

Effects of Diet on Titratable Acid-Base Excretion in Grasshoppers

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ABSTRACT

Despite the potential for diet to affect organismal acid-base status, especially in herbivores, little is known about the effects of diet on acid-base loading and excretion. We tested the effects of diet on acid-base loading and excretion in grasshoppers by (a) comparing the fecal acid-base content of 15 grasshopper species collected from the field and (b) comparing fecal acid-base excretion rates of *Schistocerca americana* grasshoppers fed vegetable diets that differed in their ashed and raw acid-base contents. The field experiments indicated that grass-feeding species excrete fairly neutral fecal pellets, while forb/mixed-feeding species vary widely in their fecal acid-base contents. In the laboratory experiment, acid-base excretion rates were positively correlated with dietary ashed base intake rates but were not correlated with the acid-base content of raw, unashed diet or feeding rate. These experiments suggest that some diets could strongly challenge the acid-base homeostasis of herbivores; in some grasshoppers, dietary acid-base loads could produce certainly lethal 1-unit changes in average body pH within 6 h if they were not excreted.

Introduction

Relatively little is known about the effects of diet on acid-base homeostasis and excretion in animals, especially nonhumans. In humans, certain baby formulas (Healy 1972; Kildgard 1972) and high-protein diets (Lutz 1984; Hu et al. 1993; Remer and Manz 1994) are associated with increased acid excretion. For

herbivores, diet may pose a particularly great challenge to their acid-base status since plants are more variable in their elemental and acid-base compositions than are animals (Slansky and Rodriguez 1987). For small herbivores such as grasshoppers, the effects of diet on acid-base homeostasis may be further magnified because of their high mass-specific metabolic rates and rates of food consumption. In this study, we investigated the effect of diet on acid-base excretion in grasshoppers (a) in the field, comparing the fecal acid-base content of grass- and forb/mixed-feeding species, and (b) in the lab, comparing the effects of different vegetable diets on the acid-base excretion of *Schistocerca americana*, the American locust.

Previous research has shown that grasshoppers closely regulate extracellular acid-base status. Grasshoppers injected with HCl or NaOH returned their hemolymph pH to normal values within 2–8 h (Harrison et al. 1992; Harrison 1995). Also, lettuce-fed grasshoppers, which excreted relatively large amounts of base, had similar hemolymph pH's as starved grasshoppers, which excreted acid (Harrison and Kennedy 1994). Injected acid or base loads ultimately are removed by the excretory system via the regulated excretion and reabsorption of titratable acid or base, ammonium, and bicarbonate (Thomson et al. 1988; Harrison et al. 1992; Phillips et al. 1994; Harrison 1995). Although acid-base excretion appears to be important to grasshopper homeostasis, nothing is known about the magnitude of acid-base loading and excretion that grasshoppers experience in the field, and little is known about dietary factors that cause acid-base loading.

Dietary acid-base loading depends on the inorganic and organic compounds present in a diet. Inorganic acids and bases can be directly titrated to determine how they challenge an organism's acid-base status. However, the effects of organic compounds on an organism's acid-base status are more complex and are determined by the extent and pathway of metabolization. The acid-base load of a completely metabolized diet can be estimated by titrating the ash that remains after the diet is completely combusted in a furnace (Joslyn 1970) or, alternatively, by calculating the difference between a diet's inorganic cations and anions (Dwyer et al. 1985). Large differences often exist between a diet's unashed and ashed titratable acid-base content. The pH of freshly homogenized (unashed) green plant tissue typically ranges between slightly acidic and neutral (Schultz and Lechowicz 1986). However, the pH of ashed green plant solutions are generally strongly basic, probably because many plants contain large quantities of organic anions such as citrate and malate, which produce base when combusted (Sherman and Gettler 1912; Joslyn 1970). Diets

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Table 1: Titratable base content of the raw (unashed) fecal pellets (TB_{RP}) from the 15 field-captured grasshopper species

Subfamily and Species	Sample Size (n)	TB_{RP} (meq dry kg^{-1}) $\bar{X} \pm SEM$
Grass consumers:		
Oedipodinae:		
<i>Leprus wheeleri</i>	11	-12.50 ± 21.42
<i>Arphia pseudoneitana</i>	6	35.41 ± 14.20
Acrididae:		
<i>Amphitornus coloradus</i>	3	7.54 ± 3.77
<i>Ageneotettix deorum</i>	4	-44.99 ± 37.28
<i>Boopedon flaviventris</i>	6	-42.20 ± 17.69
<i>Boopedon nubilum</i>	4	41.92 ± 39.30
Melanoplinae:		
<i>Phoetaliotes nebrascensis</i>	5	-67.08 ± 23.45
Forb/mixed consumers:		
Romalidae:		
<i>Brachystola magna</i>	9	127.95 ± 118.71
<i>Taeniopoda eques</i>	4	10.73 ± 24.29
Melanoplinae:		
<i>Dactylotum variegatum</i>	4	7.02 ± 86.03
<i>Melanoplus desultorius</i>	3	-690.86 ± 73.64
<i>Melanoplus aridus</i>	6	-72.62 ± 32.93
<i>Melanoplus lakinus</i>	10	641.68 ± 291.34
<i>Hesperotettix viridis</i>	4	-868.62 ± 157.77
<i>Melanoplus differentialis</i>	13	-7.53 ± 22.55

containing large ashed base contents result in base excretion or less titratable acid excretion in humans (Blatherwick 1914).

To test the effects of diet on grasshopper acid-base excretion, we first compared the titratable acid-base content of fecal pellets from grasshopper species that were captured and sampled in their natural environments. We predicted that grass-feeding species would have neutral fecal pellets in comparison to the forb/mixed-feeding species. This prediction was based on two observations. First, preliminary experiments indicated that the ashed base content of grasses tends to be less basic than the ashed base content of the forb plants. Second, grasses tend to employ structural defenses such as silica and vascularized bundle cells that make them physically challenging to consume but should not affect the acid-base status of the consumer (Caswell and Reed 1976). Forbs, however, often employ chemical defenses that the consumer must detoxify (Joern 1979). Detoxification of lipophilic toxins can result in the production of acids and bases that affect acid-base status and excretion (Foley 1992).

Field data could not unambiguously distinguish whether any differences in fecal titratable acid-base content between grasshoppers were due to dietary differences or other factors such as phylogeny. Consequently, we also performed a laboratory

experiment in which we compared the acid-base excretion rates of *S. americana* grasshoppers fed diets differing in their ashed and unashed acid-base contents. Based on previous research (Blatherwick 1914; Davidson and LeClerc 1935; Camien and Reilly 1967; Dwyer et al. 1985; Ball and Maughan 1997), we predicted that acid-base excretion would be positively correlated with the acid-base content of the ashed diet. Finally, to aid in assessing the relative magnitude of the dietary acid-base loads experienced by the grasshoppers, we measured the hemolymph and whole-body nonbicarbonate buffer values for two species.

Material and Methods

Field Experiments

Animal and Fecal Pellet Collection. We collected 92 grasshoppers (15 species) from southern Arizona on September 12, 1997, and on October 12, 1997 (Table 1). Each grasshopper was held in a plastic bag until it produced a fecal pellet. Grasshoppers that failed to produce a fecal pellet within 10 min were excluded from the study. Grasshoppers and fecal pellets were stored on dry ice until we returned to the lab and then were stored in a $-20^{\circ}C$ freezer until analyzed (about 48 h). The species were

Table 2: Dietary food types and the titratable base content of ashed diet (TB_{AD}) and raw diets (TB_{RD}) for the three experimental runs

	Number of Animals	TB _{AD} (meq dry kg ⁻¹) $\bar{X} \pm \text{SEM} (n)$	TB _{RD} (meq dry kg ⁻¹) $\bar{X} \pm \text{SEM} (n)$
Experimental run 1:			
Clover sprouts	2	446 ± 6.8 (4)	NM
Wheat grass	2	462 ± 12.8 (4)	NM
Sunflower sprouts	1	569 ± 10.5 (4)	NM
Mustard greens	1	1,512 ± 26.3 (4)	NM
Green leaf lettuce	1	981 ± 29.4 (4)	NM
Collard	2	870 ± 18.6 (4)	NM
Red chard	1	2,754 ± 92.3 (4)	NM
Experimental run 2:			
Clover sprouts	2	447 ± 35.1 (4)	27 ± .4 (2)
Wheat grass	2	323 ± 5.7 (4)	-111 ± 11.6 (2)
Sunflower sprouts	2	561 ± 16.5 (4)	-196 ± .0 (2)
Mustard greens	2	814 ± 18.7 (4)	-251 ± 1.8 (2)
Green leaf lettuce	2	1,432 ± 16.7 (4)	-176 ± 6.9 (2)
Collard	2	1,411 ± 41.2 (4)	-266 ± 47.7 (2)
Experimental run 3:			
Sunflower sprouts	3	598 ± 13.2 (4)	-293 ± 14.8 (3)
Mustard greens	5	999 ± 20.3 (4)	-135 ± 27.1 (3)
Green leaf lettuce	4	1,082 ± 33.9 (4)	-183 ± 12.8 (3)
Collard	5	1,188 ± 22.4 (4)	-203 ± 65.8 (3)
Red chard	3	3,393 ± 69.9 (4)	348 ± 73.4 (3)

Note. NM = not measured. Positive numbers indicate base content and negative numbers acid content for the ashed or raw (unashed) diet.

classified as either mixed/forb or grass consumers from literature sources (Ball et al. 1942; Otte 1981, 1984).

Fecal Pellet Titrations. Fecal pellets were dried at 45°C to constant mass, pulverized with a mortar and pestle, and titrated to determine the titratable base content of the raw (unashed) fecal pellets (TB_{RD}, meq dry kg⁻¹; Harrison and Kennedy 1994). The titrations were performed on fecal samples weighing 0.7–7 mg, with the majority of the dry pellets weighing 4–5 mg. In brief, fecal pellets were diluted with 3 mL of 100 mmol L⁻¹ KCl, and bicarbonate was removed by acidifying the solution to a pH below 4 with a measured quantity of HCl (50 mmol L⁻¹) and stirring for 4 h. We then added a quantity of NaOH (50 mmol L⁻¹) that equaled the HCl that was added. Finally, the solution was titrated to pH 7.00 with NaOH or HCl, using a Radiometer pHM 84 pH meter, a pH electrode model GK2401C TTT 80 titrator, and an ABU 80 Autoburette.

Lab Experiments

Animals. *Schistocerca americana*, *Taeniopoda eques*, and *Romalea guttata* grasshoppers were reared from eggs in culture at Arizona State University as described by Harrison and Kennedy

(1994). *Taeniopoda eques* were originally obtained from southern Arizona, while *R. guttata* were obtained from Louisiana. Male and female grasshoppers, 2–3 wk past their final molt, were used in this experiment.

Experimental Protocols. We compared the acid-base excretion of *S. americana* fed on seven different vegetables purchased locally from an organic produce store: *Trifolium sp.* (clover sprouts), *Helianthus annuus* (sunflower sprouts), *Triticum sp.* (wheat grass), *Brassica sp.* (mustard greens), *Lactuca sp.* (green leaf lettuce), *Brassica oleracea* (collard), and *Beta vulgaris* (red chard). Voucher specimens were placed in the Arizona State University herbarium. Because batches of the same vegetable differed in ashed base content, there were 18 distinct diets as characterized by their ashed base content (Table 2). One to five grasshoppers were fed the same diet in each test run.

Two days before the experiment, the grasshoppers were removed from the rearing chambers and placed individually in wire cages (15 × 15 × 30 cm). Cage temperatures were maintained at 35°C (± 2°C) during the light cycle and at 30°C during the dark cycle (14L : 10D). We provided the animals with their respective diets every 6–8 h and allowed them to acclimate to these conditions for 48 h before data collection. We measured

feeding and excretion rates and collected fecal samples from the grasshoppers over a 6-h period during three different experimental runs.

Analyses of Diet. Samples of each diet ($n = 2-4$) were either ashed and titrated or directly titrated (unashed; test runs 2 and 3 only) to determine the titratable base content of the ashed diets (TB_{AD} , meq dry kg^{-1}) and the titratable base content of the raw (unashed) diets (TB_{RD} , meq dry kg^{-1}). Since the ashed base content of the diet was the primary focus of this experiment (and all of our diets had positive ashed base contents), we defined titratable base as positive and titratable acid as negative. To determine TB_{AD} , we lyophilized representative samples of each diet to constant mass and then ground them into powder with a mortar and pestle. The powdered food was divided into 3-mg samples, then slowly heated to $550^{\circ}C$ over 2 h, and held at this temperature for 2 more hours to produce clean, white ash. The resulting ash was rinsed from the aluminum ashing pans with 3 mL of 100 mmol L^{-1} KCl. Bicarbonate was removed from the resulting solution by acidification with HCl (0.5 mol L^{-1}) to a pH below 4, and then this solution was titrated as described for the fecal pellets in the field-captured grasshoppers. To measure TB_{RD} , 5–10-mg samples of the lyophilized powdered food were diluted with 3 mL of 100 mmol L^{-1} KCl. We removed the bicarbonate and titrated the samples using the same methods described for the ashed samples.

Acid-Base Intake Rate. Ashed base intake rate ($BASE-IN_{AD}$, $\mu eq h^{-1}$) and raw (unashed) base intake rate ($BASE-IN_{RD}$, $\mu eq h^{-1}$) were calculated from the TB_{AD} , TB_{RD} , and the feeding rate (FR) for each animal. Feeding rate could not be determined accurately from the change in food mass over time due to the rapid desiccation of the food. To circumvent this problem, we weighed separate samples of each diet at the start of the experiment, dried them to constant mass, and then reweighed them to determine the dry-to-wet ratio of each diet (DWR, dry weight of diet/wet weight of diet) at the time it was given to the grasshoppers. The grasshoppers were given a weighed amount of the initial food (F_1 , wet g). At the end of the experiment any uneaten food was removed from the cages, dried to constant mass, and weighed (F_2 , dry g). The dry feeding rate (FR, mg h^{-1}) was then calculated:

$$FR = [(F_1 \times DWR) - F_2]/T,$$

where T = time (hours) over which feeding occurred. Dietary ashed base intake rate was calculated as

$$BASE-IN_{AD} = FR \times TB_{AD} \times 1/1,000.$$

Dietary raw (unashed) acid-base intake rate was calculated as

$$BASE-IN_{RD} = FR \times TB_{RD} \times 1/1,000.$$

Acid-Base Excretion. The fecal pellets were collected at the end of the experimental run, dried at $45^{\circ}C$ to constant mass, and weighed. Excretion rate (ER, dry mg h^{-1}) was calculated by dividing the total dry fecal mass by the experimental duration (6 h). To determine raw and ashed acid-base excretion rates, the fecal pellets from each grasshopper were pooled, pulverized using a mortar and pestle, and divided into two 5-mg aliquots. One fecal aliquot was titrated directly to determine the acid-base concentration of the raw pellets (TB_{RP} , meq dry kg^{-1}). These data were used to calculate raw acid-base excretion rate ($BASE-OUT_{RP}$, $\mu eq h^{-1}$):

$$BASE-OUT_{RP} = ER \times TB_{RP} \times 1/1,000.$$

The other aliquot was ashed and then titrated to determine the titratable base of ashed pellets (TB_{AP} , meq dry kg^{-1}). The ashed base excretion rate ($BASE-OUT_{AP}$, $\mu eq h^{-1}$) was calculated as

$$BASE-OUT_{AP} = ER \times TB_{AP} \times 1/1,000.$$

Hemolymph and Whole-Body Nonbicarbonate Buffer Values. We measured the hemolymph and whole-body nonbicarbonate buffer values for two North American lubber grasshoppers, *T. eques* and *R. guttata*, because the large size of these species (3–9 g individual $^{-1}$) facilitated these measurements. Hemolymph samples (200 μL) were obtained from incisions in the ventral neck region of the grasshoppers, mixed with 2,800 μL of 0.1 mmol L^{-1} KCl, and frozen. Then the grasshopper's body was powdered under liquid nitrogen, mixed with 9 mL of 0.1 mmol L^{-1} KCl, and frozen. After thawing and vortexing, 250 μL aliquots of the whole-body homogenates were diluted with 2,750 μL of 0.1 mmol L^{-1} KCl. Bicarbonate was removed from the 3-mL diluted hemolymph and whole-body samples by acidifying them to a pH below 4 with 0.5 mol L^{-1} HCl and stirring for 2 h. After adjusting the pH to the appropriate starting value, half of the samples was titrated with 0.5 mmol L^{-1} NaOH or HCl from pH 6 to 8 and back to 6, while the other half of the samples was titrated in the reverse direction. Buffer values were not affected by direction of titration. For each titration, 10 μL of NaOH or HCl were added, the solution was vigorously stirred, and pH was measured after 30 s with the Radiometer pH electrode. Titrations in a single direction took <6 min, which was important since both hemolymph and whole-body mixtures changed pH over longer time periods, probably due to metabolic activity. Plots of pH versus equivalents of acid or

base added were linear between 6 and 8, so buffer values were calculated from the slopes of these plots, adjusting for the slight buffer value of the KCl solutions and mixture dilution.

Data Analysis and Statistics

The data collected from the one to five grasshoppers fed a given diet within each test run were pooled into one data point to test our hypotheses. We believed pooling was necessary because the analysis of our data involved comparisons of base intake rate and acid-base excretion rate, and the correlations between feeding rates and excretion rates were weak for individual grasshoppers during the 6-h experiment. Presumably, there would be a tighter correlation between feeding and excretion rates if these variables were measured over a longer period of time than the 6-h test period we used. Further supporting the need for pooling were our calculated absorption efficiencies. The average dry absorption for grasshoppers fed on most of the diets was about 50%; however, on the individual level, calculated absorption ranged from -0.23 to 0.88 . It is unlikely that these extreme absorption values represent long-term average absorption; instead, they are probably due to short-term mismatches between intake and excretion. Although the same trends were evident and were statistically significant whether or not the data were pooled, we felt that pooling ameliorated discrepancies between the feeding and excretion rates obtained in the relatively short experimental period.

Unless otherwise stated, parametric statistical analysis was performed using SYSTAT (Wilkinson 1989). All data are presented as mean \pm SEM unless otherwise stated, and the Type I error was set at 0.05.

Results

How Is Diet Related to Variation in Acid-Base Excretion among Grasshopper Species in Their Natural Environments?

The fecal acid-base content of grasshoppers in their natural environment was highly variable between species. The acid-base content of the raw (unashed) fecal pellets (TB_{RP} , meq dry kg^{-1}) ranged from highly acidic (-869 meq dry kg^{-1}) in *Hesperotettix viridis* to highly basic (642 meq dry kg^{-1}) in *Melanoplus lakinus*. Trends in fecal acid-base content were evident when the grasshopper species were grouped by dietary choice or subfamily. The grass eaters, who were mostly from two subfamilies (Oedipodinae, Acrididae), had relatively neutral fecal pellets, while the forb/mixed-eating species, which also had representatives from two different subfamilies (Romalidae, Melanoplinae), had more variable fecal contents, with many of the species excreting large amounts of acid or base (Fig. 1). The only species whose dietary choice differed from its subfamily was *Phoetaliotes nebrascensis*, a grass eater in a subfamily dominated by forb/mixed feeders.

Grass and forb/mixed feeders did not differ significantly in

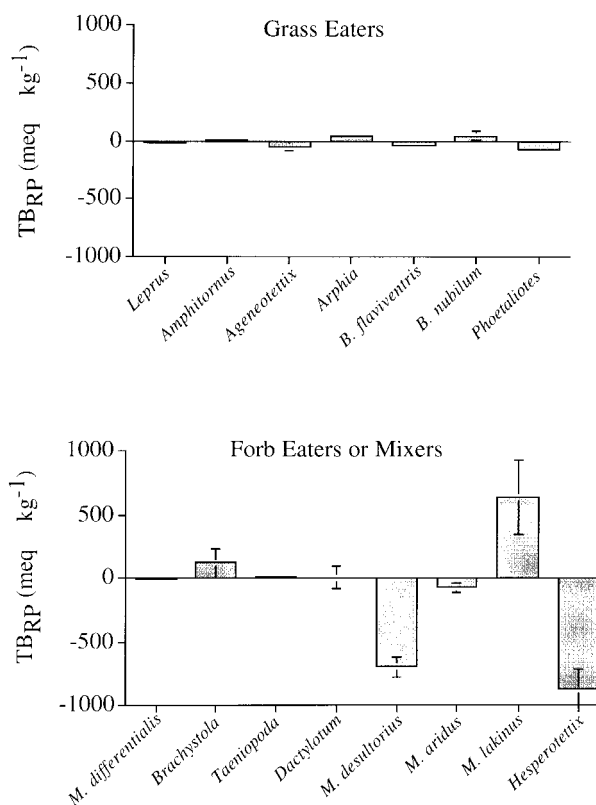


Figure 1. Fecal base content (TB_{RP}) for seven grass-eating grasshopper species (top) and eight forb/mixed-eating grasshopper species (bottom).

their mean fecal acid-base content (grass feeders: -11.7 ± 16 ; forb/mixed feeders: -106.5 ± 168 meq kg^{-1} , Welch's approximate t -test for samples with unequal variance, $t = 0.563$, $P \gg 0.05$; Sokal and Rohlf 1995). However, the forb/mixed eaters did have significantly greater variance in fecal acid-base content than the grass eaters (grass feeders: 1,762; forb/mixed feeders: 224,948, squared-ranks test for variance, $T_{7,8} = 179$, $P < 0.005$; Conover 1980). We also compared the subfamilies Acrididae and Melanoplinae since we had sufficient species in these two subfamilies for statistical analysis. Mean fecal acid-base contents of the two subfamilies were not significantly different (Acrididae: -9.5 ± 21 ; Melanoplinae: 151.1 ± 189 , Welch's approximate t -test, $t = 0.74$, $t_{0.05} = 2.46$, $P \gg 0.05$; Sokal and Rohlf 1995); however, the variance in fecal acid-base content of the two subfamilies was significantly different (Acrididae: 1,754; Melanoplinae: 248,926, squared-ranks test for equal variance $T_{4,7} = 476$, $P < 0.005$; Conover 1980).

Dietary Effects on Acid-Base Excretion in the Lab

Ashed Base Content of the Diet. $BASE-OUT_{RP}$ ($\mu eq h^{-1}$) was linearly related to $BASE-IN_{AD}$ ($\mu eq h^{-1}$), with $BASE-OUT_{RP}$

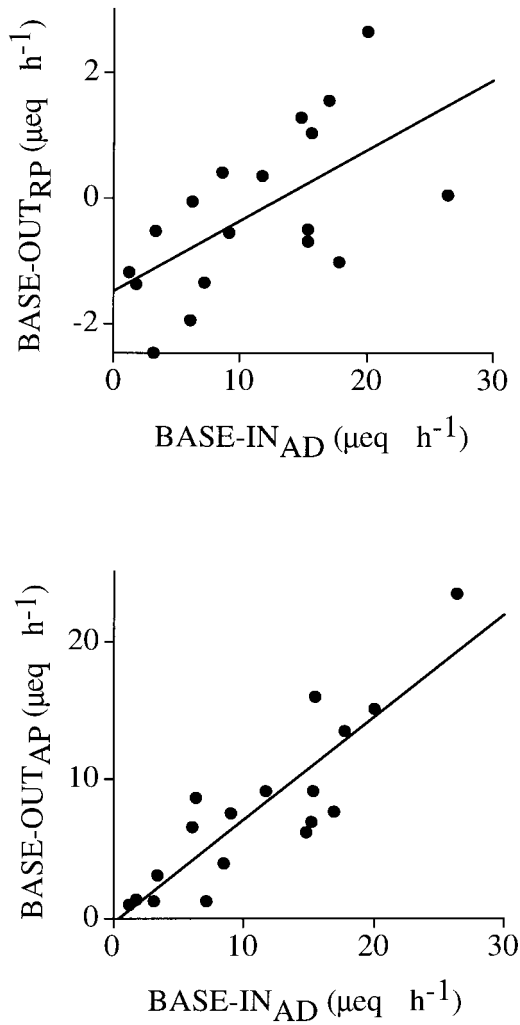


Figure 2. *Top*, the significant linear relationship between raw (unashed) base excretion rate ($BASE-OUT_{RP}$) and ashed base intake rate ($BASE-IN_{AD}$). The equation for the linear regression shown is $BASE-OUT_{RP} = 0.112 BASE-IN_{AD} - 1.494$. *Bottom*, the significant linear relationship between base excretion rate, determined from ashed fecal pellets ($BASE-OUT_{AP}$), and ashed base intake rate ($BASE-IN_{AD}$). The equation for the linear regression shown is $BASE-OUT_{AP} = 0.744 BASE-IN_{AD} - 0.352$.

accounting for about 10% of the $BASE-IN_{AD}$ ($F = 9.553$, $df = 1, 17$, $P = 0.007$, $R^2 = 0.37$; Fig. 2). Although all of the ashed diets were basic (Table 2) and there was a significant relationship between $BASE-IN_{AD}$ and $BASE-OUT_{RP}$ (Fig. 2), most grasshoppers still excreted acidic fecal pellets. When the fecal pellets were ashed before titration, $BASE-OUT_{AP}$ ($\mu\text{eq h}^{-1}$) was linearly related to $BASE-IN_{AD}$ ($\mu\text{eq h}^{-1}$), with $BASE-OUT_{AP}$ accounting for 76% of the $BASE-IN_{AD}$ ($F = 50.47$, $df = 1, 17$, $P = 0.0001$, $R^2 = 0.76$). As for the ashed diet, all of the ashed fecal pellets were basic.

Effect of Raw (Unashed) Dietary Acid-Base Content and Feeding Rate on Acid-Base Excretion. $BASE-IN_{RD}$ and $BASE-OUT_{RP}$ were not significantly correlated ($F = 0.76$, $df = 1, 10$, $P = 0.41$, $R^2 = 0.08$; Fig. 3). Multiple linear regression analysis was performed to determine whether $BASE-IN_{RD}$ significantly affected $BASE-OUT_{RP}$ after controlling for the effect of $BASE-IN_{AD}$. Again, the effect of the acid-base content of the raw diet was insignificant ($F = 2.56$, $df = 1, 10$, $P = 0.14$, $R^2 = 0.39$). When all diets were considered together, $BASE-OUT_{RP}$ was also not significantly affected by feeding rate ($F = 0.001$, $df = 1, 17$, $P = 0.98$, $R^2 = 0.000$).

Effects of Individual Diets. To determine whether some vegetables caused significantly different acid-base excretion rates than predicted by their ashed base content, we averaged the residuals for each diet from the linear regression analysis between $BASE-IN_{AD}$ and $BASE-OUT_{RP}$. An ANOVA test for unequal sample sizes of these averaged residuals indicated that the residuals did differ significantly ($F_{6,35} = 4.65$, $P < 0.001$; Fig. 4; Sokal and Rohlf 1995). This result suggests that factors other than the ashed base content of a diet play a significant role in dietary acid-base loading.

Buffer Values. Hemolymph buffer values (Table 3) were similar to those previously measured for grasshoppers using carbon dioxide tonometry (Harrison et al. 1990). Due to small sample sizes, we pooled the species for statistical analysis. Whole-body

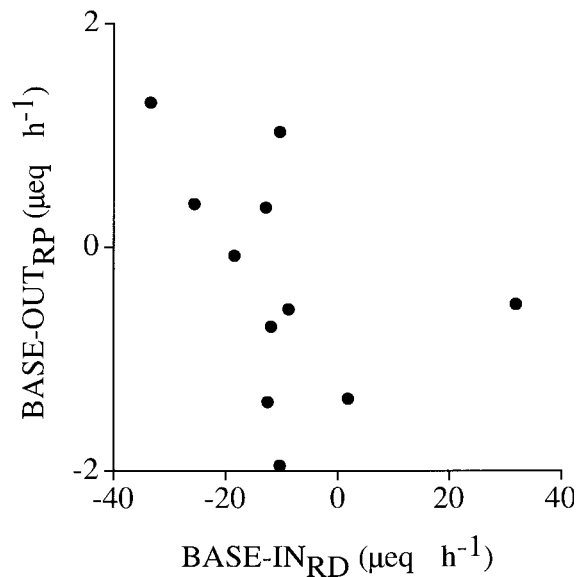


Figure 3. A plot indicating lack of significant correlation between raw (unashed) fecal acid-base excretion rate ($BASE-OUT_{RP}$) and acid-base intake rate as determined from the raw (unashed) diet ($BASE-IN_{RD}$) ($P = 0.41$, $R^2 = 0.08$).

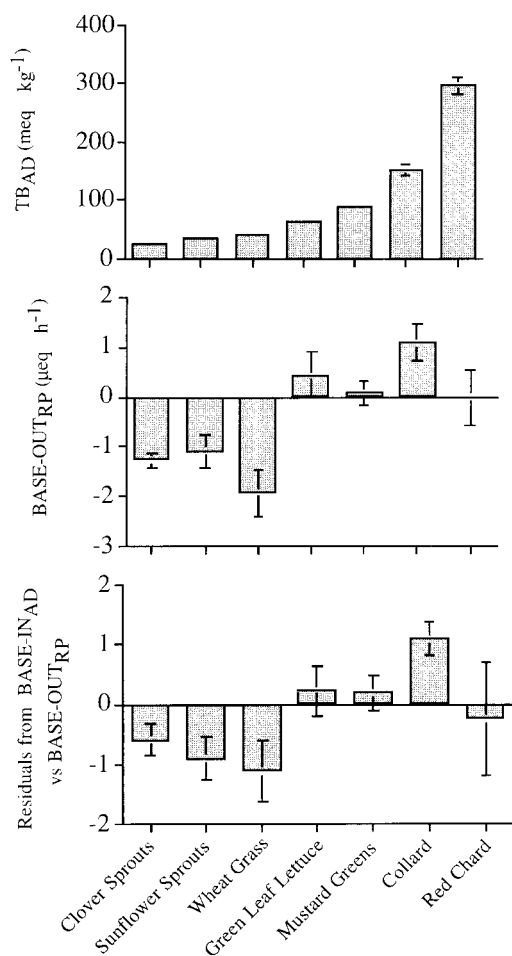


Figure 4. Ashed base content (TB_{AD}) of seven diets (top), the raw (unashed) fecal acid-base excretion rates ($BASE-OUT_{RP}$) of grasshoppers fed on these seven different diets (middle), and residuals from the linear regression analysis between acid-base excretion rates ($BASE-OUT_{RP}$) and ashed base intake rates ($BASE-IN_{AD}$; bottom).

buffer values were significantly greater than hemolymph buffer values (t -test, $T = 6.13$, $P < 0.0001$).

Discussion

Our data suggest that acid-base loading associated with plant consumption may be an important factor that affects plant-herbivore interactions; grasshoppers in their natural environments experience a wide range of acid-base loading (Fig. 1), and the excretion of large amounts of acid or base appears to be critical to the immediate survival of some of the forb-feeding grasshoppers we tested. Our lab experiments suggest that differences in acid-base excretion between different grasshopper species are partially a result of differences in the ashed base content of their diets. At present, it is unclear whether the excretion of large quantities of acid or base, as occurs in some

forb-feeding grasshopper species, requires unique physiological traits or is within the range of abilities of all insects.

Variance in Acid-Base Excretion among Grasshopper Species in Their Natural Environments

Field-collected forb/mixed-feeding grasshopper species were significantly more variable in their acid-base excretion than grass feeders. All of the grass feeders had fecal pellets that were fairly neutral, while many of the forb/mixed-eating species had fecal pellets containing large amounts of acid or base (Fig. 1; Table 1). These results were consistent with the observations that forbs have more variable ashed base contents than grasses (Table 2) and are more likely to employ chemical defenses that consumers must detoxify by conversion into conjugate acids and bases.

Given the large amount of acid-base excretion in some grasshopper species and the necessity of maintaining a relatively constant extracellular and tissue pH (Harrison et al. 1992), dietary acid-base challenges may strongly threaten pH homeostasis in some forb-feeding grasshopper species. The relative threat to pH homeostasis experienced by different grasshopper species can be quantified as the time required for a certainly lethal 1-unit change in organismal pH to occur if acid-base excretion was blocked. This value can be estimated from fecal acid-base content, whole-animal buffer values, feeding rate, and animal size (Table 4). Our calculations show that if fecal acid-base excretion were blocked and the acid-base loads were distributed throughout the body, *Hesperotettix viridis* would be acidified by 1 pH unit in 6 h, while *Melanoplus lakinus* would be similarly alkalinized in 8 h. If the dietary acid-base loads experienced by these species were confined to the less buffered hemolymph (Table 3), which contains only about 40% of total body water (Harrison 1989), hemolymph pH would change by 1 pH unit in <1 h if there were no compensatory response. In contrast, for the grass feeder *Phoetaliotes nebrascensis*, acid-base excretion could be suspended for days without large changes in whole-body pH. These calculations suggest that comparisons of the acid-base excretion capacities of these species may be worthwhile.

An important goal of future research will be to identify the specific characteristics of natural diets that cause the wide variance in acid-base excretion that we observed. Because the proportion of different plants in the diet and the location of the plant consumed can be difficult to determine for grasshoppers in the field, it will not be a trivial problem to link dietary characteristics to acid-base excretion in the field. In preliminary experiments, we attempted to circumvent these problems by analyzing the acid-base content of the crop contents of grasshoppers fed known diets. We found that crop lumen acid-base status was not correlated with the ashed or unashed dietary acid-base contents, so we do not recommend this technique. However, it would be very interesting to repeat the laboratory

Table 3: Nonbicarbonate buffer values of grasshopper hemolymph and whole-body samples

Sample and Species	Mean Buffer Value (meq kg ⁻¹ pH U ⁻¹)		<i>n</i>
	$\bar{X} \pm \text{SEM}$	Range	
Hemolymph:			
<i>Taeniopoda eques</i>	10.7 ± 1.73	3.9–15.2	3
<i>Romalea guttata</i>	15.6 ± .80	12.4–22.8	8
Whole body:			
<i>T. eques</i>	28.9 ± 3.06	23.5–34.1	3
<i>R. guttata</i>	43.1 ± 2.98	24.2–68.4	12

experiments we conducted here with plants that are known to be consumed by grasshoppers.

Can Phylogeny Affect Acid-Base Excretion?

Based on the field study, it is not clear whether the differences in titratable acid-base excretion that we observed in the animals were due to dietary differences or could be attributable to conditions unrelated to diet, such as phylogenetic history. Phylogeny could affect acid-base excretion due to differences in catabolic pathways and absorption efficiencies among species. For example, rabbits fed on rat chow produce basic urine (pH > 7), while rats fed the same rat chow produce acidic urine (Richardson et al. 1979). Since dietary choice is closely linked to phylogeny in grasshoppers (Joern 1979), it is hard to separate the effects of phylogenetic history and diet. For example, all of the species we collected in the Oedipodinae and Acrididae subfamilies were grass eaters and had fairly neutral fecal acid-base concentrations, while all Romalidae and all but one Melanoplinae (*P. nebrascensis*) were mixed/forb eaters and had highly variable acid-base fecal concentrations. It is interesting that the Melanoplinae grass feeder, *P. nebrascensis*, had fecal pellets with

the highest titratable base content of any of the grass feeders we studied (although it was relatively neutral in comparison to some of the mixed/forb feeders). This suggests that phylogeny as well as diet may affect acid-base excretion in grasshoppers. However, future studies using more species within each subfamily and controlled diets are necessary to rigorously test for phylogenetic effects on acid-base excretion in grasshoppers.

Effect of Ashed Base Content of Diet on Acid-Base Excretion

Dietary ashed base content clearly affects acid-base excretion rates in grasshoppers (Fig. 2). Similarly, in humans, diets that contained large amounts of vegetables, which tend to have higher ashed base contents, resulted in less titratable acid in the urine and higher urinary pH (Hu et al. 1993; Ball and Maughan 1997). However, most dietary compounds that have the potential to cause base loading appear to pass through the grasshopper unmetabolized, since only about 10% of the ashed base intake was excreted as titratable base. Many of the grasshoppers produced acidic fecal pellets even when fed diets with large ashed base contents; similar results have been noted in humans (Hu et al. 1993; Ball and Maughan 1997).

Table 4: Data used to estimate the number of hours it would take three grasshopper species to experience a 1-unit change in their average body pH if dietary acid-base load was not excreted

Species	Mean Adult Mass (g)	Fecal Base Content (meq kg ⁻¹)	Fecal Excretion Rate (mg h ⁻¹) ^a	Hours to 1-pH-Unit Change ^b
<i>Phoetaliotes nebrascensis</i> ^c46	-67	2.5	97.0
<i>Hesperotettix viridis</i> ^d21	-869	1.5	5.8
<i>Melanoplus lakinus</i> ^d20	+642	1.4	8.0

^a Determined assuming that log excretion rate = $a \times \log \text{mass}^{0.68}$ (Peters 1993), with $a = 4.3$, a value determined from the excretion rates of *Schistocerca americana* measured in this study.

^b Hours to 1-pH-unit change calculated as $(A \times B)/(C \times D)^{-1}$, where A = average mass for that species (kg); B = average whole-body buffer value for grasshoppers (see Table 3): 36 meq kg pH unit⁻¹; C = fecal acid-base content for that species (meq kg⁻¹; Fig. 1); and D = fecal excretion rate for that species (kg h⁻¹).

^c Grass feeder.

^d Forb/mixed feeder.

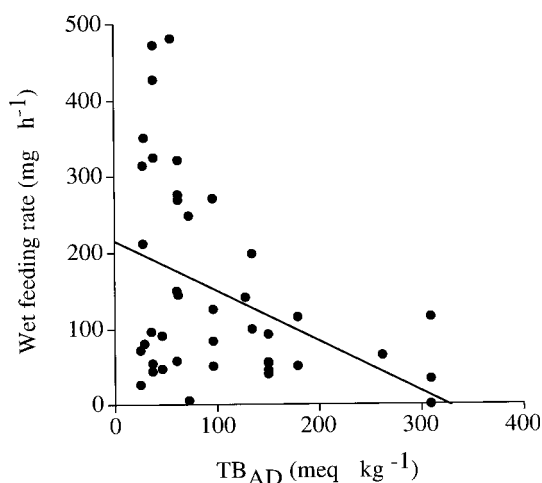


Figure 5. The significant linear relationship between wet feeding rate and the base content of the ashed diet (TB_{AD}). The equation for the linear regression shown is wet feeding rate = $-0.65 TB_{AD} + 214.6$.

Also, consumption of some diets resulted in significantly different acid-base excretion than predicted by the dietary ashed base contents (Fig. 4). There are likely to be multiple reasons why the ashed base content of the diet does not more precisely match acid-base excretion. First, combustion by organisms is not as efficient as by furnaces (Halperin 1982; Dwyer et al. 1985). For example, humans do not completely metabolize some compounds such as benzoic and quinic acids or tartrate anion. Upon ashing, these organic acids produce base, but in humans they are only partially metabolized and are excreted as organic acids (Blatherwick 1914; Blatherwick and Long 1923; Chadwick et al. 1978). Second, lipophilic plant allelochemicals such as terpenes are often detoxified by conversion into conjugate acids or bases, resulting in changes in acid excretion (Foley 1992). The conversion of allelochemicals to conjugate acids and bases cannot be predicted by ashing.

Another problem associated with ashing is the potential loss of volatile compounds such as chlorine and sulfur, which results in the overestimation of a diet's alkalinity (Davidson and LeClerc 1936; Camien and Reilly 1967). Studies of lettuce, spinach, and kale suggest that about 7% of the chlorine and 40% of the sulfur may be lost during ashing (Davidson and LeClerc 1935). Given the average quantities of sulfur (Sherman and Gettler 1912) and chlorine (Paul and Southgate 1978) in green plants, we may have overestimated dietary alkalinity by as much as 10% in some cases (calculated using the technique in Dwyer et al. 1985). However, the magnitude of this error is relatively insignificant since the ashed base contents of the diets varied by more than 10-fold.

Finally, ashed base intake might better predict acid-base excretion if fecal ammonium excretion was also measured. Since

titratable acid-base accounts for 50%–95% of the acid-base excretion in grasshoppers, and because the titratable acid content and fecal ammonium content are positively correlated in grasshopper feces (Harrison 1995), the titratable acid-base content of the fecal pellets is a good general indicator of acid-base excretion. However, because we did not account for the presence of fecal ammonium, we may have underestimated acid excretion, especially in the grasshoppers that were excreting very acidic fecal pellets.

Effects of Raw (Unashed) Dietary Acid-Base Content on Acid-Base Excretion

There was no correlation between acid-base excretion and the acid-base content of the raw (unashed) diet regardless of whether we looked at raw base intake alone or first controlled for the effects of the ashed dietary base content. The lack of correlation does not indicate that the raw acid-base content of the diet does not affect acid-base excretion; rather, it indicates that it is typically not a good predictor of acid-base loading and excretion unless the effects of dietary metabolism by the organism are considered concurrently. To illustrate this point, we will review the theoretical ingestion and metabolism of a single compound, citric acid. Citrate possesses three carboxyl groups with pK 's of 3.13, 4.76, and 6.40 (Budavari 1989). Citrate is stored in plant vacuoles, where the pH ranges between 5.0 and 6.0 (Karowe and Martin 1992); given these conditions, approximately two out of three carboxylate groups on a citric acid molecule would be protonated when consumed. When the citrate is consumed by a grasshopper, some protons would dissociate and impart an initial acid load on the grasshopper (hemolymph pH 6.5–7.3; Harrison 1988). However, if the citrate salt is then completely metabolized, about one base equivalent would be formed for every citrate molecule consumed.

It is possible that raw acid-base content might directly challenge the regulation of midgut pH (Schultz and Lechowicz 1986). At least some insects have been shown to regulate midgut pH within a fairly narrow margin (Appel and Maines 1995; Johnson and Felton 1996), and it is believed that midgut pH

Table 5: Pearson's R correlation values between wet feeding rate (WFR), dry feeding rate (DFR), dietary water content (WC), and titratable base content of the ashed diet (TB_{AD})

	WFR	DFR	WC	TB_{AD}
WFR	1.00			
DFR89 ^a	1.00		
WC46 ^a	.08	1.00	
TB_{AD}	-.40 ^a	-.25	-.48 ^a	1.00

Note. $n = 42$.

^a $P < 0.01$.

regulation is metabolically expensive. Lepidopteran larvae fed artificial diets have reduced growth on diets with low pH and high buffering capacity (Karowe and Martin 1992).

Does Dietary Acid-Base Content Affect Feeding Behavior?

The significant acid-base challenge posed by some diets suggests that the effects of plants on acid-base homeostasis may deter herbivory in grasshoppers and other insects. If this is the case, feeding rate and the ashed base content of the diet would be negatively correlated in grasshoppers. When wet feeding rates were considered, feeding rate was negatively correlated with the ashed base content of the diet (Fig. 5; $F = 7.6$, $df = 1, 40$, $P = 0.009$, $R^2 = 0.16$). However, the ashed base content of a diet was also negatively correlated with the water content of the diet, and the water content of the diet was positively correlated with feeding rate (Table 5). Thus, it is not clear from this study whether grasshopper feeding rate was responding to ashed titratable base content, water content, or some other aspect of the diets. Future studies that manipulate dietary acid-base content independently of other dietary characteristics are necessary to determine whether insects actually sense and exhibit feeding responses to dietary acid-base content.

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Literature Cited

- Appel H.M. and L.W. Maines. 1995. The influence of host plant on gut conditions of gypsy moth (*Lymantria dispar*) caterpillars. *J Insect Physiol* 41:241–246.
- Ball D. and R.J. Maughan. 1997. Blood and urine acid-base status of premenopausal omnivorous and vegetarian women. *Br J Nutr* 78:683–693.
- Ball E.D., E.R. Tinkham, R. Flock, and C.T. Vorhies. 1942. *The Grasshoppers and Other Orthoptera of Arizona*. University of Arizona Press, Tucson.
- Blatherwick N.R. 1914. The specific role of foods in relation to the composition of urine. *Arch Intern Med* 14:409–450.
- Blatherwick N.R. and M. Long. 1923. Studies of urinary acidity: the increased acidity produced by eating prunes and cranberries. *J Biol Chem* 57:815–827.
- Budavari S., ed. 1989. *The Merck Index*. 11th ed. Merck, Rahway, N.J.
- Camien M.N. and T.J. Reilly. 1967. Determination of titratable ash-acidity (Ash-TA). *Proc Soc Exp Biol Med* 126:51–55.
- Caswell H. and F.C. Reed. 1976. Plant-herbivore interactions: the indigestibility of C4 bundle sheath cells by grasshoppers. *Oecologia* 26:151–156.
- Chadwick V.S., A. Vince, V.M. Killingley, and O.M. Wrong. 1978. The metabolism of tartrate in man and the rat. *Clin Sci Mol Med* 54:273–281.
- Conover W.J. 1980. *Practical Nonparametric Statistics*. 2d ed. Wiley, New York.
- Davidson J. and J. LeClerc. 1935. A new method for the determination of the acid-base balance in food materials. *J Biol Chem* 108:337–347.
- . 1936. The variation in the mineral content of vegetables. *J Nutr* 11:55–66.
- Dwyer J., E. Foulkes, M. Evans, and L. Ausman. 1985. Acid/alkaline ash diets: time for assessment and change. *J Am Diet Assoc* 85:841–845.
- Foley W.J. 1992. Nitrogen and energy retention and acid-base status in the common ringtail possum (*Pseudocheirus peregrinus*): evidence of the effects of absorbed allelochemicals. *Physiol Zool* 65:403–421.
- Halperin M.L. 1982. Metabolism and acid-base physiology. *Artif Organs* 6:357–362.
- Harrison J.F. 1995. Nitrogen metabolism and excretion in locusts. Pp. 119–132 in P.J. Walsh and P. Wright, eds. *Nitrogen Metabolism and Excretion*. CRC, Boca Raton, Fla.
- Harrison J.F. and M.K. Kennedy. 1994. In vivo studies of the acid-base physiology of grasshoppers: the effect of feeding state on acid-base and nitrogen excretion. *Physiol Zool* 67:120–141.
- Harrison J.F., C.J. Wong, and J.E. Phillips. 1990. Haemolymph buffering in the locust *Schistocerca gregaria*. *J Exp Biol* 154:573–579.
- . 1992. Recovery from acute haemolymph acidosis in unfed locusts I. Acid transfer to the alimentary lumen is the dominant mechanism. *J Exp Biol* 165:85–96.
- Harrison J.M. 1988. Temperature effects on haemolymph acid-base status *in vivo* and *in vitro* in the two-striped grasshopper *Melanoplus bivittatus*. *J Exp Biol* 140:421–435.
- . 1989. Temperature effects on intra- and extracellular acid-base status in the American locust, *Schistocerca nitens*. *J Comp Physiol* 158B:763–770.
- Healy C.E. 1972. Acidosis and failure to thrive in infants fed Nutramigen. *Pediatrics* 49:910–911.
- Hu J.-F., X.-H. Zhao, B. Parpia, and T.C. Campbell. 1993. Dietary intakes and urinary excretion of calcium and acids: a cross-sectional study of women in China. *Am J Clin Nutr* 58:398–406.

- Joern A. 1979. Feeding patterns in grasshoppers (Orthoptera: Acrididae): factors influencing diet specialization. *Oecologia* 38:325–347.
- Johnson K.S. and G.W. Felton. 1996. Physiological and dietary influences on midgut redox conditions in generalist Lepidopteran larvae. *J Insect Physiol* 42:191–198.
- Joslyn M.A. 1970. *Methods in Food Analysis*. 2d ed. Academic Press, New York.
- Karowe D.N. and M.M. Martin. 1992. Determinants of diet quality: the effects of diet pH, buffer concentration and buffering capacity on growth and food utilization by larvae of *Manduca sexta* (Lepidoptera: sphingidae). *J Insect Physiol* 39:47–52.
- Kildegard P. 1972. Infant feeding and blood acid-base status. *Pediatrics* 49:801–802.
- Lutz J. 1984. Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. *Am J Clin Nutr* 39:281–288.
- Otte D. 1981. *The North American Grasshoppers*. Vol. 1. Acrididae: Gomphocerinae and Acridinae. Harvard University Press, Cambridge, Mass.
- . 1984. *The North American Grasshoppers*. Vol. 2. Acrididae: Oedipodinae. Harvard University Press, Cambridge, Mass.
- Paul A.A. and D.A.T. Southgate. 1978. McCance and Widdowson's "The Composition of Foods." 4th ed. Her Majesty's Stationery Office, London.
- Peters R.H. 1993. *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge.
- Phillips J.E., R.B. Thomson, N. Audsley, J.L. Peach, and A.P. Stagg. 1994. Mechanisms of acid-base transport and control in locust excretory system. *Physiol Zool* 67:95–119.
- Remer T. and F. Manz. 1994. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am J Clin Nutr* 59:1356–1361.
- Richardson R.M.A., M.B. Goldstein, B.J. Stinebaugh, and M.L. Halperin. 1979. Influence of diet and metabolism on urinary acid excretion in the rat and the rabbit. *J Lab Clin Med* 94: 510–518.
- Schultz J.C. and M.J. Lechowicz. 1986. Hostplant, larval age, and feeding behavior influence midgut pH in the gypsy moth (*Lymantria dispar*). *Oecologia* 71:133–137.
- Sherman H.C. and A.O. Gettler. 1912. The balance of acid-forming and base-forming elements in foods, and its relation to ammonia metabolism. *J Biol Chem* 11:323–338.
- Slansky F., Jr. and J.G. Rodriguez, eds. 1987. *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates*. Wiley, New York.
- Sokal R.R. and F.J. Rohlf. 1995. *Biometry*. 3d ed. W.H. Freeman, New York.
- Thomson B.R., J.M. Thomson, and J.E. Phillips. 1988. NH_4^+ transport in acid-secreting insect epithelium. *Am J Physiol* 254:R348–R356.
- Wilkinson L. 1989. *SYSTAT: The System for Statistics*. SYSTAT, Evanston, Ill.