Growth and body composition in captive *Testudo graeca terrestris* fed with a high-energy diet

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Abstract. Wild populations of *Testudo graeca* may face extinction due to large demand in the pet trade and habitat loss. Establishment of reproduction farms could alleviate the danger from collection of wild tortoises; management of such farms requires nutritional guidance. Our initial trials resulted in tortoises with soft shell syndrome and gout, due to improper nutrition. These problems were solved by using a high energy, amino acid-balanced diet for hatchlings and young tortoises. This diet also increased growth compared to wild tortoises. We determined changes in body composition with size in order to be able to formulate specific diets according to the nutritional needs of each stage of growth. Water content was about 70% in newly hatched tortoises, increased slightly till they doubled their mass, and thereafter declined gradually, stabilizing at about 60% at a body mass of 170 g. Ash content was 5% at hatching, increased gradually to 15% at 170 g, and remained stable thereafter. Lipid content was about 7% at hatching, due to the residual yolk, declined to a minimum of 2.8% at 94 g, and resumed the level of 7% from 170 g. Sulfur-amino acid levels (methionine and cysteine) were lower than those found in homeotherms; a negative correlation between sulfur and protein contents suggested a substantial sulfur compartment outside of amino acids.

Key words: Amino acids; ash; body composition; growth; lipid; minerals; nutrition; protein; *Testudo graeca terrestris*; tortoise.

Introduction

The Mediterranean spur-thighed tortoise *Testudo graeca* is rapidly becoming an endangered species, mainly because of a large demand for the pet trade and stresses caused by modern civilization: urbanization, agriculture, industry and pollution (Lambert, 1977, 1983, 1985). In order to reduce the pressure of illegal trade, the EEC Council in 1984 agreed to treat *T. graeca* according to Appendix I of the Convention on International Trade in Endangered Species (CITES). The establishment of *T. graeca* farms was suggested, to satisfy commercial demand and reduce the profits of illegal trade (Lambert, 1969). These farms may also serve as a source of animals for reintroduction into natural habitats in areas in which the natural population is totally extinct (Lambert, 1969, 1982, 1985).

Attempts to farm *T. graeca* in Europe have failed as the resulting tortoises were deformed, even when artificial incubation and heating systems were used (Kirsche, 1979; Lambert, 1980), probably because of an improper photoperiod and light spectrum. It was therefore suggested to erect tortoise farms in *T. graeca*'s natural habitats. We established a model breeding farm in Israel, the natural habitat of *T. g. terrestris,* where reproduction and growth could be followed. In the wild, hatching rate is low and survival is minimal, due to deformations, malnutrition and predation by insects and vertebrates (Andreu and Villamor, 1985; Gist and Fischer, 1993). The long life expectancy of *T. graeca* (Kirsche, 1979) compensates for the low reproductive and hatchling survival rates, allowing a stable population in the absence of interference.

The need for proper nutrition has not received much recognition in conservation biology. However, captive breeding is possible only if the animals' nutritional requirements are met, and effective habitat management also requires an evaluation of nutritional resources. It has, for example, been suggested that energy acquisition and expenditure in desert tortoises are strongly constrained by the contingencies of rainfall. This acts both indirectly through effects on the availability and quality of food, and directly through reliance on free-standing water for drinking, the latter appearing to be necessary for a net annual energy surplus (Peterson, 1996).

Dietary changes, confinement and the achievement of rapid growth were found to result in three syndromes in growing tortoises: soft shell (osteomalacia) (Wronski et al., 1992; Homer et al., 1998); lumpy shell (pyramiding), due to insufficient calcium and phosphorus; and gout (uricacidaemia), as a result of excessive protein intake (Scot, 1992). A high protein diet was suggested to solve soft shell and lumpy shell syndromes, but this diet frequently caused gout and eventual death in growing tortoises. This was also our experience in initial trials: A vegetarian diet produced the soft shell syndrome and high mortality of hatchlings, even when reared in the open exposed to natural sunlight; a high protein diet alleviated soft shell but resulted in gout and therefore also a high rate of mortality.

Body composition and weight gain is related to dietary composition (including essential amino acids) in growing homeotherms. The purpose of the present study was to secure information on the body composition of growing *T. g. terrestris* fed a high energy, amino acid-balanced diet, in order to formulate adequate diets for the different stages of their growth and reduce the mortality of captive bred individuals.

Materials and Methods

Animal care was approved by the animal ethics committee of The Hebrew University of Jerusalem. Newly hatched *T. g. terrestris* were maintained in small yards, in a greenhouse with infrared lamps for heating on cold days. The glass roof had

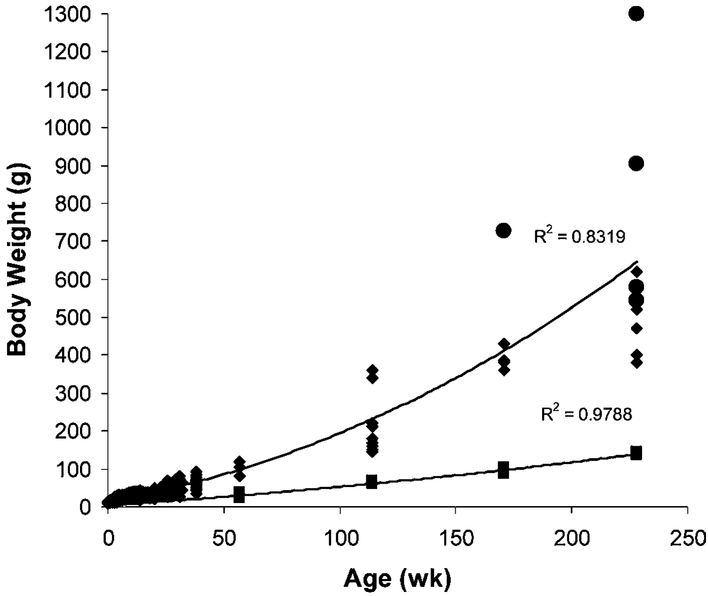

Figure 1. Growth of captive *T. g. terrestris* fed an enriched diet, compared to individuals collected from their natural habitat. Sexing was impossible prior to 171 wk and juvenile tortoises are therefore presented as one group; from 171 wk males (◆) and females (●) are shown separately. Sexing was impossible in samples collected from the wild (■). Sample sizes were 5, 9, 5, and 9 for captive bred, and 5, 2, 3, and 2 for wild-caught tortoises, at 57, 114, 171, and 228 wk, respectively; two captive tortoises were also measured at 342 wk (not shown).

openings which were only closed on cold days, but open most of the time for direct exposure to sunlight. Tortoises experienced a natural humidity (as windows were open most of the time) and photoperiod. Tortoises were divided into groups according to their body mass; they were periodically re-segregated according to body mass as their growth varied. The number of individuals weighed at different periods is shown in the legend of fig. 1. Growth was compared to that in natural conditions by collecting wild tortoises under permit, assessing age by counting rings on the scutes, weighing and releasing them back to the wild. It was not possible to sacrifice wild tortoises for body composition analysis.

The diet was composed from a commercial canned, vitamin-enriched cat food (Tuffy's, Heinz Pet Products, Kentucky, USA). 78 g of cat food was blended with 22 g starch (corn flour), to dilute the excess protein with a high carbohydrate source to prevent gout (table 1). There was no information on the vitamin A and D content of this food. Tortoises had continuous access to food and water; feeders were emptied, cleaned and refilled daily. Body composition of *T. g. terrestris* at each stage of growth was determined by sampling groups of four tortoises; on the day of hatching, and at body mass of 10 g (post-hatch), 20 g (4 wk), 30 g (12 wk), 40 g (20 wk), 50 g (27 wk), 100 g (60 wk), 170 g (90 wk), and 250 g (130 wk). These animals were deprived of food for 72 h, brought to their preferred body temperature

Table 1. Composition of the diet in terms of wet mass.

Digestible energy (kJ g^{-1})	5.94
Crude protein (%)	8.20
Lipids (ether extract) (%)	4.92
Crude fibre (%)	0.82
Ash (%)	2.87
Salt (%)	2.87
Phosphorus (%)	0.25
Calcium (%)	0.41
Water (%)	61.64

by spending 24 h in a temperature controlled room at 28°C, and then sacrificed by ether anesthesia.

Water content was measured after drying to constant weight at 105°C (Association of Official Agricultural Chemists, 1984). Samples for chemical analysis were autoclaved for 3 h at 120°C, and homogenized after cooling. They were then lyophilized, ground to a fine powder, and the following determinations were carried out. Nitrogen concentration; based on the Kjeldahl procedure, using a Kjeltec Auto 1030 Analyzer. Protein content; calculated by multiplying nitrogen concentration by 6.25. Lipid content; determined gravimetrically after chloroform-methanol (3:1) extraction (Folch et al., 1957). Energy; estimated by multiplying lipid and protein by 9.5 and 5.7, respectively (Kleiber, 1961) — glycogen stores were minimal and so not included in these calculations. Ash; determined by burning at 600°C for 3 h (Association of Official Agricultural Chemists, 1984). Minerals (Ca, P, K, Na, Mg); samples of 250-500 mg were placed for 10 min at 580 W under a pressure of up to 30 atm in microwave containers, in 5-10 ml nitric acid (65%). The resultant clear solution was analyzed by ICP (spectroflame inductively coupled plasma — atomic emission spectrometer, Spectro, Germany). Amino acids; determined by ion-exchange chromatography using an autoanalyzer (amino acid analyzer LC 5000, Biotronik), after 24 h acid hydrolysis with aqueous HCl at 115°C. Methionine and cysteine were determined in samples oxidized with performic acid (Moore, 1963).

Results

Growth

Growth was much enhanced compared to *T. g. terrestris* collected from their natural habitat (fig. 1) or to that previously described for tortoises fed mainly grass and mixed weeds with little cultivated food and no animal protein (Reid, 1986). Adult breeding activity was attained at about 170 wk in males and 230 wk in females; mean body mass at this stage was 350 g and 550 g, respectively. Fertile females (550-1300 g) were much heavier than fertile males (360-620 g) grown under similar conditions.

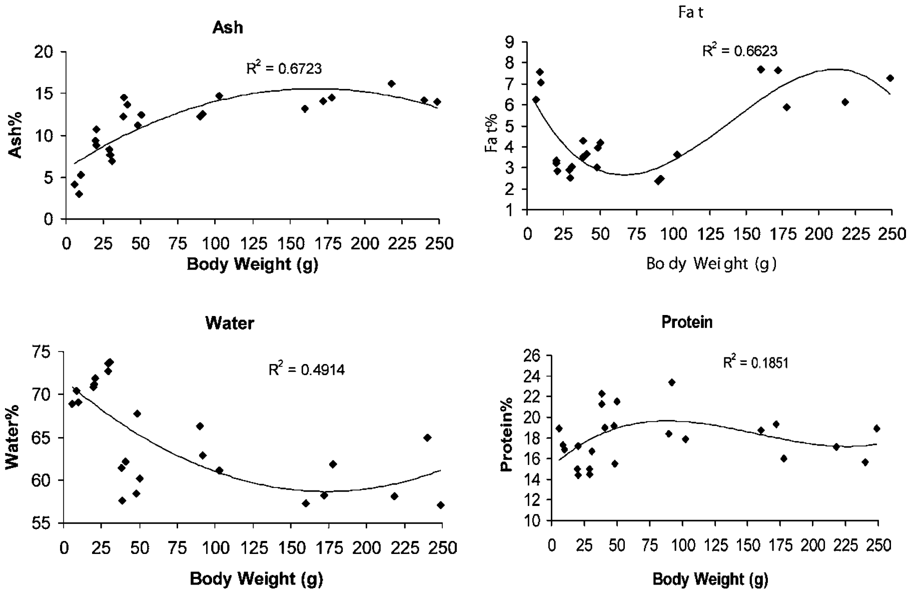

Figure 2. The effects of age (body mass) on ash, lipid, water, and protein content of captive *T. g. terrestris*. R^2 values for ash, lipid and water were highly significant ($P < 0.001$); that for protein was also significant ($P < 0.05$).

Body composition

Body composition with increasing size is shown in figs. 2 to 4. Water, ash, lipid, crude protein, and mineral contents are given as % of wet body mass. Body mass explained 50-60% of the variation of water, ash and lipids, and R^2 values were highly significant ($P < 0.001$). Water content was about 70% in newly hatched tortoises; it increased slightly till they doubled their body mass, and thereafter declined gradually and stabilized at about 60% at a body mass of 170 g (fig. 2). Ash content increased gradually from a minimum of 5% at hatching to 15% at body mass of 170 g and higher (fig. 2).

Lipid content was about 7% at hatching due to the residual yolk, decreased to a minimum of 2.8% at 94 g body mass, and resumed a level of about 7% at 170 g body mass and above (fig. 2). Body energy diminished at the post-hatch stage, from 7.1 to 5.0 kJ g^{-1}, and gradually increased to 6.3 kJ g^{-1}. The pattern of change in body energy resembled that of lipid. The R^2 between crude protein concentration and body mass was low, and body mass explained only 18% of variation of the protein level ($P < 0.05$). The slightly higher protein content at hatching (mean 17.7%) was somewhat biased by the yolk protein, which would explain the reduction in protein content in the post hatch stage (mean 15.5%).

A negative relationship was found between body sulfur and protein ($R^2 = 0.52$), so that a substantial portion of the body's sulfur was located in compartments other than sulfur amino acids. The mean protein composition for all age groups is shown

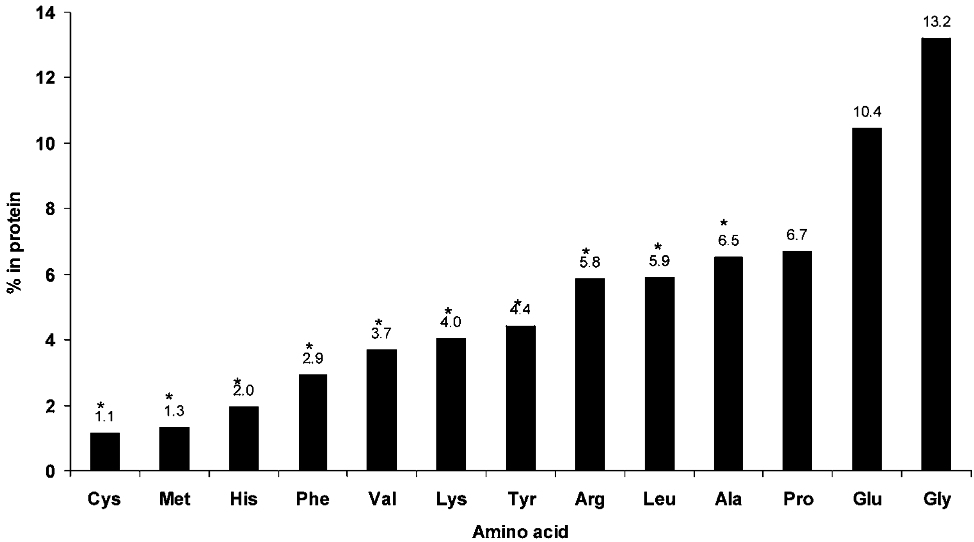

Figure 3. Amino acid composition of protein of captive *T. g. terrestris*, averaged across all age groups. Amino acids essential for avian species are marked with an asterisk.

in fig. 3, because age (body mass) effects on protein amino acid contents were minimal. The lowest concentration (close to 1%) was found for the sulfur amino acids cysteine and methionine. Glutamic acid and glycine were the highest (10.4% and 13.2%, respectively).

Calcium and phosphorus increased gradually to a plateau at body mass of 170-250 g (fig. 4). From 4 wk the Ca/P ratio was 2-2.2, but in some young animals (weighing up to 30 g) the ratio was <2. Potassium varied between 1.5-2 mg g^{-1} during the whole period without significant pattern. Sodium increased from about 2 mg g^{-1} in post hatch tortoises to 2.5-3 mg g^{-1} in heavier animals. Magnesium increased gradually from a low of 0.2 mg g^{-1} at hatching to a plateau of 0.6 mg g^{-1} in animals weighing over 100 g.

Discussion

Growth and food intake

Captive newly hatched and juvenile *T. g. terrestris* showed a high degree of physiological adaptation to a high energy, balanced protein diet in the present study. The growth of *T. g. terrestris* was very slow, even in captivity, compared to juvenile homeotherms. If the target weight is 250 g, then the growth rate was about 0.03% d^{-1} in the wild and 0.11% d^{-1} in captivity. In comparison, broiler chicks grow at a rate of 15% d^{-1}(Praharaj et al., 1996). Because of their slow growth and low metabolism, young tortoises consume less energy than homeotherms, but use a larger proportion of this for growth (Pough, 1983). It is therefore expected that

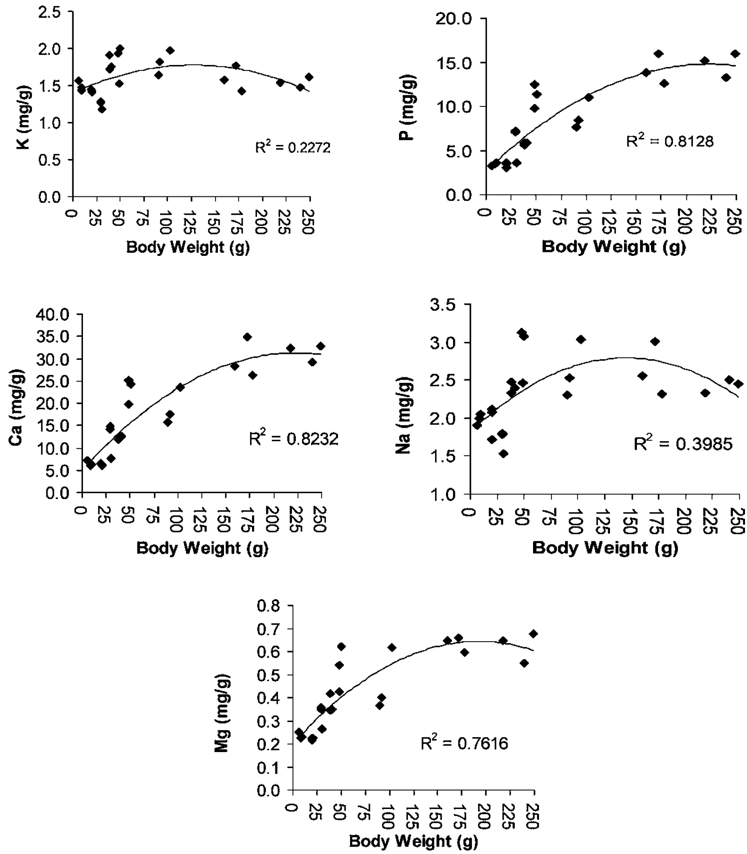

Figure 4. The effects of age (body mass) on potassium (K), phosphorus (P), calcium (Ca), sodium (Na) and magnesium (Mg) in captive *T. g. terrestris*. R^2 values for all components were highly significant ($P < 0.01$).

tortoises would be able to process a diet with much higher protein content than their natural food, and allocate this effectively to growth, as confirmed in this study.

The availability of nutrients and the absence of toxins in the prepared diet enabled much higher energy intake and faster growth than possible for wild *T. g. terrestris*. This study cannot, however, exclude some influence of higher temperature, longer activity period, or greater food availability (rather than food quality) in producing the increased growth in captivity. The digestibilities of dry matter, energy and nitrogen in *T. g. terrestris* have been proposed to be similar or superior to those of the desert tortoise *Gopherus agassizii* (Nagy et al., 1998). When determined using native plants, digestibility was quite close to that recorded for homeotherms; 63-70% for dry matter, 69-73% for energy, and 72-79% for nitrogen protein (Nagy et al., 1998). The tortoise's drive to consume high energy food in a choice situation (Nagy et al., 1998) is in accord with the assumption that energy is a limiting factor for both growth and reproduction in nature. It should be emphasized that the

T. g. terrestris were imprinted to the experimental diet immediately after hatching, because this was the only food offered to them. Previously we found that if they were exposed to a vegetarian diet after hatching, they rejected the high energy diet thereafter.

Body composition

Water, protein and lipid contents of *T. g. terrestris* were similar to those recorded in farm animals (Bondi, 1987). However, a consistent difference was observed in ash content, being much higher in the tortoise due to the bony structures of the carapace and plastron that form about 30% of the body mass. At hatching, ash content was similar to that found in farm animals (about 5%), but in adults body ash reached about 15% due to the gradual ossification of the shell. In accordance, the Ca/P ratio was similar to that of skeletal tissue throughout the experimental period.

Inorganic element contents of various farm animals are, for the most part, relatively uniform (Scott et al., 1982). Approximately 20-50% of the body's total Na and Mg is present in the bone and firmly bound in an inorganic phase. Therefore, like Ca and P, bone Na and Mg increase with age in parallel with ossification, explaining the higher Na and Mg contents of *T. g. terrestris* compared to farm animals.

The negative relationship between total body sulfur and total protein means that there are substantial alternative sulfur containing compartments, and protein synthesis may have utilized sulfur from other sites. Supplementation of mineral sulfur in the diet could thus be beneficial. The essential amino acid requirements of tortoises and of reptiles in general are not known. In the present study, high growth was obtained despite the low dietary protein content (8.2% of wet mass; table 1), suggesting that the amino acid profile of the animal protein used in the diet satisfied the tortoise's requirements. In conclusion, growth and maturity were highly accelerated in *Testudo graeca terrestris*, without symptoms of disease, by providing them with a high energy protein-balanced diet. Perhaps an even better balanced diet, adjusted to each stage of growth, can be formulated by utilizing our findings on the change in body composition with growth.

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