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## Influence of environmental humidity and dietary protein on pyramidal growth of carapaces in African spurred tortoises (*Geochelone sulcata*)

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### Summary

The carapaces of captive-raised tortoises (terrestrial chelonians of the zoological family *Testudinidae*, often develop pyramidal-shaped osseous growth centrally within the horny plates. With very few exceptions (e.g. *Geochelone elegans*, *Psammobates* sp.), this conical growth pattern is considered to be pathologic. This very common defect is believed to be an important indicator of the quality of captive tortoise management. This study was designed to examine the effect of dietary protein level and environmental humidity on the degree of pyramidal growth in the carapaces. Fifty recently hatched African spurred tortoises (*G. sulcata*) were raised for 5 months under artificial conditions of varying environmental humidity and dietary protein content (14% vs. 19% vs. 30% crude protein in dry matter). Humps of the carapaces that developed and blood values of calcium, phosphorus and haematocrit were measured and compared among groups. Dry environmental conditions (24.3–57.8% and 30.6–74.8% relative humidity) produced taller humps than humid conditions (45–99% relative humidity). Hump formation differed significantly ( $p \leq 0.001$ ) between these three groups kept under different humidity conditions. Variable dietary protein had a minor, positive impact on this pathological formation of humps (pyramidal growth syndrome, PGS). Analysis of blood (calcium, phosphorus and haematocrit) offered no further explanation as to the development of the humps.

### Zusammenfassung

Bei in Gefangenschaft gehaltenen Schildkröten entwickelt sich häufig im Zentrum der Hornplatten des Rückenpanzers ein pyramidisch geformtes Knochenwachstum. Mit wenigen Ausnahmen (z.B. *Geochelone elegans*, *Psammobates* sp.) wird dieses kegelförmige Wachstum als pathologisch angesehen und es besteht die Annahme, dass dieses Wachstumsverhalten ein Parameter zur Beurteilung der Haltungsbedingungen der Tiere ist. In vorliegendem Versuch sollte der Einfluß der relativen Luftfeuchtigkeit sowie des Rohproteingehaltes im Futter auf die Höckerbildung untersucht werden. Fünfzig Schlüpflinge von afrikanischen Sporenschildkröten (*G. sulcata*) wurden fünf Monate lang unter unterschiedlich feuchten Umweltbedingungen gehalten sowie mit Futtermitteln, welche sich durch ihren Eiweißgehalt unterschieden (14% vs. 19% vs. 30% Rohprotein in der Trockensubstanz), ernährt. Die während dieser Zeit gebildeten Panzerhöcker wurden anschließend vermessen und die Kalzium, Phosphor- und Hämatokritwerte im Blut untersucht und zwischen den Versuchsgruppen verglichen. Trockene Haltung (24.3–57.8% bzw. 30.6–74.8% relative Luftfeuchtigkeit) führte zu stärkerer Höckerbildung als feuchte Haltung (45–99% relative Luftfeuchtigkeit). Die Höckerbildung war zwischen den drei

Gruppen, die bei unterschiedlichen Bedingungen gehalten wurden, signifikant unterschiedlich ( $p \leq 0,001$ ). Der Einfluss der Fütterung auf die Höckerbildung (Pyramidenbildung) konnte keinen klärenden Beitrag leisten.

The carapaces of captive-raised tortoises (*Testudinidae*) often develop pyramidal-shaped osseous growth centrally within the horny plates (Fig. 1). With very few exceptions (e.g. *Geochelone elegans*, *Psammobates* sp.), this conical growth pattern is considered to be pathologic. This very common defect is believed to be an important indicator of the quality of captive tortoise management. This study was designed to examine the effect of dietary protein level and environmental humidity on the degree of pyramidal growth in the carapaces. Fifty recently hatched African spurred tortoises (*G. sulcata*) were raised for 5 months under artificial conditions of varying environmental humidity and dietary protein content (14% vs. 19% vs. 30% crude protein in dry matter). Humps of the carapaces that developed and blood values of calcium, phosphorus and haematocrit were measured and compared among groups. Dry environmental conditions (24.3–57.8% and 30.6–74.8% relative humidity) produced taller humps than humid conditions (45–99% relative humidity). Hump formation differed significantly ( $p \leq 0.001$ ) between these three groups kept under different humidity conditions. Variable dietary protein had a minor, positive impact on this pathological formation of humps (pyramidal growth syndrome, PGS). Analysis of blood (calcium, phosphorus and haematocrit) offered no further explanation as to the development of the humps.



Fig. 1. Aldabra giant tortoise.

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## Introduction

The carapaces of captive-raised tortoises (terrestrial chelonians of the zoological family Testudinidae) often develop pyramidal-shaped osseous growth centrally within the horny plates (Fig. 1). With very few exceptions (e.g. *Geochelone elegans*, *Psammobates* sp.) this conical growth pattern is considered pathological and occurs mostly in tortoises in captivity. This undesirable, but very common defect is believed to be an important indicator of the quality of captive tortoise management. The long history of attempts to explain this phenomenon already demonstrates this. As early as 1972, Obst and Meusel (1972) suggested that the humps were the result of a previous rickety condition. A wide range of possible causes have been mentioned in the literature, like nutritional imbalances such as excess dietary protein, deficiency of calcium and vitamin D or an improper calcium : phosphorus (Ca : P). Also rapid growth rate and deficiencies of ultraviolet radiation have been suggested as the primary cause (Frye, 1991; Highfield, 1996; McArthur, 1996 (cited by Mader, 1996); Beyon et al., 1997; Kirsche, 1997; Walls, 1997; Sassenburg, 2000; Wiechert, 2000). Most of these authors have not made an attempt to associate the development of pyramidal growth with other very common osseous diseases of chelonians such as osteodystrophia fibrosa, rickets, secondary hyperparathyroidism or metabolic bone disease (MBD). Some authors, however, have either explicitly or implicitly suggested that pyramidal growth syndrome (PGS) is, in fact, due to the same cause as these diseases (Jackson and Cooper, 1981; Gabrisch and Zwart, 1995; Köhler, 1996; Kirsche, 1997; Eggenschwiler, 2000; Sassenburg, 2000; Wiechert, 2000). A more thorough understanding

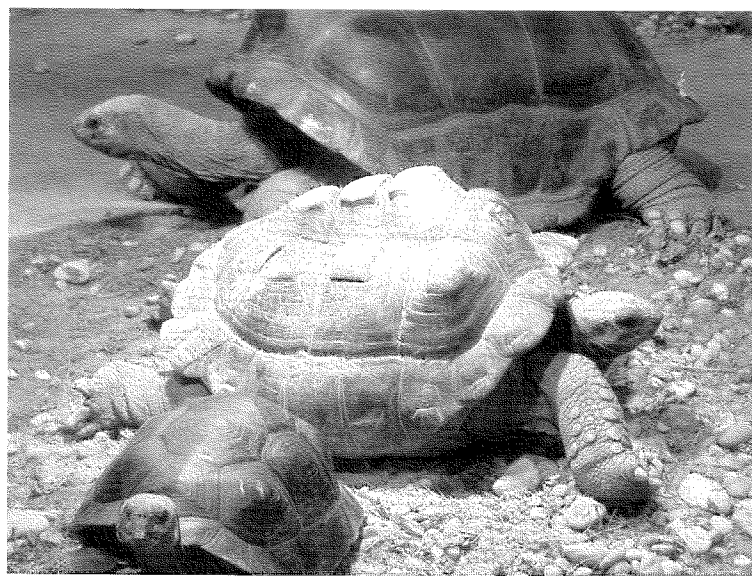


Fig. 1. Aldabra giant tortoise (*Geochelone dussumieri*) with extensive hump development

of the causes of PGS is necessary as it often appears in otherwise healthy tortoises. Furthermore, tortoises with signs of MBD like soft carapace, lordosis, or extensive growth of the costal plates, relative to the central (also named vertebral) plates, often show no signs of PGS. Although any explanation cannot be found concerning a possible influence of environmental humidity in the scientific literature, private tortoise breeder experience suggests that PGS is related to the humidity under which the tortoises are kept (Weser, 1988). In view of the above, an experiment was designed to ascertain two possible causes of hump formation in tortoises. This study was designed to examine the effect of dietary protein level and environmental humidity on the degree of pyramidal growth in the carapaces. Information gained from the study should provide the basis for advice to turtle keepers in order to avoid this problem in future.

## Materials and methods

### Experimental animals

African spurred tortoises (*G. sulcata*) were selected for this experiment as PGS is common in this species in captivity. Hatchlings of African spurred tortoises also grow very fast. As the females can produce clutches of more than 20 eggs, age and genetics can be controlled in the hatchlings. Fifty half-sibling tortoises were hatched at the farm of a non-commercial Austrian breeder (parents: two dames, one sire) during late December to late January. They were selected from three clutches of two dames containing 21, 29 and 27 hatchlings. The hatchlings were placed into transport boxes immediately after hatching. Within 2 days of hatching they were transferred to terraria at the Institute of Nutrition at the University of Veterinary Medicine, Vienna.

### Experimental design and sample collection

After excluding individuals with deformations of the carapace or the horny plates at the breeding farm, the first 50 tortoises hatched were taken for the study group. At the University, the 50 tortoises were randomly distributed to five terraria and kept under identical conditions for 5 months except for dietary protein content and environmental humidity (Table 1). At the end of the feeding trial, the formed carapace humps were measured (Fig. 2) and blood samples were taken from the vena jugularis (to measure haematocrit, calcium and phosphorus levels). Weights were measured twice a month.

### Diet

In each terrarium, water and sepia (squid-skeleton) calcium were supplied by three ceramic bowls (diameter 13 cm) *ad libitum*. Every morning, the tortoises were fed a mixture of

Table 1. Environmental humidity and dietary protein concentrations for the five experimental groups of *Geochelone sulcata*

	Group A	Group B	Group C	Group D	Group E
Environmental humidity	24.3–57.8	27.4–55.5	30.6–74.8	47.9–99	45–99
Feed	Type 1	Type 2	Type 2	Type 2	Type 3
Environmental humidity: mean of eight weekly measured values of the maximum and minimum relative humidity in percentage					

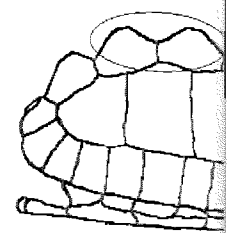


Fig. 2. Measurement of the H (height) of the central vertebral plates (2, 3).

Table 2. Nutritional composition of the feed

	Days 1–105
Crude protein	14.4
Crude fat	5.8
Crude fibre	13.4
Ca (mg/100 g)	2340
Ca : P ratio	4 : 1

dried pellets with defined nutrients (Mazda, Germany) and endive salad. Afterwards 2 : 1 until day 15. Selectively, the soaked pellet increase feed acceptance. Calcium (Table 2). This feeding program secondary hyperparathyroidism. technological reasons, values treatments, but this was not balance differences by difference.

Weende analyses were used using an atomic absorption spectrophotometer (Germany), and phosphorus was

The terraria consisted of glass 25 cm above the ground. Two front pane and at the ceiling of each terrarium, the tortoises could

in otherwise healthy tortoises. Lordosis, or extensive growth of the vertebral plates, often show no signs concerning a possible influence of the pyramidal growth experience of the tortoises are kept (Weser, 1980) to ascertain two possible causes of the pyramidal growth in the carapace to provide the basis for advice to turtle

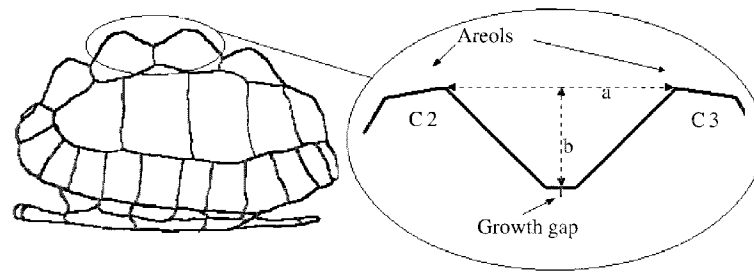


Fig. 2. Measurement of the H(ump)-index. C1–C5: Central plates 1–5; H-index = [H-value (central plates 2, 3) + H-value (central plates 3, 4)]/2; H-value =  $b/a$

is experiment as PGS is common in tortoises also grow very fast. As age and genetics can be controlled at the farm of a non-commercial breeder from December to late January. They hatch on 21, 29 and 27 hatchlings. The hatchlings are kept after hatching. Within 2 days of hatching at the University of

Table 2. Nutritional composition of the diets fed (percentage of dry matter, analysed)

	Type of feed					
	1		2		3	
	Days 1–105	Days 106–150	Days 1–105	Days 106–150	Days 1–105	Days 106–150
Crude protein	14.4	13.7	19.4	19	30.7	30.7
Crude fat	5.8	5.2	4.8	4.8	5.1	5.1
Crude fibre	13.4	13	13.4	13	11.4	10.9
Ca (mg/100 g)	2340	2411	2505	2582	2571	2649
Ca : P ratio	4 : 1	4 : 1	3.9 : 1	3.9 : 1	3.7 : 1	3.7 : 1

## collection

carapace or the horny plates at the time of the study group. At the time of the study, five tortoises were kept under the same conditions and environmental conditions. The formed carapace humps were measured from the vena jugularis (to the vena jugularis). Weights were measured twice a

They were supplied by three ceramic terraria and tortoises were fed a mixture of

concentrations for the five experimental groups

Group C	Group D	Group E
5–74.8	47.9–99	45–99
Type 2	Type 2	Type 3
Values of the maximum and minimum		

dried pellets with defined nutrient contents (prepared by sniff Spezialdiäten GmbH, Soest, Germany) and endive salad in the proportion of 1 : 1 (pellets : endive) until day 105, afterwards 2 : 1 until day 150 (all in fresh weight). To prevent the turtles from feeding selectively, the soaked pellets were mixed with thinly cut endive. Endive was used to increase feed acceptance. Calcium and phosphorus were fed at a ratio of higher than 3 : 1 (Table 2). This feeding program was designed to prevent deficiencies and pathogenesises (e.g. secondary hyperparathyroidism) which have been suggested as causes of PGS. Due to technological reasons, values of calcium and the Ca : P ratio differed slightly between treatments, but this was not considered to be crucial, as the animals could theoretically balance differences by different calcium consumption.

Weende analyses were used for the detection of crude nutrients. Calcium was analysed using an atomic absorption spectrophotometer (3030 B; Perkin Elmer, Überlingen, Germany), and phosphorus was measured with a photometer.

## Environmental conditions

### Cages

The terraria consisted of glass (100 × 80 × 80 cm) with two sliding panes at the front, 25 cm above the ground. Two air slots for ventilation were located at the bottom of the front pane and at the ceiling pane. The substrate consisted of 4–5 cm of bark humus. In each terrarium, the tortoises could hide in caves made of bricks (30 × 65 × 10 cm).

### Lighting

Each terrarium was illuminated by three different light sources. Under the top pane, a 150-W HQI-lamp (Teclumen, Castel Cofredo, Italy) was fixed, which supplied the animals with the necessary light intensity for activity. The connected intermediate unit (containing the transformer) was located outside of the terraria. At the left side of the terrarium and about 25 cm above the ground, a halogen heater lighted and heated the area below. Also at this height, an ultraviolet radiating fluorescent tube (UV-A and UV-B, Reptisun 5.0; Zoo Med Laboratories Inc., San Luis Obispo, CA, USA) was also provided at this height. The HQI-lamp was on 11 h daily from 8 am to 7 pm. Heating light and UV-tube were activated for 3 h each morning (9–12 am) and 90 min each afternoon (3–4.30 pm). At the bottom of the central area of the terrarium 7000 lux, and under the halogen lamp, 14 000–15 000 lux, were measured, respectively.

### Humidity control system

Humidity was provided by plastic-bowls (40×40×15 cm), filled with demineralized water with atomizers to produce fog. The bowls were located on top of the caves. Electric control units were used to regulate the atomizers, maintaining the level of relative humidity in the terraria. A connected sensor was located inside the caves. High humidity in terrarium D and E lead to humid soil. The humidity values provided in Table 1 were measured directly under the top pane. Values measured inside the caves of the three humid terrariums were approximately 10–15% higher.

### Measurement of humps

The wedge-shaped indentations between the second and the third, and the third and fourth central plate of the carapace were measured. In this area, hump formation is most distinct. The H-value was defined as the ratio of the depth (b) of the indentations at the growth-gap to the distance (a) between two opposite edges of areols (Fig. 2). The mean of the two H-values was considered to be the H(hump)-index to express the degree of hump formation of the tortoises. A perfectly well-shaped carapace would have a negative H-index, due to negative depths (b) and consequently negative H-values.

### Statistics

To evaluate the differences between the groups with respect to H-indices, weight, haematocrit, plasma calcium and plasma phosphorus appropriate non-parametrical tests of Kruskal–Wallis test and Mann–Whitney *U*-test were used. The selected level of significance was  $p < 0.05$ . All statistical tests were conducted by using SPSS 9.0 (SPSS, Chicago, IL, USA).

### Results

The H-indices of groups B, C and D, raised under different levels of relative humidity but identical dietary protein concentration, were compared and had formed distinctly different humps (Figs 3, 4–6; Table 3). Tortoises of group B ('dry') showed very prominent humps whereas the carapaces of tortoises of group E ('humid') were almost smooth. The humps of group C were between these two extremes. These three very different levels of hump development were significant ( $p \leq 0.001$ ). Groups A and B both raised under dry conditions, but fed different levels of dietary protein, (B: 14.4/13.7 in % of dry matter vs. A: 19.4/19; Table 2) also produced different sized humps. Although the statistical significance probability of error ( $p = 0.06$ ) of this difference was above the chosen level

### Influence of humidity

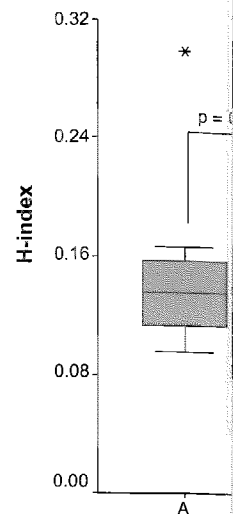


Fig. 3. This figure shows the different humps kept under different levels of relative humidity and different levels of dietary protein (groups A, B, C, D, E). The box represents the median, the 25th and 75th percentiles. '+' Value is more than 1.5 times the interquartile range.

( $p < 0.05$ ), some influence of diet was observed. Group B (raised under humid conditions but fed 14.4% protein) showed no statistically significant difference from group A (raised under dry conditions but fed 19.4% protein). At first sight, the bigger African spurred tortoise impression however reflected on the humps were actually measured. The humps of group B were higher than for groups B, C). The humps of group A (group A). As dietary protein concentration increased, the weight of groups B, C, D (same

The combination of dry environment and a nutritionally dense diet induced by a nutritionally dense diet in tortoises of this study. Humid conditions are not suitable for the development of PGS. Considering the literature regarding growth failure, they differ considerably from the reported his experience from keeping tortoises. He considers some of the natural history of tortoises have a highly variable food supply during a year and between years.

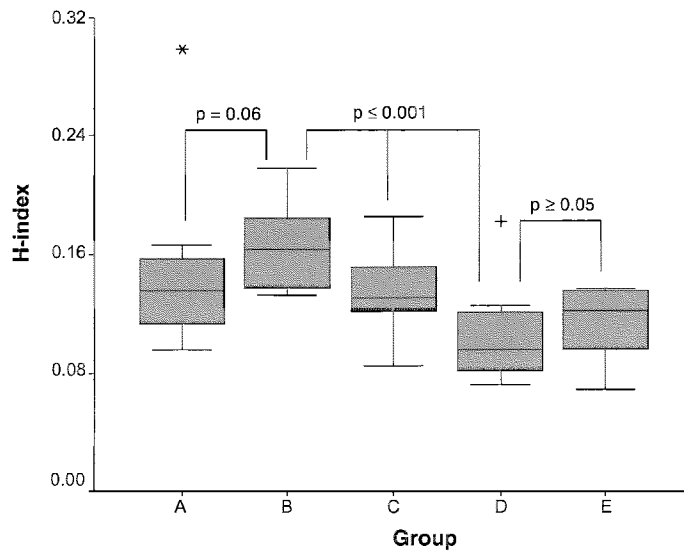
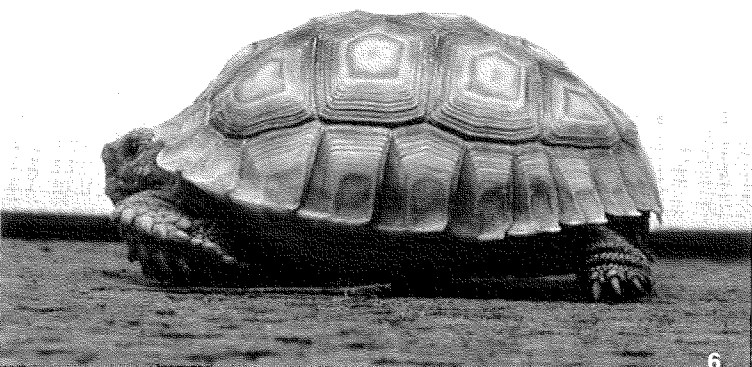
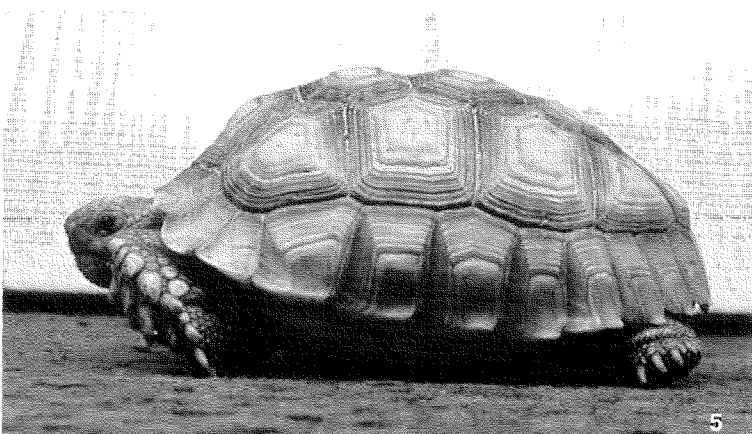
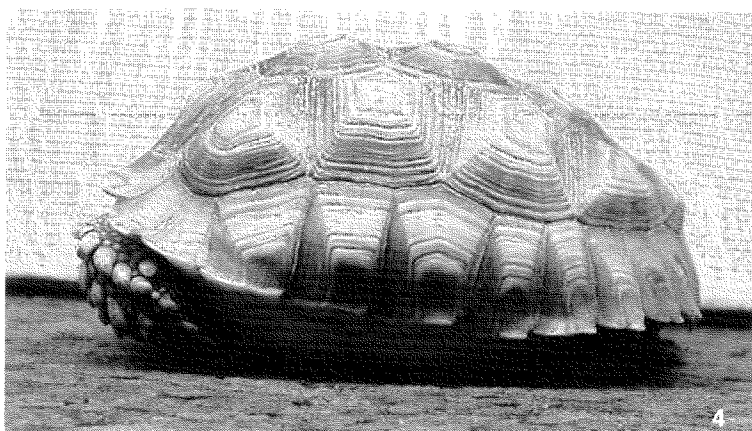


Fig. 3. This figure shows the differences in hump formation (H-indices) of the five groups of tortoises kept under different levels of relative humidity (group B, C and D; Kruskal-Wallis test) and fed different levels of dietary protein (group A vs. B, group D vs. E; Mann-Whitney *U*-test). Values shown are the median, the 25th and 75th percentiles (interquartile range) and the highest and lowest non-outlier values. '+' Value is more than 1.5-fold and '\*' value is more than threefold above the interquartile range

( $p < 0.05$ ), some influence of dietary protein is probable. Groups D and E, which were raised under humid conditions but fed different levels of dietary protein (D: 19.4/19 vs. E: 30.7) showed no statistically provable difference in hump size. It should be noted that at first sight, the bigger African spurred tortoises were believed to have bigger humps. That impression however reflected only the absolute hump size and was disproven later when the humps were actually measured. Regarding blood parameters, haematocrits did not differ among the groups but plasma calcium levels did (Ca-level of group A, D, E was higher than for groups B, C). The P-levels were between 0.82 (group C) and 0.95 mmol/l (group A). As dietary protein content increased body weight increased; however, the body weight of groups B, C, D (same level of dietary protein) did not differ.

### Discussion

The combination of dry environmental conditions and comparatively high growth rates induced by a nutritionally dense diet led to pyramidal growth in the African spurred tortoises of this study. Humid conditions suppressed the development of PGS considerably. Lowering the level of dietary protein had a questionable suppressing effect on the development of PGS. Considering the published reports in the veterinary and herpetologic literature regarding growth failure in chelonia, the results presented here are surprising as they differ considerably from the commonly propagated explanations. Only Weser (1988) reported his experience from keeping tortoises under humid condition and suspected some influence on hump formation. However, the results presented are understandable if one considers some of the natural history of wild tortoises. Nearly all species of extant tortoises have a highly variable food supply both in quality and quantity of nutritional components during a year and between years. During humid seasons, tortoises are able to select from a



Figs 4-6. Tortoises with three levels of hump formation. Figures 4-6 show one tortoise from each of group B (dry environment), C (intermediate humidity) and D (wet environment)

Table 3. Results of the weight gain

Group	Weight
A	Mean (N : 10) SD
B	Mean (N : 10) SD
C	Mean (N : 10) SD
D	Mean (N : 10) SD
E	Mean (N : 10) SD

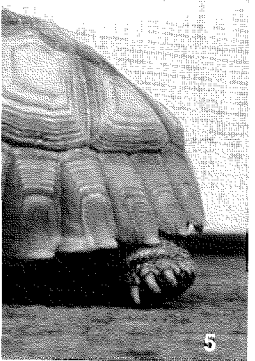
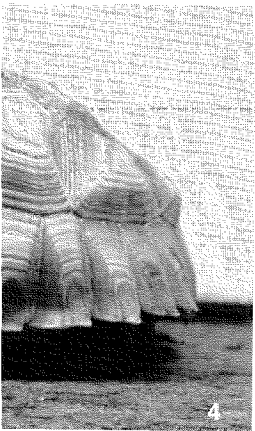
This table shows the results of the three blood values (haematocrit, calcium, and protein) for the five groups. The differences between the groups are not significant except the difference between the intermediate protein diet vs. the control group (a,b,c). Means of groups not sharing a common letter are significantly different.

variety of new growth plants, rich in calcium and phosphorus, and rapid growth. During very long periods of dry weather, when for tortoises are dry plants characteristic of the dry environment value. During these periods tortoises but grow very little. Faster-growing tortoises live most of their life higher above the soil, providing a higher level of humidity. Tortoises live on the ground surrounded by vegetation and habitats of tortoises in nature. It is understandable that the abnormal level of protein may lead to health problems. The prevalence of PGS in tortoises is very common both in private and public collections. Regarding the pathological mechanism of PGS, histological research, will it be the development of PGS and other diseases such as rickets or osteodystrophia fibrosa. It could be that dehydration reduces the cartilaginous tissue at the growing end of the bone, which could be caused by this low pressure. The bone becomes ossified and the collapse is permanent. During each period of dry weather, higher humps. But this hypothesis is not supported by the data on intake or excretion of minerals. The development of PGS. The results presented indicate that the cause of PGS, except that it may be the previously mentioned, faster growing tortoises produce bigger humps. This may be useful because of other protein levels and degree of PGS, keeping the results from this study is that, in order to

Table 3. Results of the weight gains and blood values of the five groups of sulcated tortoises

Group		Weight gain (g)	Hkt (%)	Ca (mmol/l)	P (mmol/l)
A	Mean (N : 10)	118 <sup>a</sup>	19.2	3.36	0.95
	SD	33	1.5	0.50	0.14
B	Mean (N : 10)	184 <sup>b</sup>	17.2	2.69	0.87
	SD	38	0.9	0.13	0.15
C	Mean (N : 10)	230 <sup>b</sup>	17.8	2.97	0.82
	SD	38	1.6	0.17	0.07
D	Mean (N : 10)	205 <sup>b</sup>	16.5	3.61	0.88
	SD	56	1.7	0.17	0.12
E	Mean (N : 10)	284 <sup>c</sup>	15.1	3.25	0.90
	SD	55	1.2	0.16	0.23

This table shows the results of the weight gain (end weight minus start weight of the tortoises) and three blood values (haematocrit, calcium and phosphorus values). Differences between the groups are not significant except the different weight gain of group A (low protein diet) vs. group B-D (intermediate protein diet) vs. group E (high protein diet) (Mann-Whitney *U*-test,  $p < 0.05$ ).  
<sup>a,b,c</sup> Means of groups not sharing a superscript a, b or c are significantly different



4-6 show one tortoise from each of A (dry environment) and D (wet environment)

variety of new growth plants, rich in nutritional contents corresponding to the period of rapid growth. During very long seasons of dryness and food scarcity, the main food source for tortoises are dry plants characterized by a high fibre content and low overall nutritional value. During these periods tortoises show little activity and probably osseous rebuilding, but grow very little. Faster-growing hatchlings live under the cover of grass and directly above the soil, providing a higher level of environmental humidity. Finally, many species of tortoises live most of their life hidden either in caves (e.g. *Testudo horsfieldii*, *Gopherus* sp.) or on the ground surrounded by high humidity even during arid conditions (for behaviour and habitats of tortoises in nature, see Ernst et al., 1994). Considering this, it is understandable that the abnormal combination of a dry environment and a persistent high level of protein may lead to health disorders, such as PGS. Such management conditions are very common both in private and institutional situations, which probably explains the high prevalence of PGS in tortoises in captivity. Not until further information is available regarding the pathological mechanism of how low humidity leads to PGS, such as histological research, will it be possible to evaluate any connection between the development of PGS and other diseases of the osseous tissue in turtles, such as MBD, rickets or osteodystrophia fibrosa. One possible explanation for the development of PGS could be that dehydration reduces the intracellular and intercellular pressure on the soft cartilaginous tissue at the growing gap area. A collapse of this tissue around the gap might be caused by this low pressure. If tortoises are dehydrated for a longer period, the tissue becomes ossified and the collapsed 'valleys' between the central parts of the plates are fixed permanently. During each period of growth the development of new valleys would create higher humps. But this hypothesis needs to be supported by further research. Differences in intake or excretion of minerals such as calcium or phosphorus might also help to explain the development of PGS. The results of blood assays, in the current study, neither supported or rejected such an explanation.

The results presented indicate that the level of dietary protein is probably not the main cause of PGS, except that it may predispose the tortoise to more rapid growth. As previously mentioned, faster growing tortoises can provoke the erroneous impression of forming bigger humps. This might explain the widespread belief that faster growing tortoises produce bigger humps. Even if there is no connection between dietary protein level and degree of PGS, keeping the level of dietary protein in tortoise diets low might still be useful because of other protein-related diseases, such as gout. The decisive conclusion from this study is that, in order to reduce the incidence of PGS in growing tortoises, one



needs to provide a sufficient level of environmental humidity. In particular, in order to keep the development of PGS to a minimum, areas with a relative humidity of nearly 100% for hiding should be provided to the tortoises at all times.

### Acknowledgement

We thank Prof. Dr Howard B. Hulan and Mag. Cornelia Gabler for their support and assistance. We also have to thank the World Turtle Association and the Austrian Herpetological Society (Österreichische Gesellschaft für Herpetologie) for providing financial support of this study.

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