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Digestive parameters and water turnover of the leopard tortoise

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ABSTRACT

Leopard tortoises (*Stigmochelys pardalis*) experience wide fluctuations in environmental conditions and unpredictable availability of food and water within the Nama-Karoo biome. It was hypothesised that tortoises fed two diets differing in preformed water and fibre content would have differing food intake, gut transit rate, assimilation efficiency, faecal and urinary water loss, and urine concentrations. It was predicted that tortoises fed these contrasting diets would attempt to maintain energy and water balance by altering their digestive parameters. Leopard tortoises fed lucerne (*Medicago sativa*) had a low food intake coupled with long gut transit times, which resulted in the lowest amount of faecal energy and faecal water lost. Tortoises fed tomatoes (Solanum *lycopersicum*) had higher food intake and faster gut transit times, but more energy and water was lost in the faeces. However, daily energy assimilated and assimilation efficiency were comparable between tortoises fed the two diets. Urine osmolality was significantly different between tortoises on the two diets. Results indicate that leopard tortoises can adjust parameters such as transit rate, food intake, water loss and urine osmolality to maintain body mass, water and energy balance in response to a high fibre, low water content and a low fibre, high water content diet. This study suggests that this digestive flexibility allows leopard tortoises in the wild to take advantage of unpredictable food and water resources.

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1. Introduction

Animals living in arid regions or in unpredictable environments rely on the fluctuating availability of resources and are able to adjust their diet seasonally or opportunistically according to food and water availability (Waldschmidt et al., 1987; Murphy, 1996). They may also possess a variety of morphological, physiological and behavioural characteristics that enable them to survive in an unpredictable environment (Murphy, 1996; Henen, 1997; Henen et al., 1998).

Chelonian species have been shown to relinquish maintenance of internal homeostasis on a daily basis year round, tolerating imbalances in water, energy, and salt, enabling them to cope with fluctuating resources in a fluctuating environment (Nagy and Medica, 1986; Peterson, 1996a,b; Henen, 1997). Tortoises are predominantly herbivorous and employ various mechanisms to meet their energy and nutrient demands from their herbivorous diet (Waldschmidt et al., 1987). It has been reported in various tortoise species that the digestive parameters of food intake, gut transit time and water management differed greatly when tortoises were fed on different diets (Cloudsley-Thompson, 1970; Bjorndal, 1989; Meienberger et al., 1993; Hailey et al., 1998; Spencer et al., 1998; Hatt et al., 2002). Some digestive parameters directly influence others, for example, increased food intake shortens transit time (Meienberger et al., 1993) and increases urinary water loss (Cloudsley-Thompson, 1970).

In the wild, seasonal rainfall affects tortoise digestive parameters and the osmotic balance both directly, because of the availability of free-standing water, and indirectly, by enabling tortoises to eat annuals and other plants with higher water contents (Peterson, 1996a; Hailey et al., 1998; Henen et al., 1998). In the desert tortoise (*Gopherus agassizii*), energy acquisition and expenditure were strongly constrained by the contingencies of rainfall and seasonal temperature fluctuations (Peterson, 1996a).

Leopard tortoises (*Stigmochelys pardalis*, previously *Geochelone pardalis*, Fritz and Havăs, 2006) occur throughout the savannah regions of Africa from the southern Cape up into Sudan, experiencing wide geographic variation in environmental conditions across their range, including unpredictable availability of food and water in some regions such as the Nama-Karoo (Dean and Milton, 1999; Boycott and Bourquin, 2000; Kruger, 2004). Therefore, leopard tortoises living in these areas have to cope with fluctuations in available energy and water in their environment. Leopard tortoises are considered to be generalist herbivores, feeding extensively on grass, but also utilizing a variety of

Table 1
Nutrional composition of dietary items fed to leopard tortoises in the laboratory trials

Dietary item	Protein (%)	Fat (%)	Carbon fibre (%)	Sodium (%)	Potassium (%)	Gross energy (MJ/Kg)	Preformed water (%)
Lucerne	13.62	4.33	19.36	0.43	0.60	16.61	12.22
Tomato	17.87	2.11	9.55	0.03	0.02	16.14	95.48

Protein, fat and carbon fibre percentages are given as the percentage of dry weight.

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forbs, succulents, fruit, and legumes in certain habitats or according to the seasonal availability of certain species (Milton, 1992; Rall and Fairall, 1993; Hailey, 1997; Mason et al., 1999; Kabigumila, 2001).

While leopard tortoises in the Kalahari were found to use plant foods according to seasonal abundance (Rall and Fairall, 1993), no seasonal differences were reported in the diets of leopard tortoises
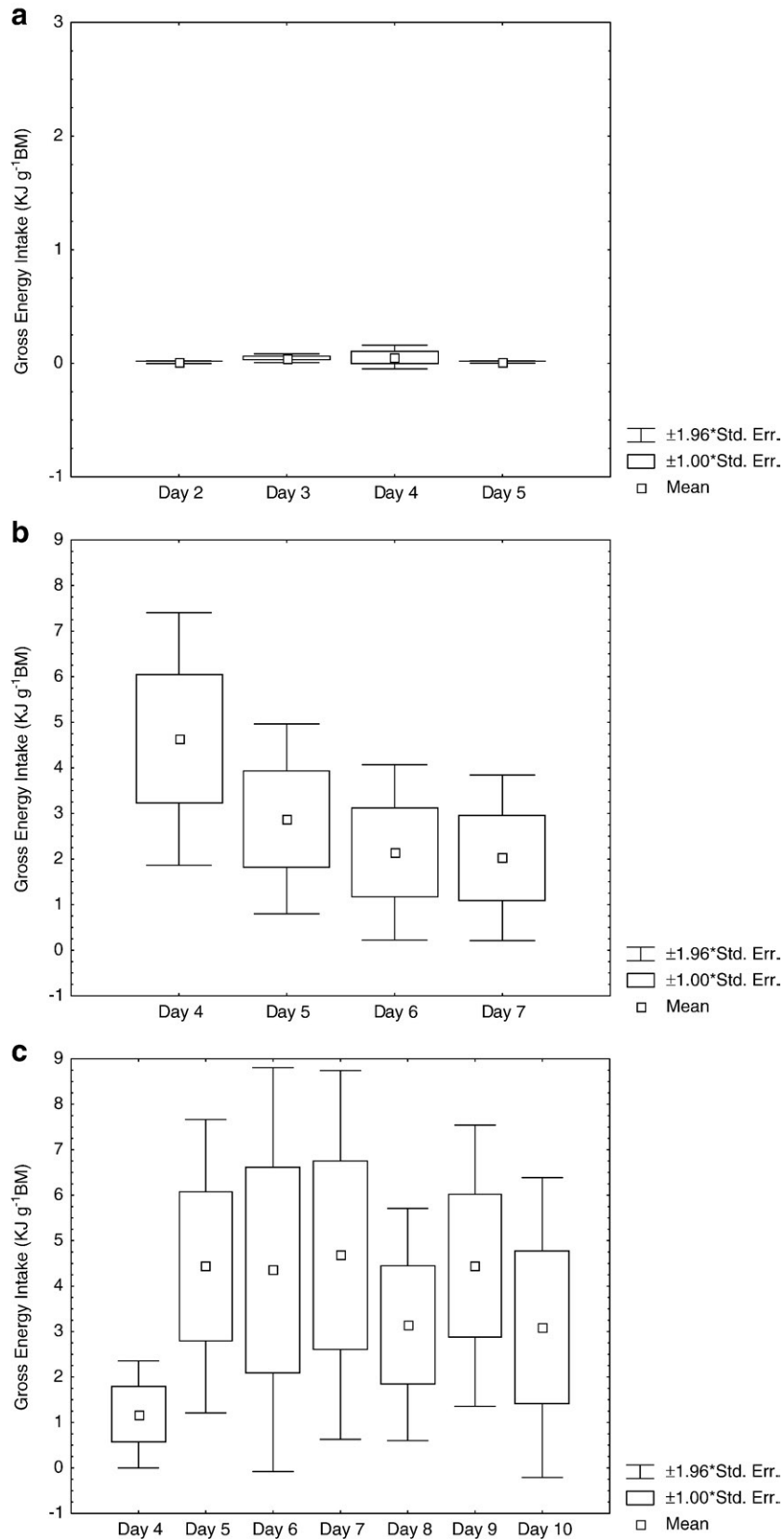

Fig. 1. Change in gross energy intake (KJ g^{-1}BM) by leopard tortoises over the days of a. trial 1 (lucerne), b. trial 2 (tomato) and c. trial 3 (tomato, outdoor).

in the southern Karoo (Milton, 1992) or Valley Bushveld in the eastern Cape (Mason et al., 1999). As a result, leopard tortoises may compromise transit times and assimilation efficiency depending on the type of food available or the seasonal change in food quality.

In this study, two diets differing in amount of metabolic and preformed water, with one low and one high, were fed to captive leopard tortoises. It was hypothesised that tortoises fed two diets differing in preformed water and fibre content would have differing food intake, gut transit rate, assimilation efficiency, and faecal and urinary water loss in an attempt to maintain energy and water balance. Greater food and preformed water intake, assimilation efficiency, and faecal and urinary water loss were predicted in tortoises that were fed the diet high in preformed water content. In addition the urine concentrating ability of leopard tortoises was examined. Urine osmolality was used as an indication of water stress and compared to seasonal variation in urine osmolality of wild tortoises.

The high fibre diet which was low in water content consisted of lucerne, while the low fibre diet which was high in water content consisted of tomatoes. All of the tortoises involved in the trials had been in captivity for at least a year, as such their dietary preferences were known. All of the individuals readily ate tomatoes, a low fibre,

high water content item which was ideally suited to the purpose of the study. Some of the individuals, probably because of the length of time in captivity, refused to eat grass. It was also difficult to obtain grass in sufficient quantities and of sufficient quality to support the trials. In addition, it was known that all of the individuals would eat lucerne, a high fibre, low water content food item. This therefore was chosen as the comparative diet to tomatoes.

2. Materials and methods

2.1. Captive tortoises

2.1.1. Maintenance of captive tortoises

Twelve captive leopard tortoises (*S. pardalis*) of various body masses were loaned for the duration of 2003. The population comprised nine adults and three juvenile tortoises (see McMaster, 2007 for morphometric details). These tortoises had been confiscated from illegal pet traders and as such could not be released back in to the wild. All had been in captivity for more than a year.

Tortoises were housed in outdoor enclosures measuring 20 m by 12 m at the Animal House of the School of Biological and Conservation Sciences, University of KwaZulu-Natal (UKZN). The enclosures were
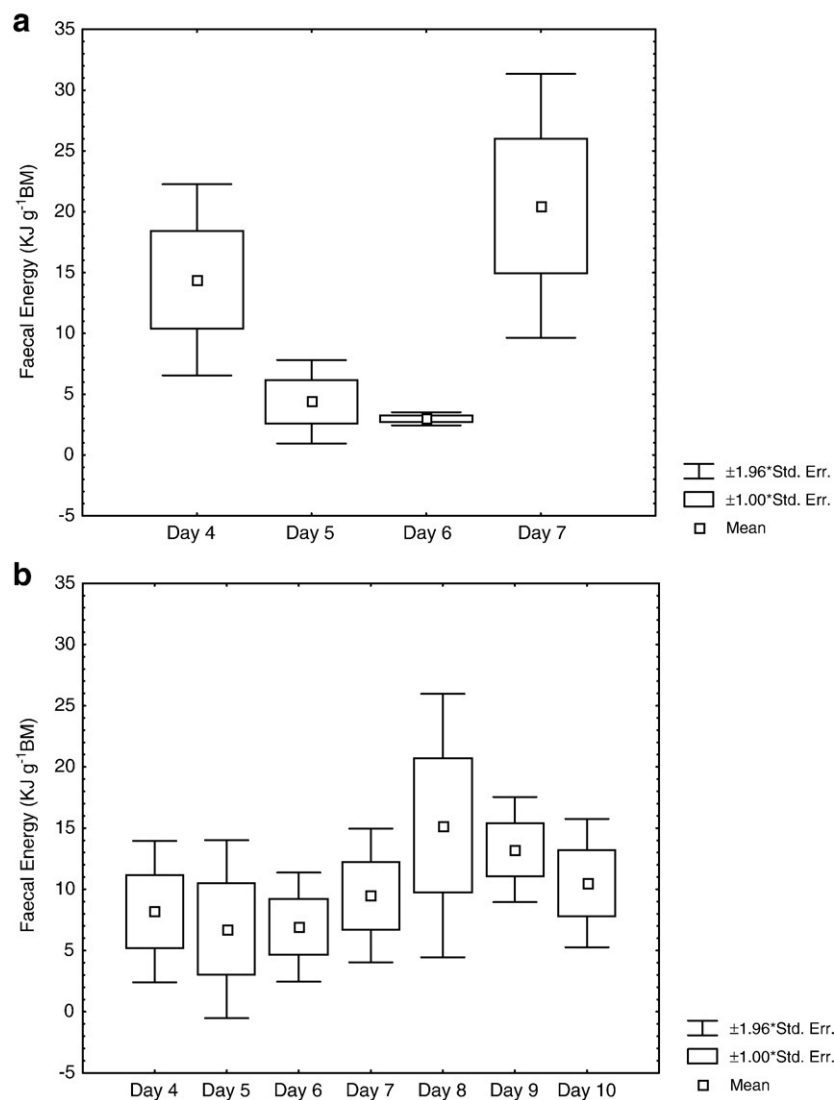

Fig. 2. Change in faecal energy (KJ g^{-1}BM) excreted by leopard tortoises over the days of a. trial 2 (tomato) and b. trial 3 (tomato, outdoor).

predominantly covered with kikuyu grass, but had patches of wild indigenous grass and weeds in them. Water was always available in shallow trays set into the ground, and shade was provided in the form of open-sided roofed shelters.

2.1.2. Digestion experiments

Two trials were conducted to examine the digestive efficiency of leopard tortoises. Both trials allowed for the controlled feeding of individual tortoises and collection of faeces, while Trial 1 additionally
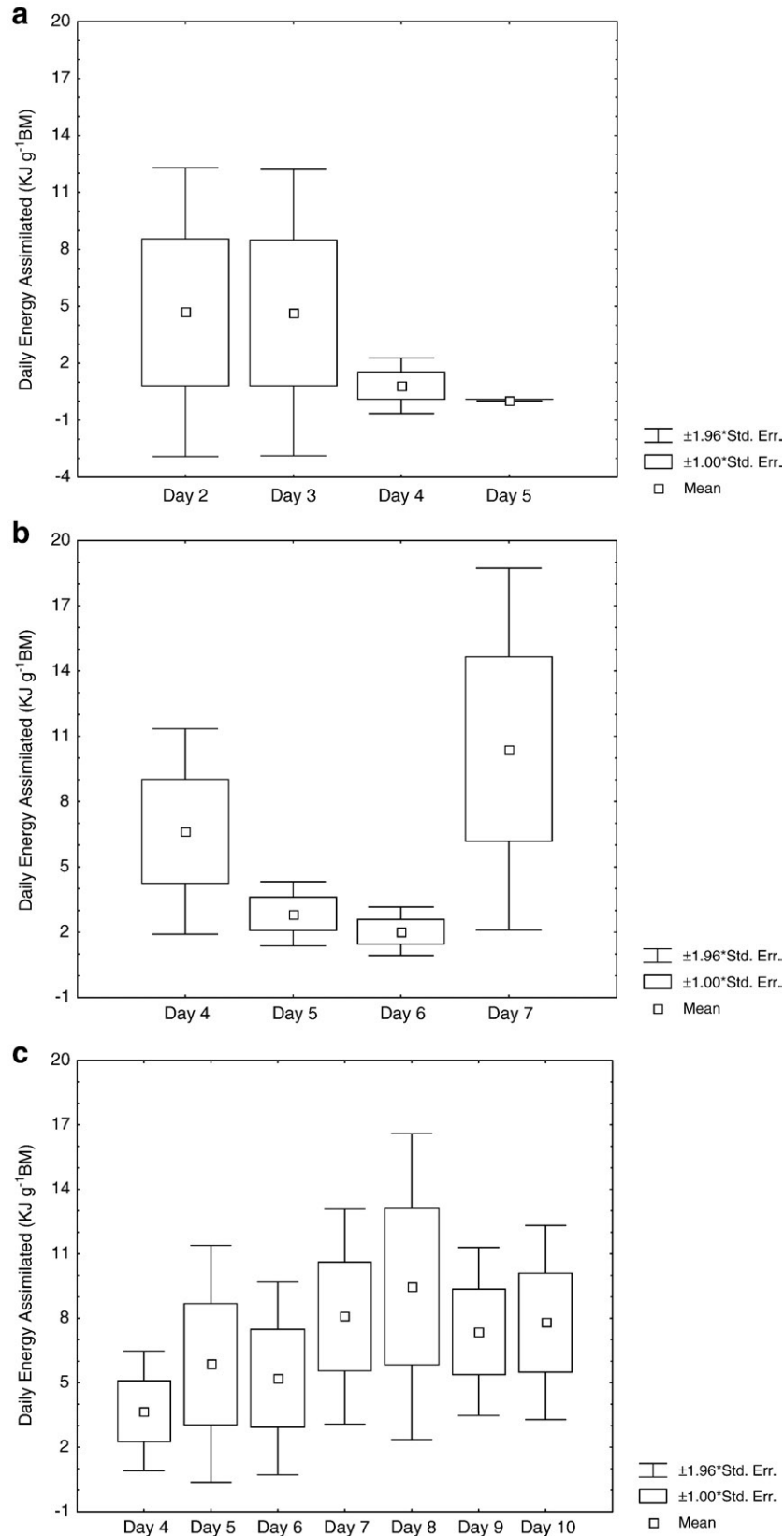

Fig. 3. Change in daily energy assimilation (KJ g^{-1}BM) of leopard tortoises over the days of a. trial 1 (lucerne), b. trial 2 (tomato) and c. trial 3 (tomato, outdoor).
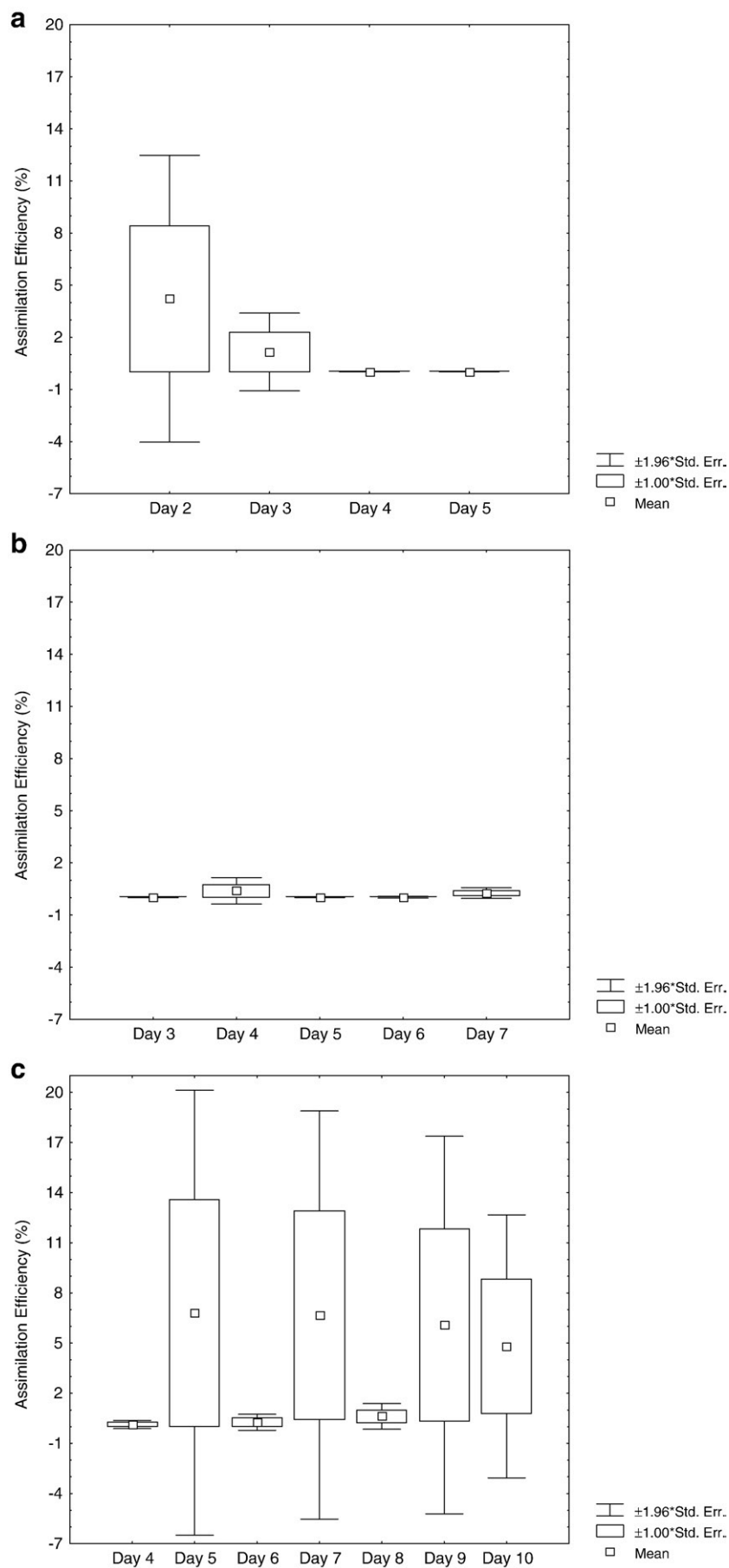
Fig. 4. Assimilation efficiency (%) of leopard tortoises over the days of a. trial 1 (lucerne), b. trial 2 (tomato) and c. trial 3 (tomato, outdoor).

allowed for urine collection. Tortoises were weighed daily to the nearest gram for the duration of the trials.

2.1.2.1. Trials 1 and 2. For Trials 1 and 2, nine adult leopard tortoises were kept in a constant-temperature room at 25 °C on a 12-hour photoperiod from 06 h00-18 h00. Tortoises were held individually in a suspended wire cage ($1 \times 1 \times 1$ m^3) with a mesh floor (2×2 cm^2). A removable frame with a finer mesh size (1×1 cm^2) was placed under the floor of the cage to allow for collection of faeces. A removable 10 cm deep tray was placed below this mesh frame to collect urine, and was filled to a depth of 2 cm with paraffin oil to prevent evaporative water loss from urine.

Tortoises did not have access to drinking water for the duration of the trials. Two diets were fed to the tortoises in separate trials. Tortoises were fed the experimental diet and kept for three days in the cages to acclimate before the trials were started. Trials then lasted a further five (Trial 1) or seven (Trial 2) days. The experimental diet consisted of 200 g of chopped lucerne (*Medicago sativa*) (Trial 1) or 800 g of quartered tomatoes (*Solanum lycopersicum*) (Trial 2) daily. The differing amounts allowed tortoises to feed *ad libitum* and ensured food was always available. At 06 h00 the food was placed in food trays and placed in the cages with the tortoises. At 12 h00, any remaining food was collected and weighed. This was then replaced with fresh food and reintroduced into the tortoise cages. At 18 h00, any remaining food items were collected and weighed. No tortoises had access to food overnight. As a control, on each day, the experimental diet was placed in a food tray, left in the constant-temperature room and the daily evaporative moisture loss calculated.

Urine was collected from under the layer of paraffin oil using syringes and the total volume of urine measured to estimate urinary water loss (UWL). Samples of urine were placed in sterile, airtight containers and frozen until analysed. Duplicate 50 μL samples were analysed for total osmolality using a Wescor vapour pressure osmometer (Electron Microscope Centre, UKZN).

2.1.2.2. Trial 3. In order to replicate a more natural environment, in Trial 3, adult and juvenile leopard tortoises were housed individually in concreted outdoor enclosures measuring 5×1.5 m^2. Shade cloth covered half the roof of the enclosures so that tortoises could seek shade as necessary. Tortoises were kept on the experimental diet in the enclosures for three days to acclimate, before the trial. Trial 3 lasting a further 10 days was then started. Tortoises did not have access to drinking water for the duration of the trial. Daily weighed amounts of quartered tomatoes (typically 800 g for adult tortoises and 100 g for juveniles) were fed to the tortoises and maintained as for Trial 2. Controls of quartered tomatoes were used on a daily basis to estimate evaporative moisture loss from food.

2.1.3. Digestive parameters

Minimum faecal transit times of food ingested were determined as the time from first digestion to the first appearance of food items in excreta. Mean faecal transit times were determined when over 50% of the faecal sample consisted of the experimental dietary item. No markers were used because tomato skins and seeds were clearly identifiable in the faeces of tortoises. Similarly, lucerne was identifiable from its flowers and stalks.

For all trials, faeces were collected daily; however faecal water loss and faecal energy loss were calculated from faecal samples that were excreted over the same number of days as the trial, delayed by the mean gut transit time. Faeces were weighed and oven-dried at 60 °C for 5 days to determine faecal water loss (FWL). Total water loss (TWL) was then determined for tortoises in Trial 1 and 2, as the sum of UWL and FWL (excluding evaporative water loss).

Samples of the different trial diets were oven-dried at 60 °C for five days to determine moisture content and, together with all faecal samples, were analyzed for Gross Energy (GE) using bomb calorimetry with a DDS Isothermal CP500 bomb calorimeter (Department of Animal Science, UKZN). In addition, diets were further analysed for fat (Soxhlet

procedure using a Buchi 810 Soxhlet Fat Extractor), crude protein (Dumas Combustion Method using LECO FP2000 Nitrogen Analyser) and fibre content (fitted glass crucible method) following Helrich (1990).

Food intake (g gBM^{-1}) of Leopard tortoises was quantified as the daily difference between total mass of food provided and total mass of food remaining, per gram body mass (BM). Gross daily energy intake (GEI) of tortoises was determined by multiplying food intake by gross energy values. Water intake (mL gBM^{-1}) was calculated as the product of food intake (g) and the percentage of preformed water in the diet. Daily faecal energy loss (FE) was subtracted from gross daily energy intake (GEI) to determine the daily energy assimilated (DEA). Apparent assimilation efficiency (%) (AE) was calculated using the following Eq.:

$$AE(\%) = \frac{GEI - FE}{GEI} \times 100$$

2.2. Free-ranging tortoises

Fieldwork was conducted on a 5500 ha area of a 26,000 ha mixed commercial sheep and game farm in the De Aar District, Nama-Karoo biome, South Africa (31°04′ S, 23°41′ E). Leopard tortoises were located opportunistically when riding transects on horseback through the study area. While taking cloacal temperatures in the field, tortoises would sometimes urinate. Samples of this urine were collected and frozen on site to -4 °C. Gender of the individual and time and season in which urine was collected were recorded. Osmolality of these urine samples was determined using the same methods as described above for urine collected in the laboratory trials. Osmolality for field urine samples was compared with those found for captive tortoises.

2.3. Statistics

All descriptive statistical tests were calculated using STATISTICA software (Statsoft, USA). Analysis of variance (ANOVA) and repeated-measures analysis of variance (RMANOVA) were used to test comparisons between the diets and trials, and between days. The results from the first three days of all trials were ignored while the tortoises were habituated. *T*-Tests were used to compare initial and final body mass of tortoises on the respective trials. Kruskal-Wallis tests for non-parametric datasets were used to compare the total osmolality between captive and free-ranging tortoises.

3. Results

3.1. Comparison of laboratory diets

Lucerne had less preformed water, less protein, but more fat, sodium, potassium and carbon fibre than tomatoes, although the gross energy of the diets was roughly the same (Table 1).

3.2. Body mass of captive tortoises

Mean initial body mass of captive leopard tortoises was adult males 12.8±2.7 kg, adult females 10.5±1.8 kg and juveniles 542.5± 203.6 g. Tortoises in all trials showed slight variations in body mass during the trials, but no significant differences between initial body mass and final body mass were found (Trial 1: $t=0.26$, $df=10$; Trial 2: $t=0.59$, $df=6$; Trial 3: $t=0.192$, $df=22$; all trials $P>0.05$).

3.3. Gut transit rates

Gut transit rates differed widely between individuals and were shorter in those individuals that had a higher food intake. Minimum transit time of lucerne through the gut was 3.0-5.5 days (mean± SE=4.25±0.5), with 50% of the faeces consisting of lucerne (mean transit time) after a mean time of 8.75 days.
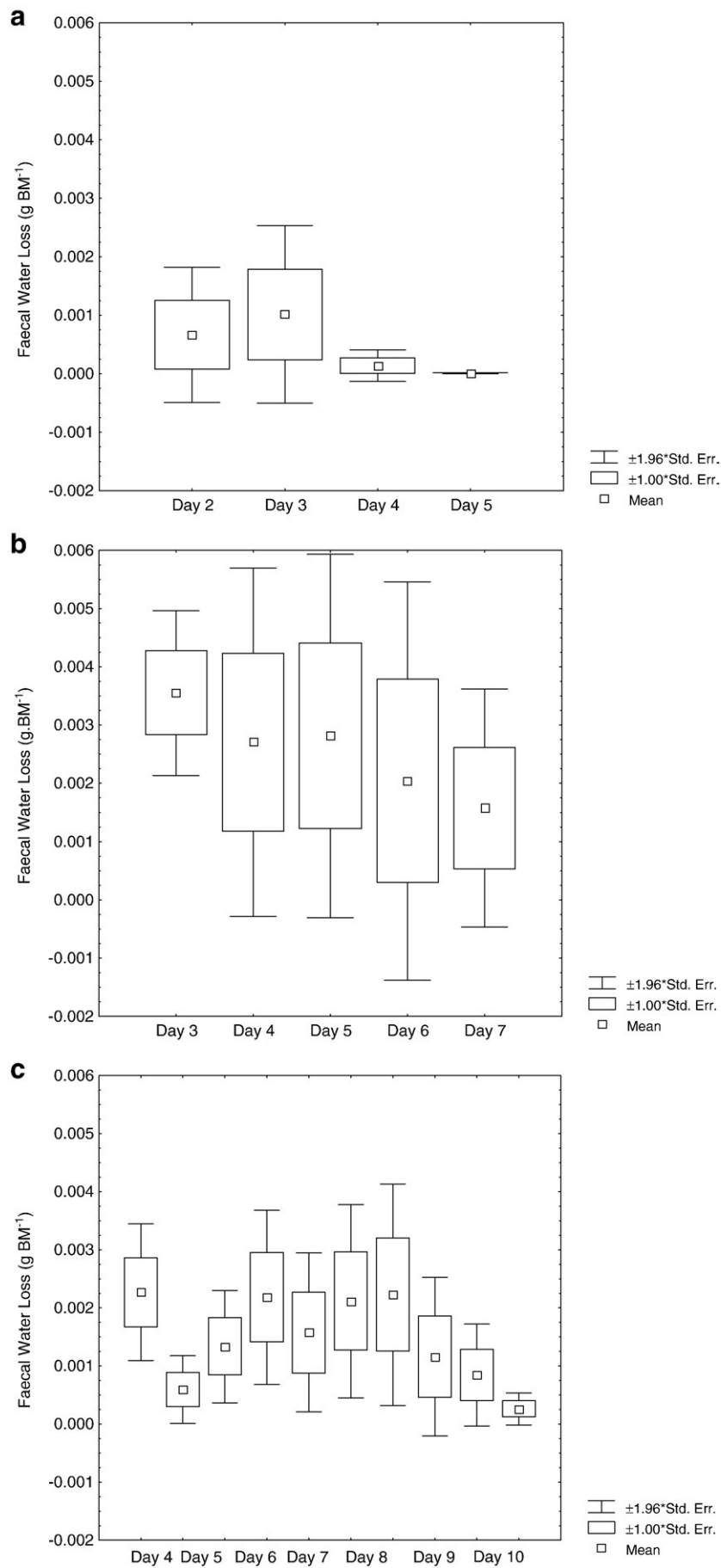
Minimum transit times of the experimental diet of pure tomato ranged from 0.75-1.86 days, with a mean±SE across individuals of 1.19±0.31 days. Mean transit time (50% of the faeces consisting of tomatoes) was much faster than in tortoises on a lucerne diet, being 1.5-3.75 days; mean±SE of 2.25±0.3.

3.4. Preformed water intake

Leopard tortoises did not have access to water for the duration of trials, and therefore, their source of water was from their food. Percentage preformed water in lucerne and tomato was 12.22% and 95.48% respectively (Table 1). Mean (±SE) daily intake of preformed water for leopard tortoises fed lucerne was $0.098 \times 10^{-3} \pm 0.0015 \times 10^{-3}$ mL gBM^{-1}. This was significantly different to the daily preformed water intake of tortoises fed tomatoes (ANOVA, $F_{(6,17)}$ =2.52, $P<0.05$) where the mean daily intake was $2.869 \times 10^{-3} \pm 0.204 \times 10^{-3}$ mL gBM^{-1}.

3.5. Food intake

Daily food intake (mean±SE) of leopard tortoises fed lucerne was $0.807 \times 10^{-3} \pm 0.091 \times 10^{-3}$ g gBM^{-1}, with no significant difference in food intake for the duration of the trial (RMANOVA, $F_{(8,2)}$=0.37, P=0.70). There was great individual variation with some individuals sometimes not eating.

Tortoises fed tomatoes had a significantly higher daily food intake (mean±SE) of $31.817 \times 10^{-3} \pm 2.062 \times 10^{-3}$ g gBM^{-1} (ANOVA, $F_{(6,17)}$= 2.32, $P<0.05$). There was no significant decrease in daily food intake over the length of the trial (RMANOVA, $F_{(5,55)}$=0.53, P=0.75).

3.6. Gross energy intake

Corresponding to amount of food eaten, daily gross energy intake (GEI) (mean±SE) for the diet trials was 0.0209±0.0023 KJ gBM^{-1} for tortoises fed lucerne (trial 1), a higher 2.922±0.012 KJ gBM^{-1} for tortoises fed tomatoes in trial 2, and a relatively high 3.621±0.012 KJ gBM^{-1} for tortoises fed tomatoes in trial 3.

There was a significant difference in the gross energy intake between tortoises fed lucerne and tortoises fed tomatoes (ANOVA, $F_{(6,17)}$= 2.61, $P<0.05$). However, there was no significant difference in the daily GEI for tortoises fed on lucerne (RMANOVA, $F_{(3,12)}$=0.57, P=0.64) (Fig. 1a), or tomatoes in trial 2 (RMANOVA, $F_{(4,20)}$=2.62, P=0.066) (Fig. 1b) or trial 3 (RMANOVA, $F_{(6,60)}$=2.1, P=0.057) (Fig. 1c).

3.7. Faecal energy loss

Faecal energy loss was significantly different between tortoises fed on the two diets (ANOVA, $F_{(6,17)}$=1.72, $P<0.05$). Leopard tortoises did not defecate every day in trial 1, so repeated measures test of changes in faecal energy loss could not be calculated. However, the mean±SE FE value obtained for trial 1 was 9.032±0.083 KJ gBM^{-1}. Daily FE loss decreased for the first few days of Trial 2, and then increased sharply again (Fig. 2a), with a mean±SE of 10.041±0.027 KJ gBM^{-1}, and no significant change over the days (RMANOVA, $F_{(5,5)}$=4.087, P=0.07).

In Trial 3, daily FE loss had a mean±SE of 10.562±0.055 KJ gBM^{-1}. Daily FE loss was fairly constant for the first three days of the trial, and then increased from the fourth day (Fig. 2b), but was not significantly different across the days (RMANOVA, $F_{(4,4)}$=0.991, P=0.50).

3.8. Daily energy assimilated

Tortoises fed lucerne had the lowest daily energy assimilation (DEA) (mean±SE of 3.847±0.254 KJ gBM^{-1}) that decreased over the

days of the trial, but not significantly so (Fig. 3a) (RMANOVA, $F_{(3,12)}$= 0.814, P=0.510). DEA decreased significantly for the first three days of trial 2, and then showed a significant increase on the last day (Fig. 3b) (RMANOVA, $F_{(4,20)}$=3.280, P=0.032), with a mean±SE of 5.481± 0.249 KJ gBM^{-1}. Tortoises fed tomatoes in trial 3 had mean±SE DEA of 6.785±0.197 KJ gBM^{-1}, with DEA increasing over the first 5 days of the trial and then decreasing for the last two (Fig. 3c), but with no significant change over the days of the trial (RMANOVA, $F_{(6,60)}$=1.038, P=0.409). DEA was significantly higher in tortoises fed tomatoes than in those fed lucerne (ANOVA, $F_{(6,17)}$=2.15, $P<0.05$).

3.9. Apparent assimilation efficiency

Apparent assimilation efficiency (taking body mass into account and expressed as a percentage) of leopard tortoises were significantly higher in those fed tomatoes to those fed lucerne (ANOVA, $F_{(6,17)}$= 1.72, P=0.017), although it was variable over the days of the trials and between individuals (Fig. 4). Tortoises in trial 1 had a mean±SE AE of 1.35±0.19%, compared with 1.43±0.11 in trial 2 and 3.63±0.31% in trial 3.

3.10. Water loss

3.10.1. Faecal water loss
Faecal water loss was relatively low on the first two days of trial 1, decreased further on the last two days (Fig. 5a), but showed no significant decrease over the trial (RMANOVA, $F_{(3,9)}$=0.99, P=0.44). The largest amount of faecal water was lost from leopard tortoises in trial 2, with the amount decreasing over the days of the trial (Fig. 5b), but the decrease was not significant (RMANOVA, $F_{(4,20)}$= 0.90, P=0.48). Daily faecal water loss of tortoises in trial 3 increased initially, and then decreased for the last three days of the trial (Fig. 5c), with the change of faecal water loss being significant over the time of the trial (RMANOVA, $F_{(6,77)}$=2.38, P=0.02). Faecal water loss was significantly lower in tortoises fed lucerne (ANOVA: $F_{(6,17)}$= 2.18, P=0.003), with a mean±SE of $4.472 \times 10^{-3} \pm 0.023 \times 10^{-3}$ mL gBM^{-1} compared with $8.126 \times 10^{-3} \pm 0.67 \times 10^{-3}$ mL gBM^{-1} and $6.263 \times 10^{-3} \pm 0.31 \times 10^{-3}$ mL gBM^{-1} for trial 2 and 3 respectively.

3.10.2. Urinary water loss
Leopard tortoises showed a steady decrease in the daily volume of water lost through urine in trials 1 and 2 (Fig. 6), with urinary water loss being greater in tortoises fed tomatoes. The mean±SE daily urinary water loss for tortoises fed lucerne was lower at 0.016± 0.08×10^{-3} mL gBM^{-1} than the mean of $0.022 \pm 0.01 \times 10^{-3}$ mL gBM^{-1} for tortoises fed tomatoes, but not significantly so (ANOVA, $F_{(4,9)}$=2.63, $P>0.05$).

3.10.3. Total water loss
Total amount of daily water lost by leopard tortoises (excluding evaporative water loss) in both trials decreased steadily (Fig. 7), but not significantly so for tortoises fed tomatoes (RMANOVA, $F_{(5,10)}$=1.84, P=0.19). There was no significant difference between the volume of total water lost by tortoises fed lucerne or tomatoes (ANOVA, $F_{(4,9)}$= 2.63, $P>0.05$), although the mean+SE $0.012 \pm 0.16 \times 10^{-3}$ mL gBM^{-1} of tortoises fed lucerne was lower compared with $0.026 \pm 0.15 \times 10^{-3}$ mL gBM^{-1} for tortoises fed tomatoes.

3.11. Osmolality of urine

Osmolality of urine from leopard tortoises fed lucerne showed an initial daily increase and then showed a sharp decrease from the third

Fig. 5. Change in the volume of faecal water lost per gram of body mass (mL gBM^{-1}) by leopard tortoises over the days of the trials where a. trial 1 (lucerne), b. trial 2 (tomato) and c. trial 3 (tomato, outdoor).

day (Fig. 8a), however there was no significant difference in urine osmolality over the days of the trial (RMANOVA, $F_{(4,12)}=0.96$, $P=0.46$). In contrast, urine osmolality of urine from tortoises fed tomatoes decreased slightly for the first five days, but then sharply increased (Fig. 8b), with a significant difference in urine osmolality of urine over the days of the trial (RMANOVA, $F_{(7,14)}=3.31$, $P=0.03$). The osmolality of urine from tortoises fed tomatoes had a higher mean±SE of 0.821± 0.318 Osmol compared with 0.376±0.120 Osmol for tortoises fed lucerne (Fig. 8), and were significantly different (ANOVA, $F_{(4,9)}=2.81$, $P=0.01$).

3.11.1. Free-ranging tortoises

Urine samples from free-ranging leopard tortoises were obtained on 26 occasions from 11 adult tortoises, 7 females and 4 males. Females weighed a mean±SE of 13.5±0.6 kg and males 12.6±0.2 kg. Osmolality (mean±SE) of urine from free-ranging tortoises in the Nama-Karoo was 0.47±0.05 Osmol, with a minimum osmolality of 0.16 Osmol, and a maximum of 1.23 Osmol. There was no significant difference between urine osmolality of females and males (Kruskal-Wallis ANOVA, $H_{(1,26)}=0.084$, $P=0.77$), between seasons (Kruskal-Wallis ANOVA, $H_{(3,26)}=2.12$, $P=0.55$), nor in relation to body mass (Kruskal-Wallis ANOVA, $H_{(9,25)}=5.40$, $P=0.79$). Osmolality of urine samples from free-ranging leopard tortoises was compared with that of captive held tortoises and no significant difference was found (Kruskal-Wallis ANOVA, $H_{(1,10)}=0.906$, $P=0.34$), however mean urine osmolality of the urine of captive tortoises (mean±SE of 0.72±0.14) was higher than that of free-ranging tortoises (mean±SE of 0.47±0.05).

4. Discussion

Over the duration of these trials, leopard tortoises showed no significant difference in loss or gain of body mass. This would indicate that the species has sufficient dietary plasticity to cope with a range of diet quality. The diet quality ranged from the high water, low fibre diet consisting of tomatoes to the low water, high fibre diet of lucerne. This
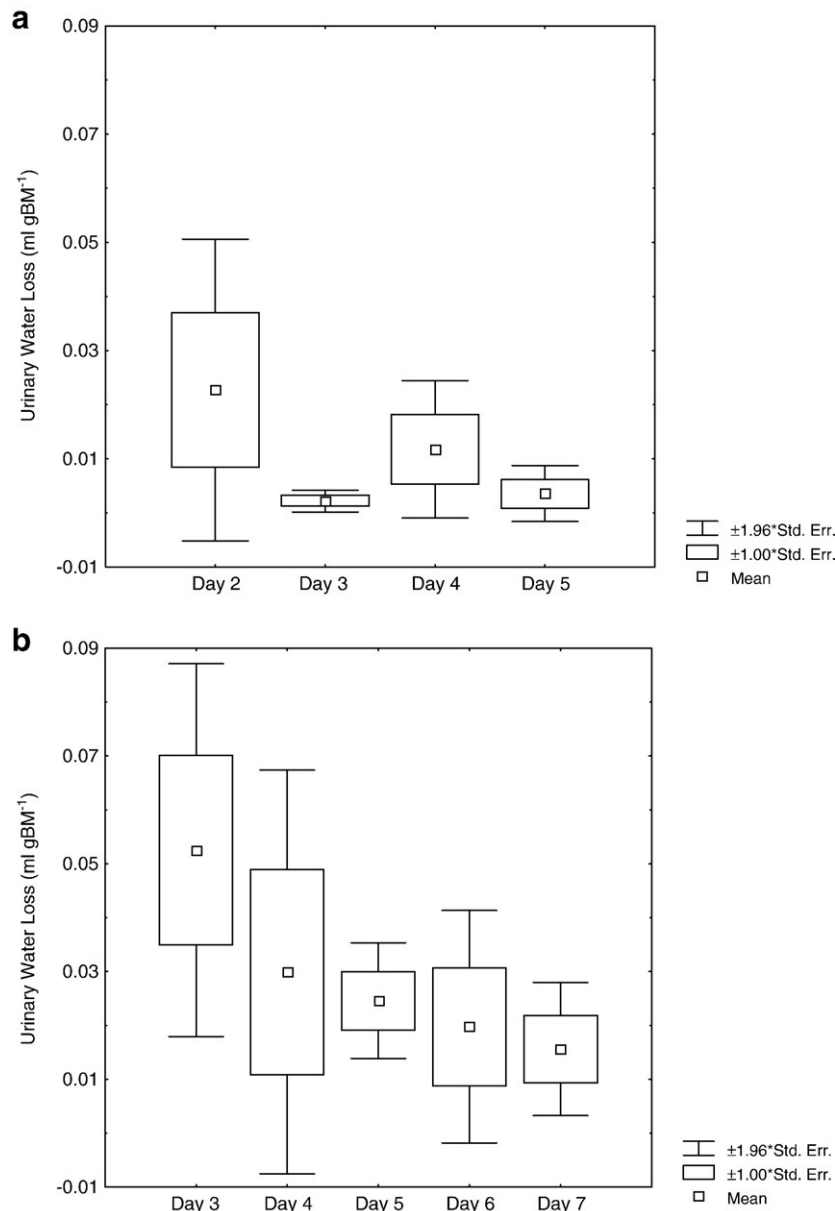

Fig. 6. Change in the volume of urinary water lost per gram of body mass (mL gBM^{-1}) by leopard tortoises over the days of a. trial 1 (lucerne), and b. trial 2 (tomato).

latter should have a longer gut transit time as the amount of fibre and preformed water in a diet influences the gut transit times, (Bjorndal, 1989). In this study, gut transit times were variable between individuals, however the mean transit time was much shorter in tortoises fed tomatoes. The gut transit time for the lucerne diet was in agreement with studies done by Taylor et al. (1996), who obtained a transit rate of 8 days for leopard tortoises, compared to this study where the mean transit time was 8.75 days in the lucerne trial. The gut transit time for the tomato diet was considerably shorter (mean transit time = 2.25 days). Hailey (1997) found minimal dietary differences in the gut transit rates of leopard tortoises, but obtained mean gut transit rates of 3.8 days when leopard tortoises were fed on kale.

Results showed differences in trials 2 and 3, although both related to tortoises on a tomato diet. Trial 2 took place inside a controlled environment room, while trial 3 took place in an outside environment. It is probable that the introduction of climatic and environmental variables in the outdoor trial stimulated the tortoises into a being more active and having a more natural feeding behaviour. This is evidenced by the increased food intake and resultant digestive processes compared to individuals kept indoors.

The ingested food, gross energy intake and faecal energy loss of tortoises on tomato diets were significantly higher than for those tortoises on a lucerne diet. However, the daily energy assimilated and assimilation efficiency between the two diets were comparable. As has already been pointed out, the gut transit time of the tortoises eating lucerne was significantly longer. Other generalist herbivores use lower food intake and increased gut transit time as a method of maximising nutrient extraction (Bjorndal, 1989). The increase in gut transit time results in greater digestive efficiencies and reduction of the amount of faecal energy lost (Bjorndal, 1987; Meienberger et al., 1993). Therefore, leopard tortoises being fed lucerne were able to assimilate comparable amounts of energy from their high fibre diet by having lower food intake and increasing gut transit time.

Water intake, faecal water loss and urine concentration of tortoises on the tomato diet were significantly higher than that of tortoises fed lucerne, however urinary and total water loss were comparable. The longer gut transit times of tortoises on a high fibre low preformed water diet allows them to use the retention time to absorb preformed water and lose significantly less faecal water (Bjorndal, 1989; Hailey, 1997). This further results in maintenance of urine concentration and the prevention of dehydration (Nagy and Medica, 1986). Tortoises on a
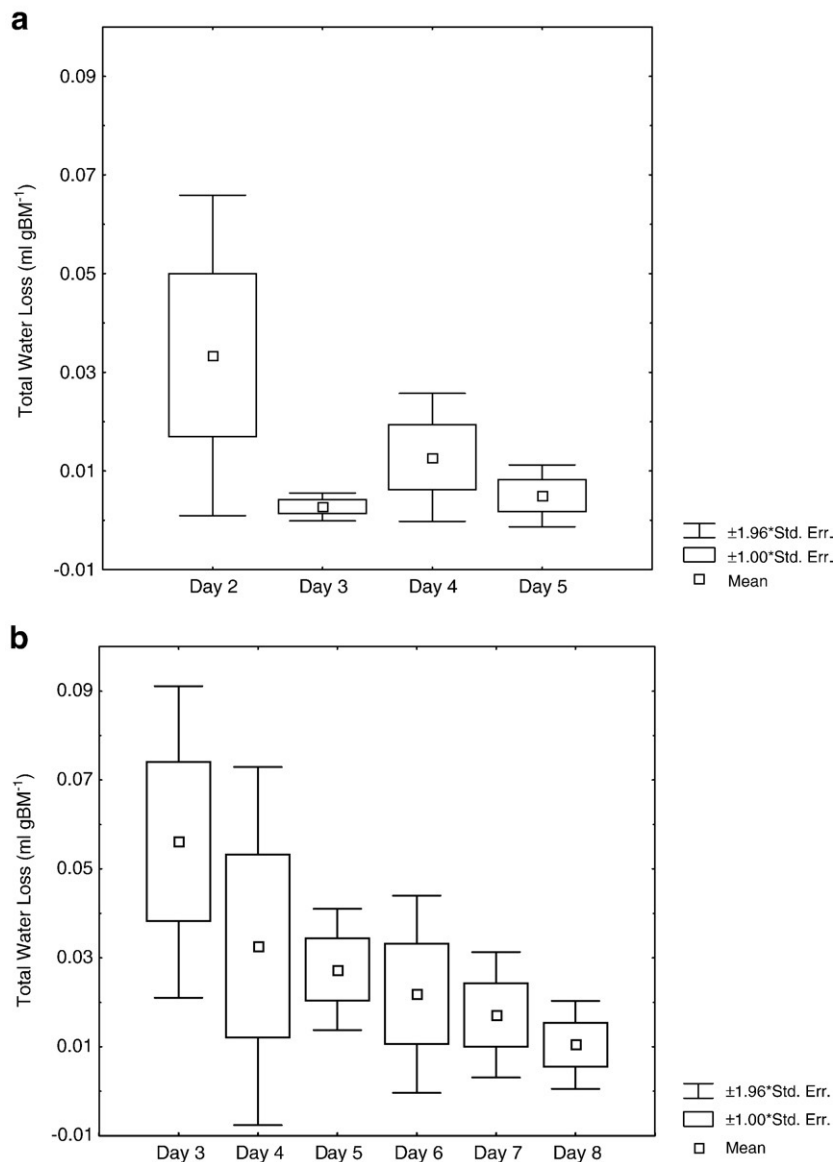

Fig. 7. Change in the total volume of water lost per gram of body mass (mL gBM^{-1}) by leopard tortoises over the days of a. trial 1 (lucerne), and b. trial 2 (tomato).
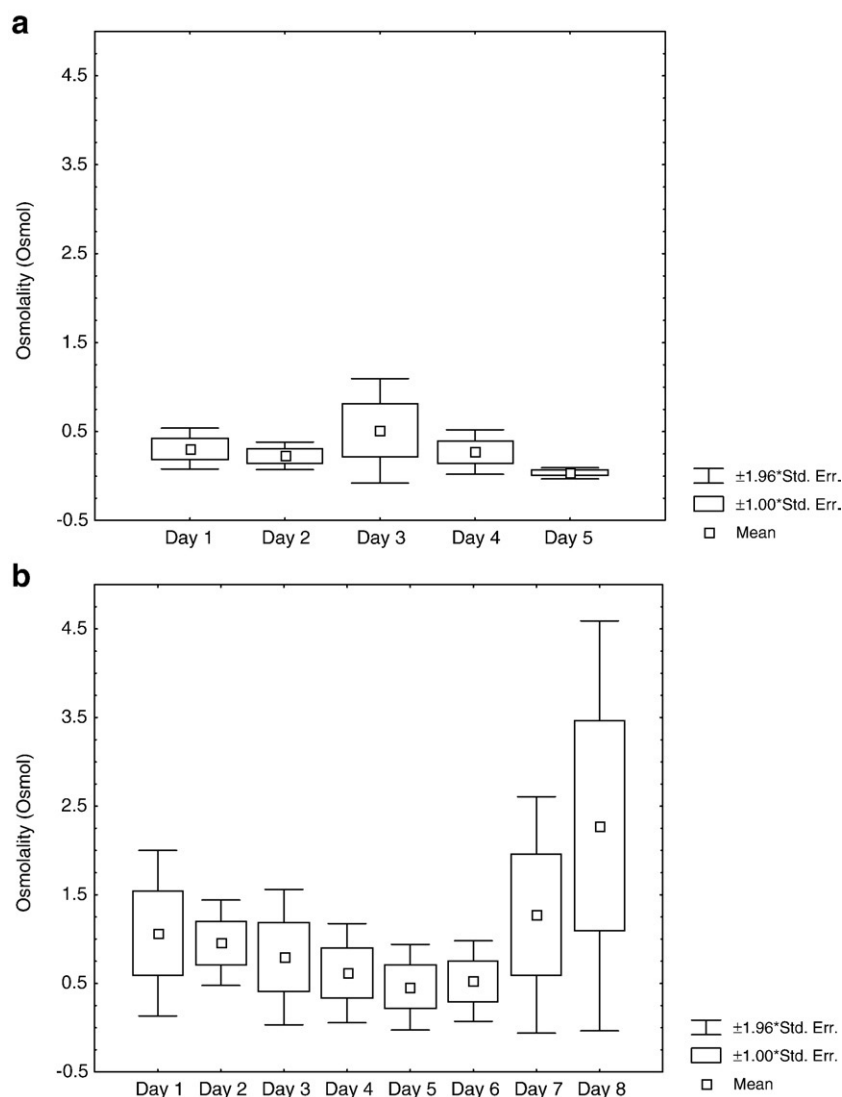
Fig. 8. Change in urine concentration (Osmol) from leopard tortoises over the days of the a. trial 1 (lucerne), and b. trial 2 (tomato).

low fibre, high preformed water content diet have less time to absorb preformed water, have a higher faecal water loss associated with the rapid gut transit times and this results in high urine concentrations (Nagy and Medica, 1986; Peterson, 1996a,b) as found in this study.

Urine osmolality in free ranging tortoises showed no significant differences with regard to body mass, between sexes or across the seasons. The lack of significant seasonal differences in osmolality implies that free ranging tortoises were able to prevent dehydration and maintain urine concentration despite seasonal variation in diet quality and water availability. This is further evidenced by the lack of significant differences between the urine osmolality of wild and laboratory tortoises. Indeed, laboratory tortoises had a mean urine osmolality that was slightly greater than wild tortoises, possibly due to the fact that their water intake was exclusively from their diets and free ranging tortoises would have had access to direct sources of drinking water.

The results show that tortoises fed two diets differing in preformed water and fibre content have significant differences in food intake, gut transit rate, assimilation efficiency, faecal and urinary water loss and urine concentration. The results further show that leopard tortoises have variable digestive parameters that can maximise water conservation depending on water availability and diet composition. This helps these tortoises to inhabit arid areas like the Nama-Karoo where

food quality and water availability are unpredictable. Henen et al. (1998) reported similar physiological and digestive flexibility in desert tortoises (*G. agassizii*) in response to unpredictable climatic variation as central to the ability of desert tortoises to survive droughts and take advantage of periods of resource abundance.

Acknowledgments

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