$+noUV− low Ca (Ca-carbonate/phosphate) + low dosage po vitamin $D_3$ without UVB light; $LCa_1LD_3$+UV− low Ca (Ca-carbonate/phosphate) + low dosage po vitamin $D_3$ + UVB light; $HCa_1$+UV− high Ca (Ca-carbonate/phosphate) + UVB light; $HCa_2$+UV − high Ca (Ca-gluconate) + UVB light

- The effect of age seems to be clinically relevant in total protein (**table 7**.)

**Table 7.** Effect of age (young: ≤ 13 month old or adult: > 13 month old) on blood parameters

| Parameters | Total | Young (≤ 13 month old) | Adult (> 13 month old) | p value |
|---|---|---|---|---|
| Ca (mmol/l) | 1,7-3,3 | 2.2 - 3.5 | 1.4 - 4.1 | <0.0001 |
| $Ca^{2+}$ (mmol/l) | 0,8 - 1,4 | 0.9 - 1.4 | 0.6 - 1.5 | 0.9201 |
| Uric acid (µmol/l) | 78,9-952,0 | 86.4 - 724 | 51.1 - 1412 | 0.012 |
| Uric acid (µmol/l) [1] | 51,1-700,5 | 87.1 - 910 | 39.8 - 588 | |
| Uric acid (µmol/l) [2] | 136,9 - 1292,1 | 126 - 709 | 101- 1469 | |
| Total protein (g/l) | 40,2 - 99,5 | 38.2 - 93.5 | 49.5 - 116 | 0.0016 |
| ALT (U/l) | 1,0 - 34,8 | 0.9 - 18.6 | 0.9 - 63.1 | 0.0002 |
| $Na^+$ (mmol/l) | 143,9 -184,2 | 143 - 176 | 138 - 186 | 0.0004 |
| $K^+$ (mmol/l) | 1,2 - 6,3 | 0.9 - 6.7 | 1.8 - 6.2 | 0.3198 |

[1]after one day of fasting; [2]without fasting

- Data summarised in **table 8** can also be used in the clinical praxis as reference values valid for healthy bearded dragons. The 24-h-long fasting before the sample takings ($2^{nd}$, $4^{th}$, and $5^{th}$) resulted significantly lower uric acid values.
- The 12-h/d-long use of UVB light significantly enhanced the plasma total Ca and $Ca^{2+}$ concentrations.

The main conclusions are the following:

- All of the treatments seem to be adequate for growing bearded dragons.
- Use of 12 h/day UVB exposure enhances both the Ca and $Ca^{2+}$ levels of the blood not only in case of oral vitamin $D_3$ supplementations but also in case of its absence. Blood uric acid concentration is significantly influenced by the fact whether animals consumed food before blood taking or not.
- To get more standard and comparable values for this parameter, we suggest taking blood samples after the 24-hours-fasting. The effect of age seems to be clinically relevant in total protein, which increases with the age. As none of our experimental lizards showed symptoms of vitamin $D_3$ overdose or symptoms of MBD in connection with Ca and vitamin $D_3$ deficiency, data in Table 4 can be used in the clinical practice as blood reference values for healthy bearded dragons.

**Table 8**. Ranges and mean values with standard deviations of the blood parameters according to the treatments

| | | $LCa_1HD_3$+noUV | $LCa_1HD_3$+UV | $LCa_1LD_3$+noUV | $LCa_1LD_3$+UV | $HCa_1$+UV | $HCa_2$+UV | Total | p value[1] | p value[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| Ca (mmol/l) | m±sd | 2.4 ± 0.3 | 2.5 ± 0.4 | 2.3 ± 0.5 | 2.6 ± 0.4 | 2.6 ± 0.3 | 2.9 ± 0.6 | 2.6 ± 0.5 | 0.0489 | 0.0082 |
| | range | 2.0 - 3.0 | 1.7 - 3.1 | 1.4 - 3.1 | 1.7 - 3.2 | 1.9 - 3.0 | 1.9 - 4.5 | 1.4 - 4.5 | | |
| $Ca^{2+}$ (mmol/l) | m±sd | 1.2 ± 0.2 | 1.2 ± 0.1 | 1.1 ± 0.2 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.2 ± 0.2 | 0.0222 | 0.4297 |
| | range | 0.8 - 1.4 | 1.0 - 1.5 | 0.5 - 1.4 | 1.1 - 1.5 | 1.1 - 1.4 | 0.9 - 1.4 | 0.5 - 1.5 | | |
| Uric acid (µmol/l) | m±sd | 334 ± 233 | 406 ± 220 | 229 ± 124 | 457.1 ± 351.2 | 400 ± 259 | 330 ± 176 | 358 ± 243 | 0.0037 | <0.0001 |
| | range | 89.1 - 979 | 82.2 - 800 | 51.1 - 453 | 115 - 1569 | 119 - 1087 | 142 - 757 | 51,1 - 1469 | | |
| TP (g/l) | m±sd | 74.2 ± 19.2 | 66.1 ± 15.5 | 58.6 ± 11.0 | 66.5 ± 11.5 | 59.5 ± 11.8 | 71.5 ± 12.5 | 66.2 ± 14.9 | 0.4774 | 0.9063 |
| | range | 39.5 - 20.6 | 41.3 - 96.1 | 37.4 - 78.5 | 42.4 - 89.2 | 40.5 - 83 | 49.8 - 93.9 | 37.4 - 120 | | |
| ALT (U/l) | m±sd | - | - | - | - | - | - | - | 0.3345 | 0.0422 |
| | range | <1- 83 | <1-13 | <1-80 | <1-13 | <1-71 | <1-24 | <1-83 | | |
| $Na^+$ (mmol/l) | m±sd | 160 ± 6.1 | 162 ± 5.9 | 163.2 ± 10.4 | 160.5 ± 8.3 | 159 ± 9.9 | 160.3 ± 6.7 | 161 ± 8.0 | 0.7325 | 0.1582 |
| | range | 144 - 173 | 151 - 178 | 143 - 187 | 148 - 185 | 133 - 180 | 148 - 174 | 133 - 187 | | |
| $K^+$ (mmol/l) | m±sd | 4.1 ± 1.0 | 4.6 ± 0.9 | 3.3 ± 0.8 | 3.9 ± 0.9 | 3.8 ± 0.9 | 3.3 ± 1.1 | 3.9 ± 1.1 | 0.1410 | 0.0001 |
| | range | 2.4 - 7.1 | 2.5 - 6.3 | 1.6 - 4.7 | 1.2 - 5.1 | 0.8 - 5.3 | 1.0 - 4.8 | 0.8 - 7.1 | | |

TP − total protein; ALT − alanin-aminotransferase;

m±sd=mean±sd;

$LCa_1HD_3$+noUV − low Ca (Ca-carbonate/phosphate) + high dosage po vitamin $D_3$ without UVB light; $LCa_1HD_3$+UV − low Ca (Ca-carbonate/phosphate) + high dosage po vitamin $D_3$ + UVB light; $LCa_1LD_3$+noUV − low Ca (Ca-carbonate/phosphate) + low dosage po vitamin $D_3$ without UVB light; $LCa_1LD_3$+UV − low Ca (Ca-carbonate/phosphate) + low dosage po vitamin $D_3$ + UVB light; $HCa_1$+UV − high Ca (Ca-carbonate/phosphate) + UVB light; $HCa_2$+UV − high Ca (Ca-gluconate) + UVB light; TP − total protein

[1] p value for UVB exposure; [2]p value for fasting

## 6. 3. VARIATIONS IN BLOOD BIOCHEMICAL VALUES OF MALE HERMANN'S TORTOISES

### 6.3.1. Materials and methods

- 2.5 year-old male Hermann's tortoises (n=12). Feeding and housing was similar to the conditions in chapter 6.1. Animals received product "A" as a dietary supplement.
- Blood taking: samples were collected 5 times every two months − beginning in February and ending in October. Due to technical problems, we could not measure AST on the second sample collection.
- Data analysis: general linear mixed models.

### 6.3.2. Main results and conclusions

- **Table 9** shows the ranges and mean values with standard deviations by each sample taking (n=12/sample). As it can be seen, except AST (p=0.153), sampling time had significant effect on the parameters (p<0.001). **Table 10**. shows the ranges, confidence intervals of the means (95%) and mean values (including standard deviation) of the examined parameters of the 12 animals (n=60/parameter).

**Table 9.** Ranges and mean values with standard deviations (SD) of blood plasma samples at the five sample takings (n = 12/sample taking)

| | p | | s1 | s2 | s3 | s4 | s5 |
|---|---|---|---|---|---|---|---|
| Ca (mmol/l) | <0.001 | range | 2.1-2.5 | 2.2-2.8 | 2.2-2.5 | 1.6-2.4 | 2.3-2.8 |
| | | m±sd | 2.3±0.1 | 2.6±0.2 | 2.4±0.1 | 1.9±0.2 | 2.5±0.2 |
| $Ca^{2+}$ (mmol/l) | <0.001 | range | 1.0-1.5 | 1.4-1.9 | 1.5-1.8 | 0.5-1.3 | 1.2-1.6 |
| | | m±sd | 1.3±0.1 | 1.7±0.1 | 1.7±0.1 | 0.7±0.3 | 1.4±0.1 |
| P (mmol/l) | <0.001 | range | 0.9-1.5 | 0.5-0.8 | 0.9-1.4 | 1.0-2.0 | 1.0-1.7 |
| | | m±sd | 1.2±0.2 | 0.7±0.1 | 1.1±0.2 | 1.3±0.3 | 1.3±0.2 |
| Ua (µmol/l) | <0.001 | range | 94.0-266.0 | 27.9-200.6 | 4.2-151.2 | 71.7-256.8 | 42.2-156.7 |
| | | m±sd | 164.3±47.8 | 87.6±46.7 | 92.4±41.3 | 168.6±48.3 | 92.8±32.0 |
| AST (IU/l) | 0.154 | range | 55.0-133.0 | - | 35.0-141.0 | 35.0-140.0 | 51.0-280.0 |
| | | m±sd | 87.2±23.3 | - | 61.2±30.2 | 66.3±30.5 | 92.5±68.5 |
| $Na^+$ (mmol/l) | <0.001 | range | 122.0-131.0 | 122.0-130.0 | 126.0-137.0 | 125.0-138.0 | 123.0-132.0 |
| | | m±sd | 125.6±2.5 | 125.3±2.6 | 132.2±3.2 | 129.7±3.5 | 125.8±3.0 |
| $K^+$ (mmol/l) | <0.001 | range | 3.5-4.6 | 3.9-5.4 | 3.9-5.2 | 4.5-6.0 | 3.9-4.9 |
| | | m±sd | 3.9±0.4 | 4.7±0.5 | 4.4±0.4 | 5.2±0.4 | 4.3±0.3 |

m±sd=mean±sd; s=sample taking, Ua=uric acid

**Table 10.** Ranges, the 95% confidence intervals of the means and mean values with standard deviations (SD) of the blood plasma parameters (n = 60/parameter)

| Blood parameters | Range | CI mean | Mean and SD |
|---|---|---|---|
| Calcium (mmol/l) | 1.6-2.8 | 2.3-2.4 | 2.3±0.3 |
| Ionised calcium (mmol/l) | 0.5-1.9 | 1.2-1.4 | 1.3±0.4 |
| Phosphorus (mmol/l) | 0.5-2.0 | 1.1-1.2 | 1.1±0.3 |
| Sodium (mmol/l) | 122.0-138.0 | 126.7-128.8 | 127.7±4.0 |
| Potassium (mmol/l) | 3.5-6.0 | 4.4-4.7 | 4.5±0.6 |
| Ca:P | 0.9-5.4 | - | 2.3±0.9 |
| $Ca^{2+}$:P | 0.3-3.6 | - | 1.4±0.8 |
| CaxP | 1.2-4.2 | - | 2.6±0.7 |
| $Ca^{2+}$xP | 0.5-2.3 | - | 1.4±0.4 |
| Uric acid (μmol/l) | 4.2-266.0 | 105.8-135.4 | 121.1±56.3 |
| AST (IU/l) | 30.0-280.0 | 63.8-89.3 | 76.8±42.8 |

CI=confidence interval, Ca:P= total calcium to phosphorus ratio, $Ca^{2+}$:P= ionised calcium to phosphorus ratio, CaxP=solubility index, $Ca^{2+}$xP=solubility index

The main conclusions are the following:

- As the indoor housing is common among hobby keepers (according to our own data collection), blood reference values provided by this article can be used by veterinarians in the clinical practice.
- Because the time of the sample taking had significant effect on most of the blood parameters for the proper diagnosis repeated sample takings are advised especially if data are in the margin of reference intervals.

## 6.3. EFFECTS OF ORAL CHOLECALCIFEROL SUPPLEMENTATION VS. UVB EXPOSURE ON PLASMA CONCENTRATION OF 25(OH)D AND CA IN BEARDED DRAGONS AND HERMANN'S TORTOISES

### 6.3.1. Materials and methods

- Housing: conditions are similar to that of the terrariums in chapters 6.1. and 6.2. The 5-year-old animals of both species were randomly divided into two groups (UV and non-UV; $n_{Pogona}$=5/group, $n_{Testudo}$=6/group, **table 11**.). Dietary supplement (containing 32 600 IU cholecalciferol/kg DM and 350 g/kg DM Ca) was given to non-UV groups by every feeding according to the label information (0.6 g supplement/100 g fresh plant +dusting the live prey; Table 1.). UVB exposure for UV groups was provided for 12 h/day (33.5±5.5μW/cm$^2$ in agamas; 15.5±2.6 μW/cm$^2$ in tortoises).

**Table 11.** Experimental setup and Ca/Vitamin $D_3$ content of the diet

| | non-UV group | | UV group | |
|---|---|---|---|---|
| | tortoise | bearded dragon | tortoise | bearded dragon |
| Ca (g/100 g food DM) | 3.2 | 6.7 | 3.2 | 6.7 |
| Vitamin $D_3$ IU/100 g food DM) | 146 | 650 | – | – |
| UVB exposure | – | – | 12 h/day | 12 h/day |

- Feeding: Bearded dragons were fed five times a week, in the morning with foods of plant origin (1/3 part dandelion or rucola, 1/3 part cucumber + 1/3 part apple and carrot), and in the early afternoon with foods of animal origin. Foods of animal origin contained mealworms *(Tenebrio molitor)* and black crickets *(Gryllus bimaculatus)*. None of the tortoises had ever received protein of animal origin; diet was based on garden weeds (80% dandelion, chickweed and plantain, cloverleaf) and additionally (20%) filed vegetables (carrot and cucumber). In winter garden weeds were replaced with iceberg lettuce and rucola. Dietary supplements were given by every feeding.
- Blood taking: Blood samples were taken 4 times with 12 weeks intervals, beginning in June and ending in February. The bearded dragons fasted for 24 hours prior to the sampling.
- Data analysis: general linear mixed models. Numerical 25(OH)D data were available only at the 2$^{nd}$ measurements of each individual; therefore Mann-Whitney-Wilcoxon test was applied to compare these measurements between UV and non-UV groups.

### 6.4.2. Main results and conclusions

- All of the animals were clinically healthy, physically active and showed no signs of MBD. **Table 12** shows the blood concentrations of Ca and 25(OH)D in tortoises. The descriptive statistics of the other biochemical parameters can be seen in **table 13**.

**Table 12.** Effects of treatments on the blood Ca and 25(OH)D concentrations of the tortoises, n=48/Ca, n=12/25(OH)D

| | Ca mmol/l | $p_{UV}$ | $p_{sample1-4}$ | 25(OH)D nmol/l | $p_{UV}$ | $p_{sample1-4}$ |
|---|---|---|---|---|---|---|
| | non-UV group | 0.0617 | 0.6023 | non-UV group | p=0.0081† | - |
| avg±sd* | 2.5±0.2 | | | 9.0±11.4 | | |
| range | 2.1-2.8 | | | 5.0-12.0 | | |
| | UV group | | | UV group | | |
| avg±sd | 2.8±0.4 | | | 23.2±7.1 | | |
| range | 2.3-3.7 | | | 11.0-33.0 | | |

*avg±sd= average±standard deviation
†2nd sample

**Table 13.** Descriptive statistics of the biochemical parameters of tortoises, n=48/parameter

| | Uric acid μmol/l | Urea mmol/l | Creatinin μmol/l | $Na^+$ mmol/l | $K^+$ mmol/l |
|---|---|---|---|---|---|
| avg±sd | 248±74.8 | 0.9±0.8 | 10.2±10.5 | 125±3.1 | 4.6±0.8 |
| range | 125-375 | 0.1-4.4 | 0.4-41.3 | 119-136 | 3.4-6.6 |
| | P mmol/l | Ca mmol/l | ALP IU/l | AST IU/l | Albumin g/l |
| avg±sd | 0.8±0.3 | 2.6±0.3 | 748±157 | 65.4±44.7 | 16.1±3.6 |
| range | 0.5-2.4 | 2.1-3.7 | 524-1120 | 22.0-237 | 8.6-26 |
| | Blood glucose mmol/l | Triglyceride mmol/l | LDH IU/l | Globulin g/l | Total protein g/l |
| avg±sd | 3.7±0.9 | 0.7±0.9 | 206±73,1 | 13.5±3.2 | 29.4±5.5 |
| range | 1.2-6.2 | 0.1-4.9 | 93-426 | 7.4-23.1 | 16.0-40.0 |

avg±sd= average±standard deviation

- **Tables 14.** shows the blood concentrations of Ca and 25(OH)D in bearded dragons. The descriptive statistics of the other biochemical parameters can be seen in **table 15**.

**Table 14.** Effects of the treatments on the blood Ca and 25(OH)D concentrations of the bearded dragons, n=40/parameter

| | Ca mmol/l | $p_{UV}$ | $p_{sample\ 1-4}$ | 25(OH)D nmol/l | $p_{UV}$ | $p_{sample\ 1-4}$ |
|---|---|---|---|---|---|---|
| | non-UV group | 0.2842 | 0.6166 | non-UV group | 0.8218 | 0.9144 |
| avg±sd* | 2.9±0.4 | | | 253±75.3 | | |
| range | 2.5-4.2 | | | 145-380 | | |
| | UV group | | | UV group | | |
| avg±sd | 2.8±0.3 | | | 264±74.5 | | |
| range | 2.4-3.8 | | | 106-350 | | |

*avg±sd= average±standard deviation

**Table 15.** Descriptive statistics of the biochemical parameters of bearded dragons n=40/parameter

| | Uric acid µmol/l | Urea mmol/l | Creatinin µmol/l | $Na^+$ mmol/l | $K^+$ mmol/l |
|---|---|---|---|---|---|
| avg±sd | 372±189 | 0.4±0.2 | 24.5±21.5 | 154.7±4.6 | 3.6±1.1 |
| range | 112-853 | 0.1-1.3 | 0.9-69.2 | 145-164 | 1.2-5.7 |
| | P mmol/l | Ca mmol/l | ALKP IU/l | AST IU/l | LDH IU/l |
| avg±sd | 1.4±0.4 | 2.9±0.4 | 265±116 | 30.3±38.6 | 438±489 |
| range | 0.8-2.9 | 2.4-4.2 | 129-587 | >1-173 | 54-2071 |
| | Blood glucose mmol/l | Triglyceride mmol/l | Albumin g/l | Globulin g/l | Total protein g/l |
| avg±sd | 9.7±1.5 | 2.7±2.2 | 22.7±3.9 | 30.1±6.5 | 52.3±8.9 |
| range | 6.8-14.6 | 0.3-9.4 | 14.7-31.5 | 17.8-46.3 | 33.2-72.2 |

avg±sd = average±standard deviation

The main conclusions are the following:

- The low plasma concentration of calcidiol and high level of ALP in tortoises suggests that they may have higher demand of UVB and oral cholecalciferol supplementation. However in the 2$^{nd}$ sampling calcidiol was higher in the UV-group, conclusions cannot be made from such limited number of data. Consequently neither that level of UVB exposure nor that amount of dietary supplement is adequate for herbivore tortoises. They require stronger UVB exposure or higher amount of dietary supplement (such as product "A" from chapter 6.1.).
- According to our results of the study, oral cholecalciferol supplementation and UVB exposure are equally adequate for adult bearded dragons to prevent MBD. We recommend a product which contains approximately 30 000 IU/kg cholecalciferol or 12 h/day UVB exposure (33.5±5.5μW/cm$^2$).

## 6.5. INVESTIGATIONS ON UVB LAMPS

### 6.5.1. Materials and methods

- The level of UVB irradiation and its changes with length of application and distance was measured by using 9 different products of 3 well known and accessible companies (**table 16**). It was a complementary study for chapter 6.2. and 6.4. Bulbs dedicated to "tropical" and "desert" species were used.

**Table 16.** The bulbs used in the study

| Company | category | output | identifier |
|---|---|---|---|
| A | "desert" | 23W | s1 |
| B | | 25W | s2 |
| B | | 25W | s3 |
| B | | 13W | s4 |
| C | | 26W | s5 |
| B | | 125 W | s6 |
| A | "tropical" | 23W | t1 |
| B | | 25W | t2 |
| C | | 26W | t3 |

- Bulbs were placed into 790x570x420 mm size transparent plastic terrariums which were covered with black plastic foil. UVB irradiation was measured 7 times with 7 weeks intervals − altogether for 49 weeks – in 100, 200, 300 and 400 mm distance from the surface of the bulb. Three measurements were made in each distance.
- Data analysis: ANOVA (data from 20 cm).

### 6.5.2. Main results and conclusions

- The level of irradiations can be seen in **table 17**. The final data in the distance of 30 cm were 6-8 μW/cm$^2$, in 40 cm it was lower with 3-4 μW/cm$^2$ in case of 4 „desert" bulbs (d1, d2, d3, d6). In the other bulbs (d4, d5, t1, t2, t3) no irradiation was measured. With two exceptions (d6-d2, t1-t2) significant differences were found even at the very beginning of the study.

**Table 17.** The initial and final levels of UVB irradiations in the different distances from the surface of the (µW/cm²)

| B | C | 10 cm | | 20 cm | | 30 cm | | 40 cm | | $p_{20cm\ initial\ data}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | initial | final | initial | final | initial | final | initial | final | |
| s1 | A | 200 | 44 | 68 | 18 | 30 | 6 | 16 | 3 | s1-s2=0,0065<br>s1-s6=0.0090<br>s2-s6=0,9999<br>others<0,001 |
| s2 | B | 220 | 61 | 75 | 24 | 35 | 8 | 22 | 4 | |
| s3 | B | 160 | 61 | 50 | 18 | 24 | 7 | 14 | 3 | |
| s4 | B | 32 | 17 | 13 | 4 | 7 | 2 | 4 | 0 | |
| s5 | C | 85 | 25 | 28 | 7 | 13 | 4 | 6 | 1 | |
| s6 | B | 130 | 35 | 75 | 20 | 45 | 8 | 25 | 4 | |
| t1 | A | 120 | 30 | 40 | 10 | 17 | 3 | 12 | 1 | t1-2=0,932<br>others<0,001 |
| t2 | B | 130 | 37 | 40 | 10 | 16 | 4 | 10 | 0,5 | |
| t3 | C | 60 | 20 | 16 | 4 | 7 | 2 | 5 | 0 | |

B=bulb; C=company; *significant difference; d="desert" bulb, t="tropical" bulb

- Independently from the type of bulb and distance of measurement it is clearly seen that the level of irradiation decreased dramatically right after putting them into operation (2nd and 3rd measurement). This meant a 30-35%, than a 50% reduction, later it was stabilized at around 70% of the initial level of irradiation.

The main conclusions are the following:

- As products with similar outputs may have different levels of irradiation, it is recommended to regularly check the irradiation of the bulbs.
- The output of the 13W bulb was very low even at the beginning; in itself it seems to be less adequate for desert species. Such product may be used in combination with dietary vitamin D supplement.

## 6.6. INVESTIGATIONS ON THE NUTRITIONAL COMPOSITION OF REPTILE FOODS

### 6.6.1. Materials and methods

- Madagascar hissing cockroach (adult, *Gromphadorhina portentosa)*, dubia cockroach (adult, *Blaptica dubia)*, rusty red cockroach (juvenile, *Blatta lateralis)* and two cricket species. African field cricket (adult and juvenile, *Gryllus bimaculatus*) and Jamaica field cricket (adult and juvenile, *Gryllus assimilis)* were analysed. Cockroaches were obtained from a commercial supplier. One part of both cricket species was obtained from a commercial supplier and the other part was raised and bred at the department. Crickets from our breed were fed with chicken starter diet ad libitum. All individuals of the cockroach species and one group of the cricket species (own raised or commercial adult and juvenile) were fasted for 24 hours to clear their gastrointestinal track of any residual food. Whole frozen and dried fish, dried Baltic prawn (*Palaemon adspersus)*, dried freshwater crab *(Gammarus roeseli)*, commercial turtle pellet and lyophilized beef heart were bought in pet shop.

- Dandelion leaves (*Taraxacum officinale*), great plantain (*Plantago major*), chickweed (Stellaria media) and tall meadow oat (*Arrhenatherum elatius*) was collected in the nature. While corn salad (*Valerianella locusta*), rucola (*Eruca sativa)* and turnip top (*Daucus carota* subsp. *sativus*) was bought.
- The samples were homogenized and then dried at 50°C during 48 hours and standard methods were used for the analysis
- Data analysis: ANOVA.

### 6.6.2. Main results and conclusions

- Nutrient composition and mineral content of the invertebrates can be seen in **table 18** and **19.** Fasting cricket had significantly lower calcium level (p=0.038; 1.7 vs. 1.3 g/kg DM). Age, origin and species did not have effect on that parameter.

**Table 18.** Nutrient composition of the cricket and cockroach species on DM basis

| | ether extract g/kg | crude protein g/kg |
|---|---|---|
| Madagascar hissing cockroach, adult | 186 | 655 |
| dubia cockroach, adult | 225 | 621 |
| rusty red cockroach, nymph | 211 | 659 |
| adult, fasted[a] | 185 | 633 |
| nymph, fasted[a] | 252 | 601 |
| adult[b] | 245 | 672 |
| nymph[b] | 115 | 689 |
| adult, fasted[b] | 258 | 721 |
| nymph, fasted[b] | 304 | 631 |
| adult, fasted[a] | 209 | 649 |
| nymph fasted[a] | 293 | 689 |
| adult[b] | 132 | 726 |
| nymph[b] | 305 | 786 |
| adult, fasted[b] | 284 | 616 |
| nymph fasted[b] | 276 | 621 |

a = commercial; b = own raised

**Table 19.** Mineral content of the cricket and cockroach species on DM basis

| | Ca g/kg | P g/kg | Ca:P | Cu mg/kg | Mn mg/kg | Zn mg/kg | Mg mg/kg | Fe mg/kg |
|---|---|---|---|---|---|---|---|---|
| Madagascar hissing cockroach, adult | 1.3 | 6.8 | 1:5.2 | 176.2 | 96.3 | 693 | 2360 | 80.7 |
| dubia cockroach, adult | 1.3 | 3.1 | 1:2.4 | 30.1 | 106.1 | 327 | 1428 | 91.1 |
| rusty red cockroach, nymph | 2.5 | 6.1 | 1:2.4 | 56.9 | 1851 | 173 | 1168 | 120.2 |
| | | | | black cricket | | | | |
| adult, fasted[a] | 1.8 | 8.7 | 1:4.9 | 22.1 | 620.2 | 239 | 1155 | 88.3 |
| nymph, fasted[a] | 0.8 | 8.7 | 1:9.9 | 29.3 | 405.3 | 256 | 1142 | 77.5 |
| adult[b] | 1.7 | 8.3 | 1:4.9 | 24.3 | 501.2 | 244 | 1053 | 94.2 |
| nymph[b] | 2.1 | 8.5 | 1:4.1 | 27.7 | 934.1 | 238 | 1289 | 112.3 |
| adult, fasted[b] | 1.5 | 8.6 | 1:5.9 | 29.9 | 52.4 | 193 | 970 | 87.6 |
| nymph, fasted[b] | 1.3 | 9.1 | 1:6.8 | 22.5 | 42.9 | 218 | 915 | 89.1 |
| | | | | banana cricket | | | | |
| adult, fasted[a] | 1.1 | 8.9 | 1:8.8 | 23.2 | 474.3 | 247 | 982 | 73.7 |
| nymph, fasted[a] | 0.7 | 7.5 | 1:9.8 | 22.2 | 412.2 | 238 | 917 | 75.9 |
| adult[b] | 1.5 | 10.1 | 1:6.7 | 23.5 | 58.6 | 230 | 1028 | 87.4 |
| nymph[b] | 1.8 | 9.4 | 1:5.2 | 20.4 | 54.7 | 202 | 969 | 88.1 |
| adult, fasted[b] | 1.4 | 8.7 | 1:6.0 | 13.8 | 58.5 | 176 | 972 | 75.6 |
| nymph, fasted[b] | 1.6 | 9.2 | 1:5.8 | 20.4 | 49.3 | 214 | 905 | 87.8 |

a = commercial; b = own raised

- Madagascar hissing cockroach has extremely high Cu content compared to other species. Cricket species had significantly lower magnesium (p=0.003; 1652 vs. 1025 mg/kg DM), copper (p=0.006; 23.3 vs. 87.6 mg/kg DM) and zinc content (p=0.026; 225.2 vs. 398.2 mg/kg DM) than the cockroaches.
- **Table 20.** shows the nutrient composition of the turtle foods. Crabs are good calcium sources, similar to fish.

**Table 20.** Nutrient composition and mineral content of the turtle feeds on DM bases

| | Ca g/kg | P g/kg | Ca:P | ether extract g/kg | crude protein g/kg |
|---|---|---|---|---|---|
| frozen whole fish | 51.3 | 32.8 | 1:0.6 | 129 | 677 |
| dried whole fish | 44.8 | 30.1 | 1:0.7 | 174 | 707 |
| dried Baltic prawn | 39.1 | 13.0 | 1:0.3 | 23.7 | 707 |
| dried freshwater crab | 59.8 | 8.5 | 1:0.1 | 52.5 | 494 |
| commercial turtle pellet | 2.7 | 4.1 | 1:1.5 | 4.9 | 273 |
| lyophilized beef heart | 24.9 | 19.8 | 1:0.8 | 100 | 647 |

- The Ca content of the dried shrimps were significantly higher (in average 49.3 vs. 1.5 g/kg, p<0.001) than that of the cricket and cockroach species, while the ether extract was much lower (3.7 vs. 23.3%, p<0.001). According to this aquatic invertebrates are much better sources of Ca than terrestrial ones. The latter contain less P (10.7 vs. 7.8 g/kg, p=0.0015). The Ca content of the whole fish and the shrimps are similar (p=0.993), but the former contains higher amount of ether extract and P (p<0.001). The commercial pellet had the lowest Ca, ether extract and crude protein content among the turtle foods (p<0.001).

- Nutritional values of the plant origin foods can be seen in table 21. The Ca content of tall meadow oat and great plantain differ significantly from other plants (p<0.001). The P, ether extract, crude protein and crude fibre levels are very similar.

**Table 21.** The nutritional values of plant origin foods on DM bases

| | Ca g/kg | P g/kg | Ca:P | ether extract g/kg | crude protein g/kg | crude fibre g/kg |
|---|---|---|---|---|---|---|
| dandelion leaf | 18.4 | 2.4 | 9:1 | 36 | 201 | 109 |
| chickweed | 12.7 | 3.6 | 4:1 | 23 | 206 | 106 |
| tall meadow oat | 4.0 | 4.1 | 1:1 | 46 | 209 | 105 |
| great plantage | 42.3 | 2.2 | 21:1 | 32 | 127 | 109 |
| rucola | 16.5 | 4.1 | 4:1 | 32 | 246 | 105 |
| corn salad | 14.6 | 3.2 | 1,7:1 | 38 | 219 | 101 |
| turnip top | 17.5 | 4.2 | 4.3:1 | 33 | 222 | 102 |

The main conclusions are the following:

- As the nutritional values of cricket and cockroach species do not show major differences – their crude protein, ether extract and mineral contents are similar – all of these can be recommended as foods for omnivore and insectivore reptile species.
- Dried shrimps are good sources of Ca, but care should be taken of vitamin D supply as well.
- Great plantain is highly recommended in summer because of its high Ca content and thick leafs which may help in the wearing of the beaks. The tall meadow oat is the least recommended because of its inadequate Ca:P ratio.

## 6.7. INVESTIGATIONS CONCERNING THE VOLUNTARY DRY MATTER INTAKE, PASSAGE TIME, AND THE NUTRIENTS' DIGESTIBILITY IN HERMANN'S TORTOISES

### 6.7.1. Materials and methods

- Housing: individually in 790x570x420 mm size transparent plastic terrariums without bedding material. Carmine red indicator (60 mg/BW kg) was added to measure gut passage time. Two weeks of preliminary feeding was applied with an extra week of acclimatization between the diets. Daily dry matter intake was measured for 3x4 days in each diet. Faeces was collected individually and stored deep frozen.
- Feeding: the three diets were given *ad libitum* (**table 22**.).
- Data analysis: ANOVA.

**Table 22.** The nutritional composition of the plant origin foods on DM basis

| | DM g/kg | ether extract g/kg | crude fibre g/kg | crude protein g/kg |
|---|---|---|---|---|
| slicing cucumber | 40.6 | 25.8 | 89.6 | 151.5 |
| dandelion leaf | 119.3 | 42.7 | 129.4 | 223.7 |
| lettuce | 55.6 | 32.4 | 113.6 | 203.5 |

### 6.7.2. Main results and conclusions

- The DM intake related to 100 g of BW was between 0.3-1.2% (**table 23**.) and it was the highest in case of lettuce while animals consumed significantly smaller amounts from cucumber and dandelion.

**Table 23.** The DM intake of the tortoises in case of the three different diets

| | | daily DM intake (g) | p daily DM intake | relative daily DM intake (g) | p relative daily DM intake |
|---|---|---|---|---|---|
| slicing cucumber | range | 0.5−2.6 | $p_{cucumber-dandelion}$ =0.732 | 0.4−1.1 | $p_{cucumber-dandelion}$ =0.673 |
| | m±SD | 1.6±0.4 | $p_{pcucumber-lettuce}$<0.001 | 0.7±0.2 | $p_{pcucumber-lettuce}$ =0.002* |
| dandelion leaf | range | 0.5−3.9 | $p_{dandelion-lettuce}$<0.001 | 0.3−0.9 | $p_{dandelion-lettuce}$ <0.001* |
| | m±SD | 1.5±0.6 | | 0.6±0.2 | |
| lettuce | range | 0.8−4.8 | | 0.8−1.2 | |

m±SD=mean±standard deviation, *significant difference

- The gut passage time was significantly faster (2-4 days) while tortoises consumed cucumber ($p<0.001$) compared to lettuce (6-13 days) and dandelion (9-14 days).
- The amount of collected faeces showed wide ranges according to the diet. This meant 0.5-2.0 g DM/day for lettuce; 0.0-1.3 g DM/7days for cucumber and 2.0-2.2 g DM/7 days for dandelion. The majority of the tortoises – independently from the diet – partly or totally consumed the faeces. That is why the investigations concerning the digestibility cannot be made.

The main conclusions are the following:

- Tortoises consume significantly higher amount of lettuce than dandelion or cucumber, this is one of the reasons why excessive feeding of lettuce should be avoided.
- The relative DM intake of the tortoises (0.4-1.2%) is much lower than that of the farm animals (1.5-5%) and the gut passage time is much slower.
- The method of total collection – if the ground is solid – cannot be used in herbivore tortoises.

## 8. New scientific results

- Studies of longer term (<10-12 months) are required for the testing of reptile dietary supplements in order to have adequate results.
- For **growing herbivore tortoises** daily application of dietary supplement containing approximately 50 000 IU/kg cholecalciferol and 150 g/kg Ca is recommended to prevent MBD (1.5 g powder/100 g fresh food). For the **adult** animals none of the methods used in chapter 6.4. are adequate. They require higher amount of dietary supplementation or stronger UVB irradiation.
- In **growing bearded dragons** either we can apply dietary cholecalciferol (~50 000 NE/kg) with Ca (150 g/kg) or Ca exclusively (300 g/kg) with UVB exposure (33,5±5,5 μW/cm$^2$, 12 h/day) to prevent MBD. The parallel application of dietary vitamin D and UVB did not cause health problems. The 12 h/day UVB exposure significantly enhances the blood Ca and $Ca^{2+}$ concentrations. According to the blood calcidiol concentrations in **adult** animals MBD can be prevented by dietary vitamin D (~ 30 000 IU/kg) and Ca (350 g/kg) or with Ca (350 g/kg) and UVB (33,5±5,5 μW/cm$^2$) exposure.

- Blood biochemical parameters described in chapter 6.2. and 6.3. can be used as reference values for healthy animals. To get more standard and comparable values for this parameter, we suggest taking blood samples after the 24-hours-fasting in omnivore or carnivore reptiles. The effect of age seems to be clinically relevant in total protein, which increases with the age.
- As the nutritional values are similar the cockroaches can be alternative preys of cricket species for omnivore or insectivore reptiles. Frequent feeding of hissing cockroach is not recommended because of its high (176.2 mg/kg) copper content. Dried crabs are good Ca sources.
- Great plantain is highly recommended in summer because of its high Ca content and thick leaves which may help in the wearing of the beaks. The tall meadow oat is the least recommended because of its inadequate Ca:P ratio.
- Tortoises consume significantly higher amount of lettuce than dandelion or cucumber, this is one of the reasons why excessive feeding of lettuce should be avoided. The relative DM intake of the tortoises (0.4-1.2%) is much lower than that of the farm animals (1.5-5%) and the gut passage time is much slower.

# 10. Publications

## 10.1. ARTICLES


- Hetényi N., Sátorhelyi T., Hullár I.: Keeping and nutrition of European tortoises. [article in Hungarian]. Review. Magyar Állatorvosok Lapja, 132:4, 223-229, 2010.
- Hetényi N.: Keeping and nutrition of the inland bearded dragon *(Pogona vitticeps)*. [article in Hungarian]. Review. Magyar Állatorvosok Lapja, 135., 72-77, 2013.
- Hetényi N., Andrásofszky E., Berta E., Hullár I.: Nutritional composition of turtle feeds and invertebrates as food for insectivores. [article in Hungarian]. Magyar Állatorvosok Lapja, 135. 467-472, 2013.
- Hetényi N., Sátorhelyi T., Kovács Sz., Hullár I.: Effects of two dietary vitamin and mineral supplements on the growth and health of Hermann's tortoise *(Testudo hermanni)*. Berliner und Münchener Tierärztliche wochenschrift, 127. 251-256, 2014.
- Hetényi N., Sátorhelyi T., Hullár I.: Metabolic bone disease in reptiles. [article in Hungarian] Review. Magyar Állatorvosok Lapja, 2015, 137. 613-623.
- Hetényi N., Sátorhelyi T., Kovács Sz., Hullár I.: Variations in blood biochemical values of male Hermann’s tortoises *(Testudo hermanni)*. Veterinaria (Sarajevo), 65. 15-21., 2016.

- Hetényi N., Andrásofszky E., Hullár I.: Investigations concerning the voluntary dry matter intake, passage time, and the nutrients' digestibility in Hermann's tortoises *(Testudo hermanni)*. [article in Hungarian]. Magyar Állatorvosok Lapja, 138. 307-312, 2016.

## 10.2. CONFERENCES

- CEELA-2009 Triannual Conference (CEELA 2009), Faculty of Veterinary Science, Szent István University, Budapest, 2009.05.23. Poster. The most common environmental and nutritional diseases of tortoises.
- Biannual Conference for researchers and lecturers of nutrition. University of Szeged Faculty of Agriculture, Hódmezővásárhely, Hungary. 2010.09.2-3. Presentation: The most common nutritional diseases of tortoises and bearded dragons.
- XXXIII. Óvár Science Day. Széchenyi István University, Faculty of Agriculture and Food Sciences, Mosonmagyaróvár, Hungary. 2010.10.07. Presentation: Keeping and nutrition of European tortoises.
- Annual conference of the Hungarian Society of Zoo and Wildlife Veterinarians. 2011.03.25-27., Budapest, Hungary. Poster. Growth of Hermann's tortoise *(Testudo hermanni)* fed two different dietary supplementations.
- Biannual Conference for researchers and lecturers of nutrition. University of Pannonia, Georgikon Faculty, Keszthely, Hungary. 2012.08.27-28. Presentation: Feeding of insectivore reptiles.
- XXXIV. Óvár Science Day. Széchenyi István University, Faculty of Agriculture and Food Sciences, Mosonmagyaróvár, Hungary. 2012.10.05. Presentation: Vitamin and mineral supply of Hermann's tortoises.
- Annual conference of the Hungarian Society of Zoo and Wildlife Veterinarians. Budapest, Hungary. 2012.03.30-04.01. Poster. Nutrient composition of some invertebrates used for feeding of reptiles.
- Annual conference of the Hungarian Society of Zoo and Wildlife Veterinarians. Budapest, Hungary. 2012.03.30-04.01. Poster. Effects of different dietary supplements on selected blood parameters and growth of bearded dragons *(Pogona vitticeps)*.
- CEELA-2015 Triannual Conference, Faculty of Veterinary Science, Szent istván University, 2015.11.28., Budapest, Hungary. Poszter. Investigations concerning the voluntary dry matter intake, passage time, and the nutrients' digestibility in Hermann's tortoises *(Testudo hermanni).*

- Biannual Conference for researchers and lecturers of nutrition. 2016.09.08-09., Debrecen, Hungary. University of Debrecen, Institute for Agricultural Research and Educational Farm. Presentation: Metabolic bone disease of reptiles.

- European Society of Veterinary & Comparative Nutrition (ESVCN), 20th Conference. Berlin, Freie Universität, Germany. 2016.09.15-17. Presentation. The effects of oral cholecalciferol supplementation vs. UVB exposure on plasma concentration of 25(OH)D and Ca of bearded dragons *(Pogona vitticeps)* and Hermann's tortoises *(Testudo hermanni).*