Plasticity in food assimilation, retention time and coprophagy allow herbivorous cavies (*Microcavia australis*) to cope with low food quality in the Monte desert

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ABSTRACT

Energy balance depends on the efficiency with which organisms make use of their trophic resources, and has direct impact on their fitness. There are environmental variations that affect the availability as well as the quality of such resources; energy extraction also depends on the design of the digestive tract. It is expected that features associated with food utilization will be subjected to selective pressures and show some adjustment to the variability of the environment. Since energetic constraints challenge animals to display digestive compensatory mechanisms, the objective of this study is to determine the physiological and behavioral responses to spatial and seasonal heterogeneity in food quality. We investigated digestive strategies (digestive efficiency and coprophagy) in cavies inhabiting two different populations, and hence naturally experiencing different levels of diet quality. Cavies under experimentally different quality diets showed changes in dry matter digestibility and intake, digesta retention time and coprophagy. Our results partially support the expectations from theory and also reveal interpopulation differences in the ability to cope with changes in food quality, and may explain the capability of *Microcavia australis* to colonize extreme habitats.

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1. Introduction

Organismal design encompasses, within certain limits, an intrinsic ability for flexibility. Numerous studies have shown that the triggers of phenotypic change are those which respond to demands during development; and those caused by limits imposed by the environment (Stearns, 1989; Piersma and Drent, 2003). The dynamic response to these changes includes behavioral, physiological and morphological ones; occurring among different taxonomic groups and is critical in the adaptive repertoire of many of them (Piersma and Lindström, 1997). Phenotypic plasticity, and its reversible version phenotypic flexibility, confer organisms a great ability to deal with environmental changes (Pigliucci, 2001; Naya and Bozinovic, 2004). It has been reported as supporting the adjustment of animals to changes in their nutritional environment (Bozinovic, 1993; Caviedes-Vidal and Karasov, 2001; Naya and Bozinovic, 2006), and is a trait for small mammals inhabiting seasonal (Sabat and Bozinovic, 2000; Merritt et al., 2001; del Valle et al., 2006) or spatially variable environments (Mangione et al., 2000; Hammond et al., 2001; Naya et al., 2008). Furthermore, under local consistent conditions, adaptation may

increase by means of genetic assimilation (Pigliucci et al., 2006), a process in which environmentally induced genetic expression of phenotypic variation becomes constitutively produced (i.e. no longer requires the environmental signal for expression). Similarly, phenotypic variants could arise during ontogeny in response to environmental signals, by means of developmental plasticity (Wilkins, 2002; West-Eberhard, 2005).

Energetic constraints challenge animals to display digestive compensatory mechanisms. Theoretically, in order to compensate for low food quality an animal with fixed ingestion events should retain the food longer, thus maximizing energy extraction. Retention time would increase, leading to greater digestion, fermentation and absorption, i.e., ground story frugivore bats (Bonaccorso and Gush, 1987). On the other hand, in animals whose foraging strategy implies several or continuous ingestion bouts (Veloso and Bozinovic, 1993), the rate at which energy is obtained is expected to remain close to the maximum. This is achieved by means of an increased ingestion rate as well as a rapid passage of food through the gut. An increase of digestive organ size with the subsequent increase in digestion and fermentation rates is also likely (Sibly, 1981). Small herbivore mammals, in particular, can evade allometric constraints by separating soluble and insoluble fractions of the diet, as noted by Foley and Cork (1992). Coupled to this, the production and re-ingestion of nutrient rich feces is a beneficial behavior for hindgut fermenters

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(Alexander, 1993; Takahashi and Sakaguchi, 2000) and is especially important for small-sized animals in which the energetic balance is challenged by low quality diets (Weiner, 1992; Karasov and Martinez del Rio, 2007).

Microcavia australis is a good animal model to test hypotheses in nutritional ecology and comparative physiology, since this species is one of the smallest rodents among cavies and is widely distributed in heterogeneous arid and semiarid zones in South America (Redford and Eisenberg, 1992). It is an herbivore that faces spatial and temporal changes in quality, availability and diversity of alimentary resources. Recently, Sassi et al. (2007) reported significant effects of season and site on the size of digestive organs among cavies from different populations and in different seasons. These authors reported that the caecum, the organ most closely related to cellulose fermentation, was significantly larger in cavies facing the lowest quality diet (Sassi et al., 2007). However, it remains to determine the physiological and behavioral responses possibly coupled with intra and interpopulation variability in morphological attributes, in order to compensate for constraints imposed by spatial and seasonal heterogeneity in food quality. Consequently, the objective of this study is to investigate digestive strategies (digestive efficiency and coprophagy) in cavies inhabiting in two different populations, and hence naturally experiencing different levels of diet quality. We tested the following hypotheses: 1) individuals compensate for low quality diets through increases in food ingestion rate and/or selective retention of digesta; 2) individuals under low quality diets have lower digestibility of matter and energy than those acclimated to higher quality diets; 3) individuals feeding on low quality diets perform coprophagy more frequently; and 4) individuals from less productive habitats are comparatively more efficient in compensating for a low quality diet.

2. Materials and methods

Our work was carried out in accordance with EC Directive 86/609/EEC for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm. The experimental animals were non-reproductive adults captured in Ñacuñán and Matagusanos, two different habitats within the Monte Desert of Argentina. The Man and Biosphere Reserve of Ñacuñán is located in the center of the Monte Desert (34°02′S, 67°58′W), at 700 m asl (Ojeda et al., 1998). The climate is semiarid, with an average annual rainfall of 322 mm which is concentrated in summer (November–March). Mean temperatures are 22.1 °C in summer and 8.7 °C in winter. The vegetation consists of open woodland dominated by *Prosopis flexuosa*, a shrub layer of *Larrea divaricata* and *Atriplex* sp, and a species-rich herbaceous layer. Matagusanos (31°14′S, 68°38′W) is in the arid extreme of the Monte Desert, at 700 m asl. Rainfall does not surpass 100 mm annually and occurs primarily during summer. Mean temperatures are 30 °C in summer and 10 °C in winter. Vegetation is xerophytic with very little plant cover. The dominant tree is mesquite (*Prosopis flexuosa*), and the shrub stratum is composed of *Larrea cuneifolia*, *Capparis atamisquea*, and a few Gramineae.

Five cavies were captured per site, brought to the laboratory and maintained on alfalfa cubes, apples and carrots with water *ad-libitum* during 4 to 6 months. Experimental diets were prepared with alfalfa cubes with water-mixed (high quality diet), and alfalfa cubes with cellulose added and water-mixed (low quality diet). The amount of cellulose added to alfalfa to obtain the low quality diet was 30%, following Sassi et al. (2007). These authors documented that at Ñacuñán and Matagusanos, *M. australis* consumes up to 38% neutral detergent fiber and 27% acid detergent fiber. Energy content of food and feces samples was estimated using a calorimeter bomb at San Luis University. Results showed that the energy content of the experimental diets was 4342 cal/g for the high quality diet and 4135 cal/g for the low quality diet. Cavies from each population were randomly assigned to one of the experimental diets and acclimated during 20 days before we conducted intake-digestibility and digestive transit

experiments. Afterwards, individuals were switched to the other diet, acclimated and tested again. Experiments were carried out in a room under constant temperature (21 °C) and photoperiod (12 h light–12 h dark). Body weight was measured at the beginning and end of each trial.

For the digestibility experiment, the animals were housed individually in steel mesh cages of $40 \times 40 \times 40$ cm, a known amount of the experimental diet was provided, and during 4 days feces and remaining food were collected daily. Our experiment did not allow determining simultaneously coprophagy and digestibility. Egestion rate was estimated as the dry weight of feces produced per day and energy from feces was measured for each individual under both treatments. Food intake was measured as the difference between the amount of food given and left per day. The intake of digestible energy was calculated for each experimental diet according to $Q_i \times D$, where Q_i is the daily rate of energy in food intake and D is the apparent digestibility of each diet, calculated as $[(Q_i - Q_e)/Q_i] \times 100\%$. Q_e is the daily rate of energy in feces production; therefore, intake of digestible energy was calculated as $Q_i - Q_e$. Digestibility is apparent because this method underestimates digestive efficiency by the contributions of metabolic wastes, non-reabsorbed secretions of the digestive system and microorganisms. Apparent digestibility was also calculated for both dry matter and energy.

For the digestive transit experiment, Co-EDTA (cobalt disodium ethylene-diaminetetraacetate, Dojin Chemical Institute, Kumamoto, Japan) was used as fluid digesta marker. We weighed 0.2 g of the marker, mixed it with apple as appetizer, and fed this to cavies at 7:00 am, after 12 h without food. Cavies had access to the marker diet for 1 h, after which they were given the experimental diet. The animals were housed in the cages described above, which allowed feces to drop through. Feces were collected every hour for 12 h after giving the digesta marker, every 12 h for the next 36 h, and then every 24 h for 72 h. Feces were dried at 60 °C for more than 24 h, weighed, and stored in aluminum bags before analysis of Co concentration. Mean retention time was estimated from the amount of cobalt released after marker ingestion. In the lab we followed a standard method to determine the total content of cobalt from the samples, performing an acid digestion. Afterwards, an EAA Perkin Elmer AAnalyst 200 was used for cobalt determination. In order to calculate mean retention time we applied the total collection method (Coombe and Kay, 1965) to the sequential change in the amount of marker excreted in the feces. In a second stage the cavies were assigned the opposite diet, acclimated for 20 days and experiments repeated. In order to compare cavies among populations, digestibility and retention time trials were designed as a Common Garden Experiment (Garland and Adolph, 1991). This provided uniform departure conditions, in order to evaluate the possible effect of site on the efficiency under different quality diets.

Since some of the animals died during the course of the study, the coprophagy trial was performed with 5 individuals from both localities, which were acclimated to the initial conditions described above. The cavies were randomly provided with high and low quality food, acclimated for 20 days and then switched to the opposite diet for the second phase of the trial, which consisted in an additional period of 20 days. The experiment consisted of recording each individual with a video camera during 36 h in an aquarium of $40 \times 40 \times 80$ cm, under constant temperature (21 °C) and photoperiod (12L–12D). Every hour the videos were digitally saved in the hard disc of a computer. The video-recording began after 1 day of adaptation to the aquarium. Due to the sensitivity of the recorder, the camera only captured movements during the light hours, which coincides with the diurnal habits of *M. australis*. The procedure allowed us to analyze each videotape by segment, as thoroughly as necessary to reveal the behavior of cavies in detail. The last twelve light hours of recording were selected to score the total number of times an individual performed cecotrophy. Since it is possible that re-ingestion also

occurs during resting periods, part of the information may be lost by disregarding the dark period. Therefore, we standardized the same period of observation for all cavies under both diets, to make them comparable.

2.1. Statistical analysis

Data on digestibility, dry matter intake, digestible energy intake, and digesta transit time were analyzed with separate General Linear Models. Site was the fixed factor, factor levels were repeated measurements under high and low quality diets, and average body weight was used as covariate, in order to control for the effect of individual weight. Additionally, a paired t-test was performed on body weight data in order to detect any effect of the experimental diets on this parameter. Data on cecotrophy were standardized to events per hour, log transformed, and analyzed with a Main Effects ANOVA, with diet quality as a fixed factor and individuals as blocks. Analyses were performed using STATISTICA software (Statsoft, 1997).

3. Results

Dry matter digestibility of cavies was significantly greater under the high quality diet ($F = 24.78$; $df = 1, 8$; $p = 0.001$). The habitat of origin had no significant effect on digestibility ($F = 0.47$, $df = 1, 8$; $p = 0.51$), and the interaction between site and diet quality was not significant ($F = 0.55$; $df = 1, 8$; $p = 0.47$, Table 1). The amount of food ingested by cavies, estimated by dry matter intake, was not affected by diet quality ($F = 0.34$, $df = 1, 8$; $p = 0.57$). Nevertheless it was significantly different between cavies from different habitats ($F = 5.96$; $df = 1, 8$; $p = 0.04$), and since this pattern was maintained under both diets, the statistical interaction between habitat and diet quality was not significant ($F = 0.17$; $df = 1, 8$; $p = 0.69$, Table 1). Digestible energy intake (DEI) provides information about the net assimilation of energy, which was not different between cavies from different populations ($F = 0.83$; $df = 1, 8$; $p = 0.39$), although it was significantly different between diet qualities ($F = 51.29$; $df = 1, 8$; $p = 0.001$); DEI was greater with the high quality diet than the low quality diet. Finally, there was a significant effect of the interaction between habitat and diet quality ($F = 7.23$; $df = 1, 8$; $p = 0.02$), due to differences a decrease of energy extraction in cavies from Ñacuñán under low quality diets. No difference in this parameter was found for Matagusanos cavies ($p < 0.05$, Table 1). Mean retention time was significantly lower for the low quality diet ($F = 34.11$; $df = 1, 8$; $p = 0.001$). The effect between habitats was not significant ($F = 1.06$; $df = 1, 8$; $p = 0.33$), though it may have been masked by interpopulation differences. In fact, the interaction between habitats and diet quality was significant ($F = 34.01$; $df = 1, 8$; $p = 0.0004$, Table 1). Body mass measured before and after trials with each diet showed that Ñacuñán cavies suffered a significant decline in weight ($t = 9.34$;

$df = 4$; $p = 0.001$). Matagusanos individuals did not modify body mass significantly.

Finally, cecotrophy occurred throughout all the light period, i.e. it was not restricted to a specific time of day. Feces were re-ingested by cavies directly from the anus at a rate significantly higher under low quality diet (3.16 ± 0.48 events/h) in comparison to high quality diet (1.55 ± 0.48 events/h) ($F = 10.65$; $df = 1, 4$; $p = 0.031$).

4. Discussion

The study of phenotypic differences among species and individuals is often used to improve the comprehension of organism design (Piersma and Drent, 2003). Our results for digestibility support the expectations from theory; there was a decline in the efficiency of energy extraction with low quality diet. Organisms should therefore compensate this decrease by different mechanisms according to their digestive structure and body size. Several studies have reported that the particular design of the hindgut (colon and caecum) makes it possible for a small herbivore to cope with low quality diets by means of post-gastric fermentation (Caughley and Sinclair, 1994; Sakaguchi, 2003) and selective retention of particles within the caecum (Holtenius and Björnhag, 1985). Our results are partially consistent with such compensatory mechanisms and could also reveal interpopulation differences.

Firstly, and contrarily to theoretical predictions, M. australis did not compensate a low quality diet through an increase in food ingestion rate. However, significant differences between habitats (i.e. Matagusanos cavies eat more than Ñacuñán cavies), agree with expectation according to interpopulation differences in the size of fermentation chambers, which are larger in Matagusanos individuals (Sassi et al., 2007). One explanation of the apparent lack of compensation by intake rate in both groups of cavies is that under the two diet qualities dry matter ingestion rate peaks at a certain level. Similar results have been reported for other small-sized species (Fernández et al., 2002; Zynel and Wunder, 2002, Martino et al., 2007), in which ingestion was hypothesized to be centrally limited by the volume of the gut.

On the other hand, energy assimilation significantly decreased in Ñacuñán and remained unchanged for Matagusanos cavies, under low quality diet. This occurred because the mean energy of feces was lower in Matagusanos individuals, suggesting that they extracted relatively more energy than Ñacuñán ones, from a low quality diet. Mean retention time showed significant effects of diet quality and habitat, since populations responded differently to different quality diets. In Matagusanos cavies this parameter changed according to predictions: a lower retention time was observed in response to a low quality diet. As suggested by Lepczyk et al. (1998), under gut volume limitations retention time decreases as digesta flow into the intestine increases, allowing a constantly high energy extraction rate. This would increase energy assimilation and is consistent with data on dry

Table 1
Effect of habitat and experimental diet quality on dry matter digestibility (DMD), dry matter intake (DM1), digestible energy intake (DEI) and mean retention time (MRT) in cavies. Covariate mean values: body mass = 321.37 g. All values are presented as mean ± standard error, plus statistical effects and interactions between factors. Means with different letters differ significantly at $p < 0.05$ based on the Tukey post hoc test.

	Ñacuñán		Matagusanos	
	High food quality	Low food quality	High food quality	Low food quality
N° individuals	5	5	5	5
DMD (%)	64.59 ± 1.57 [a]	52.87 ± 3.71 [b]	65.44 ± 1.57 [a]	56.78 ± 3.71 [b]
Effects (p)	Diet quality = 0.001	Habitat = 0.51	Habitat×Diet quality = 0.47	
DMI (g day^{-1})	23.61 ± 1.99 [a]	20.98 ± 3.23 [a]	29.24 ± 1.99 [b]	28.80 ± 3.23 [b]
Effects (p)	Diet quality = 0.57	Habitat = 0.04	Habitat×Diet quality = 0.69	
DEI (kcal day^{-1})	65.01 ± 1.88 [a]	48.75 ± 3.22 [b]	63.62 ± 1.88 [a]	56.24 ± 3.22 [a,b]
Effects (p)	Diet quality = 0.001	Habitat = 0.39	Habitat×Diet quality = 0.02	
MRT (h)	25.05 ± 2.01 [a]	25.04 ± 1.27 [a]	33.28 ± 2.00 [b]	13.40 ± 1.27 [c]
Effects (p)	Diet quality = 0.001	Habitat = 0.33	Habitat×Diet quality = 0.0004	

matter intake. By contrast, Ñacuñán cavies did not show flexibility for retention time of food, and ingested a significantly lesser amount of food compared to Matagusanos cavies, which could account for the lower intake of digestible energy. One of the parameters which could indicate the compensation of a low quality diet is body weight. In view of our data, Ñacuñán cavies appear less efficient in coping with a low quality diet, since they lost weight significantly. Although the difference in dry matter digestibility between populations was not significant, individuals from Ñacuñán displayed a lower mean dry matter intake and digestibility under the low quality diet, did not respond as expected in retention time and showed lower energy assimilation. All this concurs with the decrease in body mass.

With regard to coprophagy, we suggest the existence of a mechanism for rapid passage, consistent with a higher rate of feces production and coupled with increasing soft feces re-ingestion rate in response to a low quality diet (Takahashi and Sakaguchi, 2000; Karasov and Martinez del Rio, 2007). Among cavies, the gut presents traits associated with this behavior; a relatively large caecum with taeniae and folds, and a longitudinal furrow along the proximal colon. These structures would allow the maintenance of bacterial concentration and fermentative capacity at high levels. Holtenius and Björnhag (1985) found that at certain moments, soft feces are expelled and the cycle ends with their re-ingestion, a behavior they called cecotrophy.

Consequently, we suggest that although closely linked to the morphological and physiological levels, the behavioral phase of cecotrophy is the flexible part of a complex attribute and probably the first line of phenotypic adjustment in M. australis. In fact, the frequency of cecotrophy has been reported as very variable, remaining stable to obtain certain nutrients, increasing to increase energy profits, or decreasing in order to relax competition for space with fresh matter (Martino et al., 2007). Most studies have been performed mainly in captivity (Ouellette and Heisinger, 1980; Altuna et al., 1998; Hirakawa, 2002; Martino et al., 2007); however it appears to play a role in the response to field nutritional bottlenecks (Kenagy et al., 1999). This was argued by Taraborelli (2007) in a field comparative study, where cecotrophy was observed only in the dry season of two populations of cavies, it was also significantly more frequent in the site where M. australis faces a comparatively lower quality diet (Sassi et al., 2007). Alexander (1993) reported that when diet quality improves, coprophagy has no effect on energy yield from food. Given the methodology of the present study, coprophagy could not be tested in measurements of dry matter ingest or digestibility data. Still, it would affect the results of energy assimilation, providing a mechanistic explanation to the change in body weight with diet quality. If selective retention occurs, the increase of the hindgut would favor the concentration of fine particles within the caecum and colon, and the consequent increase of fermentation rate under low quality diets. As a result, the efficiency of energy extraction increases linked to the rapid transit of larger particles and the recycling of the more digestible phase.

Foraging behavior is intimately related to physiological processes and structures present in the gut (Bozinovic and Martínez del Río, 1996). Among other parameters, the variation in diet quality permits the study of this relationship from an ecological perspective. Thus, in explaining habitat differences, we suggest that the nutritional environment in Matagusanos imposes costs due to comparatively poor quality food (Sassi et al., 2007), low abundance and diversity of food items (Sassi et al., in rev.). In Matagusanos, the amount of fiber in the diet of cavies is constant throughout the year, at a concentration higher than both seasons in Ñacuñán (Sassi et al., 2007). Lepczyk et al. (1998) suggested that short term compensation is unlikely, since digestion of food represents a tradeoff between processing rates of digesta and the thoroughness of digestion (Robbins, 1993). In this regard we propose that a larger hindgut (Sassi et al., 2007) and an increased digesta passage rate are present in the Matagusanos

population as the result of a constant signal from the environment. This could lead to local adaptation to consistent trophic conditions by means of genetic accommodation of a novel phenotype from a previously ample reaction norm (West-Eberhard, 2005; Pigliucci et al., 2006). Alternatively, differences could arise during development triggered by environmental factors and maternal effects, and eventually remain in the population (i.e. developmental recombination, West-Eberhard, 2005). Certainly, such hypotheses remain to be tested by long term studies dealing with heritability estimates, genetic correlation and fitness implications of plasticity in digestive traits (Naya et al., 2008).

In conclusion, the responses found within and between populations under experimental diet changes support our predictions regarding the acclimatization ability of M. australis. In fact, the small cavy along its geographic range undergoes spatial and seasonal changes in the quality of trophic resources, which is typical of arid environments. The variation in phenotypic traits may increase fitness under heterogeneous conditions (Piersma and Lindström, 1997; DeWitt et al., 1998; Naya and Bozinovic, 2004), and could explain the versatility of M. australis in terms of habitat colonization and use.

Acknowledgments

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