Journal of Herpetology, Vol. 39, No. 2, pp. 321–324, 2005

Microbial Fermentation in Juvenile and Adult Pond Slider Turtles, *Trachemys scripta*

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ABSTRACT.—**Herbivorous reptiles use microbial gut symbionts to digest plant material. These symbionts ferment cell wall components, producing short-chain fatty acids (SCFA), which the host uses as an energy source. In reptiles, fermentation usually occurs in the large intestine; however, the freshwater Florida Red-Bellied Cooter, *Pseudemys nelsoni*, has both small and large intestine fermentation. Although small intestine fermentation has not been found in other chelonians, no other freshwater turtles have been examined. We measured SCFA concentrations in the digestive tracts of juvenile and adult Pond Sliders, *Trachemys scripta*. Like many other turtles, *T. scripta* experiences an ontogenetic diet shift from carnivory to herbivory, and it is unknown whether juveniles can digest plant material. We determined whether (1) this species harbors small intestine fermentation, (2) juveniles possess SCFA concentrations comparable to other herbivorous reptiles, and (3) a change in relative fermentation chamber capacity accompanies the diet shift. We fed turtles a plant diet for five weeks and then measured SCFA concentrations in their gastrointestinal tracts and the mass of gastrointestinal tract contents. Both juveniles and adults had SCFA concentrations comparable to other herbivorous reptiles; however, they did not have significant small intestine fermentation. Additionally, there was no difference between the relative masses of juvenile and adult fermentation chamber contents. Therefore, the ontogenetic diet shift in *T. scripta* is not accompanied by a change in relative gut capacity.**

Microbial gut symbionts play a critical role in the digestive physiology of herbivorous reptiles (Zimmerman and Tracy, 1989; Stevens and Hume, 1995; Bjorndal, 1997). These symbionts ferment the cell wall constituents of plants, producing short-chain fatty acids (SCFAs) that the host absorbs and uses as an energy source. For reptiles that rely on fermentation to help meet their energy requirements, the capacity of the fermentation chamber must be sufficiently large or passage of digesta through the fermentation chamber must be delayed by negative peristalsis or morphological structures so that cell wall constituents can be digested and microbes can reproduce. In herbivorous reptiles, fermentation chamber capacity (as measured by mass of fermentation contents) scales with body size according to the equation: capacity = 0.0926 body size$^{0.9919}$ (Bjorndal, 1997).

In most herbivorous reptiles studied to date, fermentation occurs primarily in the large intestine (Troyer, 1984b; Stevens and Hume, 1995; Bjorndal, 1997). The only known exception is the freshwater Florida Red-Bellied Cooter, *Pseudemys nelsoni*, which maintains significant fermentation in both the small and large intestines (Bjorndal and Bolten, 1990). The small intestine is typically where carbohydrates and proteins are digested by endogenous enzymes of the turtle (Stevens and Hume, 1995). Fermentation in this region is, therefore, surprising because endogenous enzymes must compete with microbial symbionts for these high quality nutrients. However, Bjorndal and Bolten (1990) concluded that the capacity of the large intestine alone did not provide an adequate fermentation chamber for an herbivore the size of *P. nelsoni*. They hypothesized that, because of limited space within the turtle shell, expansion of the large intestine would necessarily involve a decrease in small intestine size. Although such a trade-off would have potentially little consequence for an herbivore relying on fermentation, it could compromise juvenile digestive efficiency if young *P. nelsoni* are carnivorous like the young of many other freshwater turtle species (Sexton, 1959; Clark and Gibbons, 1969; Moll, 1976; Hart, 1983). Reduced juvenile digestive efficiency has fitness consequences because both survivorship and future reproductive output can be linked to rapid juvenile growth (Congdon and Gibbons, 1983; Bodie and Semlitsch, 2000).

Small intestine fermentation does not occur in other chelonians investigated to date, including the Green Seaturtle, *Chelonia mydas* (Bjorndal, 1979), Red Foot Tortoise, *Geochelone carbonaria* (Guard, 1980), and Desert Tortoise, *Gopherus agassizii* (Barboza, 1995). However, no freshwater species other than *P. nelsoni* have been examined. One freshwater turtle that could harbor small intestine fermentation is the Pond Slider, *Trachemys scripta*. As juveniles, these turtles are carnivores that feed on aquatic invertebrates, but, as they mature, they become opportunistic omnivores that feed primarily on aquatic plants (Clark and Gibbons, 1969; Hart, 1983). Adult *T. scripta* presumably maintain active microbial fermentation because they can digest plant cell wall constituents (Bjorndal, 1991, 1997). However, the presence of SCFAs and the location of the fermentation chamber have not been evaluated.

Additionally, it is not known whether significant microbial fermentation occurs in juvenile *T. scripta*. Because gut tissue is energetically expensive (Cant et al., 1996) and because digestion of animal matter requires a smaller gut capacity than digestion of plants, juveniles may possess relatively smaller gut capacities

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TABLE 1. Nutrient composition of duckweed, *Lemna valdiviana*, fed to juvenile and adult turtles, *Trachemys scripta*. All values except energy are presented on a percent dry matter basis.

	Juvenile trial	Adult trial
Organic Matter (%)	86.4	86.4
Fiber (%)		
Neutral detergent fiber (cellulose, hemicellulose, lignin, and cutin)	41.2	46.7
Acid detergent fiber (cellulose, lignin, and cutin)	19.7	29.9
Nitrogen (%)	5.0	2.7
Energy ($kJ \cdot g^{-1}$ dry matter)	18.49	16.56

than do adults. Such an ontogenetic shift in gut capacity could allow juveniles to allocate more energy to growth, maximizing survivorship and future reproduction (Congdon and Gibbons, 1983; Bodie and Semlitsch, 2000). If relative gut capacity increases with age, juveniles may not possess a sufficiently large fermentation chamber to maintain microbial fermentation when fed a plant diet.

The purpose of this study was to measure SCFA concentrations in the digestive tracts of juvenile and adult *T. scripta* to determine whether (1) this species harbors small intestine fermentation, (2) juveniles possess significant concentrations of SCFAs compared with other reptiles known to rely on microbial fermentation, and (3) the ontogenetic diet shift is accompanied by changes in fermentation chamber capacity.

MATERIALS AND METHODS

Juvenile *T. scripta* were obtained from a commercial turtle farm in Pt. Mayaka, Florida in mid-June 2000. These turtles were the offspring of breeding adults collected from northern Florida, Georgia, and South Carolina. Feces from adult freshwater turtles caught in a local pond in Gainesville, Florida, were fed to juveniles after their arrival in the laboratory to ensure turtles acquired gut symbionts (Troyer, 1984a). Adult turtles were collected in May 2001 from Kathwood Ponds located in the Audubon Society's Silver Bluff Sanctuary in Aiken County, South Carolina. Before the experiment, turtles were maintained on a mixture of aquatic plants and invertebrates collected from Gainesville area ponds.

The trial with juveniles was conducted from 9 November to 14 December 2000, and the trial with adults from 24 September to 1 November 2001. At the onset of the study, juveniles and adults had a mean mass of 12.3 ± 0.5 g ($N = 13$, range = 9.1–15.8 g) and 770.5 ± 92.8 g ($N = 7$, range = 375.2–1183.0 g), respectively. Adults ($N = 7$) were housed individually in Nalgene tanks (45 × 60 cm) equipped with a 75-W floodlight and a 20-W full spectrum natural light fluorescent bulb. Juveniles were divided in two groups ($N = 6$ and 7) and housed in Nalgene tanks equipped with the same lighting as adult tanks. During both trials, turtles experienced a 12-h photoperiod and temperatures between 25 and 26 °C.

For five weeks, all turtles fed ad libitum on duckweed, *Lemna valdiviana*, a small, floating aquatic plant consumed by adult *T. scripta* throughout much of their range (Parmenter and Avery, 1990). Although juveniles are primarily carnivorous in the wild, they will consume *L. valdiviana* in the laboratory. For the trial with juveniles, duckweed was collected from a pond in Gainesville, Florida. Because this pond dried up before the onset of the trial with adults, duckweed for that trial was obtained from a local aquarium shop. Table 1 describes the nutrient composition of duckweed from each source (for analytical methods see Bouchard, 2004).

After five weeks, turtles were euthanized with sodium pentobarbital. Turtles were dissected, digestive tracts removed, and digesta samples collected. In adults, samples were taken from five gut sections (stomach, anterior and posterior small intestine, and anterior and posterior large intestine; mean sample wet mass = 0.80 g) and preserved in 20% phosphoric acid, which stopped fermentation and the production of SCFAs. The remaining contents of these gut regions were removed, weighed, and dried at 60 °C. Because juvenile guts were so much smaller than those of adults, all digesta in each gut region was used for analysis of SCFAs. Samples were collected and preserved from four gut regions (stomach, small intestine, and anterior and posterior large intestine; mean sample wet mass = 0.31 g) because there was not enough digesta in the small intestine for analyzing anterior and posterior sections separately. Samples from both age classes were centrifuged, and SCFA concentrations of the supernatants were measured using a Shimadzu gas chromatograph (Model GC-9AM) with a Perkin Elmer Computing Integrator (LCI-100). Peak retention times and areas were compared to known standards run under identical conditions. Sample peak responses were within the linear range of the detection system and within fivefold of the area of the standard. After SCFA concentrations were determined, water was evaporated from vials. Dry mass of samples was determined with corrections made for the quantity of phosphoric acid present.

SCFA concentrations were compared between small and large intestines using a paired *t*-test. In juveniles, the composite small intestine sample was compared to the anterior large intestine sample, whereas in adults, the posterior small intestine sample was compared to the anterior large intestine sample. One adult turtle was not included in this analysis because SCFA concentrations in its digestive tract were over four standard deviations below the mean. Because duckweed fed to adults and juveniles varied in fiber content, no comparisons were made between SCFA concentrations in adults and juveniles. However, in both age classes, SCFA concentrations indicated that the large intestine was the main fermentation chamber. Therefore, the mass of large intestine contents was compared between juveniles and adults with a *t*-test. This comparison was performed on a mass-specific basis because fermentation chamber capacity scales isometrically with body mass in reptiles (Bjorndal, 1997). A paired *t*-test was used to compare mass of large intestine contents of juveniles and adults with values predicted for herbiv-

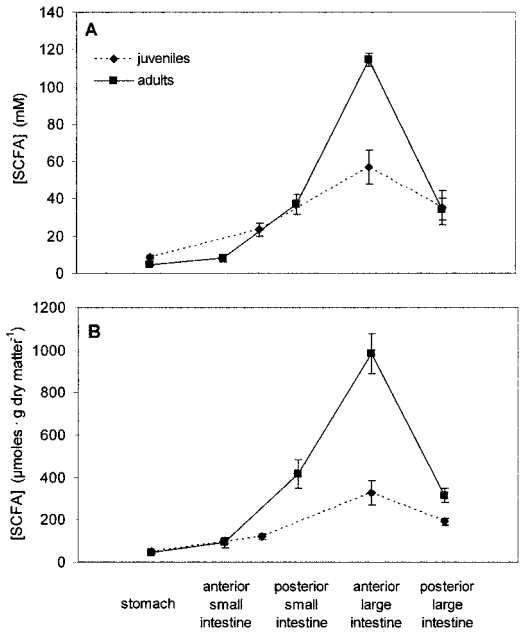

FIG. 1. Mean concentrations of total short-chain fatty acids in the digestive tracts of juvenile ($N = 13$) and adult ($N = 6$) *Trachemys scripta*. Bars represent standard errors. Note that small intestine values for juveniles are from the entire small intestine and are not divided into anterior and posterior regions as in adults. (A) Concentrations are presented on molar basis. (B) Concentrations are presented on dry matter basis.

orous reptiles of the same size based on the equation in Bjorndal (1997). This equation is based on interspecific comparisons of six species of herbivorous reptiles and is not significantly different from the well-established relationship described for herbivorous mammals by Parra (1978). Means are given ± 1 SE.

RESULTS

The gastrointestinal tracts of *T. scripta* were relatively simple, consisting of a stomach, small intestine, and large intestine with a slight eccentric dilation at the proximal end as described in other reptiles by Guard (1980). SCFAs were found in the digestive tracts of both juveniles and adults with concentrations peaking in the anterior large intestines of both age classes (Fig. 1). In juveniles, concentrations in the anterior large intestines were 148% higher on a molar basis and 172% higher on a dry matter basis than in the composite small intestine (molar basis: $t_{12} = 3.096$, $P = 0.009$; dry matter basis: $t_{12} = 3.460$, $P = 0.004$). For adults, concentrations in the anterior large intestine were 210% higher on a molar basis and 136% higher on a dry matter basis than in the posterior small intestine (molar basis: $t_5 = 5.21$, $P = 0.006$; dry matter basis: $t_5 = 3.57$, $P = 0.023$). In the anterior large intestine of both juveniles and adults, acetate was produced in the highest proportions followed by propionate and butyrate (Table 2).

Mean mass-specific wet mass of fermentation contents (i.e., large intestine contents) did not vary between age classes (juveniles: 49.49 ± 2.21 g; adults: 54.14 ± 6.65 g; $t_{18} = 0.87$, $P = 0.40$). The mean mass of juvenile fermentation contents (0.64 ± 0.05 g) was approximately half that predicted for herbivorous reptiles of the same size (1.22 ± 0.05 g; $t_{12} = 21.91$, $P = 0.001$). The mass of adult fermentation contents (40.14 ± 5.21 g) was also significantly less than predicted (71.47 ± 8.55 g; $t_6 = 4.309$, $P = 0.005$).

TABLE 2. Molar percentages of individual short-chain fatty acids in the anterior large intestines of juvenile and adult *Trachemys scripta*. Values are means ± SE. Molar percentages for other gastrointestinal tract regions can be found in Bouchard (2004).

	Juveniles ($N = 13$)	Adults ($N = 6$)
Acetate	81.9 ± 2.1	75.9 ± 3.7
Propionate	12.0 ± 1.0	11.2 ± 2.9
Butyrate	3.4 ± 0.4	10.2 ± 1.6
Isobutyrate	2.7 ± 2.1	1.1 ± 0.1
Valerate	0.0 ± 0.0	0.4 ± 0.3
Isovalerate	0.0 ± 0.0	1.2 ± 0.3

DISCUSSION

Concentrations of total SCFAs in the large intestines of both juvenile and adult *T. scripta* are indicative of an active microbial fermentation and are within the range found in the hindguts of other herbivorous reptiles (range: 51–807 μmoles · mL^{-1}; Bjorndal, 1997) and mammals (range: 18–236 μmoles · mL^{-1}; Stevens and Hume, 1995). Although direct comparisons of concentrations between age classes were not made because of differences in diet nutrient composition, relative proportions of SCFAs in both juveniles and adults were consistent with the pattern found in most reptiles: acetate > propionate > butyrate > valerate (Bjorndal, 1997).

SCFAs were found in the small intestine of *T. scripta*; however, they were at significantly lower levels than in the anterior large intestine, indicating that the main fermentation chamber has not expanded to include the small intestine. This is somewhat surprising because large intestine contents in *T. scripta* are approximately half the mass of fermentation contents expected for an herbivorous reptile of that size. This contrasts with *P. nelsoni*, which had equally high SCFA concentrations in the small and large intestines, resulting in a fermentation chamber capacity equivalent to that predicted for that size reptile (Bjorndal and Bolten, 1990). Adult *T. scripta* are much more omnivorous than *P. nelsoni* (Bjorndal and Bolten, 1993) and probably rely less on fermentation as an energy source than do *P. nelsoni*. Consequently, there may be less selective pressure for *T. scripta* to expand their fermentation chamber capacity.

Differences in relative fermentation chamber size between *T. scripta* and *P. nelsoni* probably account for differences in digestive performance observed between these species. Bjorndal and Bolten (1993) found that both turtles had the same daily gain of energy and nitrogen on a diet that required limited microbial fermentation, the duckweed *Spirodela punctata*. However, on a diet requiring more extensive fermentation (the aquatic plant *Hydrilla verticillata*), *P. nelsoni* had

greater daily gains than did *T. scripta*. Similarly, the large intestine contents of the omnivorous tortoise, *Kinixys spekii*, were about half the mass of fermentation contents expected for an herbivorous reptile of that size (52.8 g vs. 101.8 g), whereas the mass of large intestine contents of the herbivorous tortoise, *Geochelone pardalis*, better approximated the expected value (285.6 g vs. 311.7 g; Hailey, 1997). Both species digested a low fiber diet of kale, *Brassica oleracea*, to the same extent. However, the omnivore consumed only minimal quantities of a more fibrous, grass diet, *Lolium* sp., which the herbivore readily consumed and digested.

Comparisons between the large intestine contents of juveniles and adults indicated that the wet mass of *T. scripta* fermentation contents scales isometrically with body size, as has been found in mammals (Parra, 1978) and other reptiles (Troyer, 1984a; Bjorndal, 1997). Thus, the ontogenetic diet shift from carnivory to herbivory experienced by these turtles is not associated with a change in relative fermentation chamber capacity. Juveniles might, therefore, be capable of digesting and subsisting on plant material, despite the fact that they are primarily carnivorous in the wild.

Acknowledgments.—Funding for this work came from the Archie Carr Center for Sea Turtle Research, Brian Riewald Memorial Fund, Linnaeus Fund of the Chelonian Research Foundation, Sigma Xi, and Grants in Herpetology of the Society for the Study of Reptiles and Amphibians. P. Coehler, D. Connelly, J. Congdon, and B. Hopkins assisted with the collection of turtles by providing equipment and access to ponds. C. Baker, L. Barrow, and R. Marcus assisted with care and maintenance of turtles and with dissections. D. Chynoweth provided access to the gas chromatograph, and P. Haley assisted with the short-chain fatty acid analyses. L. Chapman, C. Lanciani, D. Levey, and N. Frazer provided constructive comments on the manuscript. The University of Florida Institutional Animal Care and Use Committee approved this research.

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Accepted: 18 February 2005.