

CELLULOSE DIGESTION AND VOLATILE FATTY ACID PRODUCTION IN THE GREEN TURTLE, *CHELONIA MYDAS*

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Abstract—1. Cellulose is digested as efficiently in the green turtle, *Chelonia mydas*, as it is in ruminants and the dugong, *Dugong dugon*.

2. The production of volatile fatty acids (VFA) in the cecum of the green turtle was estimated from *in vitro* studies.

3. Acetate, butyrate and propionate are the VFAs produced, listed in order of decreasing concentrations.

4. Organic acids produced in the cecum provide 15.2% of the green turtle's daily energy balance.

5. Hydrogen is the major gas evolved during *in vitro* fermentation of cecum contents.

INTRODUCTION

Until recently reptilian herbivores were thought to be nutritionally dependent upon easily soluble, non-fibrous plant fractions because it was assumed that they lacked the intestinal specializations necessary for fiber (cellulose and hemicellulose) digestion (Szarski, 1962; Sokol, 1967; Berry, 1974; Wilson & Lee, 1974; Harlow *et al.*, 1976). The unaltered appearance of high-fiber vegetation in reptile scats supports this assumption. An exception is the herbivorous green turtle, *Chelonia mydas*, whose feces appear well digested. The contrast between the appearance of the scats of green turtles and those of other herbivorous reptiles (e.g. *Conolophus pallidus*, *Geochelone elephantopus*, *Gopherus agassizi* and *Gopherus polyphemus*) prompted me to study the digestive physiology of the green turtle. During a 12 month study conducted with green turtles feeding on the seagrass *Thalassia testudinum*, their natural food, in an impounded bay on Great Inagua, Bahamas, high apparent digestibility coefficients for cellulose and hemicellulose were found throughout the year in four size classes of turtles. A rich cellulolytic microflora in their gut is responsible for the fiber digestion (Bjorndal, in preparation).

These data raise the questions of where along the gut the cellulose breakdown occurs, what end products are formed, their rates of formation and their degrees of absorption. The opportunity to pursue these questions was provided by an invitation from the Seagrass Study Group to join their *Alpha Helix* cruise in November 1977 to the Miskito Cays, Nicaragua, where the major feeding grounds of the green turtle in the Caribbean are located. The results of that study are reported here.

METHODS

While on board the R.V. *Alpha Helix*, I obtained the complete digestive tracts of two green turtles (50 and 82 kg) which had been caught the night before by Nicaraguan turtles and were later consumed by them. Seven sections along the gut were sampled: the esophagus, stomach, small intestine, "cecum", anterior colon, mid colon and

rectum. The "cecum" in the green turtle is distinct from the rest of the colon. It is a section of the continuous gut tube, rather than a blind out-pocketing of the gut, and therefore not anatomically a cecum. However, it has several characteristics that make it functionally a cecum. It lies just posterior to the ileo-colic valve, and is wider and has a much higher fluid content than the rest of the gut. In starved adults, the entire gut is empty except the "cecum" which always contains a dark green fluid with small pieces of vegetation. In embryonic and hatchling green turtles, the "cecum" is apparent as an enlarged section of the intestine which contains a green paste, while the rest of the tract is empty (Bjorndal, in preparation; Mortimer, 1976).

The length and pH of each gut section were measured (Table 1), and two sets of samples were taken. One set was dried at 80°C to constant weight and stored in plastic bags until my return to the University of Florida for acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, organic matter and energy content determinations. The second set were liquid samples obtained by squeezing gut contents through two layers of cheese cloth and preserved with NaOH for later volatile fatty acid (VFA), ethanol and lactate analyses.

Organic matter was determined by ashing samples at 600°C for 3 hr. Energy content was measured in a Parr bomb calorimeter (Parr, 1960). ADF and NDF analyses were carried out according to Van Soest (1963) and Van Soest & Wine (1967). Sulfuric acid lignin analysis is from

Table 1. Lengths and pH of each section of the digestive tract from two green turtles

	Turtle 1 (50 kg)		Turtle 2 (82 kg)	
	Length (cm)	pH	Length (cm)	pH
Esophagus	48	6	41	6.25
Stomach	41	4	59	3.85
Small Intestine	312	7	297	7.0
Cecum	42	5.5-6.0	52	5.9
Anterior Colon		5.5-6.0		5.6
Mid Colon	611	5.5-6.0	850	5.9
Rectum		7		7.2

Van Soest (1963). The liquid samples were analyzed for VFAs and ethanol on a Packard 800 gas chromatograph. Lactate levels were determined using the lactate test kit prepared by Mannheim Boehringer.

When I collected the above two sets of samples, I also strained the contents of the cecum from each turtle through two layers of cheese cloth. Five 100 ml samples were put into 250 ml bottles. In order to determine the rates of VFA, ethanol and lactate production, four of these samples were gassed with CO₂, sealed and incubated at 30°C, the average body temperature of green turtles (Hirth, 1962; Mrosovsky & Pritchard, 1971). One bottle was removed from the incubator each hour for 4 hr, and a sample of the liquid was preserved in NaOH and analyzed as above for VFAs, ethanol and lactate. Time-zero rates of production were calculated (Carroll & Hungate, 1954).

To determine what gases were evolved during fermentation, and their relative amounts, the fifth sample bottle was gassed with N₂ and incubated at 30°C for 5 hr. At the end of the incubation period, the bottle contents were shaken to equilibrate the dissolved and free gas phases. The gas was transferred to a tube, sealed and later analyzed for CO₂, H₂ and CH₄ on a Packard 800 gas chromatograph.

Fermentation in the first turtle was killed, probably from mishandling, so no data were obtained on VFA, ethanol and lactate production rates or gas evolution. The data presented in this paper on these parameters are from the second turtle only.

Replicates of organic matter, energy content, nitrogen, VFA and lactate determinations were acceptable with 1% error. Replicates of ADF, NDF and lignin were accepted within a range of 2%.

Apparent digestibility coefficients (ADC) were calculated using a lignin ratio. Lignin is often used as an indigestible marker to calculate changes in nutrient content (Van Dyne, 1968) using the formula:

$$\text{ADC} = 1 - \left[\frac{\text{lignin content of food}}{\text{nutrient content of food}} \times \frac{\text{nutrient content of } X}{\text{lignin content of } X} \right] \times 100.$$

The values of the esophagus sample are used as the food values; the nutrient is either cellulose, hemicellulose, organic matter or joules; and *X* refers to the section of the gut being considered. During the Inagua study, zero digestibility of lignin was found in a total collection trial from one green turtle.

Values for cellulose were obtained by subtracting sulfuric acid lignin values from ash-free ADF; hemicellulose figures are ash-free NDF minus ash-free ADF. The results from a study of the dugong, *Dugong dugon* (Murray *et al.*, 1977), were recalculated as percentage of organic matter, and cellulose and hemicellulose values were calculated as above in order to facilitate comparison with the green turtle.

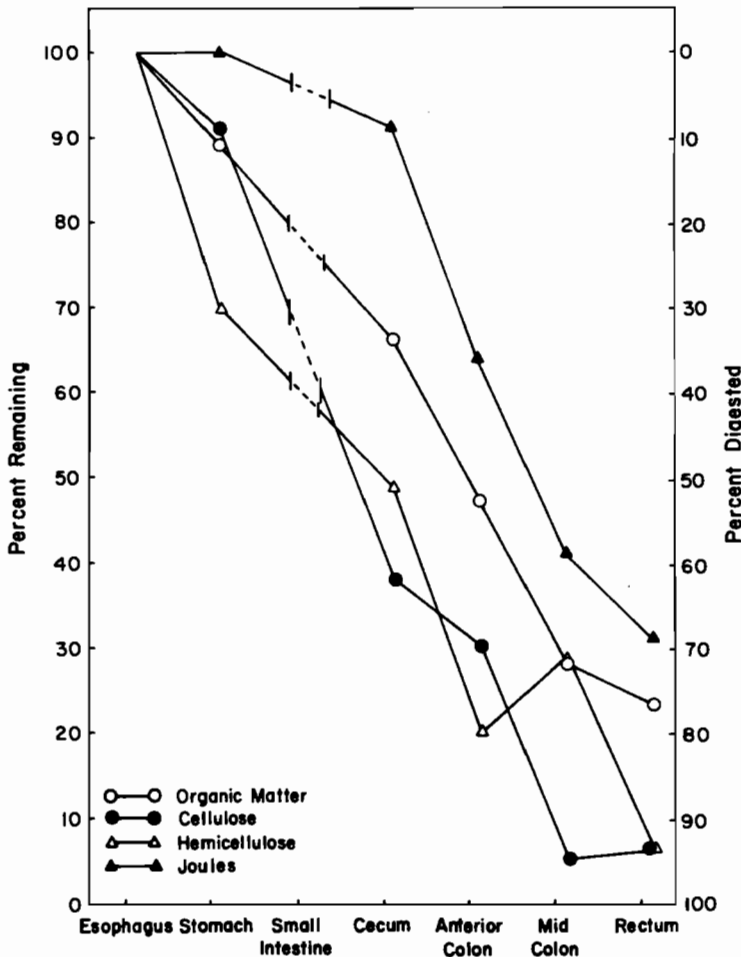


Fig. 1. The amount of nutrient remaining in each region of the digestive tract of Turtle 1 from 1 g (ash-free) of nutrient ingested, calculated by a lignin ratio.

RESULTS

The results from the cellulose, hemicellulose, organic matter and energy content analyses are shown in Figs 1 and 2. The data are presented as the amount remaining in each region of the gut from one ash-free gram of ingested nutrient, calculated with a lignin ratio. Although these values are based on only two turtles, the ADCs of organic matter, cellulose, hemicellulose and joules fall within the range of values obtained from 15 turtles during the year's study on Great Inagua (Bjorndal, in preparation).

The data for the two turtles presented in Figs 1 and 2 are similar except for the lower apparent digestibility coefficients of turtle 2 and the differences between the turtles in the nutrient values for the ceca. Individual variation accounts for some of the difference between the ADCs. Also, these data are not based on one food bolus passing through the gut but are calculated, using a lignin ratio, from successive samples along the gut. Although both digestive tracts contained leaves of *Thalassia testudinum* throughout their entire lengths, the variation between ADC values could result in part from differences in the lignin content, since lignin increases with age in *T. testudinum* leaves. Turtles select young leaves, and therefore low lignin food, so that the entire range of lignin

values for *T. testudinum* would not be found in a turtle's gut (Bjorndal, in preparation).

The difference between the cecum values results from differences in the degree of digestion. The contents of the cecum of turtle 1 had been there long enough for a considerable amount of digestion to occur; the cecum fluid and contents were well-mixed and resembled the colon contents. In contrast, turtle 2's cecum contents resembled the contents of its small intestine rather than the more digested contents of the colon. The cecum values for turtle 1 represent food that has already undergone cecum digestion, while the values for cecum nutrients in turtle 2 represent pre-cecum digestion (as does the small intestine value).

To determine percentage of total digestion that occurs posterior to the ileo-colic valve, the rectal nutrient value is subtracted from the cecal nutrient and divided by the total ADC. The data for turtle 2 show that 83% of digested organic matter, 82% of the digested cellulose and 58% of the digested hemicellulose are digested in the cecum and large intestine. The data for turtle 1 are inappropriate for these calculations, because of the advanced state of cecal digestion, which would result in an underestimate of the percentage of digestion that occurs in the cecum and

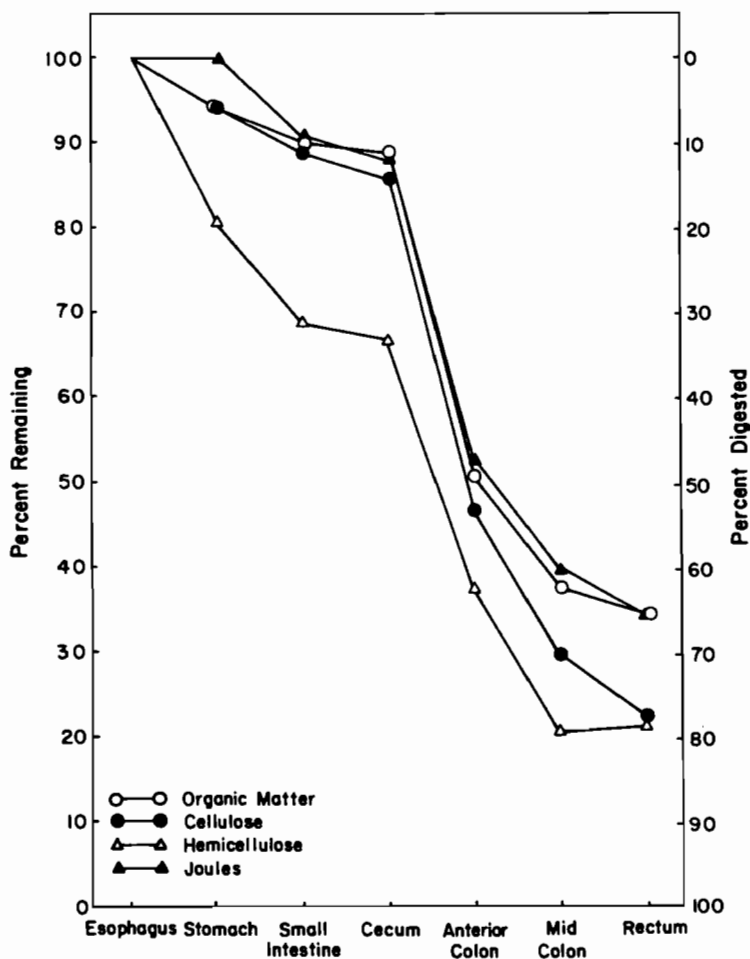


Fig. 2. The amount of nutrient remaining in each region of the digestive tract of Turtle 2 from 1 g (ash-free) of nutrient ingested, calculated by a lignin ratio.

beyond. The morphological characteristics of the cecum and these results demonstrate that the cecum is the initial site of cellulolytic activity.

DISCUSSION

Fiber digestion

The dugong, *Dugong dugon*, is a non-ruminant, herbivorous marine mammal which also feeds on sea-grasses and has an active post-gastric fermentation (Murray *et al.*, 1977). These similarities provide the basis for a useful comparison of digestive efficiencies. Comparing the data in Figs 1, 2 and 3 shows that a 50 kg turtle with an average body temperature of 30°C (Hirth, 1962; Mrosovsky & Pritchard, 1971) digests cellulose and hemicellulose as efficiently as a 250 kg dugong with a temperature regulated in the upper 30s (Blair Irvine, personal communication for manatees—dugong data not available).

Comparing green turtles and ruminants is more difficult because of the differences in their food. Since variations in diet (e.g. lignin and fiber content) can cause the apparent digestibility coefficients of individual animals to change (Hungate, 1966), comparisons

between animals on different diets must be made with caution. On a diet of orchardgrass, *Dactylis glomerata*, which has a cellulose and lignin content similar to that of *T. testudinum* (Keys *et al.*, 1969; Bjorndal, in preparation), sheep have an apparent digestibility coefficient of 67.4% for cellulose and 76.1% for hemicellulose (Keys *et al.*, 1969). These data, when compared with the green turtle data in Figs 1 and 2, show that the green turtle is at least as efficient as a ruminant with respect to fiber digestion.

Unfortunately, there are no fiber digestibility coefficients available for reptiles. There is, however, a paper that reports cellulase activity in the colon of the herbivorous lizard *Sauromalus obesus* to be as high as in the rumen of a cow (Nagy, 1977). Unfortunately, the enzyme activities for both the lizard and the cow were determined at about 23°C, giving misleading and uninterpretable results. The same paper presents data which suggest that there is no decrease in organic matter along the large intestine. Thus, cellulose is apparently not digested to any detectable degree, as it would be reflected in a decrease of organic matter, regardless of whether the end products were assimilated.

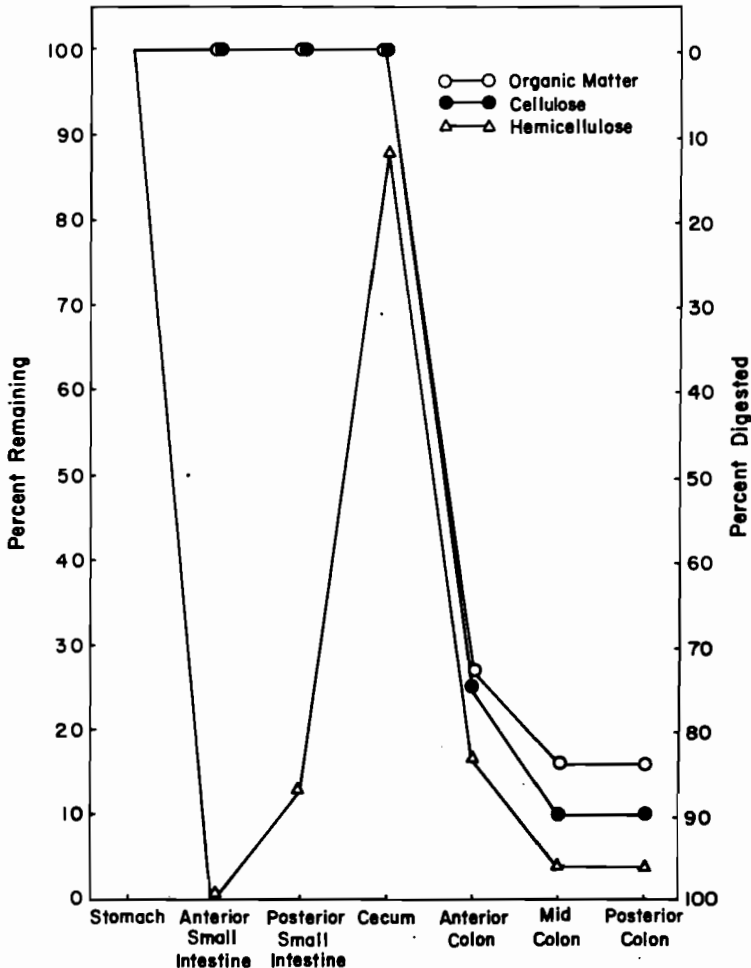


Fig. 3. The amount of nutrient remaining in each region of the digestive tract of a dugong from 1 g (ash-free) of nutrient ingested, calculated by a lignin ratio. Data are from Murray *et al.* (1977).

Joules

Turtle 1 and turtle 2 had ADCs of 69 and 64%, respectively, for joules. These values are rather high for an herbivorous reptile, particularly one with a 30°C body temperature. Harlow *et al.* (1976) showed a clear relationship between temperature and energy ADCs in the desert iguana, *Dipsosaurus dorsalis*, when fed rabbit pellets. At 33°C, the iguanas had an ADC of 54.3%. At 28°C, their digestive system failed, and they died as a result of massive distension of the esophagus and stomach. The group of iguanas cycled between 41 and 28°C, the regime most similar to natural conditions, had an ADC of 57%. Nagy & Shoemaker (1975) give an energy ADC of 56% for free-ranging *Sauromalus obesus*, an herbivorous lizard with a preferred body temperature of 37°C. They also give a value of 57% energy ADC for *D. dorsalis*, the same as Harlow *et al.* (1976). The higher energy ADCs of green turtles are probably due to the fermentation and breakdown of fiber in their gut.

Fermentation end products

The concentrations of VFAs and lactate found along the gut at the time of death are shown in Table 2. Ethanol was not found and so is not included in the table. As can be seen, VFA and lactate levels are low in the esophagus, stomach and small intestine, showing a low level of carbohydrate digestion. In the cecum and colon the levels rise significantly, indicating a great increase in fermentation in that region. The sharp drop in VFA and lactate concentrations from the mid colon to the rectum indicates that most of the VFA and lactate end products are absorbed in the cecum and large intestine. Acetate is by far the major VFA component, followed by butyrate and propionate. Isobutyrate was present in only trace amounts and is not included here.

No other reptile is known to possess an active gut fermentation, and there are no data on reptilian VFA production with which to compare these data. However, the quantities of VFAs, particularly acetate, in the cecum and colon of the green turtle are greater than those found in the hindgut of the horse (Church,

1971) or the rumen of sheep and cattle (Moir, 1968). The data in Table 2 for the dugong from Murray *et al.* (1977), correspond closely to the values for the green turtle.

The most common relative molar concentrations of VFAs found in microbial fermentations in the guts of vertebrates is acetate > propionate > butyrate (Hungate, 1966). The pattern of proportions in the green turtle (acetate > butyrate > propionate) is, however, shared with the dugong (Murray *et al.*, 1977), the quokka, *Setonix brachyurus*, a small marsupial (Moir, 1968), and the porcupine, *Erethizon dorsatum* (Johnson & McBee, 1967). The low proportion of propionate in the green turtle may reflect a substrate low in soluble carbohydrates, as rumen microbes produce high levels of propionate when the diet is high in soluble carbohydrates (Hungate, 1966). The relatively high butyrate levels may be a result of end product inhibition of the butyrate to acetate breakdown caused by the very high acetate concentration.

The ratio of % acetate:% butyrate for rates of production in the cecum is much lower than the same ratio for initial concentrations in the cecum: 3.20 and 16.64, respectively. This difference suggests that either butyrate is absorbed more rapidly from the cecum than acetate, or that a significant quantity of acetate is converted to butyrate *in vitro*. If the former explanation is correct, the rates of absorption from the cecum are butyrate > propionate > acetate. This sequence is the same as that for rock ptarmigan, *Lagopus mutus* (Gasway, 1976a), willow ptarmigan, *Lagopus lagopus*, (Gasaway, 1976b), sheep and cattle (Church, 1971).

The increase in proportions of propionate and butyrate relative to acetate in successive sections of the colon indicates a probable differential absorption rate in that region of acetate > butyrate > propionate. This pattern is different from that suggested for the cecum. The changes in VFA proportions in the lower colon might also result from a change in the VFAs produced in this region. But, as the change is an increase in propionate, and as the contents of the lower colon are high in fiber and low in soluble

Table 2. Concentrations of VFAs and lactate along the digestive tract at time of death

	Lactate mM/l	Total VFA mM/l	Acetate molar %	Propionate molar %	Butyrate molar %
Green Turtle					
Esophagus	0.40	30.57	75.17	21.98	2.85
Stomach	0.69	7.64	96.86	3.14	0
Small Intestine	0.93	57.78	92.46	1.99	5.52
Cecum	2.79	156.13	92.69	1.74	5.57
Anterior Colon	2.75	191.36	82.89	2.07	14.95
Mid Colon	2.00	206.96	78.41	7.54	14.05
Rectum	0.60	62.28	70.46	11.17	18.37
Dugong					
Stomach		16	82	6	12
Small Intestine		18	84	6	10
Cecum		183	57	17	25
Large Intestine		236	50	17	32

Dugong data are from Murray *et al.* (1977)

carbohydrates, the former explanation of differential absorption is more plausible than the latter explanation of differential production.

McBee & West (1969) found ethanol to be one of the major fermentation end products in most of the willow ptarmigan, *Lagopus lagopus*, that they sampled, and they found low levels of lactate in several of the willow ptarmigan. No ethanol was found in any of the samples from the green turtles. Lactate was found in low levels and is certainly not a significant energy source.

Table 3 shows the time-zero rates of production of VFAs and lactates in 1 l. of cecum fluid per hour. The percentage contribution of acetate, butyrate and propionate to the total VFA production are also given. The amount of energy a green turtle gains from the VFAs and lactate produced in the cecum can be estimated from the data for turtle 2. An 82 kg green turtle defecates approx 74 g of organic matter each day (Bjorndal, in preparation). Using the 65% ADC of organic matter and the 65% ADC of joules for turtle 2, an absorption value of 2419875 joules/day is obtained. Turtle 2's cecum contained 1 l. of fluid. If we assume that the rate of fermentation in the cecum is constant throughout the day, we arrive at a figure of 368277 joules/day being produced in the cecum. If, as the data in Table 2 indicates, most of the organic acid end products are absorbed, the cecum provides 15.2% of the green turtle's daily energy budget.

The gut fermentation in the green turtle provides more energy than the value calculated for the cecum. The colon is very long in green turtles, and there is active fermentation along much of its length, as evidenced by both the increase in VFA concentration past the cecum and the rapid swelling of the gut due to accumulated gases once the intestine is removed from the turtle. Therefore, the cecum value is a great under-estimate of the total contribution of the gut fermentation to the energy balance of the green turtle.

The gas sample following the 5 hr incubation of cecum fluid contained the following: CH₄—0.035%, H₂—2.1%, CO₂—1.9%. The gases evolved during cecum fermentation are not in the proportions normally found in gut fermentation. The CH₄ value is quite low and the H₂ value is high. The low pH of the cecum blocks the methanogenic pathway, resulting in the accumulation of free H₂. Further along the colon, as the pH rises, the fermentation may become methanogenic.

The proportions of gases produced are similar to those reported for the quokka, *Setonix brachyurus*, a macropod marsupial (Moir, 1968). Moir found that the gas produced soon after fermentation began had a much higher hydrogen content and lower methane content than the rumen gas of cattle. Gasaway (1976c) found that methane production in rock ptarmigan

was not an accurate estimate of cecum fermentation, as it is in cattle, and suggested that hydrogen might also be given off, although he had not tested for the presence of that gas.

I have shown that in the green turtle cellulose is digested as efficiently as it is in a ruminant, but it is unclear the extent to which this breakdown of cellulose benefits the turtle in terms of its energy balance. More work is needed to determine the nature of this benefit, and the amount of energy and vitamins gained.

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Table 3. Time-zero rates of production of total VFAs (sum of acetate, propionate and butyrate) and lactate in green turtle cecum fluid

Lactate mM/1·hr	Total VFA mM/1·hr	Acetate molar %	Propionate molar %	Butyrate molar %
0.46	11.97	75.10	1.42	23.48

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