

# Effects of Anatomy and Diet on Gastrointestinal pH in Rodents

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## ABSTRACT

The pH of the gastrointestinal tract can have profound influences on digestive processes. Rodents exhibit wide variation in both stomach morphology and dietary strategies, both of which may influence gut pH. Various rodent species have evolved bilocular (or semi-segmented) stomachs that may allow for more microbial growth compared to unilocular (single-chambered) stomachs. Additionally, herbivory has evolved multiple times in rodents. The high dietary fiber typical of an herbivorous diet is known to induce secretion of bicarbonate in the gut. We predicted that stomach segmentation might facilitate the separation of contents in the proximal chamber from that of the gastric stomach, facilitating a chemical environment suitable to microbial growth. To investigate the effect of stomach anatomy and diet on gut pH, several species of rodent with varying stomach morphology were fed either a high or low-fiber diet for 7 days, and pH of the proximal stomach, gastric stomach, small intestine, and cecum were measured. We discovered that rodents with bilocular stomach anatomy maintained a larger pH gradient between the proximal and gastric stomach compartments, and were able to achieve a lower absolute gastric pH compared to those with unilocular stomachs. Dietary fiber increased the pH of the small intestine, but not in any other gut regions. The stomach pH data supports the century old hypothesis that bilocular stomach anatomy creates an environment in the proximal stomach that is suitable for microbial growth. Additionally, the alkaline small intestinal pH on a high fiber diet may enhance digestion. *J. Exp. Zool.* 319A:225–229, 2013. © 2013 Wiley Periodicals, Inc.

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The gastrointestinal tract is a chemically complex mixture of macromolecules, electrolytes, and enzymes that interact to supply nutrients to the animal. However, certain physicochemical characteristics, such as pH, can alter digestive processes, including the efficiency of digestive enzymes (Cornish-Bowden, '95), nutrient transporters (Thwaites and Anderson, 2007), and microbial fermentation (Erfle et al., '82). Therefore, vertebrates tightly regulate the pH of their gastrointestinal tract through the secretion of HCl from the stomach, and bicarbonate from the pancreas, intestine, and cecum (Schulz, '80; Hopfer and Liedtke, '87; Canfield, '91; Stevens and Hume, '95).

However, variation in gastrointestinal anatomy may alter the pH of gut regions. For example, some species have gastric glands spread through the entirety of the stomach, while others have them reduced to the distal portion (Kararli, '95). Rodents tend to follow the latter condition, yet still exhibit variation in stomach anatomy. Some rodents, such as laboratory mice, exhibit a

unilocular stomach, where the stomach exists as a single chamber (Stevens and Hume, '95). Others, such as New World mice (*Peromyscus* spp.), woodrats (*Neotoma* spp.), and voles (*Microtus* spp.) have a bilocular stomach, where a deep invagination near the

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esophageal opening slightly separates two regions of the stomach, with the proximal segment extending above the esophageal opening (Carleton, '73; Stevens and Hume, '95). Although the morphology of the bilocular stomach anatomy in rodents was described over a century ago, its function remains unknown. It has long been proposed that the separation might allow for growth of symbiotic microbes in the proximal chamber (Toepfer, 1891). However, the chemical environments of these chambers have not been investigated in rodents with bilocular stomachs with respect to their suitability for microbial growth. This anatomy may aid in separating the proximal contents of the stomach from the gastric stomach.

Diet is another factor that may influence gastrointestinal pH. Rodents exhibit a wide range of dietary habits, with herbivory having evolved multiple times independently (Samuels, 2009). Dietary fiber increases pancreatic secretion of bicarbonate in a number of mammals, including rodents (Stock-Damge et al., '83; Sommer and Kasper, '84; Zebrowska and Low, '87). Additionally, microbes throughout the gut can produce short-chain fatty acids from easily fermentable carbohydrates, which may locally lower pH (Lupton et al., '88; Yoshioka et al., '94). Thus, diet is likely to alter the gastrointestinal pH of rodents.

Here, we investigated how variation in stomach anatomy and diet might influence gastrointestinal pH. We predicted that species with bilocular stomachs would exhibit different pH values between stomach chambers due to a more enhanced anatomical separation. Additionally we predicted that dietary fiber would increase the pH in the gastrointestinal tract. To test these predictions, we maintained several species of rodents with varying stomach anatomy on both high fiber and low fiber diets, and measured the pH of various gut regions.

## MATERIALS AND METHODS

### Animals

We conducted diet trials on one species of rodent with unilocular stomachs, the house mouse (*Mus musculus*), and two species with bilocular stomachs [deer mouse (*Peromyscus maniculatus*); desert woodrat (*Neotoma lepida*)] (Carleton, '73). To investigate the effect of diet on gut pH, individuals of all three species were fed either a high fiber diet (Harlan Teklad 2031, Madison, WI, USA), or a low fiber diet (Harlan Teklad 2018), ad libitum for 7 days. Diets are meant to replicate "herbivorous" and "omnivorous" diets, respectively. Though the largest difference between the diets is the content of fiber and easily digestible carbohydrates, they differ in other nutrients as well, namely the low fiber diet contains slightly more protein and fat (Table 1). House mice ( $n = 4$ /diet) originated from captive, outbred individuals under IACUC #10-07012. Deer mice ( $n = 4$ /diet) were captive bred individuals under IACUC#11-01007. Desert woodrats ( $n = 3$ /diet) were collected in nature (Lytle Ranch, Washington Co., UT, USA) and maintained in the laboratory under IACUC #10-01013. All animals used were

**Table 1.** Macronutrient composition of experimental diets (% dry matter).

	High fiber <sup>a</sup>	Low fiber <sup>b</sup>
Crude fiber	21.8	3.5
Crude protein	14.8	18.6
Fat	2.3	6.2
Ash	8.3	5.3

<sup>a</sup>Composed primarily of alfalfa, soybean hulls, and oats.<sup>b</sup>Composed primarily of wheat and corn.

adults of both sexes. Food intake in this experiment was not measured.

We also collected samples from two species without conducting diet treatments. Samples were collected from montane voles (*Microtus montanus*), which have bilocular stomachs (Stevens and Hume, '95) and common spiny mice (*Acomys cahirinus*). Previous reports on the stomach anatomy of a closely related species (*A. spinosissimus*) show varying descriptions (Perrin and Curtis, '80; Boozaier, 2012), and thus we aimed to document the stomach anatomy of *A. cahirinus*. Voles ( $n = 3$ ) were wild-caught individuals, dissected in the field, from Big Creek Canyon, Lander Co., NV, USA, collected under IACUC #09-02004. Traps were baited with just a few seeds and placed on obvious runways of voles. Common spiny mice ( $n = 3$ ) were from breeding colonies at the Department of Biology and Environment at the University of Haifa, Oranim, and fed ad libitum rodent chow (Koffolk 19510, Tel Aviv, Israel) and whole, fleshy fruit of *Ochradenus baccatus*. The experimental protocols were approved by the Committee of Animal Experimentation of the University of Haifa (permit number 096/08).

Individuals of all species were euthanized under CO<sub>2</sub> and immediately dissected. All animals were nocturnal and were dissected within 5 hrs of the beginning of the daylight cycle, and thus had likely completed daily feeding recently. However, voles were an exception as they were dissected directly from traps with limited food, and so may have consumed very little food during the evening. Complete contents of the proximal stomach, distal stomach, small intestine, and cecum were collected, frozen, and transported to the University of Utah. Large intestinal pH was not measured. Gastrointestinal contents were thawed to room temperature, and pH was measured using an Omega Soil pH electrode (PHH-200), which compensates for temperature.

### Statistics

For those species in which a diet comparison was conducted (house mouse, deer mouse, and woodrat), we used a repeated measures ANOVA model with species, diet, and gut region as variables. Data were tested for sphericity, and if any violations occurred, Huynh-Feldt corrections were used to compare

treatments. To test whether pH varied by stomach compartment, we conducted post-hoc, paired *t*-tests within each species. Additionally, we tested for diet effects of specific gut region pH values by conducting post-hoc *t*-tests on each gut region, within each species. A Bonferroni corrected value of  $\alpha = 0.025$  was used for all post-hoc tests. For species that lacked a diet treatment (spiny mouse, vole), we simply conducted paired *t*-tests between the pH values of stomach regions.

## RESULTS

Upon dissection, we learned that *Acomys cahirinus* exhibits bilocular stomach anatomy (Fig. 1).

The data used in the repeated measures ANOVA model violated the assumption of sphericity (Mauchly's Test of Sphericity,  $P = 0.047$ ), and so degrees of freedom were modified by a Huynh-Feldt correction of  $\epsilon = 0.98$  to determine final *P*-values. The gut pH values differed between species, and the pH of contents differed significantly by gut region (Table 2, Fig. 2). The pH of gut regions also varied across species, and gut regions responded differently to diet treatments (Table 2, Fig. 2). Specific pH values for all regions and treatments can be found in Supplementary Table 1.

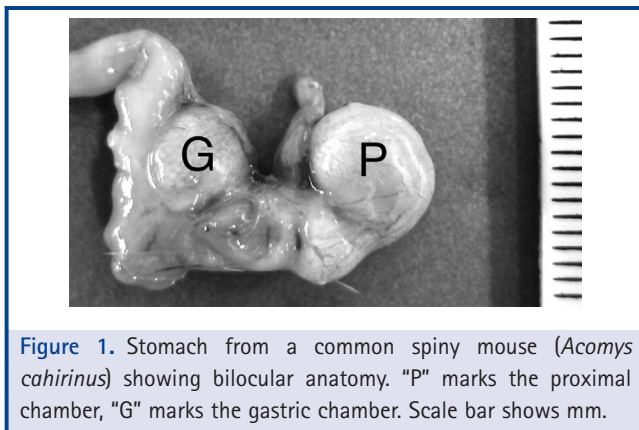


Figure 1. Stomach from a common spiny mouse (*Acomys cahirinus*) showing bilocular anatomy. "P" marks the proximal chamber, "G" marks the gastric chamber. Scale bar shows mm.

Table 2. Statistical results from repeated measures ANOVA of gastrointestinal pH.

Effect	<i>F</i>	<i>df</i>	<i>P</i>
Species	9.54	2,16	<b>0.0019</b>
Diet	2.33	1,16	0.15
Species × diet	1.66	2,16	0.22
Gut region <sup>a</sup>	831.45	2,9,47.3	<b>&lt;0.0001</b>
Gut region × species <sup>a</sup>	28.48	5,9,47.3	<b>&lt;0.0001</b>
Gut region × diet <sup>a</sup>	3.25	2,9,47.3	<b>0.031</b>
Gut region × species × diet <sup>a</sup>	1.71	5,9,47.3	0.13

Significant differences are in bold.

<sup>a</sup>Degrees of freedom have been transformed with Huynh-Feldt correction.

Post-hoc tests investigating regional differences in pH within the stomach revealed the importance of anatomy. Paired *t*-tests for all species with bilocular stomachs (deer mouse, woodrat, vole, and spiny mouse) showed significant differences between the proximal and gastric stomach pH ( $P \leq 0.002$  for all species, Figs. 2 and 3). In contrast, the only species with a unilocular stomach (house mouse), showed no differences between proximal and distal stomach pH ( $P = 0.58$ , Fig. 2).

Post-hoc tests investigating the effect of diet on gut pH revealed that the only region that differed was the small intestine. The high fiber diet significantly increased small intestinal pH in the house mouse ( $P = 0.005$ ) and deer mouse ( $P = 0.006$ ) and showed a trend for increased pH in the woodrat ( $P = 0.049$ , Fig. 2). All other

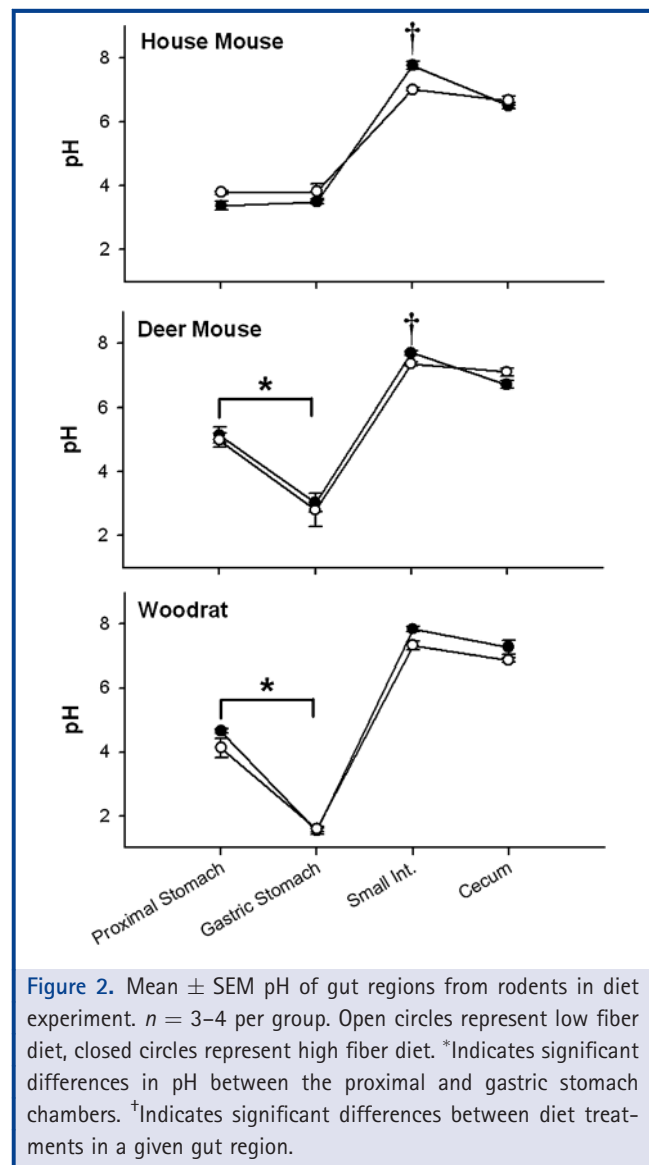
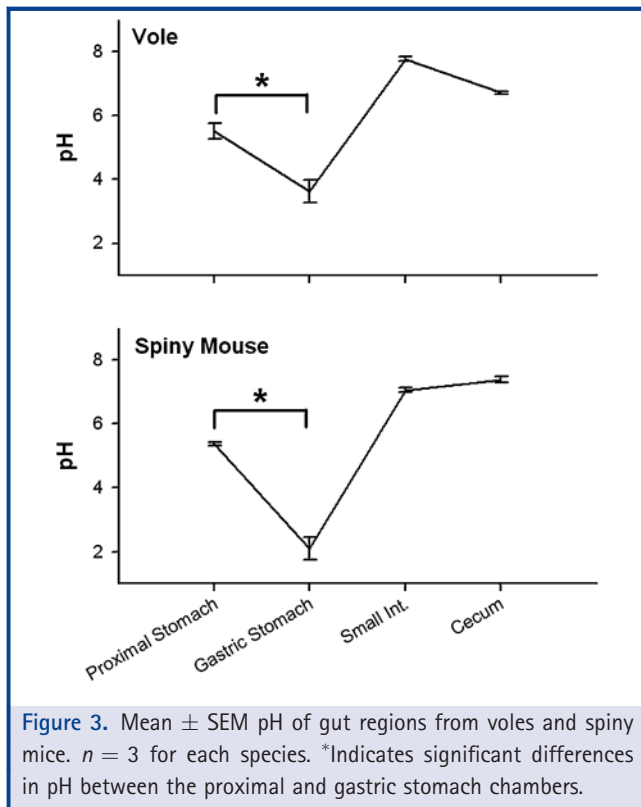


Figure 2. Mean  $\pm$  SEM pH of gut regions from rodents in diet experiment.  $n = 3-4$  per group. Open circles represent low fiber diet, closed circles represent high fiber diet. \*Indicates significant differences in pH between the proximal and gastric stomach chambers. <sup>†</sup>Indicates significant differences between diet treatments in a given gut region.



**Figure 3.** Mean  $\pm$  SEM pH of gut regions from voles and spiny mice.  $n = 3$  for each species. \*Indicates significant differences in pH between the proximal and gastric stomach chambers.

gut regions lacked significant differences in pH based on diet treatment.

## DISCUSSION

We explored the gastrointestinal pH of rodents with differing stomach anatomy fed both high and low fiber diet treatments. We found that overall gastrointestinal pH differed between species. The results revealed that a bilocular stomach anatomy seems to allow rodents to maintain a pH gradient between stomach chambers. Diet seemed to have little effect on gastrointestinal pH, as we found few differences in pH due to a high fiber diet. The exception was the small intestine, where a high fiber diet caused a more alkaline pH. Below we discuss mechanisms and possible consequences of these findings.

The functional significance of bilocular stomach anatomy in rodents was proposed over a century ago to allow growth of symbiotic microbes in the proximal chamber (Toepfer, 1891). Indeed, we have documented that relatively diverse microbial community resides within this proximal chamber (Kohl and Dearing, 2012). Our study represents the first thorough investigation in to the stomach pH of rodents with bilocular stomachs. Here, we report that this anatomy aids in maintenance of differential pH between chambers. This finding is further supported when the results herein are placed in the context of pH values from previously studied rodents (Table 3). Rodents with bilocular

**Table 3.** Comparison of stomach pH between regions from species with bilocular and unilocular stomachs.

	Proximal stomach	Gastric stomach
<b>Bilocular species</b>		
Hamster <sup>a,b</sup>	6.9	2.9
Deer mouse	5.1	2.9
Woodrat	4.4	1.6
Spiny mouse	5.4	2.1
Vole	5.5	3.6
<b>Unilocular species</b>		
Guinea pig <sup>a,c</sup>	4.5	4.1
Rat <sup>a</sup>	5.0	3.3
Mouse <sup>a</sup>	4.5	3.1
Gerbil <sup>a,d</sup>	5.5	3.8
Mouse	3.6	3.7

<sup>a</sup>Stomach pH data from Kararli ('95).  
<sup>b</sup>Anatomy assigned based on Carleton ('73).  
<sup>c</sup>Anatomy assigned based on Potter et al. ('56).  
<sup>d</sup>Anatomy assigned based on Naumova et al. (2011).

stomachs maintain a larger pH gradient between regions, and achieve an overall lower pH in the gastric region. The only exception is the high gastric pH of the vole, which is likely due to the length of time since feeding in our study. Low gastric pH facilitates digestion of protein and protection against ingested pathogens (Giannella et al., '72; Stevens and Hume, '95), and so bilocular stomachs may function better in these respects compared to unilocular stomachs. These hypotheses, though, remain to be explored in further studies.

We found that when feeding on the high fiber diet, rodents tend to maintain an elevated small intestinal pH. Feeding on a high fiber diet increased the small intestinal pH by 0.35–0.75 pH units, which corresponds to a roughly 2- to 5.5-fold increase in the concentration of hydrogen ions. An increase in small intestinal pH in response to dietary fiber has been documented in cattle (Russell et al., '81). This result is unlikely to be an artifact of the pH of the contents entering the small intestine from the stomach, as no differences in pH based on diet were observed in stomach contents. A larger sample size may have allowed us to detect a difference due to the high fiber diet in other gut regions. Another possible mechanism for lower intestinal pH could be higher microbial production of short-chain fatty acids from easily fermentable carbohydrates in the low fiber diet (Lupton et al., '88; Yoshioka et al., '94). However, this is unlikely to be occurring as the small intestine has the lowest microbial density (Savage, '77), and we did not observe an effect of diet on cecal pH, where the most microbial activity occurs. Differences in rates of coprophagy between animals fed different diets (Franz et al., 2011) or the higher ion binding

capacity of dietary fiber (Eastwood, '92) might also drive this response. With these mechanisms, though, we would expect differences in other gut regions, not only the small intestine. Thus, rodents likely physiologically regulate the luminal intestinal environment at high pH values. Intestinal pH is regulated largely by secretion of bicarbonate ( $\text{HCO}_3^-$ ) by the pancreas and intestine (Schulz, '80; Hopfer and Liedtke, '87). Secretion of bicarbonate is an energy-requiring, active process (Schulz, '80). Therefore, there may be some adaptive significance for the increased intestinal pH exhibited by rodents consuming high fiber diets. Alterations in pH can cause differential ionization of nutrients, enzymes, transporters, and secondary chemicals (Cornish-Bowden, '95; Kararli, '95; Thwaites and Anderson, 2007). It could be that when on a high fiber diet, rodents regulate at a higher pH to yield a more beneficial suite of traits, such as selecting for optimal activity of certain enzymes or transporters. Herbivorous species, given their constant high fiber diet, may have evolved enzymes or transporters that work optimally at this higher pH. However, these hypotheses, like those regarding stomach morphology, remain to be investigated.

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