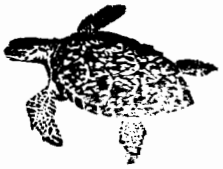

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# DIGESTIVE FERMENTATION IN GREEN TURTLES, *CHELONIA MYDAS*, FEEDING ON ALGAE

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The green turtle, *Chelonia mydas*, is the only herbivorous marine turtle (Mortimer, 1982). When feeding on the seagrass *Thalassia testudinum*—the primary diet of the green turtle in the Caribbean (Mortimer, 1982)—the green turtle relies on a microbial fermentation in its hindgut to degrade the plant cell walls (Bjorndal, 1979). The major end products of this fermentation, volatile fatty acids (VFA), provide an important energy source to the green turtle (Bjorndal, 1979).

Green turtles in the waters around the Ogasawara Islands, Japan, feed on algae. Kurata et al. (1978) recorded 34 species of marine algae in the diet of green turtles there. To determine whether green turtles harbor a microbial fermentation when their diet is predominantly algae, we examined the digestive tracts of green turtles from the Ogasawara Islands.

## METHODS

Digestive tracts were obtained from five green turtles killed in a legal harvest in the Ogasawara Islands, Japan. One juvenile male with body mass of 10.4 kg and straight carapace length (SCL) of 42.8 cm was in the sample. The other turtles were adults, one male and three females, with a range in mass from 105.5 to 135.5 kg and range in SCL from 86.0 to 99.8 cm. All turtles had been feeding on a mix of algae species. All sections of all digestive tracts contained digesta.

In all but one adult female, digesta samples from seven regions of the digestive tract were removed soon after death and preserved with meta-phosphoric acid for later analysis for volatile fatty acids (VFA). In each section, pH was measured to 0.1 pH units with indicator sticks (ColorpHast, E. Merck Co.). Length of each region of the gut was measured.

Samples for VFA analysis were centrifuged, and the supernatant and pellet were separated for analyses. The supernatant was analyzed for concentrations of VFA (acetate, propionate, butyrate,

i-butyrate, valerate and i-valerate) on a Hewlett Packard Model 5880A chromatography system with auto-sampler and electronic integrator. For this study, values for the two forms of butyrate and valerate were combined.

The pellet was washed with water, centrifuged and dried at 105°C to allow VFA concentration to be expressed on a per gram dry matter basis. VFA concentrations are also expressed on a total volume basis. These values should not be confused with, and will be much lower than, values reported in the literature expressed on the basis of the volume of the fluid fraction only.

VFA concentrations and molar percentages (after arcsine transformation) among regions of the digestive tract were compared with repeated measures ANOVA to control for inter-individual variation (SAS, 1982; Zar, 1984). Unless otherwise stated, alpha = 0.05.

## Results and Discussion

The high VFA concentrations in the hindgut of the green turtles in this study indicate that an active microbial fermentation occurs in that region (Table 1, Fig. 1). Concentrations of VFA are expressed on both a dry matter and volume basis to facilitate comparisons with other studies (Table 1). Mean VFA concentrations are significantly higher in the cecum, anterior colon and mid colon than in the other sections of the digestive tract (ANOVA, Tukey's test). The low concentrations of VFA (predominantly acetate) in the esophagus and stomach (Table 1) are probably derived from the plant matter itself, not microbial activity. The VFA concentration in the posterior colon (the last 30 cm of the colon) is significantly

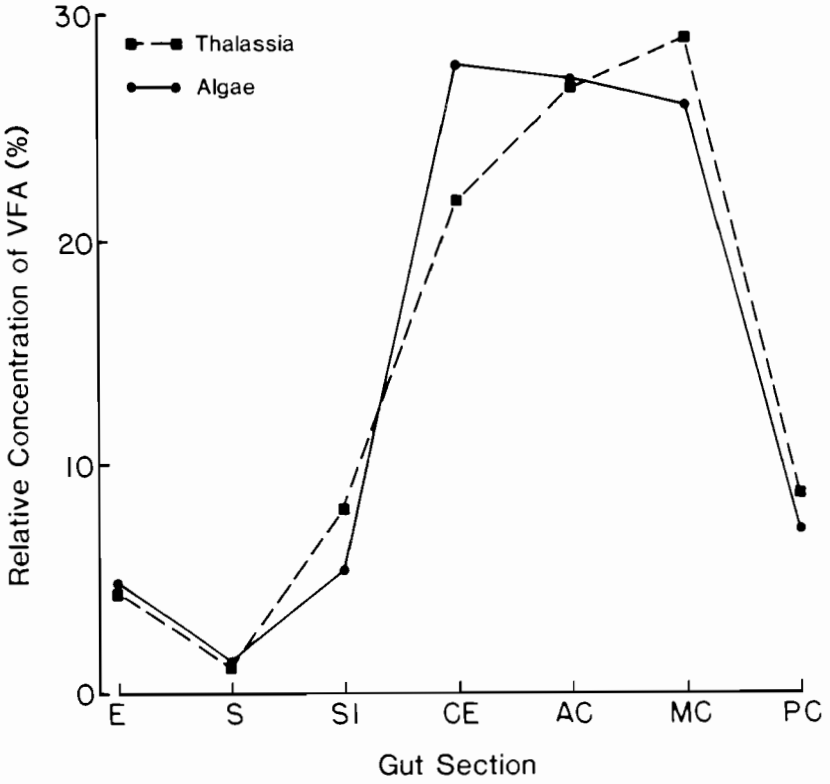


Figure 1. Relative concentrations of VFA for green turtles feeding on algae (this study) and on the seagrass *Thalassia testudinum* (data from Bjorndal, 1979). Relative concentration in each gut section is percentage of the sum of mean concentrations for all gut sections. E is esophagus, S is stomach, SI is small intestine, CE is cecum, AC is anterior colon, MC is mid colon, and PC is posterior colon.

Table 1. Mean ± standard deviation of pH, and VFA concentration on a volume basis ($\mu M \cdot ml^{-1}$ total sample volume) and on a dry matter (DM) basis ($\mu M \cdot g^{-1}$ DM). VFA means within each column with different superscripts are significantly different (ANOVA, Tukey's test). Sample size for pH values is 5; sample size for VFA concentrations is 4

| | | VFA concentration | |
|---|---|---|---|
| | pH | $\mu M \cdot ml^{-1}$ | $\mu M \cdot g^{-1}$ DM |
| Esophagus | 6.6 ± 0.9 | $^{A}12 \pm 9$ | $^{A}86 \pm 23$ |
| Stomach | 2.6 ± 1.3 | $^{A}4 \pm 4$ | $^{A}164 \pm 154$ |
| Small intestine | 6.6 ± 0.5 | $^{A}13 \pm 20$ | $^{A}203 \pm 106$ |
| Cecum | 6.4 ± 0.5 | $^{B}67 \pm 22$ | $^{B}1,174 \pm 339$ |
| Anterior colon | 6.8 ± 0.8 | $^{B}66 \pm 18$ | $^{B}1,220 \pm 319$ |
| Mid colon | 6.4 ± 0.5 | $^{B}63 \pm 30$ | $^{B}870 \pm 521$ |
| Posterior colon | 6.2 ± 0.4 | $^{A}17 \pm 5$ | $^{A}134 \pm 118$ |

lower than in other sections of the large intestine (Table 1) for two reasons. First, much of the fermentable substrate has been degraded before the digesta reaches that region. Second, the VFA produced in more anterior regions of the colon are absorbed across the gut wall and do not accumulate in the gut contents. If VFA were not absorbed, the pH of the digesta would decrease as the acids accumulated (Table 1).

Because of differences in methods of collection and preservation, the VFA concentrations in this study cannot be compared directly with those measured in green turtles on a *Thalassia* diet (Bjorndal, 1979). However, relative concentrations of VFA along the digestive tracts in green turtles feeding on algae and on *Thalassia* are similar (Fig. 1). Thus, the relative microbial activity among gut sections are the same on two diets. The pH values for each gut section (Table 1) are also similar to those of green turtles on a *Thalassia* diet (Bjorndal, 1979). The VFA concentrations for green turtles feeding on algae or on seagrass fall within the range of values measured in herbivorous mammals with foregut and/or hindgut fermentations (Parra, 1978; Bjorndal, 1979) and in the herbivorous freshwater turtle *Pseudemys nelsoni* (Bjorndal and Bolten, in press).

VFA molar percentages (Fig. 2) did not vary significantly among regions (ANOVA on arcsine-transformed data). In regions of active fermentation, the relative amounts of VFA are acetate > propionate > butyrate > valerate (Fig. 2). This sequence is the most common in gut fermentations in vertebrates (Van Soest, 1982). However, it differs from the relative proportions of acetate > butyrate > propionate found in green turtles feeding on *Thalassia* (Bjorndal, 1979). This difference between diets is not surprising. In mammals, molar percentages of VFA are affected by diet and status of methanogenic bacteria and protozoa (Van Soest, 1982). In the gopher tortoise, *Gopherus polyphemus,* the relative proportions of VFA in feces differed between two diets (Bjorndal, 1987).

Molar percentages of VFA must be interpreted with care. In the posterior colon, the relative proportion of propionate is larger and that of acetate is smaller than in more proximate sections (Fig. 2). This shift does not represent an increase in propionate concentration in the posterior colon. Rather, propionate concentration is relatively constant along the colon; the shift in molar percentages in the posterior colon results from a sharp decline in the concentration of acetate in that region.

The importance of the large intestine in the nutrition of the green turtle is underscored by the relative intestine lengths. The ratio of large intestine length to small intestine length is 2.4 (±0.6) in this study. Mean ratio of total intestine length (both small and large) to straight carapace length was 10.6 (±1.5). The

relative investment in large intestine tissue to small intestine tissue is not significantly different (Kruskal-Wallis, $P = 0.371$) in green turtles feeding on algae or seagrasses: 2.4 (this study), 2.9 ($\pm 0.6$, N = 4) for algae-eating green turtles in Australia (Thompson, 1980)[1], and 2.5 ($\pm 0.4$, N = 5) for *Thalassia*-eating green turtles in Nicaragua (Bjorndal, 1985; Mortimer, unpubl.).

Bjorndal (1985) hypothesized that microbial populations in the digestive tracts of green turtles may affect diet selection. This theory was suggested by several lines of evidence. First, as reviewed by Mortimer (1982), in many areas where both seagrasses and algae are present, green turtles feed on either algae or on seagrasses, not on a mixture. Second, in turtles (and dugongs) that feed primarily on seagrasses, algae will appear totally undigested in the feces in contrast to the very digested appearance of the seagrass (Bjorndal, 1980). Conversely, in green turtles that feed primarily on algae, blades of *Thalassia* in the posterior colon appear undigested, again in contrast to the very digested appearance of algae surrounding them in the colon (Bjorndal, pers. obs.).

The complex carbohydrates that comprise the cell walls of seagrasses are quite different from those in marine algae; structural carbohydrates also vary consid-

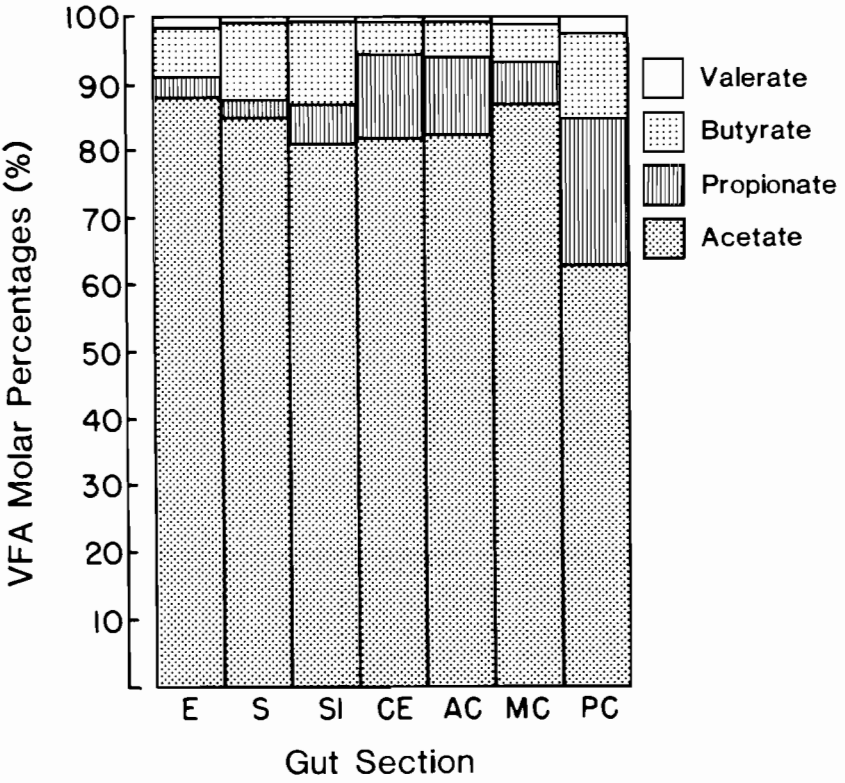


Figure 2. Mean molar percentages of VFA in each section of the digestive tract. There was no significant difference among regions (ANOVA on arcsine-transformed data). Abbreviations are given in Figure 1.

[1] Thompson, S. M. 1980. A comparative study of the anatomy and histology of the oral cavity and alimentary canal of two sea turtles: the herbivorous green turtle *Chelonia mydas* and the carnivorous loggerhead *Caretta caretta*. Master's thesis, James Cook University of North Queensland, Australia, 314 pp. Unpublished.

erably among algae. The gut microflora that would develop in green turtles feeding on seagrasses would almost certainly be different than that of green turtles feeding on algae. Bjorndal (1985) suggested that this specificity of microflora could affect diet selection because turtles with gut flora adapted to algae would digest seagrasses less efficiently and vice versa. Although gut microflora adapt to long-term diet shifts by varying the number and relative abundance of microbial species (Hungate, 1966), turtles would digest food less efficiently if they made successive, short-term diet shifts. There is a parallel situation in Orkney sheep on North Ronaldsay Island (Greenwood et al., 1983a; 1983b; Orpin et al., 1985).

However, the extent of the restriction on diet selection in green turtles that Bjorndal (1985) hypthesized has been over-emphasized by researchers working with sea turtles. Green turtles, like all organisms, forage to fill their digestive tracts with food that will yield the most nutrition for the least investment in search and handling costs. Specificity of the microflora may be one component in the optimal foraging strategy of the green turtle, but it will not overwhelm all others. When vast pastures of seagrass and/or algae are available, the optimal forage for green turtles may well be that to which its gut flora is adapted (either all seagrass or all algae). However, where food is limited or where food types are more dispersed, the greater search and handling costs of seeking either an all-algae or all-seagrass diet may be greater than the energy gain from more efficient digestion. In this case, the turtle would ingest a mixed diet.

Animals consistently ingesting a mixed diet would almost certainly develop a microflora capable of degrading the various complex carbohydrates. In some areas of Australia, green turtles ingest both seagrasses and algae and, in the feces, both components have the appearance of being equally digested (C. Limpus, pers. comm.). However, the microbial populations in these turtles would have to be constantly adapting to the digesta as the proportions of seagrass to algae, and the proportions of the various algae, change. Although green turtles feeding on a mixed diet may have a lower digestive efficiency as a result, their nutrient gain may well be maximized by the ability to ingest a greater quantity of the mixed diet more rapidly.

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