Research article

Effect of environmental temperature and diet on the digestive response of red-footed tortoise *Chelonoidis carbonaria* hatchlings

Pierina Mendoza[1,2], Camila Furuta[1], Ellen S. Dierenfeld[3,4] and Aulus C. Carciofi[5]

[1]Department of Animal Science, São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, 14884-900, Jaboticabal, São Paulo, Brazil
[2]Wildhunger-Wildlife Nutrition Advisory, Lima, Peru
[3]School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Nottingham, United Kingdom
[4]Zootrition Consulting, St. Louis, Missouri, USA
[5]Department of Clinic and Veterinary Surgery, São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, 14884-900, Jaboticabal, São Paulo, Brazil

Correspondence: Aulus C. Carciofi, email; aulus.carciofi@gmail.com

Abstract
Temperature effect on digestive response is still unknown in most reptile species as is the case with the red-footed tortoise (*Chelonoidis carbonaria*). Hatchlings were fed with two diets, one high in fiber (14.16% crude fiber, 39.20% neutral detergent fiber dry matter basis, DMB) and one high in starch (27.71% DMB), housed at 30°C or 20°C, to evaluate the temperature effect on food intake (FI), digesta passage, apparent digestive efficiency (Da), and growth. At 30°C the animals showed higher FI and digestible energy (DEI), as well as metabolic mass-specific intake of digestible nutrients and energy (DEImm, 99.48±14.30 versus 43.18±17.26 kJ kg-0.86 day-1; P<0.001); daily gain (0.98±0.26 versus 0.32±0.11 g day-1; P<0.001), and growth of carapace length (0.25±0.05 versus 0.09±0.02 mm day-1; P<0.001) and width (0.15±0.03 versus 0.05±0.01 mm day-1; P<0.001). DEI at 30°C was expressed: (R2=0.67). Non-diet effect was observed on digesta passage, however, at 20°C the transit (5.50±1.36 versus 3.60±1.05 days; P<0.01) and retention times (13.80±1.29 versus 8.90±1.15 days; P<0.001) were longer than at 30°C. Animals housed at colder conditions also presented lower gut content (30.39±13.39 versus 40.45±9.76 g Kg-1; P<0.05) and gut fill time (0.08±0.01 versus 0.02±0.01 g day-1; P<0.001). Da were similar between temperatures but due to the diet effect, hatchlings fed the high starch diet presented higher DM and energy coefficients. Environmental temperature influences the digestive response and growth of *C. carbonaria*. Overall digestive efficiency was temperature-independent but rather influenced by diet quality and composition.

Introduction

The red-footed tortoise *Chelonoidis carbonaria* is an opportunistic omnivorous species and its diet is strongly influenced by seasonality of food availability (Merchan et al. 1998; Moskovits 1985; Moskovits and Bjorndal 1990). The nutritional requirements of most reptiles have not been defined in detail, and research in this area has been limited (Puga-Torres et al. 2017). Although the red-footed tortoise consumes large quantities of fibre in free-ranging conditions, in captivity these animals are often fed diets rich in easily assimilated carbohydrates (simple sugars and starch), which are linked with faster growth rates and can increase the risk of developing metabolic disorders such as obesity, pyramiding growth or hepatic diseases (Ritz et al. 2010).

Physiological and morphological adaptations at a digestive level should be reflected in three interrelated animal responses: digestion efficiency, food intake, and digesta passage time, and this combination determines the 'digestive strategy' (Milton 1981). The successful ingestion of energy and its effective allocation are essential to an organism's activity, growth and reproduction (Nagy 1983; Secor 2001). Ectotherm activity patterns and physiological functions are generally related to climatic conditions, with temperature one of the principal factors (Lillywhite 1987; Peterson et al. 1998; Skoczylas 1970). In this context, the digestion rate, gut passage speed, absorption rate, biochemical pathways and secretory processes are sensitive to temperature in diverse reptile species (Dandrifosse 1974; Harlow et al. 1976).

Studies in different species of lizards, geckos, iguanas, snakes, and turtles have described that higher body temperatures can increase the frequency and amplitude of peristaltic contraction, digestive efficiency of dry matter and energy (Beaupre et al. 1993; Du et al. 2000; Harlow et al. 1976; Mackay 1968; Mader 1996). Also, species such as pond slider turtles *Trachemys scripta* presented faster growth rates and greater body size after thermal impact, explained by the diet quality and higher food intakes at higher temperatures (Parmenter 1980). On the other hand, low temperatures were linked with a decrease in appetite (Wang et al. 2002), slow or arrested peristalsis, and regurgitation cases (Regal 1966). Digestive efficiencies are linked with physiological and ecological aspects of the energy budget (Bedford and Christian 2000), and in some reptile species are significantly influenced by temperature (Beaupre and Zaidan 2012; Harwood 1979; McKinon and Alexander 1999; Qu et al. 2011; Stevenson et al. 1985), while in other species there is no temperature effect (Karasov and Diamond 1985; Tracy et al. 2006; Waldschmidt et al. 1986; Zimmerman and Tracy 1989). The characteristic appears to be species-dependent (McKinon and Alexander 1999).

The purpose of this study was to determine the thermal dependence of appetite, digestive response and growth rate of red-footed tortoise hatchlings. These parameters were evaluated at two thermal regimens with animals fed with two different diets by analysing food intake, minimum and maximum retention times, apparent digestive efficiency of nutrients and energy, body mass gain, and carapace growth (width and length). It was hypothesised that lower temperatures would decrease the appetite and in consequence the food intake, causing longer retention times. Thus, the ingested food would be exposed for a longer time to enzymes and/or fermentation during the digestive process, which would result in higher digestive efficiencies if enzyme secretion is not inactivated or downregulated. Due to reduced intake at low temperatures, however, it was anticipated that animals would show slower growth rates, independent of diet quality.

Materials and methods

All procedures, animal care, animal use and treatment were performed in accordance with the Animal Ethics and Use Committee of the Sao Paulo State University (UNESP, for its acronym in Portuguese), and the protocol of the study (no. 006095/19) was approved by this committee.

Experimental animals and husbandry
Twelve hatchlings (2–4 months of age), hatched in captivity and of unknown sex, were used; average body mass was 84±30 g. Animals were individually housed in cages measuring 0.29 × 0.13 × 0.40 m in two acclimatised rooms of 1.10 m³, with six cages in each room. An electric heater and a portable air conditioning system linked to thermostats were used to keep a constant temperature, and fluorescent lamps were connected to a timer to maintain the animals under a 12 h light:12 h dark photoperiod.

Temperature treatments
By considering that the red-footed tortoise shows better adaptation at 20–35°C with high moisture (Costa and Bérnils 2018; Wang et al. 2011), and different reptile species at 20°C display a slow digestion rate (Pafilis et al. 2007; Skoczylas 1978) and/or arrested peristalsis (Harwood 1979), two thermal regimens were evaluated: 20°C and 30°C. The thermostat in each acclimatised room was fixed at one thermal regimen. Internal temperature and moisture of each acclimatised room was registered twice a day with a digital hygro-thermometer. The study was separated into two 60-day experimental periods in a crossover design, with an interval of 3 days of rest between periods for animals to take an outdoor sunbath. The first week of each experimental period was used to acclimatise animals to the evaluated temperatures.

Experimental diets and feeding
Two gross energy isocaloric dry pelleted diets were evaluated, which presented similar nutritional composition with the exception of starch and fibre content: Diet 1 had a high level of crude fibre and neutral detergent fibre (NDF), and Diet 2 was high in starch content (Table 1). External inert indigestible markers of chromium oxide (Cr_2O_3; Merck, Darmstadt, Germany) and ferric oxide (Fe_2O_3; 99.5% Êxodo Cientifica, São Paulo-SP, Brazil) were added to experimental diets following different protocols. Digestibility was evaluated by adding Cr_2O_3 (green colouring) at 0.25% of the offered diets; animals were continually fed with chrome-labelled diets throughout all experimental periods. After one week of chromium diet adaptation, Fe_2O_3 (red colouring) was incorporated at 2.5% of the daily chrome-labelled offered food to study passage of the digesta (minimum and maximum retention times). A single dose of ferric-labelled food was given during the initial (day 8) and final (day 54) weeks of the digestibility evaluations. Throughout all trials, tortoises could eat and drink freely.

Hatchlings were randomly distributed across these two diets (six animals per diet), and hatchlings on each diet were divided in two groups to be maintained at 30°C and 20°C (three animals per diet per temperature in each experimental period) in a repeated-measures design where all animals were under both thermal regimens in different experimental periods. Animals were fed once daily, 2.50% or 1.13% of body mass per day at 30°C and 20°C, respectively. To obtain the daily dry matter intake (FI, g animal⁻¹ day⁻¹) and intake relative to metabolic body mass (IMM, %), the amount of offered and residual food was weighed daily with a scale (5 kg±0.01 g capacity).

Table 1. Chemical composition of dry matter (DM) of experimental diets for growing tortoises with different starch and fibre contents. 4.184 kilojoules (kJ)=1 kcal. Ingredient list: soybean meal 45%, wheat bran, sugarcane fibre, corn starch, poultry meal, wheat flour, beet pulp, alfalfa hay, corn grain, poultry fat, liquid palatant, cane yeast, whole flaxseed, dicalcium phosphate, vitamin-mineral premix, calcium carbonate, choline chloride, common salt, mould inhibitor, DL-methionine, antioxidant. Passage time evaluation: ferric oxide Fe_2O_3 was incorporated at 2.50% of the offered food with palatant.

Nutrients (%)	High fibre (HF)	High starch (HS)
Crude protein	25.0	26.0
Crude fibre	14.2	6.6
Neutral detergent fibre	39.2	24.9
Starch	16.1	27.7
Acid hydrolysed fat	7.0	6.5
Calcium	1.1	1.1
Phosphorus	0.7	0.7
Mineral matter (ash)	8.4	7.7
Gross energy (kJ g DM⁻¹)	19.5	18.9
Chromium oxide (Cr_2O_3)	0.25	0.25

Response variables
Apparent digestibility

Faecal collection started when the first red-marked faeces from the first addition of ferric oxide for digesta passage evaluations were observed. Collection was performed for at least 1 month and samples were stored at −20°C. Faeces were evaluated and scored from 1 (liquid) to 7 (hard, dry) (Mendoza et al. 2022 Supplementary material).

Diet and faecal samples were analysed to determine moisture content, dry matter, crude protein, acid-hydrolysed fat, starch and crude fibre (Association of Official Analytical Chemists 1995); unfortunately, faecal sample size was insufficient to analyse NDF content. The NDF analysis of the diets included a pre-treatment of the sample with heat-stable alpha-amylase and was expressed exclusive of residual ash (aNDFom; Udén et al. 2005). Concentrations of chromium oxide in diets and faecal samples were calculated by chromium determination via visible spectrometry (Labquest Bio 2000, Labtest Diagnóstica S.A., Lagoa Santa, Brazil) at 450 nm. In addition, gross energy content of faeces and diets were determined through bomb calorimetry on an automated diabetic calorimeter (Model 1281, Parr Instrument, Moline, USA).

Apparent digestibility (Da) coefficients of dry matter (Da_{DM}), acid-hydrolysed fat (Da_{FAT}), starch (DaST), crude fibre (Da_{CF}), crude protein (Da_{CP}) and gross energy (Da_{GE}) were calculated through the following equations (Harshaw 2012):

Da (Dry Matter,%)=100- (100 × (%Cr_2O_3 food) / (%Cr_2O_3 faeces))

Da (Nutrient,%)= 100 - (100× (%Nutrient in faeces × %Cr_2O_3 food)/ (%Nutrient in food× %Cr_2O_3 faeces))

Daily food intake and apparent digestibility coefficients were used to calculate and express the daily intake of digestible energy (DEI, kJ animal^{-1} day^{-1}), and mass-specific intake per gram of metabolic mass of digestible protein (DPI, g kg-0.86 day^{-1}), digestible acid-hydrolysed fat (DAI, g kg-0.86 day^{-1}), digestible starch (DSI, g kg-0.86 day^{-1}), digestible fibre (DFI, g kg-0.86 day^{-1}) and digestible energy (DEI_{mm}, kJ kg-0.86 day^{-1}). The reptile allometric exponent of 0.86, described by Bennett and Dawson (1976), was used for the metabolic body mass calculation.

Passage time, gut content and gut fill time
The first trial of passage time was initiated at the time when animals started eating the ferric-labelled food. We determined the minimal retention time as the period between when the marked food was eaten and the moment when the first red-marked faeces were observed, and maximal retention time was defined as the interval until resumption of Cr_2O_3 (green) coloured faeces (Van Weyenberg et al. 2006). The total gut content (g kg^{-1}) per gram of body mass in dry matter was calculated following the Holleman and White (1989) equations, and was expressed in relation to body mass:

V_N=F×RT

V=(V_N-(V_N/(1-A)))/(ln(1-A))

Where F=daily dry faeces output (g day^{-1}), RT=maximum retention time (days), VN=indigestible gut content, V=gut content and A=fractional digestibility of dry matter in the diet. Also, the gut fill time (g day^{-1}) was estimated by the following ratio:

Gut fill time=(FI×DC_{DM})/RT.

Biometry and weighing
To measure growth rates, straight length and width of the carapace (±0.01 mm) and body mass (±0.01 g) of the hatchlings were recorded weekly. For each tortoise, the biometric measurements were determined as carapace straight length, measured on the midline of the shell from the anterior midpoint of the nuchal scute to the posterior tip of the longest of the pair of posterior marginal scutes, and carapace straight width between the fifth and sixth marginal scutes.

Statistical analysis
A factorial design was used with a two-factor repeated measures model. A two-way ANOVA was tested: environmental temperatures (20°C and 30°C) and diet (high starch and high fibre). The effects of both factors and their interactions were evaluated on FI and DEI; Da of nutrients; mass-specific intake of digestible nutrients in terms of metabolic body mass (g kg-0.86 day^{-1}); minimum and maximum retention time (days); gut content (g kg^{-1}) and gut fill time (g day^{-1}); final body mass (g); daily gain of body mass (g day^{-1}); and daily growth of carapace length and width (mm day^{-1}). Also, the body mass effect was evaluated as a covariate in the analysis of FI, DEI, mass-specific intake of digestible energy (DEI_M), and minimum and maximum retention times. Da_{GE}, DEI and DEI_M—transformed by log_{10}—were expressed in terms of body mass (BM) by linear regression to determine the allometric exponent b adjusted at the integral of the aBM_b equation (b×log_{10} BM+a). All analyses were conducted in R Studio Software (version 3.2.3). A P value less than 0.05 was considered significant.

Results

Environmental temperature and food, nutrients and energy intake
The means of environmental temperature during both experimental periods for each thermal treatment were 28.7±0.4ºC and 20.3±1.4°C, close to the target temperatures of 30ºC and 20ºC.

The effects of temperature, diet and their interaction on FI, IMM (%), DEI, and mass-specific intake of digestible energy and nutrients (DEI_{mm}, DPI, DAI, DSI and DFI) in relation to metabolic body mass are given in Table 2. All these variables were significantly affected by the temperature, with all variables 55.7–56.9% lower at 20°C.

As expected, in addition to the temperature effect, DSI and DFI were influenced by diet; hatchlings fed with the high fibre diet consumed 36.20% less starch and 104.44% more fibre than those fed with the high starch diet. No factor interaction effects were observed.

Passage time, gut content, gut fill time and apparent digestibility
Data on passage times, gut content and gut fill time for both diets at each evaluated thermal regimen are given in Table 3. At the lower temperature (20°C), the speed of digesta passage was slower, which resulted in longer minimum and maximum retention times in both diets, without a significant effect of diet. In addition, a relatively high negative relationship between FI and digesta passage time was observed with correlation coefficients of −0.65 and −0.71 for minimum and maximum retention times, respectively. At 20°C the gut content and gut fill times were lower, and a diet effect on the gut content was observed; animals fed with the high fibre diet presented higher values.

The Da of evaluated nutrients, energy and dry matter, and faecal characteristics are presented in Table 4. Temperature and diet significantly influenced daily production of faeces (both fresh and dry matter), where hatchlings at 30°C and/or fed with the

Table 2. Mass-specific intake of dry matter and digestible nutrients and energy of growing tortoises *C. carbonaria* fed with diets containing different starch and fibre content at two different environmental temperatures (mean±SD). Mean value of 53 days at each diet and ambient temperature. 4.184 kilojoules=1 kcal. Digestible energy determined in vivo (kJ g DM^{-1})=high fibre 13.84 and high starch 14.76; Metabolic mass= body mass$^{0.86}$ (Bennett and Dawson 1976). *ns: not significant (P>0.05). †insufficient faecal sample: high starch at 20°C, n=3.

Variable	Temperature	Diet High fibre (n=6)	High starch (n=6)	Mean±SD	P-value Diet	Temperature	Interaction
Dry matter (g animal^{-1} day^{-1})	30°C	1.2±0.2	1.1±0.2	1.1±0.2	ns*	<0.001	ns
	20°C	0.5±0.3	0.5±0.2	0.5±0.2			
	Mean	0.9±0.4	0.8±0.4				
Digestible dry matter (g kg$^{-0.86}$ day^{-1})	30°C	5.6±1.0	5.7±0.7	5.6±0.8	ns	<0.001	ns
	20°C	2.4±1.0	2.5±1.0	2.4±0.3			
	Mean	4.0±1.9	4.1±1.9				
Food intake (%)	30°C	2.1±0.4	1.9±0.2	2.0±0.3	ns	<0.001	ns
	20°C	0.9±0.4	0.9±0.4	0.9±0.4			
	Mean	1.5±0.7	1.4±0.6				
Digestible protein (g kg$^{-0.86}$ day^{-1})	30°C	1.7±0.3	1.5±0.2	1.6±0.3	ns	<0.001	ns
	20°C	0.7±0.3	0.7 ±0.3	0.7±0.3			
	Mean	1.2±0.6	1.1±0.5				
Digestible fibre (g kg$^{-0.86}$ day^{-1})	30°C	0.5±0.1	0.2±0.0	0.4±0.2	<0.001	<0.001	0.05
	20°C	0.2±0.1	0.1±0.0	0.2±0.1			
	Mean	0.4±0.2	0.2±0.1				
Digestible starch, (g kg$^{-0.86}$ day^{-1})	30°C	1.3±0.3	1.8 ±0.2	1.5±0.3	0.02	<0.001	ns
	20°C	0.6±0.3	0.7±0.0†	0.6±0.2			
	Mean	1.0±0.5	1.5±0.6				
Digestible acid-hydrolysed fat (g kg$^{-0.86}$ day^{-1})	30°C	0.3±0.1	0.3±0.1	0.3±0.1	ns	<0.001	ns
	20°C	0.1±0.1	0.1±0.0	0.1±0.1			
	Mean	0.2±0.1	0.2±0.1				
Digestible energy (kJ animal^{-1} day^{-1})	30°C	15.3±2.7	15.6±2.7	15.4±2.6	ns	<0.001	ns
	20°C	6.3±2.8	6.7±1.9	6.5±2.3			
	Mean	10.8±5.4	11.1±5.2				
Digestible energy intake (kJ kg$^{-0.86}$ day^{-1})	30°C	98.6±17.5	100.3±12.0	99.5±14.3	ns	<0.001	ns
	20°C	42.2±18.5	44.2±17.6	43.2±17.3			
		70.3±34.1	72.2±32.6				

Table 3. Minimum and maximum retention times of growing tortoises *C. carbonaria* fed with diets containing different starch and fibre content at two different environmental temperatures (mean ± SD). *ns: not significant (P>0.05)

Variable	Temperature	Diet High fibre (n=6)	High starch (n=6)	Mean±SD	P-value Diet	Temperature	Interaction
Minimum retention time (days)	30°C	4.1±1.0	3.2±0.9	3.6±1.1	ns*	0.002	ns
	20°C	5.6±1.6	5.4±1.2	5.5±1.4			
	Mean	4.8±1.5	4.3±1.6				
Maximum retention time (days)	30°C	8.3±1.2	9.5±1.1	8.9±1.2	ns	<0.001	ns
	20°C	17.0±1.4	12.3±1.2	13.8±1.3			
	Mean	11.0±1.5	10.8±1.2				
Gut content (g kg^{-1})	30°C	45.5±10.5	35.4±6.1	40.5±9.8	<0.01	<0.05	ns
	20°C	53.5±35.3	22.7±10.1	30.4±13.4			
	Mean	42.9±10.7	29.0±10.4				
Gut fill time (g day^{-1})	30°C	0.1±0.0	0.1±0.0	0.1±0.0	ns	<0.001	ns
	20°C	0.0±0.0	0.0±0.0	0.0±0.0			
	Mean	0.1±0.0	0.1±0.0				

Table 4. Coefficients of total tract apparent digestibility and faecal characteristics of experimental diets for growing tortoises fed with different starch and fibre content at two different environmental temperatures (mean±SD). Digestible energy determined in vivo (kJ g DM^{-1})=high fibre 13.84 and high starch 14.76. *ns: not significant (P>0.05). †insufficient faecal samples: high starch at 20°C, n=3. ‡Non parametric Kruskal-Wallis test.

| Variable | Temperature | Diet | | Mean±SD | P-value | | |
		High fibre	High starch		Diet	Temperature	Interaction
Coefficient of total apparent digestibility (%)							
Dry matter	30°C	71.1±3.4	79.5±1.7	75.3±5.1	<0.001	ns*	ns
	20°C	70.5±4.4	78.1±3.7	74.3±5.5			
	Mean	70.8±3.8	78.8±3.0				
Acid-hydrolysed fat	30°C	59.7±10.0	56.5±6.6	59.8±9.7	ns	ns	ns
	20°C	60.8±5.1	52.3±8.9	57.4±7.7			
	Mean	60.2±7.6	56.9±10.1				
Starch	30°C	99.9±0.1	99.9±0.1	99.9±0.1	ns	ns	ns
	20°C	99.9±0.1	99.9±0.0†	99.9±0.1			
	Mean	99.9±0.1	99.9±0.1				
Crude fibre	30°C	42.8±9.1	41.0±1.81	42.0±6.6	ns	ns	ns
	20°C	44.0±7.9	37.6±13.1	42.4±8.8			
	Mean	43.4±8.1	40.1±5.8				
Crude protein	30°C	84.8±1.4	82.5±2.3	83.6±2.2	0.003	ns	ns
	20°C	84.2±3.2	80.9±4.0	82.6±3.9			
	Mean	84.5±2.3	81.7±3.2				
Gross energy	30°C	71.2±3.5	79.0±1.9	75.1±4.9	<0.001	ns	ns
	20°C	70.7±4.0	77.6±3.8	74.1±5.2			
	Mean	70.9±3.6	78.3±2.9				
Faecal characteristics							
Dry matter (DM, %)	30°C	46.5±7.7	44.5±6.3	45.5±6.8	ns	ns	ns
	20°C	48.6±7.5	40.1±8.5	44.4±8.9			
	Mean	47.6±7.4	42.3±7.5				
Faecal score[1]	30°C	1.8±0.4	2.2±0.4	2.0±0.4	<0.01	ns	ns
	20°C	2.0±0.2	2.9±0.6	2.4±0.6			
	Mean	1.9±0.3	2.6±0.6				
Faeces production (g day^{-1}; dry matter basis)	30°C	0.3±0.1	0.2±0.1	0.3±0.1	<0.001	<0.001	ns
	20°C	0.2±0.1	0.1±0.0	0.1±0.0			
	Mean	0.2±0.1	0.1±0.1				
Faeces production (g day^{-1}; as collected)	30°C	0.7±0.2	0.4±0.1	0.5±0.2	<0.001	<0.001	ns
	20°C	0.4±0.2	0.2±0.1	0.3±0.2			
	Mean	0.5±0.3	0.3±0.1				

high fibre diet excreted more faeces. In this context, animals at 20°C produced about half as much faeces (47.72%) as those at the higher temperature. Problems of insufficient faecal samples were reported in animals housed at 20°C and fed with high starch levels. Temperature had no significant effect on dry matter content of faeces, faecal score, nutrients or energy digestibility coefficients.

Diet influenced the Da$_{DM}$, Da$_{GE}$, Da$_{CP}$ and faecal score. The first two parameters were lower in hatchlings fed with the high fibre diet; these animals showed a better faecal score with firm faeces that was not hard, but was pliable with little or no residue left on the ground after collection. Also, these animals presented a higher Da$_{CP}$. No effect was detected for the interaction between temperature and diet factors.

Body mass effect
Initial and final body mass, daily gain of body mass, and daily growth of carapace (length and width) of hatchlings fed with both diets at each temperature are shown in Table 5. The initial and final body mass did not show a significant effect of temperature or diet. The daily gains of body mass and carapace growth were, however, significantly affected by temperature independent of the diet, with values at 20°C only about two-thirds (67.35–64.00%) those at the higher temperature. A non-significant effect was determined for factor interaction.

FI, DEI and Da$_{GE}$ showed a weak positive correlation with body mass (0.23–0.36), while DEI$_{mm}$ presented weak negative correlation with body mass (−0.26). In terms of passage time, the

Table 5. Initial and final body weight of growing tortoises C. carbonaria fed with diets of different starch and fibre content at two different environmental temperatures (mean±SD). Mean value of 53 days. *ns: not significant (P>0.05).

Variable	Temperature	Diet High fibre	Diet High starch	Mean±SD	P-value Diet	Temperature	Interaction
Initial body mass (g)	30°C	89.1±1.5	89.1±1.4	89.1±1.4	ns*	ns	ns
	20°C	93.3±1.4	107.1±1.3	100.0±1.3			
	Mean	91.6±1.4	97.7±1.3				
Final body mass (g)	30°C	148.2±60.6	143.9±34.4	135.4±30.8	ns	ns	ns
	20°C	124.6±39.1	123.9±24.7	124.3±31.1			
	Mean	124.9±32.0	133.9±30.4				
Body mass gain (g day⁻¹)	30°C	1.0±0.3	1.0±0.2	1.0±0.3	ns	<0.001	ns
	20°C	0.3±0.1	0.3±0.1	0.3±0.1			
	Mean	0.7±0.4	0.6±0.4				
Straight carapace length (mm day⁻¹)	30°C	0.3±0.1	0.3±0.0	0.3±0.1	ns	<0.001	ns
	20°C	0.1±0.0	0.1±0.0	0.1±0.0			
	Mean	0.2±0.1	0.2±0.1				
Straight carapace width (mm day⁻¹)	30°C	0.1±0.0	0.2±0.0	0.2±0.0	ns	<0.001	ns
	20°C	0.1±0.0	0.1±0.0	0.1±0.0			
	Mean	0.1±0.1	0.1±0.1				
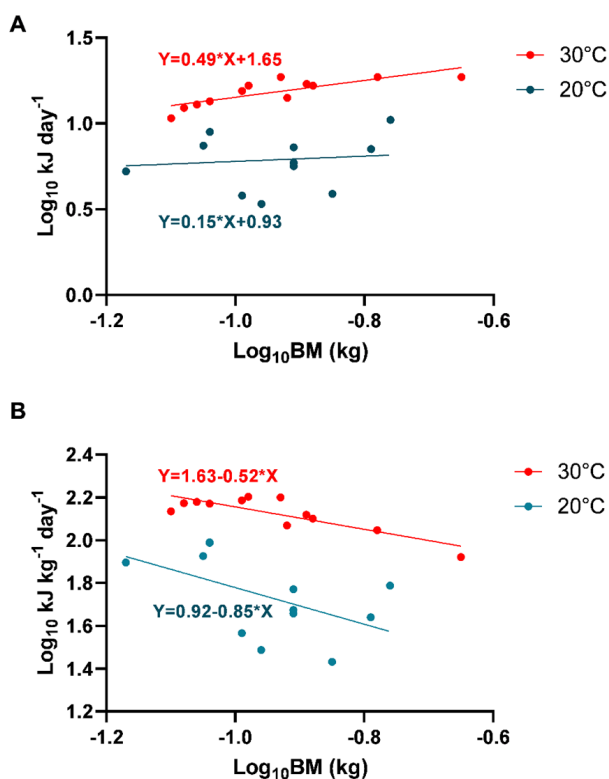
Figure 1. Linear regression of body mass (BM, kg) with the log10 of A. the daily intake of digestible energy (kJ day⁻¹) and B. mass-specific intake of digestible energy (kJ kg⁻¹ day⁻¹), at both evaluated temperatures in hatchlings from two to four months of age.

retention time of digesta presented a weak negative correlation (−0.30). When these variables were analysed with body mass as a covariate, it was possible to observe significant influences on Da_{GE} (P=0.004), DEI (P=0.013), DEI_{mm} (P=0.003) and retention time (P=0.031). In addition, the equations obtained from linear regressions of log_{10} DEI, DEI_M and BM at 30°C were:

log_{10} DEI (kJ day⁻¹)=0.49×log_{10} BM (kg)+1.65 (adjusted R^2=0.67; P=0.001), log_{10} DEI_M (kJ kg⁻¹) day¹=-0.52×log^{10} BM (kg)+1.63 (adjusted R^2=0.70; P=0.001).

At 20°C, body mass, DEI and DEI_M had no interaction (adjusted R^2=0.01 and 0.28, respectively; P>0.05) (Figure 1).

Discussion

The obtained daily food intakes related to body mass (0.9 to 1.5%) at both temperatures were higher than reported in some adult tortoises (0.17% to 0.22%; Bjorndal 1987; Carlos et al. 2016). This difference can be explained by age, because younger animals have greater mass-specific metabolic rates which result in higher food intake in terms of body mass, in comparison to adults. Most previous studies that evaluated the temperature effect force-fed the animals, making it impossible to determinate voluntary food intake (Harlow et al. 1976; Hazard et al. 2009; Zimmerman and Tracy 1989). Studies of lizard, tortoise and turtle species (Carlos et al. 2016; De La Ossa et al. 2009; McConnachie and Alexander 2004; Waldschmidt et al. 1986) described significantly greater food intakes at higher temperatures due to increased metabolism (Bentley and Schmidt-Nielsen 1966), similar to the results reported in the current study with regard to mass-specific daily intakes of food, digestible nutrients and digestible energy in terms of metabolic body mass.

Contrary to reports in other reptile species (Harwood 1979; McConnachie and Alexander 2004), cases of distress, reduced food intake or death were not observed at the higher temperature (30°C). Also, at 20°C, no negative effects were noted, compared to other reptile species where despite declining animal activity and appetite, cases of regurgitation and inanition were observed (Beaupre et al. 1993; De La Ossa et al. 2009; Harwood 1979; Stevenson et al. 1985), which has been interpreted as a mechanism to avoid a possible putrefaction process in the gut (Regal 1966). However, a reduced passage time was observed at the higher temperature, similar to reports in snakes (Bedford and Christian 2000), tortoises (Sadeghayobi et al. 2011), and lizards (McConnachie and Alexander 2004; McKinon and Graham 1999; Pafilis et al. 2007). Reductions of 34.54% and 35.51% of minimum and maximum retention times, respectively, were associated with an increase of 10°C, which is lower than differences described in tortoises (40.9%) and lizards (74%) with increases of 5°C and 10°C (Sadeghayobi et al. 2011; Waldschmidt et al. 1986). The higher gut fill time and lower gut content observed at 30°C may be related to greater digestion rates, explained by the increase in the frequency and amplitude of peristaltic contractions at higher temperatures (Angilletta 2001; Meienberger et al. 1993; Riddle 1909). On the contrary, in ectotherms, low temperature does not stimulate gut peristalsis (Meienberger et al. 1993) due to the absence or low level of food intake, which could cause longer passage times at 20°C.

Despite the temperature effect, the mean minimum retention time (4.6 days) was within the range reported for *C. carbonaria* (3.6–6.1 days; Madera-Vergara et al. 2010). In relation to maximum retention time, the mean value (10.9 days) was close to values reported for herbivorous reptiles (from 6.2 to 14.3 days; Barboza 1995a; Bjorndal 1987; Hatt et al. 2005; Hazard et al. 2009).

In addition to temperature, the passage time is affected by different factors such as composition, quality and volume of the diet (Bjorndal 1989; Brand et al. 1999). Contrary to longer retention times reported in red-footed tortoises fed diets with high fibre content (Bjorndal 1989), no diet effect was observed in the current study. However, greater gut content and lower digestibility efficiencies were observed in animals fed with the high fibre diet, consistent with previous reports in desert *Xerobates agassizii* and yellow-footed *Geochelone denticulata* tortoises (Barboza 1995b; Bjorndal 1989). Although the fibre represents a physical disruption due to mastication challenges (Barboza 1995b; Bjorndal et al. 1990; Throckmorton 1973), fibre digestibility was similar to that in herbivorous reptiles (37–86%; Tracy et al. 2006) and was not affected by diet at either temperature. On the other hand, the high level of fibre showed a significantly lower gross energy digestibility. However, animals on both diets showed similar digestible energy intake even when they consumed similar dry matter, which could be explained by the slightly greater gross energy content in the high fibre diet (19.5 kJ g DM^{-1} versus 18.9 kJ g DM^{-1}). This gross energy difference compensated for the lower digestion efficiency produced by the high fibre content. Fermentation in the hindgut covers between 15% and 30–40% of energy requirements in herbivorous reptiles (McBee and McBee 1982; Bjorndal 1987), which explains the importance of fibre for energy balance in this species.

Basic requirements to maintain an efficient gut microflora are body temperature (preferably high), constant food supply, slow passage of digesta, anaerobic conditions, gut pH control and removal of fermentation waste products (Bjorndal 1987). Greater fluctuations in environmental temperatures and long periods of hibernation and/or aestivation in some reptiles may make it difficult to maintain efficient microflora under those conditions, and re-inoculation by coprophagic behaviour may be necessary (Bjorndal 1987; Hazard et al. 2009), although the prevalence of

this remains unknown. Temperature effects of enzymatic affinity have been described, such as increased proteolytic activity at preferred temperatures in carnivorous reptiles (Diefenbach 1975), decreased secretion of gastric juice at declining temperatures in herbivorous reptiles (Wright et al. 1957), and greater apparent digestive efficiencies of sugars and lipids at higher temperatures due to sugar-digesting enzymes and lipase activity (Beaupre et al. 1993; Harwood 1979) and bile acids secretion (Pafilis et al. 2007) in lizards. All of this demonstrates that digestive efficiency is clearly temperature-dependent (Harlow et al. 1976; Ruppert 1980; Troyer 1987). However, the standard digestive efficiency of the majority of ectotherms has not been studied, nor have the effects of temperature on these processes (McKinon and Alexander 1999).

Sibly (1981) reported that in tortoises, digestive efficiency at lower temperatures could actually be improved due to the increase in fermentation time in the hindgut associated with longer digesta retention time (Mader 1966; Wallach and Boever 1983). Also, proteolytic enzymatic actions require extended time to complete the digestion of proteins (Skoczylas 1978). However, the obtained thermal independence of the digestive efficiency of nutrients in the present study is similar to reports in reptiles with both different (Beaupre et al. 1993; Beaupre and Zaidan 2012; Harwood 1979) and similar feeding habits (Carlos et al. 2016; McConnachie and Alexander 2004; McKinon and Alexander 1999). The decrease in digesta passage speed at lower temperatures may explain this independency. As McConnachie and Alexander (2004) expressed, the digestion and absorption process happen at slower rates and the digesta passage occurs at a corresponding rate, which allows animals to be able to more completely digest the food. At higher temperatures the gut content is lower and the gut fill time is shorter, so even when less enzymatic activity is needed, the effectiveness of digestive enzymes increases as the exposure time of food to enzymatic action decreases (Harwood 1979); thus, at lower temperatures, in spite of reduced enzymatic efficiency, the animal is able to compensate through longer retention time of digesta.

A positive effect of temperature was observed on the daily growth rates of carapace length and width, and daily body mass gain, similar to faster growth rates of body mass and length in some tortoises and snakes (Beaupre and Zaidi 2012; Mitchell et al. 2012). As described, at higher temperatures, animals consumed significantly more digestible energy and nutrients, which explains the faster growth rates for body mass and length. The conversion rate shows that hatchlings at 30°C were more efficient and required less digestible energy to gain 1 g of body mass (15.72 kJ day^{-1} g^{-1}) and 1 mm increase in carapace length (61.64 kJ day^{-1} mm^{-1}) and width (102.73 kJ day^{-1} mm^{-1}) than those at 20°C (20.28 kJ day^{-1} g^{-1}, 72.11 kJ day^{-1} mm^{-1}, and 129.8 kJ day^{-1} mm^{-1}).

Humidity means of 52% and 58% were recorded in the 30°C and 20°C chambers, respectively. However, both chambers showed great variability (±15%). Although humidity and temperature are related, it is not possible to elucidate whether the variables that were affected by temperature, such as carapace growth (length and width), were also affected by humidity because humidity was not controlled during the experiment.

The relationships of food intake and passage time with body mass are not clearly established. The results show a weak positive correlation of body mass with food intake, and a negative relationship with minimum and maximum retention times, a pattern also seen in lizards and tortoises (Hamilton and Coe 1982; McConnachie and Alexander 2004; Meienberger et al. 1993), but contrary to previous studies that reported longer passage times in juveniles than in hatchlings (Barboza 1995b; Meienberger et al. 1993; Tracy et al. 2006; Zentek and Dennert 1997). Despite those correlations, a non-significant effect of body mass on minimum

retention time was observed, which agrees with reports in red and yellow-footed tortoises (Bjorndal 1987, 1989). Multiple interacting variables must be considered in evaluating these conflicting results across various reptile species. A significant body mass effect on digestion efficiency was observed, similar to adult and juvenile desert tortoises (Hazard et al. 2009), but contrary to the non-significant effect of body size on digestibility for organic matter, energy, nitrogen, and cell wall constituents described in red-footed tortoises (Bjorndal 1989). Also, body mass presented a weak positive correlation and significant effect on DEI, and a negative relationship with DEIM, in direct opposition to reports of juvenile red and yellow-footed tortoises (Bjorndal 1989). As mentioned previously, older animals (juveniles versus hatchlings) have lower mass-specific metabolic rates, which reduces the energy demand related to the body mass and may underlie these findings. Nutrient partitioning may vary in different tissues at different growth stages; juveniles, for example, may gain greater proportions of less metabolically active tissue (i.e. carapace and plastron mass) compared with hatchlings, leading to varying energy demands. Further detailed research is needed to better understand these interpretations.

Nagy et al. (1999) evaluated 55 diverse species of reptiles in free-ranging conditions and indicated allometric slopes of 0.889 (insectivorous, omnivorous, herbivorous, and carnivorous) and 0.813 (herbivorous) for predicting rates of daily energy expenditure and daily food requirements. At 20°C, b values of 0.80, 0.77 and 0.86 have been reported for lizards, snakes, and turtles, respectively (Bennett and Dawson 1976) in terms of metabolic rate. Although these values are higher than the obtained allometric exponent of DEI at 30°C (0.49), some reptile species such as lizards and squamates have shown lower allometric exponents ranging from 0.609 to 0.824 (Andrews and Pough 1985; Roe et al. 2005). Use of general allometric formulas have resulted in overestimated energy requirements, such as in juvenile leopard tortoises *Geochelone pardalis*, where the average metabolic energy intake was 70% of that predicted for herbivorous reptiles (Higgins and Edwards 2009). Studies of digestive physiology of ectotherms are critical to the construction of physiologically structured models (DeAngelis et al. 1991; Dunham 1993) which require specific information about energy relations of individuals, including energy processing capacity and the factors that can affect it (Beaupre et al. 1993).

In conclusion, the present study indicates that at higher temperatures, red-footed tortoise hatchlings increase their food intake, speed of digesta passage, daily body mass gain and growth rates of carapace (length and width). Contrary to the hypothesis, digestive efficiency was not affected by temperature. Gut content was lower and gut fill time faster at the higher temperature; this appears to be temperature-independent and suggests a non-significant effect on enzyme activity. This indicates that this species has a very flexible digestive strategy, to keep a constant digestion efficiency throughout thermal variations, even at lower temperatures when the food intake decreases. However, the metabolic efficiency is affected, showing a slower growth rate and more digestible energy required to support growth per mass and length unit. Nonetheless, high content of dietary crude fibre significantly decreased the dry matter and energy digestive efficiency.

Acknowledgements

References

Andrews R.M., Pough F.H. (1985) Metabolism of squamate reptiles: Allometric and ecological relationships. *Physiological Zoology* 58(2): 214–231. doi:10.1086/physzool.58.2.30158569

Angilletta M.J. Jr. (2001) Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* 82(11): 3044–3056. doi:10.1890/0012-9658(2001)082[3044:TAPCOE]2.0.CO;2

Association of Official Analytical Chemists (1995) *Official Methods of Analysis, 16th edition*. Washington DC: Association of Official Analytical Chemists.

Barboza P.S. (1995a) Nutrient balances and maintenance requirements for nitrogen and energy in desert tortoises (*Xerobates agassizii*) consuming forages. *Comparative Biochemistry and Physiology Part A* 112(3–4): 537–545. doi:10.1016/0300-9629(95)02023-3

Barboza P.S. (1995b) Digesta passage and functional anatomy of the digestive tract in the desert tortoise (*Xerobates agassizii*). *Journal of Comparative Physiology Part B* 165: 193–202. doi:10.1007/BF00260810

Beaupre S.J., Dunham A.E., Overall K.L. (1993) The effects of consumption rate and temperature on apparent digestibility coefficient, urate production, metabolizable energy coefficient and passage time in canyon lizards (*Sceloporus merriami*) from two populations. *Functional Ecology* 7(3): 273–280. doi:10.2307/2390205

Beaupre S.J., Zaidan F. (2012) Digestive performance in the timber rattlesnake (*Crotalus horridus*) with reference to temperature dependence and bioenergetic cost of growth. *Journal of Herpetology* 46(4): 637–642. doi:10.1670/11-108

Bedford G.S., Christian K.A. (2000) Digestive efficiency in some Australian pythons. *Copeia* 2000(3): 829–834. doi:10.1643/0045-8511(2000)000[0829:DEISAP]2.0.CO;2

Bennett A.F., Dawson W.R. (1976) Metabolism. In: Gans C., Dawson W.R. (eds.). *Biology of the Reptilia Vol. 5*. Physiology. London, England: Academic Press, 127–223.

Bentley P.J., Schmidt-Nielsen K. (1966) Cutaneous water loss in reptiles. *Science* 151(3717): 1547–1549. doi:10.1126/science.151.3717.1547

Bjorndal K.A. (1987) Digestive efficiency in a temperate herbivorous reptile, *Gopherus polyphemus*. *Copeia* 1987(3): 714–720. doi:10.2307/1445664

Bjorndal K.A. (1989) Flexibility of digestive responses in two generalist herbivores, the tortoises *Geochelone carbonaria* and *Geochelone denticulata*. *Oecologia* 78: 317–321. doi:10.1007/BF00379104

Bjorndal K.A., Bolten A.B., Moore J.E. (1990) Digestive fermentation in herbivores: Effect of food particle size. *Physiological Zoology* 63(4): 710–721. doi:10.1086/physzool.63.4.30158172

Brand S.J., Lanyon J.M., Limpus C.J. (1999) Digesta composition and retention times in wild immature green turtles, *Chelonia mydas*: A preliminary investigation. *Marine and Freshwater Research* 50(2): 145–147. doi:10.1071/MF98033

Carlos N., Sassi P., Kubisch E., Cona M., Campos C. (2016) ¿La temperatura afecta la eficiencia digestiva en la tortuga terrestre argentina (*Chelonoidis chilensis*)? I Congreso Argentino-Paraguayo de Herpetología 2016 [Conference paper].

Costa H.C., Bérnils R.S. (2018) Répteis do Brasil e suas Unidades Federativas: Lista de espécies. *Herpetologia Brasileira* 7(1): 11–57.

Dandrifosse G. (1974) Digestion in reptiles. In: Florkin M., Scheer B.T. (eds.). *Chemical Zoology. Amphibia and Reptilia Vol. 9*. New York, New York: Academic Press, 249–270.

DeAngelis D.L., Godbout L., Shuter B.J. (1991) An individual-based approach to predicting density-dependent dynamics in smallmouth bass populations. *Ecological Modelling* 57(1–2): 91–115. doi:10.1016/0304-3800(91)90056-7

De La Ossa J.V., Vogt R.C., Schneider L., De La Ossa A.L. (2009) The influence of temperature on the feeding behaviour of *Peltocephalus dumerilianus* (Testudines Podocnemidae). *Revista MVZ Córdoba* 14(1): 1587–1593.

Diefenbach C.O. da C. (1975) Gastric function in *Caiman crocodilus* (Crocodylia: Reptilia)—I. Rate of gastric digestion and gastric motility as a function of temperature. *Comparative Biochemistry and Physiology Part A* 51(2): 259–260. doi:10.1016/0300-9629(75)90369-2

Du W.G., Yan S.J., Ji X. (2000) Selected body temperature, thermal tolerance and thermal dependence of food assimilation and locomotor performance in adult blue-tailed skinks*, Eumeces elegans. Journal of Thermal Biology* 25(3): 197–202. doi:10.1016/S0306-4565(99)00022-4

Dunham A.E. (1993) Population responses to environmental change: Operative environments, physiologically structured models, and population dynamics. In: Karieva P.M., Kingsolver J.G., Huey R.B. (eds.). *Biotic Interactions and Global Change*. Sunderland, Massachusetts: Sinauer Associates, 95–119.

Hamilton J., Coe M. (1982) Feeding, digestion and assimilation of a population of giant tortoises (*Geochelone gigantea* Sehweigger)) on Aldabra atoll. *Journal of Arid Environments* 5(2): 127–144. doi:10.1016/S0140-1963(18)31545-3

Harlow H.J., Hillman S.S., Hoffman M. (1976) The effect of temperature on digestive efficiency in the herbivorous lizard, *Dipsosaurus dorsalis. Journal of Comparative Physiology Part B* 111: 1–6.

Harshaw L.T. (2012) Evaluation of the Nutrition of Florida Manatees (*Trichechus manatus latirostris*). PhD thesis. University of Florida.

Harwood R.H. (1979) The effect of temperature on the digestive efficiency of three species of lizards, *Cnemidophorus tigris, Gerrhonotus multicarinatus* and *Sceloporus occidentalis. Comparative Biochemistry and Physiology Part A* 63(3): 417–433. doi:10.1016/0300-9629(79)90613-3

Hatt J.M., Clauss M., Gisler R., Liesegang A., Wanner M. (2005) Fiber digestibility in juvenile Galapagos tortoises (*Geochelone nigra*) and implications for the development of captive animals. *Zoo Biology* 24(2): 185–191. doi:10.1002/zoo.20039

Hazard L.C., Shemanski D.R., Nagy K.A. (2009) Nutritional quality of natural foods of juvenile desert tortoises (*Gopherus agassizii*): Energy, nitrogen, and fiber digestibility. *Journal of Herpetology* 43(1): 38–48. doi:10.1670/07-160R1.1

Higgins C., Edwards M.S. (2009) Application of allometric field metabolic rate equations to predict energy and food requirements of leopard tortoises (*Geochelone pardalis*). In: Ward A., Treiber K., Schmidt D., Coslik A., Maslanka M. (eds.). *Proceedings of the Eighth Conference on Zoo and Wildlife Nutrition, AZA Nutrition Advisory Group*, Tulsa, OK. [Conference paper]. Retrieved from https://nagonline.net/2177/application-allometric-field-metabolic-rate-equations-predict-energy-food-requirements-leopard-tortoises-geochelone-pardalis/

Holleman D.F., White R.G. (1989) Determination of digesta fill and passage rate from nonabsorbed particulate phase markers using the single dosing method*. Canadian Journal of Zoology* 67(2): 488–494. doi:10.1139/z89-071

Karasov W.H., Diamond J.M. (1985) Digestive adaptations for fueling the cost of endothermy. *Science* 228(4696): 202–204. doi:10.1126/science.3975638

Lillywhite H.B. (1987) Temperature, energetics, and physiological ecology. In: Siegel R.A., Collins J.T., Novak S.S. (eds.) *Snakes: Ecology and Evolutionary Biology*. New York, New York: McGraw-Hill, 422–477.

Mackay R.S. (1968) Observations on peristaltic activity versus temperature and circadian rhythms in undisturbed *Varanus flavescens* and *Ctenosaura pectinata. Copeia* 1968(2): 252–259. doi:10.2307/1441751

Mader D.R. (ed.) (1996) *Reptile Medicine and Surgery.* Philadelphia, USA: WB Saunders.

Madera-Vergara L., Núñez-Hernández M., De La Ossa J. (2010) Tránsito digestivo en *Geochelone carbonaria* (Morrocoy) con diferentes dietas experimentales. *Revista Colombiana de Ciencia Animal* 2(2): 263–269. doi:10.24188/recia.v2.n2.2010.275

McBee R.H., McBee V.H. (1982) The hindgut fermentation in the green iguana, Iguana iguana. In: Burghardt G.M., Rand A.A. (eds.). *Iguanas of the World: Their Behavior, Ecology, and Conservation.* Park Ridge, New Jersey: Noyes Publications, 77–83.

McConnachie S., Alexander G.J. (2004) The effect of temperature on digestive and assimilation efficiency, gut passage time and appetite in an ambush foraging lizard, *Cordylus melanotus melanotus. Journal of Comparative Physiology Part B* 174: 99–105.

McKinon W., Alexander G.J. (1999) Is temperature independence of digestive efficiency an experimental artifact in lizards? A test using the common flat lizard (*Platysaurus intermedius*). *Copeia* 1999(2): 299–303. doi:10.2307/1447475

Meienberger C., Wallis I.R., Nagy K.A. (1993) Food intake rate and body mass influence transit time and digestibility in the desert tortoise (*Xerobates agassizii*). *Physiological Zoology* 66(5): 847–862.

Mendoza P., Furuta C., Garcia B., Zena L.A., Artoni S., Dierenfeld E.S., Bícego K.C., Carciofi A.C. (2022) Starch and fiber intake effects on energy metabolism, growth, and carapacial scute pyramiding of red-footed tortoise hatchlings (*Chelonoidis carbonaria*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 265: 111131. doi:10.1016/j.cbpa.2021.111131

Merchan M., Fidalgo A., Pérez C. (1998) Biología, distribución y conservación del morrocoy o tortuga carbonera en los llanos de Venezuela. *Reptilia* 4(15): 30–38.

Milton K. (1981) Food choice and digestive strategies of two sympatric primate species. *The American Naturalist* 117(4): 496–505. doi:10.1086/283730

Mitchell N.J., Jones T.V., Kuchling G. (2012) Simulated climate change increases juvenile growth in a Critically Endangered tortoise. *Endangered Species Research* 17(1): 73–82.

Moskovits D.K. (1985) *The Behavior and Ecology of the Two Amazonian Tortoises,* Geochelone carbonaria *and* Geochelone denticulata*, in Northwestern Brazil.* PhD thesis. University of Chicago.

Moskovits D.K., Bjorndal K.A. (1990) Diet and food preferences of the tortoises *Geochelone carbonaria* and *G. denticulata* in Northwestern Brazil. *Herpetologica* 46(2): 207–218.

Nagy K.A. (1983) Ecological energetics. In: Huey R.B., Pianka E.R., Schoener T.W. (eds.). *Lizard Ecology: Studies of a Model Organism.* Cambridge, Massachusetts: Harvard University Press, 24–54.

Nagy K.A., Girard I.A., Brown T.K. (1999) Energetics of free-ranging mammals, reptiles, and birds. *Annual Review of Nutrition* 19: 247–277. doi:10.1146/annurev.nutr.19.1.247

Pafilis P., Foufopoulos J., Poulakakis N., Lymberakis P., Valakos E. (2007) Digestive performance in five Mediterranean lizard species effects of temperature and insularity. *Journal of Comparative Physiology Part B* 177: 49–60. doi:10.1007/s00360-006-0108-5

Parmenter R.R. (1980) Effects of food availability and water temperature on the feeding ecology of pond sliders (*Chrysemys s. scripta*). *Copeia* 1980(3): 503–514. doi:10.2307/1444528

Peterson C.C., Walton B.M., Bennett A.F. (1998) Intrapopulation variation in ecological energetics of the garter snake Thamnophis sirtalis, with analysis of the precision of doubly labeled water measurements. *Physiological Zoology* 71(4): 333–349.

Puga-Torres B.H., Morales-Arciniega S.M., Núñez L.F.N., Carvajal-Mora O., Santander-Parra S.H., Aragón-Vásquez E.F. (2017) Comparison of the effect of three food diets provided to giant tortoises (*Chelonoidis guntheri* and *Chelonoidis vicina*) bred in captivity at Isabela Island – Galápagos. *Journal of Veterinary Science and Animal Husbandry* 5(2).

Qu Y., Li H., Gao J., Xu X., Ji X. (2011) Thermal preference, thermal tolerance and the thermal dependence of digestive performance in two *Phrynocephalus* lizards (Agamidae), with a review of species studied. *Current Zoology* 57(6): 684–700. doi:10.1093/czoolo/57.6.684

Regal P.J. (1966) Thermophilic response following feeding in certain reptiles. *Copeia* 1966(3): 588–590. doi:10.2307/1441092

Riddle O. (1909) The rate of digestion in cold blooded vertebrates—The influence of season and temperature. *American Journal of Physiology* 24(5): 447–458. doi:10.1152/ajplegacy.1909.24.5.447

Ritz J., Hammer C., Clauss M. (2010) Body size development of captive and free-ranging leopard tortoises (*Geochelone pardalis*). *Zoo Biology* 29(4): 517–525. doi:10.1002/zoo.20273

Roe J.H., Hopkins W.A., Talent L.G. (2005) Effects of body mass, feeding, and circadian cycles on metabolism in the lizard *Sceloporus occidentalis. Journal of Herpetology* 39(4): 595–603. doi:10.1670/75-05A.1

Ruppert R.M. (1980) Comparative assimilation efficiencies of two lizards. *Comparative Biochemistry and Physiology Part A* 67(3): 491–496. doi:10.1016/S0300-9629(80)80028-4

Sadeghayobi E., Blake S., Wikelski M., Gibbs J., Mackie R., Cabrera F. (2011) Digesta retention time in the Galápagos tortoise (*Chelonoidis nigra*). *Comparative Biochemistry and Physiology Part A* 160(4): 493–497. doi:10.1016/j.cbpa.2011.08.008

Secor S.M. (2001) Regulation of digestive performance: A proposed adaptive response. *Comparative Biochemistry and Physiology Part A* 128(3): 563–575. doi:10.1016/S1095-6433(00)00325-1

Sibly R.M. (1981) Strategies of digestion and defecation. In: Townsend P.R., Calow P. (eds.). *Physiological Ecology: An Evolutionary Approach to Resource Use*. Oxford, England: Blackwell Scientific Publications, 109–139.

Skoczylas R. (1970) Influence of temperature on gastric digestion in the grass snake *Natrix natrix L. Comparative Biochemistry and Physiology* 33(4): 793–804. doi:10.1016/0010-406X(70)90028-9

Skoczylas R. (1978) Physiology of the digestive tract. In: Gans C., Gans K.A. (eds.). *Biology of the Reptilia Vol. 8.* New York, New York: Academic Press, 587–717.

Stevenson R.D., Peterson C.R., Tsuji J.S. (1985) The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiological Zoology* 58(1): 46–57. doi:10.1086/physzool.58.1.30161219

Throckmorton G. (1973) Digestive efficiency in the herbivorous lizard *Ctenosaura pectinata*. *Copeia* 1973(3): 431–435. doi:10.2307/1443106

Tracy C.R., Zimmerman L.C., Tracy C., Bradley K.D., Castle K. (2006) Rates of food passage in the digestive tract of young desert tortoises: Effects of body size and diet quality. *Chelonian Conservation and Biology* 5(2): 269–273. doi:10.2744/1071-8443(2006)5[269:ROFPIT]2.0.CO;2

Troyer K. (1987) Small differences in daytime body temperature affect digestion of natural food in a herbivorous lizard (*Iguana iguana*). *Comparative Biochemistry and Physiology Part A* 87(3): 623–626. doi:10.1016/0300-9629(87)90371-9

Udén P., Robinson P.H., Wiseman J. (2005) Use of detergent system terminology and criteria for submission of manuscripts on new, or revised, analytical methods as well as descriptive information on feed analysis and/or variability. *Animal Feed Science and Technology* 118(3–4): 181–186. doi:10.1016/j.anifeedsci.2004.11.011

Van Weyenberg S., Sales J., Janssens G.P.J. (2006) Passage rate of digesta through the equine gastrointestinal tract: A review. *Livestock Science* 99(1): 3–12. doi:10.1016/j.livprodsci.2005.04.008

Waldschmidt S.R., Jones S.M., Porter W.P. (1986) The effect of body temperature and feeding regime on activity, passage time, and digestive coefficient in the lizard *Uta stansburiana*. *Physiological Zoology* 59(3): 376–383. doi:10.1086/physzool.59.3.30156109

Wallach J.D., Boever W.J. (1983) Reptiles and amphibians. In: Wallach J.D., Boever W.J. (eds.). *Diseases of Exotic Animals*. Medical and Surgical Management. Philadelphia: Saunders, 979–1047.

Wang T., Zaar M., Arvedsen S., Vedel-Smith C., Overgaard J. (2002). Effects of temperature on the metabolic response to feeding in *Python molurus*. *Comparative Biochemistry and Physiology Part A* 133(3): 519–527. doi:10.1016/S1095-6433(02)00250-7

Wang E., Donatti C.I., Ferreira V.L., Raizer J., Himmelstein J. (2011) Food habits and notes on the biology of *Chelonoidis carbonaria* (Spix 1824) (Testudinidae, Chelonia) in the southern Pantanal, Brazil. *South American Journal of Herpetology* 6(1): 11–19. doi:10.2994/057.006.0102

Wright R.D., Florey H.W., Sanders A.G. (1957) Observations on the gastric mucosa of Reptilia. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* 42(1): l–14. doi:10.1113/expphysiol.1957.sp001224

Zentek J., Dennert C. (1997) Fütterung von Reptilien: Praxis und Probleme. *Tierarztliche Praxis* 25: 684–688.

Zimmerman L.C., Tracy C.R. (1989) Interactions between the environment and ectothermy and herbivory in reptiles. *Physiological Zoology* 62(2): 374–409. doi:10.1086/physzool.62.2.30156176