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Temperature effect on the digestive efficiency of the main organic compounds in two Mediterranean lizards

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ABSTRACT: The effect of temperature on apparent digestive efficiency (ADE) and gut passage time (GPT) was investigated in two endemic lizard species (*Lacerta graeca* and *Podarcis peloponnesiaca*) of the Peloponnese (Greece) in a typical Mediterranean ecosystem. The two taxa have the same thermal preferenda but different thermoregulatory strategies. All animals were force-fed exclusively on mealworms (to eliminate the influence of the diet). The experiment was repeated at three different temperatures (20°C, 25°C and 30°C) in order to detect the influence of temperature. ADE was calculated separately for different nutrients of the food (lipids, sugars and proteins) using classical biochemical methods. Our results demonstrate that in both species GPT changes inversely with increasing temperature. ADE for proteins followed the same pattern. In contrast, ADE for lipids and sugars increased with temperature, but in a distinct way for each species. The performance of the digestion is lower in the case of *L.graeca* in comparison to *P.peloponnesiaca*.

1 INTRODUCTION

The majority of physiological and behavioral performances in reptiles are temperature dependent. Thermal optima have been demonstrated in reptiles for many different physiological functions (Huey 1982) including digestion. Especially for lizards, food passage time through the gastrointestinal tract varies from few hours to several days (Christian et al. 1984, Troyer 1984, Karasov et al. 1986). It is generally accepted that in most cases the increase in body temperature (Tb) causes an increase in the rates of various digestive processes (Cowles and Bogert 1944). Nevertheless, the effect of body temperature on the digestive efficiency of lizards is not clear (McKinon and Alexander 1999). Some species exhibit a direct temperature-dependent digestive performance (Harwood 1979, Ruppert 1980, Troyer 1987, Xiang et al. 1996) while others are either slightly (Waldschmidt et al. 1986) or not dependent. (Karasov and Diamond 1985, Christian 1986, Zimmerman and Tracy 1989).

Digestive efficiency is defined as the percentage of the ingested energy that is absorbed from the gut (Kleiber 1961, Johnson and Lillywhite 1979). However this term is rather factitious since faeces contain other additional materials such as nitrogenous wastes, intestinal cells, secreted proteins, bacteria and undigested food. Thus the term apparent digestive efficiency (ADE) is more frequently used instead of digestive efficiency (with the concession that none of the excretory products are separated from the faecal waste) (Beaupre et al. 1993, McKinon and Alexander 1999).

The effect of temperature on digestive efficiency has been approached until now using the quantitative method of calorimetry. However, there are no data on the qualitative analysis of digestion performance, i.e. no sufficient information is available on the digestion of different prey biochemical components. Calorimetric methods provide only a summary estimation of digestive efficiency as a percentage of calories absorbed from ingested food material. But the main organic components of the lizard diet (lipids, proteins and sugars) are characterized by different nutrient values. What happens with each of these components?

We investigated (a) the effect of temperature on digestive efficiency and on gut-passage time in the examined species (b) whether there are any differences in the way that a species digests the separate biochemical components of prey.

2 METHODS

Five lizards from each species were tested in three different temperatures (20 °C, 25 °C and 30 °C). Food was withheld from the animals for 3-4 days, until no faeces were found in the terrarium. Mealworms were weighed to the nearest 0.1 mg and matched for mass to create pairs of similar food items. One individual of each pair then was force-fed to the lizard every second day while the other half of the pair was used for biochemical analysis. The ADE was computed according to the following equation:
ADE = (I-E/I) x 100, where I= total fuel (lipids, sugars or proteins) energy ingested and E= total fuel (lipids, sugars or proteins) energy remaining in the fecal material.

2.1 *Animals*

Lacerta graeca and *Podarcis peloponnesiaca* are similar-sized (70-85mm, mass 5-7g) lizards with different habitat requirements (a rock specialist and a generalist species respectively), endemic and sympatric to the Peloponnese peninsula (Greece).
Both taxa have similar thermal preferences. The mean annual body temperature of *L.graeca* is 31.76°C and of *P. peloponnesiaca* is 31.15°C (Maragou et al. 1997).

We used in total 15 *P. peloponnesiaca* and 15 *L. graeca* in this study. The collection of specimens was carried out according to Presidential Degree 67/81 that permits sampling of endemic species only for scientific research. None of the animals were killed for this study.

All animals used in this study (all adults of both sexes, 8 males and 7 females *P.peloponnesiaca* and 10 males and 5 females *L.graeca*) were collected in the Stymfalia Lake area (22, 28´ N, 37, 51´ W) in the Northern Peloponnese. The animals were collected during the non-reproductive period (October 1998) because reproductive effort, especially for the females, has been shown to influence study results (Xiang and Brana 2000, Wapstra and Swain 2001).

After an acclimatization period of 30 days in the lab (ambient temperature of 25°C), each of the 30 animals was transferred to an individual clear plastic terrarium and was placed in a constant temperature chamber. Choice of these thermal and photoperiod conditions was based on previous work with these species (Maragou 1997).

2.2 *Gut-passage time*

Mealworms were marked by introducing a small piece (3x2x0.1 mm) of plastic material (PVC) as an indigestible marker (Van Damme et al. 1991) into their abdomen and afterwards were force-fed to the lizards. Faeces production was monitored continuously (in time intervals of 60 min) throughout day and night, and faeces from each individual were collected immediately. Faecal material was placed in liquid nitrogen immediately after collection and was stored in the ultra high freezer till the biochemical analysis. Each faex was searched for the plastic marker, and the time of

collection was recorded to estimate passage time. Gut-passage time was defined as the time from feeding to first defecation and the appearance of the plastic tag in the faeces.

2.3 *Biochemical analysis*

Lipids determination was realised according to the method described by Alexis and Papapoutsoglou (1986). Determination of total protein values was performed by the typical Biuret method. Finally the determination of total sugars was performed according to the method described by Dubois et al. (1956).

3 RESULTS

3.1 *Gut passage time*

Temperature had a large and significant effect on gut passage time in both species (Table 1). Gut passage time decreased with an increase of temperature from 20°C to 30°C. (ANOVA, *P. peloponnesiaca*: $F_{2, 33}$=532.23, P<0.05, *L.graeca*: $F_{2, 33}$=92.95, P<0.05). In the case of *L.graeca* GPT is faster than in *P.peloponnesiaca* at 20° C and 25°C (t-test, t=10.24, P<0.05, df=22 and t=5.57, P<0.05, df=22 correspondingly) but there is no significant difference between the two species at 30° C (t-test, t= 0.89, P=0.39 df=39).

Table 1. Mean gut-passage time for P.peloponnesiaca and L.graeca at the three experiment temperatures. Numbers in parentheses: number of lizards tested. Means are followed with standard deviation.

	GPT (hours)		
	20°C (12)	25°C (12)	30°C (12)
P.peloponnesiaca	38.83± 1.89	23.41± 2.31	14.25± 1.21
L.graeca	27.16± 1.34	18.33± 2.14	13.83± 1.11

3.2 *ADE for lipids*

Temperature alteration had different effects on lipid absorption in the two species (Table 2). The mean ADE in the case of *P. peloponnesiaca* changed when temperature increased (ANOVA $F_{2, 22}$=5.18, P=0.014). There were significant differences in ADE when temperature increased from 25° C to 30° C (Tukey post-hoc test, P<0.05). On the contrary it seems that there was no impact of the temperature in the mean ADE in *L.graeca* ($F_{2, 21}$=0.36, P=0.69).

In both species the increase of temperature had no effect on total lipid concentration in the faeces and in the larvae that animals were fed (ANOVA, *P.peloponnesiaca*: larvae, $F_{2, 22}$=0.50, P=0.61, faeces, $F_{2, 22}$=2.21, P=0.13, *L.graeca*: larvae, $F_{2, 21}$=1.17, P=0.32, faeces, $F_{2, 21}$=0.96, P=0.90).

With an exception at 20°C when the mean ADE did not differ between the two examined species (t-test, t=0.44, P=0.66 df=15), *P.peloponnesiaca* showed a larger ADE than *L.graeca* at 25°C and 30°C (t-test, 25°C: t=3.23, P=0.005 df=15, 30°C: t=2.20, P=0.046, df=13).

Table 2. Concentration of total lipids in larvae and faeces, as well as total lipids Apparent Digestive Efficiency at three experiment temperatures Numbers in parentheses: number of lizards tested. Means (mg/g wet tissue) are followed with standard deviations.

	P.peloponnesiaca			*L.graeca*		
	20°C (8)	25°C (9)	30°C (8)	20°C (9)	25°C (8)	30°C (7)
Larv.	195.56±18.83	192,36±20.61	202.92±26.30	192.13±17.99	204.14±21.54	208.97± 29.23
Faeces	51.09± 16.60	38.27± 10.62	40.01± 12.63	53.23± 18.43	56.71±17.82	53.21± 18.89
ADE	74.23± 6.08	80.32± 3.38	80.66± 3,80	72.82± 6.77	72.74± 6.04	75.19± 5.74

3.3 *ADE for sugars*

In both examined species ADE was affected by temperature augmentation as shown in Table 3 (ANOVA, *P.peloponnesiaca*: $F_{2, 19}$=25.26, P<0.05, *L. graeca*: $F_{2, 13}$=11.3, P=0.001). ADE in both species increased significantly when we raised temperature from 20°C to 25°C (Tukey post-hoc test P<0.05) but did not change significantly from 25°C to 30°C (Tukey post-hoc test P>0.05). The sugar concentration of the larvae, did not differ between the three trials in either species (ANOVA, *P.peloponnesiaca*: $F_{2, 19}$=0.61, P=0.54, *L.graeca*: $F_{2, 13}$=0.34, P=0.71). On the contrary the sugar concentration of the feaces seemed to be affected by temperature only in the case of *P.peloponnesiaca* (ANOVA, $F_{2, 19}$=8.29, P=0,002) but not in *L. graeca* (ANOVA, $F_{2, 13}$=11.3, P<0.05). In all trials the ADE did not differ between the examined species (t-test, 20°C: t=0.64 P=0.36, df=10, 25°C: t=1.1, P=0.26 df=11, 30°C: t=0.48, P=0.63, df=11).

Table 3. Concentration of sugars in larvae and faces, as well as sugars Apparent Digestive Efficiency at three experiment temperatures Numbers in parentheses: number of lizards tested. Means (mg/g wet tissue) are followed with standard deviations.

	P.peloponnesiaca			*L.graeca*		
	20°C (7)	25°C (7)	30°C (8)	20°C (5)	25°C (6)	30°C (5)
Larvae	39.80± 7.33	38.83± 5.81	41.98± 3.40	40.45± 3.35	39.00± 5.02	40.92± 3.10
Faeces	11.35± 2.02	8.77± 1.21	8.55± 0.96	11.12± 0.75	8.05± 0.53	8.50± 0.53
ADE	71.37± 1.68	77.18± 2.92	79.57± 2.06	72.40± 2.09	79.06± 2.80	78.33± 2.70

3.4 *ADE for proteins*

In both species the rise in temperature affected negatively ADE of proteins (Table 4, ANOVA, *P.peloponnesiaca*: $F_{2, 27}$=27.52, P<0.05, *L. graeca*: $F_{2, 20}$=13.69, P<0.05). This decrease was gradual in the case of *P.peloponnesiaca* with increasing temperature (Tukey post-hoc test between 20°C-25°C and 25°C-30°C P<0.05). In contrast in the case of *L.graeca* the drop in the values of ADE was only between 25°C and 30°C (Tukey post-hoc test P>0.05). As expected the protein concentration of larvae remained stable in all trials for both species (ANOVA, *P.peloponnesiaca*: $F_{2, 27}$=0.79, P=0.46, *L.graeca*: $F_{2, 20}$=0.55, P=0.58), but the temperature effected the faeces protein concentration (ANOVA, *P.peloponnesiaca*: $F_{2, 27}$=35, P<0.05, *L.graeca*: $F_{2, 20}$=7.62, P=0.03). In all experiment temperatures the mean values of ADE did not differ between the two species (t-test, 20°C: t=1.97 P=0.06, df=15, 25°C: t=0.96, P=0.34, df=16, 30°C: t=1.29, P=0.21, df=16).

Table 4. Concentration of proteins in larvae and faeces, as well as proteins Apparent Digestive Efficiency at three experiment temperatures Numbers in parentheses: number of lizards tested. Means (mg/g wet tissue) are followed with standard deviations.

	P.peloponnesiaca			*L.graeca*		
	20°C (9)	25°C (10)	30°C (8)	20°C (9)	25°C (10)	30°C (11)
Larvae	33.29± 8.82	32.29± 6.59	36.43± 8.09	33.64± 5,54	37.53± 9.40	37.75± 10.42
Faeces	10.55± 1,88	13.42± 2,93	10.55± 2.61	12.32± 2.69	14.36± 3.20	18.85± 3.94
ADE	67.55± 4.36	57.94± 7.79	44.29± 8.04	63.54± 3.93	61.21± 6.15	49.05± 6.76

4 DISCUSSION

From our results it is clear that gut-passage time is clearly temperature dependent. In both species GPT decreased with increasing temperature. This pattern is rather typical in reptiles (Bedford and Christian 2000, Alexander et al. 2001) suggesting that higher temperature has a stimulatory effect on gut motility and absorption. GPT is a general indicator of the digestive process and does not provide detailed information about effectiveness of digestion or about the pure assimilation of

energy occurring in the gastrointestinal tract. However GPT measurement does provide an estimator of the time that food remains in the gastrointestinal tract, which is prerequisite for digestive efficiency. For example longer gut passage times may allow gastric enzymes more time to act on ingested food (Alexander et al. 2001).

Avery (1971, 1973) and Van Damme et al. (1991) carried out similar studies with *Lacerta vivipara,* the only other project to our knowledge that focused on lacertid lizards. Avery (1973) estimated the time for stomach evacuation at 11 h at 30^0 C and at 24h at 20^0 C. Aforementioned data are very close to the figures given by Van Damme et al. (1991) (18,8±1.9 at 20^0C, 13.5±1.3 at 25^0C and 11.2±0.8 at 30^0C). According to our data *L.graeca* has digestive behaviours similar to these of *L.vivipara,* which is not surprising given the common taxonomy of the two species (genus *Lacerta*).

Lipid digestion poses special problems for animals because lipids are only slightly soluble in water whereas lipolytic enzymes are water-soluble. So lipids have to undergo therefore a complex and time-consuming catabolic procedure reactions. As fats tend to separate out into an oily phase, they usually are emptied from the stomach later than the other gastric contents. Hence it is logical to presume that the longer they remain in the intestinal tract (so as their digestion accomplished) the more energy will be released. However as we have demonstrated in this study, the time that food remains in the digestive track decreases with the increase of temperature. In contrast ADE for lipids increases with temperature. The answer for these apparently conflicting data may lie in changing enzyme activity. Licht (1964) found that the activity of some intestinal enzymes reaches a maximum at 42^0C in different lizard species, regardless of differences in their preferred levels of body temperatures. Lipolytic enzymes (following this general rule for enzymes) are also much more effective at higher temperatures (Stryer 1988). Consequently, despite the longer period that food remains in the digestive track at low temperatures, ADE is lower because thermal conditions do not favor enzyme activity.

Sugars are the most important component of the diet of lizards since they are found in abundance in their invertebrate diet (Chapman 1998). It is necessary that ADE for this component has to remain invariable in order to ensure the smooth alimentation of the animals. Thus it seems that the specialized enzymes for sugars (sacharitases) are rather independent to the changes of temperature. Sugars are the every-day meal of lizards and the most direct energy-source. If sacharitases were temperature-depended, ADE for sugars will fall dramatically with a fatal impact on animal's energy input.

In normal and healthy lizards all ingested proteins are digested and absorbed. Most of the proteins in digestive secretions and exfoliated cells are also digested and absorbed. The small amount of proteins in the faeces is derived principally from colonic bacteria and exfoliated colonic cells. In this study a significant fall in the ADE of proteins occurred in both species when we increased temperature, that rise caused a decrease of GPT and more rapid defecation. Most likely, at higher temperature regimes the time that food remains in the digestive track is not sufficient for the proper digestion of proteins.

From the study of the data it is obvious that changes in the performance of the digestion with temperature are smaller in *L.graeca* than in *P.peloponnesiaca*. Furthermore we observe that *P.peloponnesiaca* has adopt the same digestive pattern at the temperatures of 25 ^{0}C and 30 ^{0}C while *L.graeca* shows this tendency for the temperatures 20^0C and 25^0C (in the case of sugars for example) or its ADE remain constant (the case of lipids). Apparently *L.graeca* shows a preference in cooler temperatures. Van Damme et al. (1991) reported exactly the same pattern for *L.vivipara* where the ADE did not change from 20 ^{0}C to 25 ^{0}C.

The above data suggest that digestion in *L.graeca* is more insensitive to changes in the thermal environment and appears more stable at lower temperatures. *L.vivipara* shows a similar thermal independence as mentioned above (Avery 1971, 1973, Van Damme at al. 1991). This fact reinforces the hypothesis that *L.graeca* is a relict species in Peloponnese, with a previously northern

distribution (Maragou 1997). On the other hand, *P.peloponnesiaca* most likely evolved in Peloponnisos and is adapted to the Mediterranean conditions. It is a more thermophilic species, which is clearly more influenced by changes in the temperature. It would be rather dicey to state that phylogeny exclusively affects ADE performance. However, further research in more species of the family, is necessary to elucidate the real influence of phylogenetic history on digestive performance. A plethora of data about ADE is known by the literature: Licht and Jones (1967) reported an assimilation efficiency of 88.9% for lizards fed mealworms, Harwood (1979) 89%, 49%-55.7% in iguanas by Troyer (1987), 30% for herbivorous species (Ruppert 1980) and 85%-93% for *Klauberina riversiana* (Johnson and Lillywhite 1979). The problem is that all the previous authors estimated ADE using calorimetric methods; hence it is difficult to compare our data. Yet, as we show, ADE changes with temperature in different way for lipids, sugars and proteins. Future directions of study need to focus on the activity of digestive tract enzymes.

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