

Flexibility of digestive responses in two generalist herbivores, the tortoises *Geochelone carbonaria* and *Geochelone denticulata*

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Summary. To test the prediction that digestive responses (digestibility, intake and passage time) – of generalist herbivores vary with different diets, feeding trials were conducted in Venezuela with two sympatric tortoise species, *Geochelone carbonaria* and *G. denticulata*. Three single-species diets (two fruit, one foliage) were fed to both species. For a given diet, digestibility, mass-specific intake and passage time did not differ between the two tortoise species, nor did they vary by sex or body mass within each species. However, the digestive parameters varied for tortoises feeding on the different diets. The responses ranged from nearly abandoning cell wall fermentation and depending entirely on extraction of cell contents to relying heavily on cell wall fermentation. Therefore, these generalist herbivores have flexible digestive responses that are influenced by diet, not fixed digestive responses that limit the diet, as previously observed in other generalist herbivores. A three-part classification of herbivores (specialist, specialized mixed feeder and opportunistic mixed feeder) is suggested as an approach to understanding flexible and inflexible digestive strategies in herbivores.

Key words: Digestion – Frugivory – Herbivory – Nutrition – Tortoise

Generalist herbivores – herbivores that consume a mixture of foliage, fruit and flowers – select their diet from a great array of plants of different types and nutrient composition. Physiological and morphological adaptations to a varied diet should be reflected in three animal responses: 1) digestibility, the net loss of a nutrient from digesta as it moves through the digestive tract; 2) intake, the amount of food consumed each day; and 3) passage time, the time it takes food to pass through the digestive tract. The three parameters are interrelated. For a given diet, higher intakes generally result in shorter passage times and lower digestibilities. However, when comparing among diets, although the inverse relationship between intake and passage time continues to hold, no predictable relationship exists between intake or passage time and digestibility (Van Soest 1982; Robbins 1983).

The combination of these three animal responses determines the “digestive strategy” (sensu Milton 1981) of an organism. An “efficiency-velocity” continuum has been described (Milton 1981; Robbins 1983), along which herbi-

vores can be placed according to whether their digestive strategy is to maximize digestive efficiency or to maximize velocity of food processing. An often cited example is that of the ruminant as an efficiency maximizer and the equid as a velocity maximizer (Bell 1971; Janis 1976). One would expect that specialist herbivores, such as many ungulates that are strict folivores, would have a more restrictive digestive strategy, and thus could be classified in this manner (Van Soest 1982; Robbins 1983; Demment and Van Soest 1985).

The situation is not clear with generalist herbivores – herbivores that continually feed on an ever changing mixture of fruit, flowers and foliage. One would predict that generalist herbivores would have a more flexible digestive strategy to allow the animal to utilize its varied diet most efficiently depending on its composition, and that they would not have a fixed location on the efficiency-velocity continuum. However, in a study of passage times in two generalist herbivores with similar adult body mass (the mantled howler monkey, *Alouatta palliata*, and the black-handed spider monkey, *Ateles geoffroyi*), Milton (1981) concluded that both species were fixed on their respective locations on the efficiency-velocity continuum. Irrespective of diet, *A. palliata* always had long passage times – and thus, by inference, higher digestibility – whereas *A. geoffroyi* passed food rapidly, with presumably lower digestibility. Milton (1981) surmised that these inflexible digestive strategies determined the diet choice of the two species rather than the reverse. She attributed the inflexibility of the digestive responses to gut morphology. The more voluminous colon of *A. palliata* required slow passage of the digesta, regardless of its nature. The smaller colon of *A. geoffroyi* did not allow prolonged retention of digesta, resulting in necessarily rapid passage rates for all diet types.

Milton’s (1981) results contradict the above prediction that generalist herbivores should have flexible digestive responses. To determine whether other generalist herbivores are restricted to a fixed digestive strategy on the efficiency-velocity continuum, a series of feeding trials was conducted with two sympatric tortoises *Geochelone carbonaria* and *G. denticulata* in Venezuela. Both tortoise species are similar in body mass and have relatively simple gut morphology (Guard 1980; Bjorndal, unpublished work) with no obvious morphological characteristics that might restrict digestive flexibility. Tortoises were selected for this study because they select a variable diet. In Brazil, *G. carbonaria* and *G. denticulata* consume fruits and flowers from at least 33 spe-

cies (representing 16 families) and foliage from many additional species (Moskovits 1985); their diets have a wide range in chemical composition (Moskovits 1985). Also, both species feed selectively; that is, the relative abundance of food items in their diets does not reflect the relative availability of those food items in the environment (Moskovits 1985).

The feeding trials were designed to investigate the interactions of the three primary animal responses – digestibility, intake and passage time – and the effect that diet type (foliage or fruit) and nutrient content have on these responses in generalist herbivores. It is difficult to ascertain the extent to which diet determines digestive strategy or digestive strategy determines diet in free-ranging animals. In controlled feeding trials with captive animals, the potential range of animal responses can be measured. If animal responses in feeding trials vary significantly with diet, it would suggest that, in the wild, the digestive strategy is not fixed and can be affected by diet choice, rather than diet choice being determined by a fixed digestive strategy.

Methods

Feeding trials. Tortoises (males and females) were obtained in the state of Monagas, Venezuela. Body mass ranged from 0.4 to 4.8 kg for *G. carbonaria* and from 0.5 to 6.6 kg for *G. denticulata*.

Diets were selected: 1) to represent the range of chemical composition of the tortoises' natural diet (Moskovits 1985); 2) to be readily available and relatively constant in chemical composition throughout the fairly long period of the feeding trial (at least 5 weeks); and 3) to be palatable to the tortoises, because I relied on free-feeding, not force-feeding of, animals. The three diets selected – guava fruits (*Psidium guajava*), mango fruits (*Mangifera indica*), and lantana foliage (*Lantana urticifolia*) – were fed to both species. The two fruit species are not native to Venezuela but have become naturalized in many areas; *L. urticifolia* is native to Venezuela (R. Sanders, Fairchild Tropical Garden, pers. comm.).

Tortoises were placed in individual pens out of sight of each other. The pens were shaded by a roof so that the tortoises would not overheat and rain would not damage the food. Air temperatures 20 cm above the ground in the shaded pens ranged from 22 to 32° C during the trials.

The full range of body mass (given above) for each species was represented in each feeding trial. No change in body mass was recorded for any tortoise during the experiments.

A separate feeding trial was conducted for each diet; the three trials were run sequentially. Following a one-week adjustment period to the test diet, tortoises were presented a weighed amount of food each morning of sufficient quantity to ensure ad libitum feeding. The amount consumed each day was determined by subtracting the amount of food remaining after 8 h from the amount presented. Corrections were made for water loss by measuring loss of mass in a sample of diet placed in an adjacent, empty pen. The intake trial was run for 12 days, and mean daily intake was determined for each individual.

At the end of the intake trial, the digestibility trial was started. Each animal was fed a portion of diet dyed with fluorescent pigment, a marker of the particulate digesta;

the number of days until the dye first appeared in feces (determined by visual inspection) was used as a measure of passage time. Every day, each animal was fed an amount of food equal to 85% of its mean daily intake to ensure consistent feeding (Schneider and Flatt 1975); all offered food was consumed. Two days after the dye had passed in the feces, cloth bags were attached to the animals by threads passed through 1 mm holes drilled in the anterior carapace and plastron, and feces were collected daily for two weeks.

Digestive efficiencies were calculated by the equation

$$[(\text{intake of X} - \text{output of fecal X}) / \text{intake of X}] \times 100$$

where intake and output are measured in grams, except energy is in kJ, and X is a diet component (e.g., organic matter, energy, cell walls). This equation yields apparent digestive efficiencies, or the net loss of a nutrient from digesta as it passes through the digestive tract.

Sample preparation and chemical analyses. Feces were removed once a day for two weeks after the collection bags were attached. Daily fecal production was relatively constant with the consistent feeding regime. Uric acid pellets were removed from the feces to prevent contamination with urinary nitrogen so that digestibility of nitrogen could be calculated. The feces were dried to constant mass at 60° C. Diet samples were collected daily as the diets for the tortoises were prepared. The fresh mass of the diet sample was recorded, and the sample was dried to constant mass at 60° C.

Analyses for chemical composition of diet and fecal samples were conducted to determine the chemical composition of the diets and to allow calculation of digestibilities. Daily fecal samples were combined for individual tortoises. Dried diet and fecal samples were ground through a 1 mm screen in a Wiley mill. A portion of each sample was dried at 105° C to determine percent dry matter (DM) and then ashed in a muffle furnace for 3 h at 500° C to determine percent organic matter (OM). Percentage of cell walls [cellulose, hemicellulose and lignin, or neutral detergent fiber ash-free (NDF_A)] was measured by the Van Soest technique (Goering and Van Soest 1970) with decalin and sodium sulfite omitted (Golding et al. 1985). Use of amylase in the determination of cell walls (Robertson and Van Soest 1977) was tested and found not to be necessary for the fruit diets. Analyses for percentages of acid detergent fiber (a sub-fraction of cell walls which is approximately equal to the cellulose and lignin content) and potassium permanganate lignin followed Goering and Van Soest (1970). Energy content of food and feces was determined in a bomb calorimeter following standard procedure (Parr Instr. Co. 1960). Percent concentration of total (Kjeldahl) nitrogen was measured with a block digester (Gallaher et al. 1975) and an automated Technicon analyzer (Hambleton 1977).

Results

Digestibilities of four diet components (organic matter, energy, cell walls and nitrogen), ad libitum intakes and passage times for three diets in both *Geochelone carbonaria* and *G. denticulata* are presented in Table 1. The high digestibilities of cell walls in mango fruits and lantana foliage indicate that both tortoise species harbor microflora that are capable of degrading the cellulose and hemicellulose

Table 1. Results from *Geochelone* feeding trials. Digestibilities are expressed as percentages; intake is in g dry matter per kg fresh body mass per day; and passage time is in days. Carb = *G. carbonaria*; dent = *G. denticulata*. Values are mean \pm standard deviation; n = sample size

	Guava fruit		Mango fruit		Lantana foliage	
	carb	dent	carb	dent	carb	dent
Digestibilities (n)	4	5	6	5	4	5
Organic matter	36 ± 1.3	36 ± 0.8	69 ± 6.1	71 ± 3.4	38 ± 3.5	41 ± 5.6
Energy	32 ± 0.5	32 ± 0.7	65 ± 4.6	67 ± 4.1	34 ± 3.1	37 ± 4.8
Cell walls	7 ± 1.5	7 ± 0.5	40 ± 6.3	42 ± 4.3	37 ± 3.3	41 ± 2.3
Nitrogen	-3 ± 5.2	-1 ± 1.4	-12 ± 8.9	-14 ± 6.7	26 ± 7.8	31 ± 10.0
Intake (n)	4	5	7	8	4	5
	8.6 ± 0.5	10.5 ± 4.2	7.2 ± 1.2	8.2 ± 1.2	2.1 ± 0.8	2.3 ± 1.0
Passage time (n)	4	5	7	8	4	5
	2.6 ± 1.0	3.6 ± 0.9	6.6 ± 1.2	4.8 ± 1.3	9.5 ± 1.7	8.7 ± 1.9

Table 2. Chemical composition of test diets. All components are expressed as percent organic matter (OM) except dry matter is percent of fresh diet and energy is kJ per g OM

	Guava fruit	Mango fruit	Lantana foliage
Dry matter	20.6	12.6	30.8
Organic matter	95.7	95.7	92.3
Energy	19.7	20.7	21.7
Cell walls	58.3	28.6	53.0
Acid detergent fiber	46.6	18.9	40.3
Lignin	8.1	5.0	15.8
Nitrogen	0.95	0.65	2.99

in their diet. As in all other herbivorous reptiles studied (see Bjorndal 1987), microbial fermentation in *G. carbonaria* and *G. denticulata* occurs in the hindgut (Bjorndal, unpublished).

Intakes of the three diets varied greatly and varied inversely with passage time (Table 1). The low intake of lantana foliage compared to the two fruit diets do not reflect abnormal feeding; other turtles and tortoises feeding on foliage diets have similar intakes (Bjorndal 1985).

For each of the diets, the two tortoise species did not differ significantly in digestibility of the four components measured, intake, or passage time [Kolmogorov-Smirnov tests using chi-square distribution (Seigel 1956), $\alpha = 0.05$]. Analyzing the results for the two species separately, sex did not have a significant effect on any of the digestive responses [Kolmogorov-Smirnov tests using chi-square distribution (Seigel 1956), $\alpha = 0.05$]. Body mass did not have a significant effect in either species on digestibility of the four components, mass-specific intake or passage time (Spearman rank correlation, $\alpha = 0.05$), but did

have a significant effect on absolute intake in both species ($P < 0.01$, Spearman rank correlation).

As predicted, the different diets had a significant effect on all digestive parameters for each tortoise species: digestibilities ($P < 0.05$, Kruskal Wallis one-way ANOVA), absolute intake ($P < 0.05$, Kruskal Wallis one-way ANOVA), mass-specific intake ($P < 0.01$, Kruskal Wallis one-way ANOVA), and passage time ($P < 0.05$, Kruskal Wallis one-way ANOVA). As intended, the three diets varied greatly in chemical composition (Table 2) and fell within the range of chemical composition of natural food plants measured for the two tortoise species in Brazil (Moskovits 1985).

Discussion

Digestive responses to different diets

All plant tissues can be divided into two fractions – cell contents and cell walls. Cell contents (soluble carbohydrates, protein and lipids) are readily digested by the herbivore's endogenous enzymes and are rapidly absorbed. Cell walls are comprised of structural carbohydrates: cellulose, hemicellulose and lignin. The cell wall constituents cannot be digested by the herbivore's endogenous enzymes but can be degraded, to a variable extent and at a variable rate, by symbiotic gut microflora (Van Soest 1982). Generally, in non-ruminants, the microbial fermentation of cell walls is a slow process that occurs in the large intestine, whereas enzymatic digestion and absorption of cell contents is rapid, primarily occurring in the small intestine.

One would expect that generalist herbivores would adjust to the variety of diet items (with different ratios of cell walls to cell contents) by varying intake, passage rate and digestibility so that different diets would be processed to maximize the total efficiency of nutrient extraction. The results of the feeding trials indicate that this is the case with tortoises and that they thus have flexible digestive strategies (Table 1). Diets high in cell contents or low in cell wall digestibility, which are digested primarily enzymatically (e.g., guava), have high intakes and short passage times. Diets with high cell wall digestibilities requiring relatively slow microbial fermentation (e.g., lantana) have low intakes and long passage times.

Guava fruits have a low digestibility of cell walls, as expected from the short passage time, but digestibilities of cell walls in mangos and lantana are similar, despite the difference in passage times. However, the similarity in cell wall digestibility in mangos and lantana is misleading. Because the percent of cell walls is low in mango fruit (Table 2), the primary nutritional gain is from enzymatic digestion of the high percentage of cell contents. In the two tortoise species, the mean percentage of digested organic matter that is cell walls is 11% for guava fruits, 17% for mangos and 52% for lantana foliage (calculated by multiplying percent cell walls by percent cell wall digestibility and dividing by percent organic matter digestibility). These values of cell wall digestion fit the predicted pattern of an inverse relation between cell wall digestion and passage time.

The apparent energy gain (intake \times energy content \times % energy digestibility) of tortoises on the lantana diet is low compared with the two fruit diets. However, both fruit diets have negative nitrogen digestibilities; that is, the tortoises are in negative nitrogen balance on either fruit diet. This

observation supports the idea that generalist herbivores can feed on fruit to meet energy needs and on foliage to gain necessary nitrogen, which underscores the need to consider factors other than energy in studies of diet choice.

Gut morphology

A flexible digestive strategy requires a gut morphology that permits differential processing of various types of digesta and does not fix the rate of passage. Both tortoise species studied here have a relatively simple gut morphology. The cecum is only an eccentric dilation of the proximal colon, and the small intestine is roughly equal in length to the large intestine (Guard 1980; Bjorndal, unpublished). The expanse of the small intestine is sufficient to deal with a diet that is digested primarily or exclusively by the herbivore's endogenous enzymes, and the large intestine is not so expansive as to cause unnecessary delay in passage of such a diet. However, the large intestine is capable of retaining digesta long enough to allow for extensive microbial activity.

Reverse or anti-peristalsis may aid tortoises in adjusting passage rates to different types of food (Hukuhara et al. 1975). Varying the ratio of reverse to positive peristaltic contractions could adjust the passage rate of digesta in the large intestine.

Geochelone carbonaria and *G. denticulata* have small and large intestines of nearly equal length to maintain a flexible digestive strategy. One would expect that herbivores specializing in diets that are either digested largely by endogenous enzymes or largely by a hindgut microbial fermentation should have a higher proportion of either small intestine or large intestine, respectively. Evidence for this prediction is found in the green turtle, *Chelonia mydas*, an herbivore that specializes on a folivorous diet and depends on a symbiotic microflora in its hindgut (Bjorndal 1982). Green turtles have a large intestine about three times as long as the small intestine (Bjorndal 1979, 1985).

Digestive responses of generalist herbivores

The extremes in digestive processing observed in the present data set for single-species diets may not be common in free-ranging tortoises because their diets are generally a mixture of plant types. Mixed diets could decrease the range of digestive responses because of associative effects among different ingested foods that affect the digestibility, intake or passage rate of other foods (Van Soest 1982; Robbins 1983).

Clearly, the tortoises are not locked into one digestive strategy or one position on the efficiency-velocity continuum as Milton (1981) reported for *A. palliata* and *A. geoffroyi*. Thus the present study demonstrates that Milton's model of diet selection is inappropriate for some generalist herbivores. It remains to be determined whether the majority of generalist herbivores has flexible or inflexible digestive strategies, and, thus, whether their diet choice is dictated by fixed digestive responses or their digestive responses are determined by diet choice.

A three-part classification may be useful when considering diet selection and digestive responses of herbivores: specialists, specialized mixed feeders and opportunistic mixed feeders. Specialists are those herbivores that feed only within a narrow range of plant types, e.g., folivores. Specialized

mixed feeders – herbivores that ingest a mixed diet, but with relatively constant ratio of foliage to fruit – may be represented by *A. palliata* and *A. geoffroyi*, based on feeding habits reported by Milton (1981). Both specialists and specialized mixed feeders should have more inflexible digestive responses than the third group, the opportunistic mixed feeders. These herbivores, represented by *G. carbonaria* and *G. denticulata*, ingest the most variable diet and should have the widest range of digestive responses.

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