# Nitrogen Requirements of the Adult Prairie Vole (Microtus ochrogaster)

ABSTRACT.—We determined maintenance nitrogen requirements of the adult prairie vole (*Microtus ochrogaster*) using a nitrogen balance trial approach. Prairie voles required 401 mg kg$^{-0.75}$ day$^{-1}$ of nitrogen to meet maintenance requirements and met requirements on diets containing approximately 4.4% crude protein. Dry matter intake did not vary with percentage of nitrogen in the diet, suggesting that animals on nitrogen-insufficient diets do not display appreciable compensatory intake. Body mass change was only moderately associated with nitrogen balance, indicating that fluctuations in body mass during the trial were likely associated with factors other than nitrogen intake.

## INTRODUCTION

Episodic deficiencies in food quantity or quality may be linked to population fluctuations in microtine and other small mammal species (Taitt and Krebs, 1985; Lochmiller and Dabbert, 1993). Specifically, nitrogen (*e.g.*, protein) has been identified as the most common limiting nutrient of herbivore populations (White, 1978, 1993). However, nitrogen requirements for most microtine rodents have not been determined experimentally (National Research Council, 1995), and values must be inferred from requirements published for other rodent species. The limited research on dietary nitrogen requirements of microtine species suggests that requirements may vary with species, age and diet composition (Robbins, 1993). Lynch and Keys (1968) reported that adult meadow voles (*Microtus pennsylvanicus*) fed a wood pulp-based diet maintained or increased body mass on diets containing ≥8% crude protein, whereas Shenk (1976) found that weanling meadow voles maintained body mass (no growth) on cereal grain-based diets containing as little as 2.8% crude protein. Shenk *et al.* (1970) found that weanling meadow voles had normal growth rates on casein-based diets with 11% crude protein. Field voles (*M. agrestis*) have been shown to maintain body mass on oat-based diets containing as low as 8% crude protein (Spears and Clarke, 1987). Nitrogen requirements of the prairie vole (*M. ochrogaster*), a microtine inhabitant of the central U.S., have not been determined experimentally. Because nitrogen requirements may vary with species, age, or diet, the use of inferred nitrogen requirements to interpret prairie vole data may lead to erroneous conclusions. As a result, we examined the maintenance nitrogen requirements of adult, male prairie voles and compared them to values published for other species.

## MATERIALS AND METHODS

We used adult, male prairie voles weighing approximately 28–40 g (32.2 ± 2.0) from a laboratory colony maintained by the Department of Zoology, Oklahoma State University (Stillwater). Our colony was started in 1994 with animals from a 20-yr-old colony at Kirksville College of Osteopathic Medicine (Kirksville, Mo.). Laboratory animals were used to eliminate experimental variation caused by stresses common to free-ranging animals held in captivity. To determine maintenance nitrogen requirements, we conducted two nitrogen balance trials with 21 animals per trial. During each trial, animals were housed individually in 20 by 25 cm mesh-wire cages under a 14L:10D photoperiod at 24 C with water provided ad lib. Each trial consisted of a 7-day acclimation period followed by a 5-day experimental period. Before the acclimation period of the first trial, animals were maintained on a 23% crude protein rodent chow (Diet no. 5001; PMI Feeds, Inc., St. Louis, Mo.). Animals were fed a casein-based, 16% crude protein diet for 7 days before the acclimation period of the second trial. All animals were fed their experimental diet ad lib during the acclimation and experimental periods. Experimental diets were isocaloric (4.35–4.38 kcal g$^{-1}$), casein-based rations and were formulated by mixing specified quantities of 4% and 16% crude protein diets (United States Biochem, Cleveland, Ohio). We attempted to formulate diets of 4, 6, 8, 10, 12, 14 and 16% crude protein, but the actual protein levels of the diets were slightly less (Table 1). Although casein-based rations may be unsuitable for long-term experimental studies (Shenk *et al.*, 1971), their consistent amino acid composition, which mirrors that of mammalian muscle tissue, permits the flexibility of formulating diets with fairly low nitrogen content. This is not always possible with high protein forages (alfalfa, etc.). Each animal was randomly assigned to one of the experimental rations (n = 3 per diet; six animals were placed on the 10.63% N diet because both trials had diets with 10.63% N), which was fed in a powdered form using ceramic crucible

TABLE 1.—Body mass, dry matter intake, and nitrogen intake, excretion and balance of prairie voles (*Microtus ochrogaster*) fed varying levels of dietary crude protein

| Dietary protein (%) | n | Mean body mass[a] (g) | | Dry matter intake[b] (g) | | Nitrogen intake (mg kg$^{-0.75}$ day$^{-1}$) | | Nitrogen excretion (mg kg$^{-0.75}$ day$^{-1}$) | | Nitrogen balance[c] (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | SE | $\bar{x}$ | SE | $\bar{x}$ | SE | $\bar{x}$ | SE | $\bar{x}$ | SE |
| 3.38 | 2 | 29.38 | 1.78 | 42.63 | 1.87 | 230.2 | 10.1 | 363.9 | 43.0 | −0.047 | 0.017 |
| 3.56 | 3 | 30.55 | 2.63 | 50.71 | 5.82 | 289.1 | 33.2 | 242.2 | 47.1 | 0.021 | 0.029 |
| 5.19 | 3 | 29.25 | 2.06 | 48.32 | 5.06 | 401.1 | 42.0 | 374.3 | 86.5 | 0.008 | 0.017 |
| 5.56 | 2 | 33.60 | 4.20 | 39.69 | 1.43 | 353.3 | 12.8 | 417.6 | 5.8 | −0.026 | 0.010 |
| 7.31 | 2 | 31.03 | 1.73 | 54.25 | 0.31 | 634.7 | 3.6 | 278.2 | 10.6 | 0.132 | 0.003 |
| 7.56 | 3 | 29.07 | 1.01 | 50.46 | 0.58 | 610.6 | 7.0 | 522.5 | 26.0 | 0.032 | 0.013 |
| 8.00 | 3 | 33.88 | 3.74 | 52.81 | 2.51 | 676.1 | 32.1 | 476.7 | 72.6 | 0.082 | 0.027 |
| 9.06 | 3 | 32.25 | 1.45 | 41.32 | 3.93 | 599.2 | 57.0 | 589.2 | 28.9 | 0.005 | 0.031 |
| 10.63 | 6 | 35.01 | 1.62 | 53.03 | 1.27 | 901.5 | 21.6 | 685.0 | 28.2 | 0.088 | 0.009 |
| 12.06 | 3 | 31.43 | 3.19 | 51.00 | 4.04 | 984.3 | 78.0 | 696.6 | 128.1 | 0.105 | 0.063 |
| 12.44 | 3 | 30.37 | 3.45 | 45.41 | 3.60 | 903.7 | 71.7 | 808.2 | 31.1 | 0.038 | 0.025 |
| 13.69 | 3 | 31.23 | 0.47 | 55.97 | 3.36 | 1225.8 | 73.5 | 621.1 | 158.7 | 0.226 | 0.082 |
| 14.13 | 3 | 33.95 | 0.28 | 42.98 | 2.77 | 971.4 | 62.5 | 691.0 | 81.2 | 0.111 | 0.012 |

[a] Mean body mass was calculated as the mean of the body mass measurements at the start and end of the experimental trial

[b] Dry matter intake, and nitrogen balance values are for the entire experimental period (5 days)

[c] Nitrogen balance was calculated by subtracting nitrogen excretion from nitrogen intake

feed bowls with metal washers inserted to minimize spillage. We collected orts (leftovers) and subtracted their weight from that of food offered to determine dry matter intake.

We collected all feces and urine excreted together in drop pans containing 10 ml of 10% $H_2SO_4$ and dried each composite (feces plus urine) for 5 days at 50 C as described by Hammond and Wunder (1991). We determined nitrogen content of dried composites and samples of each experimental diet in duplicate using a nitrogen analyzer (Perkin Elmer 2410 Series II, Norwalk, Conn.). To ensure that nitrogen loss from our samples due to the 50 C drying process was minimal, we collected additional fecal/urine composites from voles fed a 23% crude protein diet. Pairs of samples were dried either in a freeze-dryer for 24 h or for 5 days at 50 C. Nitrogen content of heat-dried samples were 95.7% the value (4.3 % less) of freeze-dried samples, but differences were not statistically different (P = 0.115).

We determined nitrogen intake by multiplying percent nitrogen of the diet by dry-matter intake; nitrogen excretion was determined by multiplying dry mass of fecal and urine composites by their nitrogen content. We estimated nitrogen balance (g N during the 5-day experimental period) by subtracting nitrogen excretion from nitrogen intake. We used simple linear regression (SAS Institute, 1985) to determine the relationships between nitrogen balance, nitrogen intake (mg $kg^{-0.75}$ $day^{-1}$), percent dietary nitrogen, nitrogen excretion (mg $kg^{-0.75}$ $day^{-1}$), and percent change in body mass. We estimated the maintenance nitrogen requirement to be the level of nitrogen intake at zero nitrogen balance. Data from both trials were combined for all statistical analyses because we did not find differences between slopes or intercepts (P > 0.05) of the two trials. We ran regression analyses with trial inserted as an indicator variable (Zar, 1984) to test for differences between experimental trials.

## Results and Discussion

One animal died due to unknown causes on the 8% crude protein diet during the first trial and two animals were removed from the second trial (one each on the 4% and 6% CP diets) due to excessive spillage of excreta (experimenter error), resulting in a total sample size of 39. The relationship between nitrogen balance ($N_B$) and nitrogen intake ($N_I$) was described by the equation $N_B = 0.208(N_I) - 84.197$ ($r^2 = 0.55$; 38 df; P < 0.001; Fig. 1a), which yielded an estimated maintenance nitrogen requirement for adults of 401.0 ± 1.0 mg $kg^{-0.75}$ $day^{-1}$ (±95% CI) (2.5 g $kg^{-0.75}$ $day^{-1}$ of crude protein). Species-specific requirements vary due to differences in diet and digestive physiology (Robbins, 1993). Kam and Degen (1988) estimated that adult fat sand rats (*Psammomys obesus*) required 242.3 mg $kg^{-0.75}$ $day^{-1}$ of nitrogen to maintain positive nitrogen balance. The maintenance nitrogen requirement of adult antelope ground squirrels (*Ammospermophilus leucurus*) varied seasonally from 481 mg $kg^{-0.75}$ $day^{-1}$ in the autumn to 505 mg $kg^{-0.75}$ $day^{-1}$ during spring (Karasov, 1982). Additionally, Karasov (1982) estimated that antelope ground squirrels required a dietary nitrogen level of ca. 1.4% (8.75% crude protein) to maintain nitrogen balance.

To estimate the percentage of nitrogen in the diet that yielded a nitrogen balance of zero, we regressed $N_B$ against percent nitrogen of the diet ($N_D$); $N_B = 8733.69(N_D) - 61.86$ ($r^2 = 0.34$; 38 df; P < 0.001; Fig. 1b). From this equation, nitrogen balance was achieved on a diet of 0.710 ± 0.004% nitrogen (±95% CI; 4.4% crude protein). Nitrogen excretion ($N_E$; mg $kg^{-0.75}$ $day^{-1}$) was positively associated with $N_I$ and was described by the equation $N_E = 0.445 (N_I) + 228.3$ ($r^2 = 0.44$; 38 df; P < 0.001; Fig. 1d). The combined value for excreted metabolic fecal nitrogen and endogenous urinary nitrogen was estimated from the intercept of this equation to be 228.3 mg $kg^{-0.75}$ $day^{-1}$. This value is similar to those reported for other small mammal species (Karasov, 1982; Kam and Degen, 1988; Meyer and Karasov, 1989).

Dietary nitrogen did not explain a significant amount of the variation associated with dry matter intake ($r^2 = 0.013$; 38 df; P = 0.483), suggesting that animals on submaintenance nitrogen diets did not increase dry matter intake to compensate for a diet lower in nitrogen concentration. Mean dry matter intake for our animals was 48.9 ± 1.1 g $kg^{-0.75}$ $day^{-1}$ (x̄ ± SE). This is contrary to Robbins (1993) suggestion that dry matter intake should increase with decreasing dietary nutritive value. Because the formulated diets essentially had a fiber content approaching 0%, it is possible that dry matter digestibility was great enough to allow all animals to have dry matter intake values approaching or equal to their physical capacity to ingest dry matter, which would explain similar intake values across diets (Table 1).

Percent change in body mass ($W_\Delta$) was weakly associated with nitrogen balance [$N_B = 995.3 (W_\Delta)$
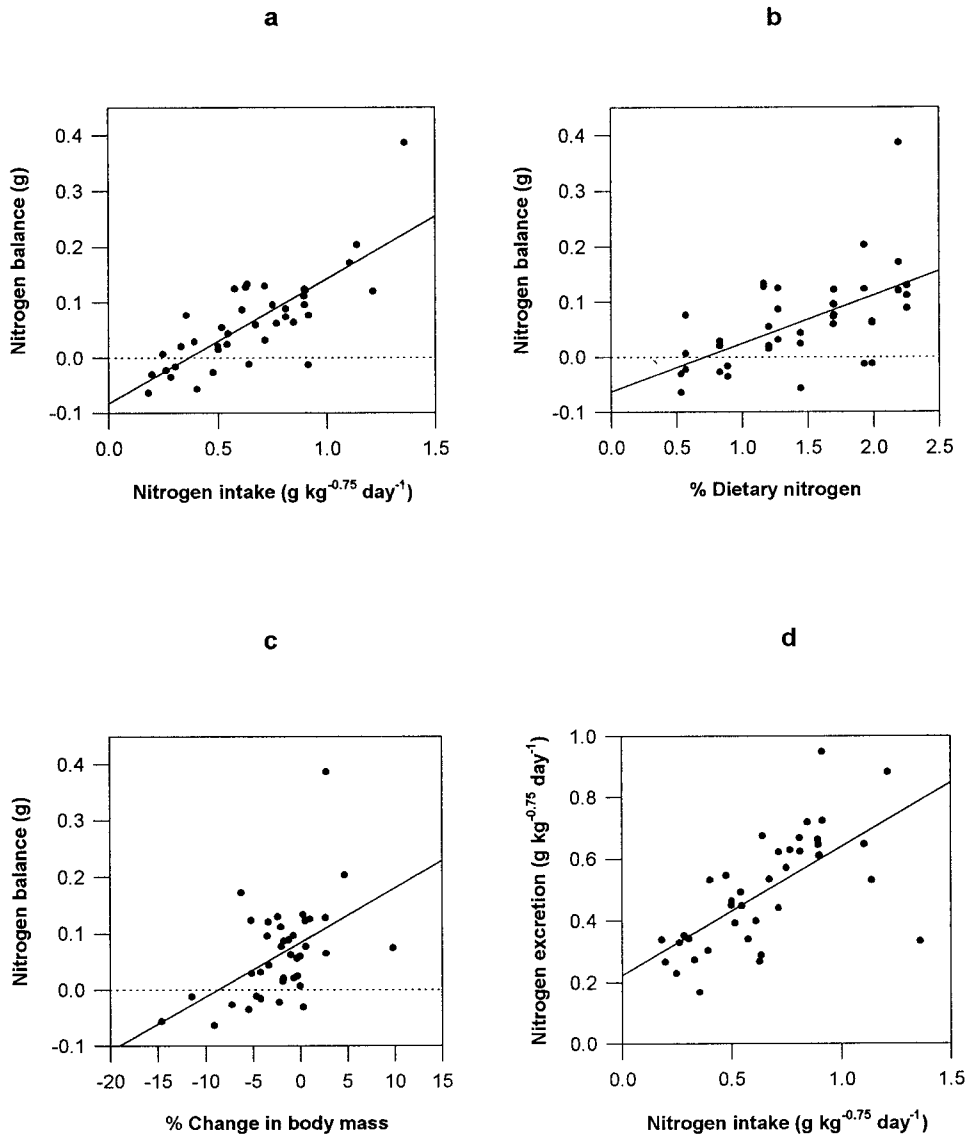
**a**

**b**

**c**

**d**

FIG. 1.—Nitrogen balance of prairie voles (*Microtus ochrogaster*) regressed as a function of (a) nitrogen intake; (b) percent dietary nitrogen, and (c) percent change in body mass. Nitrogen excretion is also depicted as a function of nitrogen intake (d)

+ 83.11 ($r^2 = 0.25$; 38 df; $P < 0.002$; Fig. 1c)]. Animals at zero nitrogen balance lost an estimated 8% of their original body mass over the 5-day trial. Nitrogen intake was related to percent change in body mass [$0.0031(N_I) - 0.1699$ ($r^2 = 0.27$; 38 df; $P < 0.001$)]. These relationships suggest that fluctuations in body mass were primarily associated with energy intake rather than protein since the latter is linked to tissue maintenance and replacement in adult animals rather than growth (Robbins, 1993). Our

observation that dry matter intake was not related to nitrogen balance suggests that there was adequate energy intake to support nitrogen metabolism.

Our study suggests that diets containing 4.4% crude protein meet the apparent minimal daily nitrogen requirements for maintenance in the adult prairie vole. This value is less than previously published values (8% crude protein) for field voles and meadow voles, suggesting that future studies involving prairie voles should consider the possibility that dietary nitrogen values <8% crude protein may be suitable for maintenance. However, we advise that care should be taken when extrapolating this value to free-ranging prairie voles. It has been demonstrated that differences in physiological characteristics (Nelson, 1985) and behavior (Huck *et al.*, 1986) may exist between captive and free-ranging wildlife, and although it has not been documented, it stands to reason that differences in nutritional requirements may exist as well. As a result, future research should aim at estimating the nitrogen requirements of free-ranging prairie voles to determine if differences exist between captive and free-ranging populations.

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STEPHEN S. DITCHKOFF, Department of Zoology and Oklahoma Cooperative Fish and Wildlife Research Unit, Oklahoma State University, Stillwater 74078, CHAD S. BOYD, Department of Zoology, Oklahoma State University, Stillwater, 74078, EDGAR R. WELCH, JR., Department of Zoology and Oklahoma Cooperative Fish and Wildlife Research Unit, Oklahoma State University, Stillwater 74078, and JOSHUA B. RAGLIN AND ROBERT L. LOCHMILLER, Department of Zoology, Oklahoma State University, Stillwater 74078. *Submitted 9 June 1997; accepted 23 February 1998.*