NUTRITIONAL RESTRICTION AND ACID-BASE BALANCE IN WHITE-TAILED DEER

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ABSTRACT: We examined the effect of progressive nutritional restriction on acid-base balance in seven captive, adult white-tailed deer (*Odocoileus virginianus*) from 4 February to 5 May 1988 in north central Minnesota (USA). Metabolic acidosis was indicated by low mean blood pH (7.25 to 7.33) in deer throughout the study. Mean urinary pH values declined ($P = 0.020$) from a mean ($\pm$SE) baseline of 8.3 ± 0.1 to 6.7 ± 0.3 as restriction progressed. Acidemia and aciduria were associated with significant variations in mean blood CO_2 ($P = 0.006$) and pO_2 ($P = 0.032$), serum potassium ($P = 0.004$) concentrations, and with a significant ($P = 0.104$) handling date $\times$ group interaction in urinary potassium : creatinine values. Mean bicarbonate : carbonic acid ratios were consistently below 20:1 during nutritional restriction. Mean packed cell volume increased ($P = 0.019$) and serum total protein decreased ($P = 0.001$); thus there was evidence for progressive dehydration and net protein catabolism, respectively. Blood pCO_2, serum sodium, and urinary sodium : creatinine were stable throughout the study. We propose that acidosis and aciduria are metabolic complications associated with nutritional restriction of white-tailed deer.
Key words: Acid-base, blood gases, electrolytes, *Odocoileus virginianus*, serum pH, urinary pH, white-tailed deer.

INTRODUCTION

Nutritional restriction is a natural and common occurrence in white-tailed deer (*Odocoileus virginianus*) and other ungulates on northern ranges (Mautz, 1978; Nelson and Leege, 1982). Because nutrition is closely linked to reproduction, survival, and most other aspects of *Odocoileus* spp. ecology, understanding physiological effects of nutritional restriction in free-ranging white-tailed deer is essential to improving future management. Fluid and electrolyte balance are critically involved in regulation of acid-base balance (Carlson, 1989); thus, nutritional restriction may profoundly affect acid-base status. Most enzymatic reactions have narrow ranges of optimum pH; the vital limits of blood pH for mammals are considered to be 7.0 and 7.8 (Benjamin, 1981; Houpt, 1984). Therefore, alterations of acid-base balance in deer may directly affect rates of enzymatic reactions, and thus, a variety of biological processes (Carlson, 1989). There has been little study of the relationship

between nutrition and the acid-base status of deer. Our objective was to document the effects of nutritional restriction on the acid-base balance of blood and urine pH, and other closely associated metabolic characteristics and to provide reference values.

MATERIALS AND METHODS

During January to May 1988, seven adult (>1.5 yr) deer (four pregnant females, three males) were maintained individually in outdoor pens (15.5 × 30.0 m) near Grand Rapids, Minnesota (USA, 47°14'N, 93°31'W). Monthly mean maximum and minimum ambient temperatures were −9.7, −6.8, 2.1, 12.7, and 24.1 C and −23.3, −23.8, −9.9, −2.8, and 7.2 C, respectively (National Oceanic and Atmospheric Administration, 1988).

Until 11 February, all deer were fed a high protein-high energy pelleted ration (DelGiudice et al., 1990). On 4 February 1988, two males and two females were randomly assigned to an experimental group, and one male and two females to a control group. We anesthetized deer with 100 to 150 mg xylazine HCl (Rompun, Haver-Lockhart Laboratories, Shawnee, Kansas, USA) and 200 to 650 mg ketamine HCl

TABLE 1. Blood and urinary characteristics unaltered during nutritional restriction of seven captive white-tailed deer in north central Minnesota, 4 February to 5 May 1988.

Characteristic[*]	Number of samples	$\bar{x}$	SE	Range
Blood				
HCO_3^- (mEq/l)	35	23.8	0.85	14.4–31.7
pCO_2 (mm Hg)	35	50	1.6	33–69
Serum				
Na (mEq/l)	57	146	0.4	134–157
Urine				
K:Cr (mEq:mg) × 1,000	57	87.0	7.6	3.6–303
Na:Cr (mEq:mg) × 1,000	57	36.2	6.5	0.2–263

[*] HCO_3^- = bicarbonate, pCO_2 = carbon dioxide partial pressure, Na = sodium, K:Cr = potassium : creatinine ratio, and Na:Cr = sodium : creatinine ratio.

meter (Beckman Instruments, Inc., Brea, California, USA) within 24 hr of collection. Urinary electrolyte data were compared as ratios of creatinine (Cr) to control for extraneous variability associated with differences in hydration (Hove and Jacobsen, 1975; Coles, 1980).

Split-plot, repeated measures analysis of variance (ANOVA) was employed to analyze urine and blood data; treatment was diet group, whole-plot was deer, and individual daily measures on each deer were subplots (Mead, 1988). Greenhouse-Geisser adjusted F-tests were used as a conservative measure to guard against apparent violation of the sphericity assumption (Milliken and Johnson, 1984). Analyses by ANOVA were confined to dates when serum and urine samples were collected from all seven deer so as to avoid confounding effects of time with those of diminished sample size. Sample sizes were not adequate to analyze for the effects of sex; however, data from the males fell within the bounds of variability of the females. To increase statistical power, while maintaining low probability of a Type I error, significance was accepted at $P \leq 0.10$. Non-overlapping simultaneous confidence limits were computed with an estimate of the pooled variance (i.e., mean square error) at alpha = 0.10 to determine differences of means between dates.

RESULTS

There was no significant difference in PCV ($P = 0.20$); serum total protein (TP, $P = 0.11$), Na ($P = 0.52$), and K ($P = 0.77$); or blood pH ($P = 0.39$), pCO_2 ($P = 0.47$), and pO_2 ($P = 0.29$) between the control and restricted groups. However, mean PCV ($P = 0.019$), TP ($P = 0.001$), and K ($P = 0.004$) varied significantly over time (Fig.

1). Mean PCV exhibited an increasing trend, whereas serum TP steadily decreased. Mean serum K concentrations were highest ($P \leq 0.10$) on 31 March. There was no significant handling date × group interaction for PCV ($P = 0.56$) or any serum characteristic ($P \geq 0.48$). Serum Na remained stable ($P = 0.24$) during the study (Table 1).

Blood pH was low and stable ($P = 0.20$) during most of the study (Fig. 2A). Thirty-one (74%) of 42 blood samples had pH values ≤7.35. Mean CO_2 ($P = 0.006$) and pO_2 ($P = 0.032$) varied over time (Fig. 2B, C). There was a significant ($P = 0.0001$) handling date × group interaction for blood CO_2; mean values were similar until 11 April when there was a significant ($P \leq 0.10$) increase in blood CO_2 in restricted deer compared to its first measurement on 23 February. Mean pO_2 was lowest ($P \leq 0.10$) on 11 April. Highest CO_2 concentrations (67 and 70 mmole/l), accompanied by low pO_2 (23 and 30 mm mercury [Hg]), occurred in the two deer that died of undernutrition within the following week. Mean HCO_3^- ($P = 0.43$) and pCO_2 ($P = 0.56$) concentrations remained unaltered from 23 February to 11 April.

There was no significant difference in urinary pH ($P = 0.40$), K:Cr ratio ($P = 0.96$), or Na:Cr ratio ($P = 0.51$) between the control and experimental deer. Urinary pH exhibited a decreasing ($P = 0.020$) trend as nutritional restriction progressed
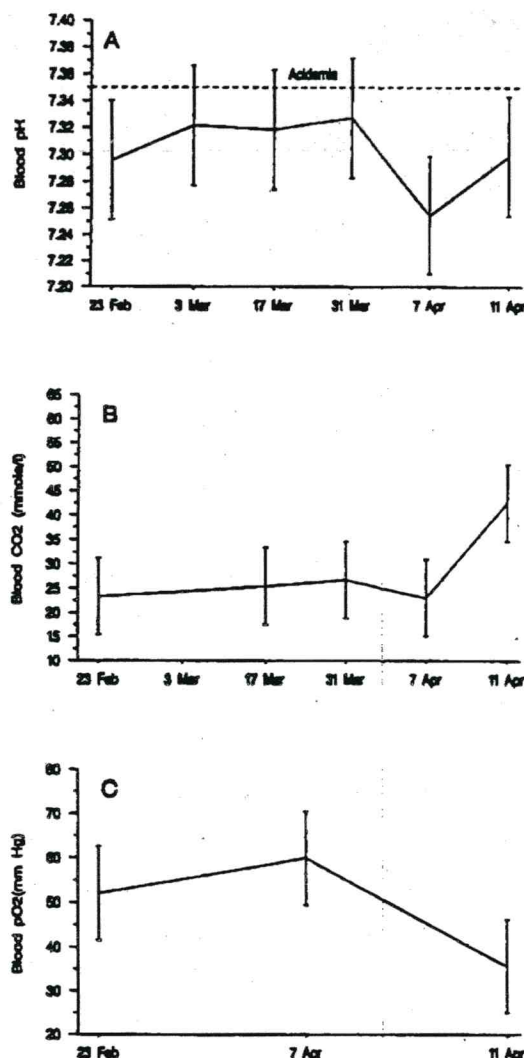
FIGURE 2.   Mean (with 90% confidence limits) values of blood pH (A), CO_2 (B), and pO_2 (C) in seven captive, adult white-tailed deer during nutritional restriction in north central Minnesota, 23 February to 11 April 1988. The pH threshold below which acidemia occurs is from Benjamin (1981).
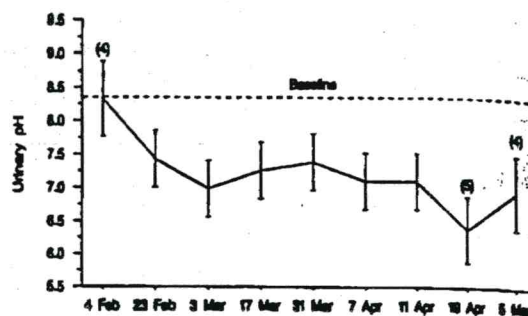

FIGURE 3.   Mean (with 90% confidence limits) values of urinary pH in seven captive, adult white-tailed deer during nutritional restriction in north central Minnesota, 4 February to 5 May 1988. The mean ($\pm SE$) baseline was 8.3 (± 0.1). Sample sizes were seven unless otherwise indicated in parentheses.

(Fig. 3). Mean baseline urinary pH on 4 February was 8.3 $\pm$ 0.1 (range = 8.2 to 8.6), and its mean value decreased (16%) significantly ($P < 0.10$) by the third handling date (3 March; $\bar{x}$ = 7.0 $\pm$ 0.2, range = 6.5 to 8.0) and remained below baseline for the remainder of the study. By 19 April, mean urinary pH (6.7 $\pm$ 0.3, range = 5.7 to 7.2) was 19% below the baseline value. There was no handling date × group in-

teraction effect for urinary pH ($P = 0.15$). Urinary pH was inversely related to mass loss in all seven deer ($R^2 = 0.34$, Y = 7.678 $-$ 4.237e^{-2x}; where Y = urinary pH, x = cumulative percent mass loss, and e = natural logarithm base). Furthermore, urinary pH decreased (11 to 20%) in all control deer from 7.3 $\pm$ 0.4 on 11 April to 6.3 $\pm$ 0.3 by 19 April after 4 days of acute nutritional restriction.

There was a significant ($P = 0.104$) handling date × group interaction for urinary K:Cr ratios (Table 1). Values were low and similar in restricted and control deer until 11 April (125 $\pm$ 9.4 mEq:mg [×1,000]) when the K:Cr ratio increased 70% from 7 April (73.5 $\pm$ 11.0 mEq:mg [×1,000]) in the restricted deer, but remained low in control deer. Mean K:Cr ratios continued to increase in restricted deer as nutritional deprivation progressed; on 5 May it was 237 $\pm$ 67 mEq:mg (×1,000). Mean urinary Na:Cr ratio tended to decrease by 23 February, then remained low and variable throughout the study (Table 1). There was no interaction effect ($P = 0.15$).

DISCUSSION

Condition deterioration in both deer groups was reflected by mass loss as the study progressed (DelGiudice et al., 1994). Since 4 February, peak mean ($\pm SE$) mass loss was 23 $\pm$ 3% (range = 16 to 29%) for

restricted deer (by 11 April) and $14 \pm 3\%$ (range = 7 to 17%) for control deer (by 19 April). Northern deer voluntarily reduce food intake during winter (Ozoga and Verme, 1970); DelGiudice et al. (1987a) reported body mass loss $(10.7 \pm 1.6\%)$ in captive deer even when fed an unnaturally high quality diet *ad libitum* during winter. The fact that condition deteriorated in both restricted and control groups, along with small sample sizes (low statistical power), probably accounted for the absence of significant differences in most of the blood and urine characteristics measured in this study.

Mean blood pH <7.35 probably was indicative of metabolic acidosis in our deer (Benjamin, 1981) as early as 23 February (12 days after nutritional restriction began), and it became most severe by 7 April. By 23 February, mean ($\pm$SE) body mass loss was $8.1 \pm 1.1\%$. The vital limits of blood pH for domestic mammals are 7.0 to 7.8 (Houpt, 1984), and values >7.45 are indicative of alkalosis in domestic animals (Benjamin, 1981). Accelerated net catabolism of endogenous proteins and accumulation of organic acids as nutritional restriction progresses contribute to metabolic acidosis (Houpt, 1984).

Endogenous protein loss has been directly associated with body mass loss in chronically undernourished deer. (Torbit et al., 1985; DelGiudice et al., 1990). Based on the significant decrease in serum TP in our deer, we believe that an accelerated net protein catabolism occurred; this was supported by a direct relationship between percent mass loss and urinary urea nitrogen:Cr ratios (DelGiudice et al., 1994). The steady 22% increase in mean PCV to its peak value (45%) by 19 April is consistent with a plasma volume deficit of 25 to 30%, attributable to the dehydration that accompanies nutritional deprivation and mass loss (Carlson, 1989). Progressive dehydration favors catabolic processes (Coles, 1980).

Metabolic acidosis is due primarily to a HCO_3^- deficit (Benjamin, 1981; Carlson,

1989). The lowest mean HCO_3^- concentration in our deer (21.8 mEq/l) was at the low end of normal ranges reported for bovines and ovines, 20 to 27 mEq/l and 20 to 25 mEq/l, respectively (Schotman, 1971; Kaneko, 1989). Tasker (1967) reported decreased plasma HCO_3^- and pH in horses after 8 days of food and water deprivation; however, average pH did not decline to <7.35. Similarly, acidosis was not induced by 5 days of fasting in dairy cattle (Dale et al., 1954).

More informative than HCO_3^- concentration alone, however, is the HCO_3^- : carbonic acid (H_2CO_3) ratio (Houpt, 1984). Calculating H_2CO_3 concentrations by multiplying pCO_2 by 0.03 (solubility constant for CO_2 in plasma) (Carlson, 1989), HCO_3^-: H_2CO_3 ratios in our deer (range = 14.5 to 16.7) were 15 to 30% below the accepted normal of 20:1 (Houpt, 1984). The HCO_3^-- H_2CO_3 buffer system responds immediately to acidosis; clinically and physiologically, it is considered the most important component of the body's buffering capacity (Carlson, 1989). The mean pCO_2 values associated with the prolonged nutritional restriction tended to be elevated compared to the normal concentration of 40 mm Hg for domestic animals (Houpt, 1984). These concentrations probably were maintained by the persistent low base concentrations reflected by diminished HCO_3^-:H_2CO_3 ratios (Houpt, 1984). The increase in blood CO_2 concentration by 11 April may be explained by renal handling of hydrogen and HCO_3^- ions and reabsorption of CO_2 (Houpt, 1984). Further, based on the elevated CO_2 and diminished pO_2, we believe that respiratory compensation was inadequate to correct the chronic acid-base imbalance induced by prolonged nutritional restriction.

Baseline (4 February) urine samples of our deer exhibited alkaline pH values, consistent with findings for healthy domestic herbivores (Finco, 1989); however, progressive aciduria accompanied the acidemia. Acute aciduria occurred in all control deer (pH = 5.7 to 6.7) following the 4 days
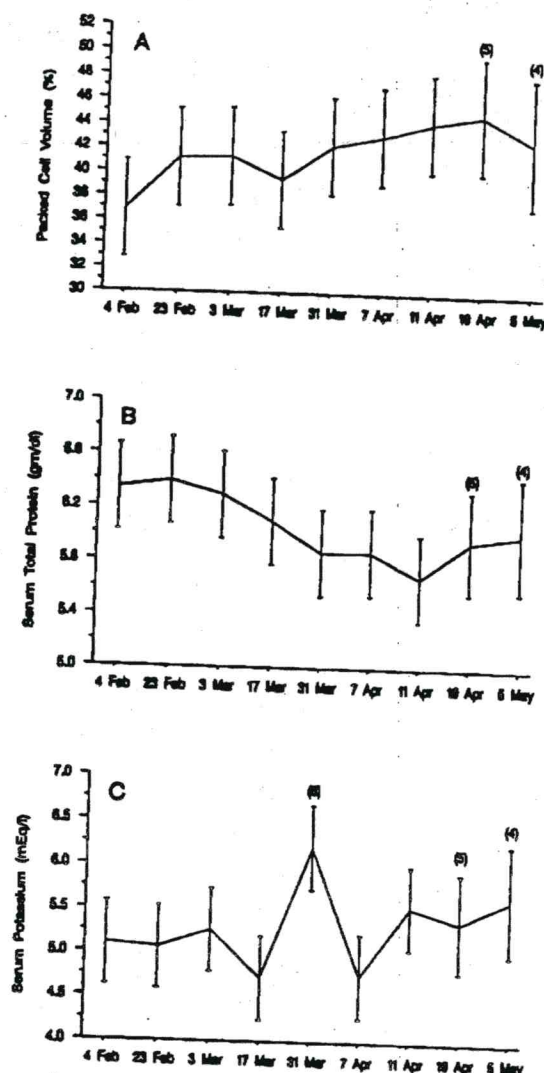
FIGURE 1.   Mean (with 90% confidence limits) values of packed cell volume (A), serum total protein (B), and potassium (C) in seven captive, adult white-tailed deer during nutritional restriction in north central Minnesota, 4 February to 5 May 1988. Sample sizes were seven unless otherwise indicated in parentheses.

(Ketaset, Bristol Laboratories, Syracuse, New York, USA) via pole syringe. Blood was collected by jugular venipuncture into serum tubes and ethylenediamine tetraacetic acid (EDTA) vials, and urine was collected by catheterization or cystocentesis (Kreeger et al., 1986). Deer were weighed by spring scale to the nearest 0.5 kg, and rectal temperatures were monitored throughout immobilizations. Anesthesia was reversed by intravenous injection of 15 mg of yohimbine HCl (Sigma Chemical Co., P.O. Box

14508, St. Louis, Missouri, USA). Serum and urine samples were stored frozen until laboratory analyses were conducted.

Beginning 11 February, the experimental group was fed a maximum of 0.2 to 1.0 kg deer/day of a low protein (7.0% crude protein), low energy (1,900 kcal digestible energy [DE]/kg) pelleted diet (LPLE, E. J. Houle, Inc., Forest Lake, Minnesota). The diet was 90% dry matter and included 92% ground ear corn, 4.6% liquid molasses, 1.6% calcium carbonate, 0.9% dicalcium phosphate, 0.7% trace mineral salt, and 0.1% multiple vitamin supplement. Control deer were fed the same LPLE diet ad libitum until 15 April. To document the effect of acute nutritional restriction on the control animals, all deer were limited to 0.2 kg of feed per day from 15 to 18 April; ad libitum feeding of controls was resumed on 19 April until 5 May (DelGiudice et al., 1994). Daily measurements of feed not taken permitted calculation of food consumption. Mean daily mass-specific digestible energy intake was greater in control deer (79 to 117 $kcal/kg^{0.75}$) than in experimental deer (28 to 73 $kcal/kg^{0.75}$), except when both groups were restricted from 15 to 18 April (mean = 50 $kcal/kg^{0.75}$) (DelGiudice et al., 1994). All deer were dependent upon snow for water until late February; subsequently water was provided ad libitum.

From 23 February through 5 May, between 0800 and 1200 hours, we again chemically immobilized and handled deer at primarily 1 to 2 wk intervals (eight handling dates). Mean (±SE) times between induction of anesthesia and blood and urine sampling were 10.2 ± 0.7 and 61.6 ± 17.4 min, respectively. On six of the eight handling dates, blood also was collected by vacutainer into heparin tubes by jugular venipuncture for analysis for pH, CO_2, and pCO_2. Samples were analyzed for pO_2 on three handling dates.

Blood in EDTA tubes was analyzed for packed cell volume (PCV) and sera were analyzed for sodium (Na), potassium (K), and total protein (TP) as described by DelGiudice et al. (1990). Heparinized blood was analyzed for pH, CO_2, pCO_2, and pO_2 within 1 hr of collection by a pH and blood gas analyzer (Model 170, Ciba-Corning, Medfield, Massachusetts, USA) at Itasca Medical Center, Grand Rapids, Minnesota; samples were kept cool on ice until analysis. Blood pH and gas analyses were conducted opportunistically depending on availability of the analyzer. We calculated bicarbonate (HCO_3^-) by the Henderson-Hasselbalch equation (Carlson, 1989).

Analyses of urinary creatinine, Na, and K were by the methods of DelGiudice et al. (1990). Urinary pH was measured on a Beckman pH

(15 to 18 April) of feed restriction; this partially accounted for the further decline in the overall mean value by 19 April (Fig. 3). Full correction of acid-base balance can be achieved only by renal excretion of hydrogen ions (Houpt, 1984). We have observed a decline in mean ($\pm SE$) urinary pH in white-tailed deer from 8.1 (± 0.3) to 6.6 (± 0.6) and 6.0 (± 0.3) after 2 and 4 wk of fasting, respectively (G. D. Del-Giudice, unpubl.).

Renal compensation in response to metabolic acidosis has been characterized by increased acid excretion, base preservation by enhanced Na-hydrogen (H) exchange, and increased HCO_3^- reabsorption (Coles, 1980). In fact, the maintenance of stable serum Na and blood HCO_3^- concentrations in our deer most likely is explained by renal restoration of Na and HCO_3^- to the blood by the ion-for-ion exchange between tubular H and Na ions; the Na ions are paired with HCO_3^- ions (Houpt, 1984).

Serum K concentrations and urinary K:Cr ratios appeared to be quite responsive to the variable feed consumption, as well as accelerated protein catabolism. Except for serum K concentrations on 31 March, which followed a brief period of increased available feed for restricted deer, the low mid-study values of serum K and urinary K:Cr ratio were consistent with progressive K depletion. Hypokalemia and decreased urinary K:Cr ratio have been reported for white-tailed deer fasted for 4 wk (DelGiudice et al., 1987a, b). An increasing trend of urinary K:Cr ratio after 31 March was associated with high mass losses and accelerated protein catabolism of several of the restricted deer (Tepperman, 1980; DelGiudice et al., 1987b, 1991), two of which died from undernutrition during 11 to 19 April. Progressive K depletion promotes low intracellular K concentrations and increased exchange of K with H ions, contributing to acidosis and aciduria (Houpt, 1984).

We documented alteration of acid-base balance, specifically acidosis and aciduria, as one of the metabolic complications associated with nutritional restriction of white-tailed deer. Our reported blood and urine values should serve as useful reference values for the additional research needed in this area.

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