

Effect of Ruminal Glucose Infusion on Dry Matter Intake, Urinary Nitrogen Composition, and Serum Metabolite and Hormone Profiles in Ewes^{1,2}

M. S. Brown, D. M. Hallford³, M. L. Galyean⁴, C. R. Krehbiel, and G. Duff

Department of Animal and Range Sciences, New Mexico State University, Las Cruces 88003-0003

ABSTRACT: Twelve 18-mo-old Debouillet ewes were used to determine the effect of ruminal glucose infusion on DMI, on urinary ammonium (NH₄⁺) and urea N (UUN) concentrations, and on serum metabolite and hormone profiles. Ewes were limit-fed a 90% concentrate diet for 30 d, stratified by BW into three groups (average BW = 82.6 ± 1.1 kg), and assigned randomly to receive 0, 5, or 10 g of glucose/kg of BW via esophageal intubation. Urine was collected hourly for 12 h and blood (jugular venipuncture) at 30-min intervals for 12 h. After 12 h, ewes were housed individually, allowed free access to the diet, and DMI was recorded for 5 d. Venous blood pH averaged 7.49, 7.48, and 7.48 at 0 h and decreased (linear [L], $P < .01$) at 12 h (7.41, 7.36, and 7.26) with increasing glucose. Serum glucose increased (L, $P = .06$) at 3 and 6 h. Serum L(+)-lactate increased (L, $P = .08$) at 3, 6, and 9 h, whereas serum D(-)-lactate increased linearly ($P = .09$) at 6 and 9 h and quadratically ($P < .10$) at 12 h. After the glucose challenge, DMI decreased (L, $P < .05$). Urinary pH and

NH₄⁺ were not influenced by glucose infusion; however, UUN increased at 3 (quadratic [Q], $P < .05$), 4, 5, 6 (L, $P = .03$), and 7 h (Q, $P < .05$) and decreased at 11 and 12 h (L, $P = .09$). As glucose infusion increased, serum creatinine increased at 9 (L, $P < .01$) and 12 h (Q, $P = .02$). Generally, serum Na and P increased ($P = .09$), whereas K decreased ($P < .05$), with glucose infusion. Lactate dehydrogenase activity increased with glucose infusion (Q, $P < .10$) at 3, 6, 9, and 12 h. Increasing glucose infusion increased serum globulin (Q, $P = .06$), albumin, and total protein (L, $P = .08$). Serum prolactin and vasopressin were not influenced ($P = .22$) by glucose infusion. Serum insulin and aldosterone increased quadratically ($P = .08$), whereas serum growth hormone decreased linearly ($P = .08$) as a result of increasing glucose infusion. Results suggest that UUN, serum insulin, aldosterone, and several serum constituents may serve as markers of organic acid load in ruminants fed high-concentrate diets.

Key Words: Acidosis, Serum, Urea Nitrates, Hormones

©1999 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 1999. 77:3068–3076

Introduction

Previous reports have provided extensive reviews of the etiology and pathophysiology of acidosis (Huber, 1976; Slyter, 1976; Owens et al., 1996) and related secondary disorders in ruminants (Brent, 1976; Brink et al., 1990; Nagaraja and Chengappa, 1998). Obvious visual signs characterize acute acidosis (Elam, 1976),

whereas animals experiencing subacute acidosis may appear normal (Owens et al., 1996) but display decreased and/or erratic feed intake (Fulton et al., 1979). Although feed intake seems to be a sensitive marker of subacute acidosis in individually fed animals, decreased feed intake by a small number of animals among a larger group in a pen is likely not discernible (Stock et al., 1995). Identification of sensitive markers to indicate the progression of acidosis may aid in defining the point at which animal performance is adversely affected.

Biological and microbiological alterations in the ruminal environment have largely been the focus of previous acidosis research efforts. However, ruminal measures do not directly reflect the peripheral impact of organic acids. The process of sensing and signal transduction of blood CO₂ concentration, osmolality, or blood and/or urine H⁺ concentration likely involves humoral signals. Additionally, evidence of cellular damage (Patra et al., 1993) and altered urinary N composition (Telle and Preston, 1971) resulting from an excessive

¹Research supported by the New Mexico Agric. Exp. Sta., Las Cruces.

²The authors gratefully acknowledge the assistance of J. Hernandez, M. J. Brown, H. Al-Tamimi, K. Kane, and J. Richards. Appreciation is also expressed to the NHPP and NIDDK for supplying assay materials.

³To whom correspondence should be addressed: Dept. 3-I, Box 30003 (phone: 505/646-1004; fax: 505/646-5441; E-mail: dhallfor@nmsu.edu).

⁴Dept. of Anim. Sci. and Food Tech., Texas Tech. Univ., Lubbock. Received December 14, 1998.

Accepted May 21, 1999.

Table 1. Basal diet ingredient and chemical composition

Item	% of DM
Ingredient composition	
Sudangrass hay	9.89
Whole corn	9.68
Steam-flaked corn ^a	65.09
Soybean meal	3.66
Urea	1.00
Ammonium sulfate	.25
Molasses	4.81
Yellow grease	3.07
Limestone	.75
Dicalcium phosphate	.50
Salt	.30
Premix ^b	1.00
Chemical composition	
ADF	8.5
CP	13.5
Ash	4.6
Starch	49.1
Available starch ^c	47.8

^aDegree of processing = 360 g/L bulk density.

^bPremix contained (DM basis): CaI₂, .008%; CoCO₃, .011%; CuSO₄, .098%; FeSO₄, .583%; MgO, .893%; MnO, .208%; ZnSO₄, .845%; wheat middlings, 96.30%; mineral oil, .119%; vitamin A, .665% (30,000 USP/g); vitamin E, .270% (500,000 IU/kg).

^cAvailable starch as a percentage of total dietary starch content.

organic acid insult have been reported. Few data are available describing systemic biochemical and endocrine changes during metabolic acidosis. Therefore, our objective was to evaluate the effect of ruminal glucose infusion designed to induce an increasing severity of metabolic acidosis on DMI, urinary N composition, and serum metabolite and hormone profiles in sheep.

Materials and Methods

Animals and Diet. Twelve 18-mo-old Debouillet ewes (82.6 ± 1.1 kg) were group-fed a 90% concentrate diet (Table 1) twice daily during a 30-d adaptation period. Animals were handled and cared for according to a protocol approved by the New Mexico State University Institutional Animal Care and Use Committee. Feed intake was slightly restricted (2.4% of BW) in an attempt to simulate "clean" bunk management systems used in commercial feedlots. Diet samples were collected daily and composited weekly. Before the afternoon feeding on d 30, animals were weighed and subsequently fitted with Foley catheters (14 French) attached to urine collection bags (West Texas Medical Specialties, El Paso, TX) to facilitate urine collection. Urine bags were secured on animals by attaching fecal collection bags. Urine bags were emptied once during the night, because approximately 14 h elapsed between the time of catheter placement and the beginning of sample collection. Ewes were stratified by BW into three groups (average BW = 82.6 ± 1.1 kg) and assigned randomly to receive 0, 5, or 10 g of glucose/kg of BW via esophageal intubation.

Sample Collection. On the morning of the glucose challenge (d 31), urine bags were emptied 1 h before feeding. Just before feeding (0 h), urine and whole blood (via jugular venipuncture) samples were collected, and animals were allowed 30-min access to fresh feed. After 30 min, feed was removed, and each ewe was dosed with an equal volume (2 L) of a glucose solution or distilled water as per treatment. Urine was collected hourly for 12 h after infusion. Urinary pH was determined immediately after collection using a pH meter (Model No. HI 9024C, Hanna Instruments, Woonsocket, RI) equipped with a combination electrode, and a 15-mL aliquot was stored at -20°C.

Venous whole blood (5 mL) was collected as follows: 1) into heparinized tubes (15 U/mL of blood) at 0 and 12 h for blood pH determination; 2) at 0, 3, 6, 9, and 12 h for serum metabolite analyses; and 3) at 30-min intervals for 12 h for serum insulin, GH, prolactin, aldosterone, and vasopressin analyses. Blood pH was determined immediately after sample collection using a pH meter equipped with a combination electrode (Model No. 01048, Denver Instrument Co., Denver, CO). Serum metabolite and hormone samples were allowed 30 min to clot at room temperature and centrifuged at 1,000 × g for 15 min at 4°C, and serum was stored at -20°C. Following the 12-h glucose challenge, animals were housed individually in outdoor pens (2.7 × 2.7 m) and allowed ad libitum access to the basal diet to monitor DMI for 5 d. Feed refused was weighed and discarded before each feeding (once daily). Daily DMI variation for each of the 5 d also was calculated (Stock et al., 1995).

Laboratory Methods. Diet samples were ground in a Wiley mill to pass a 1-mm screen and analyzed for ADF (Goering and Van Soest, 1970), DM, ash, CP (AOAC, 1990), and total and available starch (Xiong et al., 1990; Table 1). Urine samples were analyzed for urea N (UUN; No. 545-B, Sigma Chemical Co., St. Louis, MO) and ammonium concentration (NH₄⁺) using the titration method of Chan (1972). A commercial laboratory (Southwest Medical Laboratories, Las Cruces, NM) assayed serum metabolites, except lactate. Eight replicates of a serum pool from roughage-fed sheep were interspersed among the other samples to calculate CV for each constituent. All CV were less than 12%. Serum was deproteinized according to the procedure of Krehbiel et al. (1995a) and was used to determine L(+)-lactate (Gutmann and Wahlefeld, 1974; Engel and Jones, 1978) and D(-)-lactate concentrations (Gawehn and Bergmeyer, 1974; Brandt et al., 1980). Serum prolactin and GH were determined with RIA according to the procedures of Spoon and Hallford (1989) and Hoefler and Hallford (1987), respectively. Commercially available RIA kits were used to assay insulin, aldosterone (Diagnostics Products Corp., Los Angeles, CA), and vasopressin (Vasopressin Direct, American Laboratory Products Co., Windham, NH). The vasopressin kit had been previously validated for sheep (Senn et al., 1995). All hormone intraassay CV were less than 18%.

Data Analyses. Data were analyzed as a split-plot in time (Gill and Hafs, 1971) using the GLM procedure of SAS (1985). Animal within treatment served as the error term to test treatment effects, whereas residual error was used to test time and treatment \times time. Data were analyzed within time when a significant ($P < .10$) treatment \times time interaction was detected. Serum hormone data also were subjected to area under the curve analysis using trapezoidal summation. The 0-h values for each response variable were evaluated for their use as covariates, and they were retained in the model when significant ($P < .10$). Treatment sums of squares were partitioned into linear and quadratic components.

Results and Discussion

Our interest in the present trial involved inducing an increasing severity of metabolic or systemic acidosis to identify sensitive markers of metabolic acidosis. Krehbiel et al. (1995a) produced an increasing severity of acidosis by feeding wethers a 50% concentrate diet and ruminally infusing 6 to 18 g of glucose/kg of BW, whereas Montaña et al. (1999) fed steers a 90% concentrate diet and ruminally infused 3.3 g of glucose/kg of BW. Krehbiel et al. (1995a) reported a nadir in ruminal pH of approximately 4.9 and 4.5 by 8 h after glucose infusion for lambs infused with 6 or 12 g/kg of BW and 18 g/kg of BW, respectively. A nadir in ruminal pH of 5.11 by 3 h after glucose infusion was reported by Montaña et al. (1999). Although ruminal pH was not determined in the present trial, glucose infusion levels used resulted in an increased systemic acid load, as indicated by a decrease in blood pH (treatment \times time, $P < .01$) from 7.49, 7.48, and $7.48 \pm .03$ before feeding and glucose infusion ($P > .10$) to 7.41, 7.36, and $7.26 \pm .03$ at 12 h (linear, $P < .01$) for animals treated with 0, 5, and 10 g of glucose/kg of BW, respectively. However, no overt signs indicative of acute acidosis (i.e., diarrhea, labored breathing, and incoordination) were evident during or after the glucose challenge.

Patra et al. (1993) used a pH meter to measure blood pH of mature ewes deprived of feed for 24 h and offered 90 g of reconstituted whole wheat/kg of BW (actual consumption not reported). Jugular blood pH averaged 7.42 before feeding and decreased to 7.22 at 48 h. Krehbiel et al. (1995a) used a blood gas/pH analyzer and reported blood pH at 12 h of 7.44, 7.39, 7.34, and 7.29 for wethers intraruminally dosed with 0, 6, 12, and 18 g of glucose/kg of BW, respectively.

A treatment \times time interaction ($P = .04$) occurred for serum D(-)- and L(+)-lactate. Glucose infusion did not affect L(+)-lactate concentration at 0 or 12 h (Figure 1). At 3, 6, and 9 h, L(+)-lactate increased linearly ($P < .10$) with increasing glucose infusion. Generally, L(+)-lactate for each treatment was greatest at 0 h, decreased at 3 h, and gradually increased thereafter, but it did not exceed 0-h concentrations. Serum D(-)-lactate was not altered by glucose infusion through 3 h (Figure

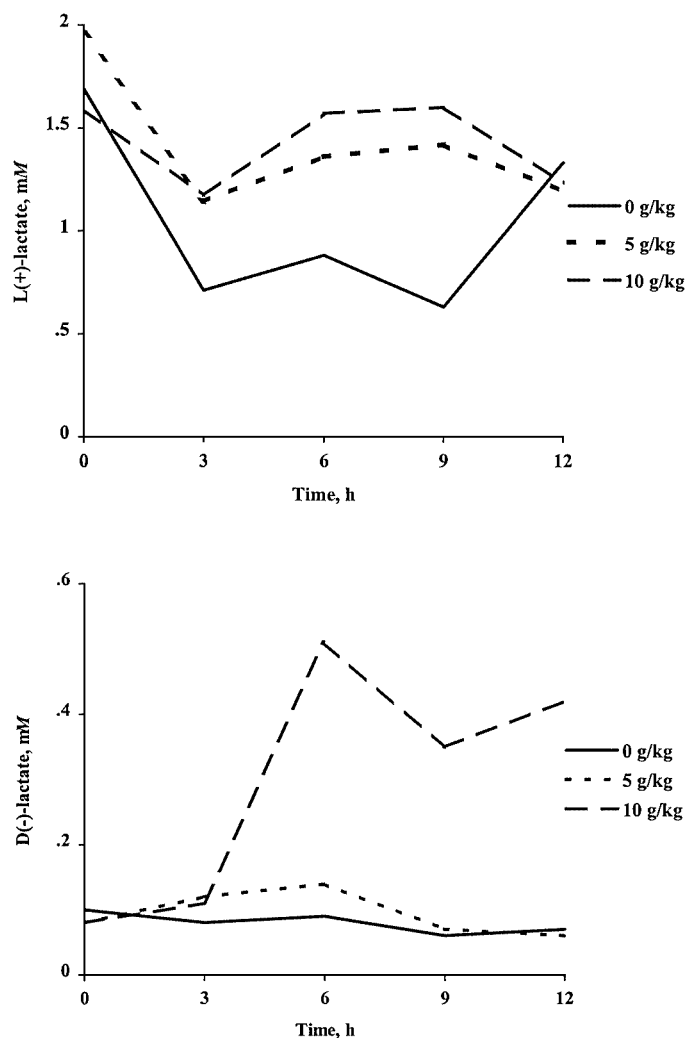


Figure 1. Effect of ruminal glucose infusion on serum L(+)-lactate (upper graph) and D(-)-lactate (lower graph) concentration. For both lactate isomers, the treatment \times time interaction was significant ($P < .04$). There were four observations per data point, and the SEM across time was .26 and .14 for L(+)- and D(-)-lactate, respectively. Serum L(+)-lactate concentrations did not differ ($P > .10$) at 0 and 12 h. At 3, 6, and 9 h, serum L(+)-lactate concentration increased linearly ($P < .08$) with increasing glucose infusion. Serum D(-)-lactate concentration did not differ through 3 h. Serum D(-)-lactate increased linearly ($P < .01$) at 6 and 9 h. At 12 h, serum D(-)-lactate increased quadratically ($P < .06$) with increasing glucose infusion.

1), but it increased linearly at 6 and 9 h ($P < .10$) and quadratically at 12 h ($P < .06$).

Cao et al. (1987) reported that plasma total lactate concentration was increased from 1.78 to 2.72 mM at 12 h by dosing mature goats with 16 g of sucrose/kg of BW. Patra et al. (1993) indicated that whole blood total lactate concentration increased from 1.29 to 4.26 mM by 12 h after consumption of whole wheat. Krehbiel et al. (1995a) reported that plasma L(+)-lactate was not influenced in wethers adapted to a 50% concentrate

diet and ruminally dosed with up to 18 g of glucose/kg of BW; plasma L(+)-lactate was approximately .6 mM before the glucose challenge and peaked at approximately 1 mM by 4 to 8 h. Present trends within time for D(-)-lactate are in agreement with those of Krehbiel et al. (1995a). In their study, plasma D(-)-lactate increased linearly at 4, 8, and 12 h, whereas D(-)-lactate concentrations across time for lambs infused with 0 or 6 g/kg of BW were less than .25 mM. Lambs given 12 g of glucose/kg of BW had approximately .4, 1.2, and 3.0 mM D(-)-lactate at 4, 8, and 12 h, respectively. In contrast, D(-)-lactate of ewes given 10 g/kg of BW in the present experiment peaked at 6 h and maintained this plateau through 12 h (Figure 1).

Dry Matter Intake. No interaction for DMI was observed ($P = .39$) between glucose infusion level and time. Average DMI 5 d after glucose infusion decreased linearly ($P < .05$) with increasing glucose infusion (1.39, 1.34, and $.72 \pm .20$ kg/d, respectively). A numerical trend ($P > .10$) for increasing daily DMI variation (.10, .25, and $.36$ kg², respectively) was noted with increasing glucose infusion. Stock et al. (1995) defined daily DMI variation as the variation in daily DMI residuals for each day among animals within the same treatment. Earlier work by Fulton et al. (1979) indicated that subacute acidosis was characterized by decreased and erratic DMI by individually fed steers. Decreased DMI followed by a decrease in gain and gain efficiency of pen-fed finishing cattle has been attributed to subacute acidosis (Stock et al., 1990). Krehbiel et al. (1995b) reported that replacing dry-rolled corn with 35, 86.5, or 94.5% wet corn gluten feed in starter diets fed to steers decreased daily DMI variation and, thereby, the incidence of subacute acidosis during adaptation (24 d) to a finishing diet.

Urinary pH and Nitrogen Composition. Urinary pH of ewes responded similarly across time regardless of treatment (treatment \times time, $P = .80$) and was not influenced ($P = .34$) by glucose infusion (6.96, 7.60, and $7.15 \pm .30$). Patra et al. (1993) observed a decrease in urinary pH from 7.95 to 6.84 by 12 h after administering 90 g of wheat/kg of BW, with the nadir in urinary pH at 48 h (6.05). Cao et al. (1987) reported that urinary pH was unchanged through 12 h (7.7, 7.9, 8.1, and $7.1 \pm .8$ at 0, 4, 8, and 12 h, respectively) in female goats given 16 g of sucrose/kg of BW, and reaching a nadir of 5.5 at 24 h. Urinary pH at 0 h in the present experiment was 7.92, 7.89, and 7.73 for animals given 0, 5, and 10 g of glucose/kg of BW, respectively. The lack of a decrease in urinary pH with increasing glucose infusion in the present experiment may have resulted from one control animal responding atypically. The urinary pH of this ewe reached a low point similar to that of animals given 10 g of glucose/kg of BW (pH 5.34). One possible explanation for this atypical response may be overconsumption of feed during the 30-min access period on the morning of the glucose challenge before individual DMI monitoring had begun.

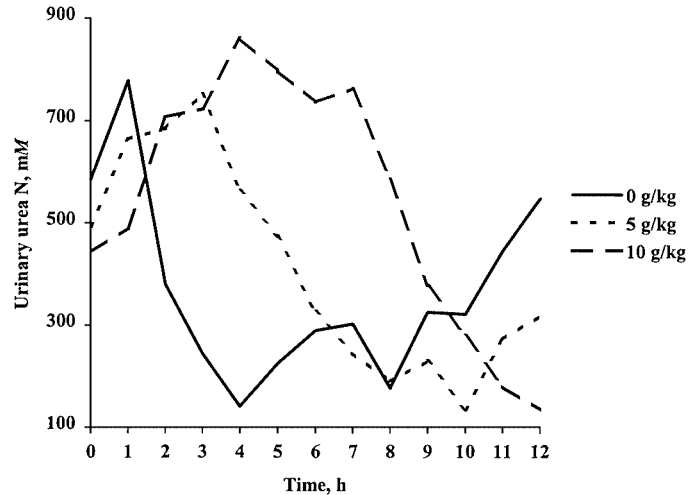


Figure 2. Effect of ruminal glucose infusion on urinary urea nitrogen concentration. The treatment \times time interaction was significant ($P < .001$). Across time, the SEM = 164, with four observations per data point. Urinary urea nitrogen concentration increased quadratically ($P < .05$) at 3 h, linearly ($P < .04$) at 4, 5, and 6 h, and quadratically ($P < .05$) at 7 h. At 11 and 12 h, urinary urea nitrogen concentration decreased linearly ($P < .04$) with increasing glucose infusion.

Urinary NH_4^+ responded similarly across time ($P = .21$) and was not influenced ($P = .17$) by infusing 0, 5, or 10 g of glucose/kg of BW (12.3, 9.1, and 22.5 ± 4.8 mEq/L, respectively). There was a general trend for UUN to peak later and subsequently decrease with increasing glucose infusion (Figure 2), as evidenced by a treatment \times time interaction ($P < .05$). At 3 h, UUN increased quadratically ($P < .05$) such that control animals had lower UUN than those given 5 or 10 g of glucose/kg of BW. Increasing UUN in response to increasing glucose infusion became linear ($P = .04$) at 4, 5, and 6 h. At 7 h, UUN increased quadratically ($P < .05$), and ewes given 0 or 5 g of glucose/kg of BW had a lower concentration than those given 10 g/kg of BW. At 11 and 12 h, increasing glucose infusion resulted in a linear decrease ($P = .04$) in UUN.

Alterations in urinary N composition during acute or subacute metabolic acidosis seem to depend on the compound used for induction and/or resulting type of metabolic acidosis. Subacute hyperchloremic acidosis from supplementing HCl or NH_4Cl has resulted in increased renal extraction of glutamine by rats (Welbourne et al., 1986) and sheep (Heitmann and Bergman, 1978) and increased urinary NH_4^+ excretion in rats (Parry and Brosnan, 1978; Welbourne et al., 1986; Boon et al., 1996) and dairy cows (Wang and Beede, 1992). Inducing subacute acidosis in rats by feeding HCl has markedly decreased urea excretion, whereas feeding NH_4Cl has only slightly decreased urea excretion (Welbourne et al., 1986; Boon et al., 1996). Hepatic ureagenesis in rats seems to be decreased regardless of the

compound used for induction of acidosis, and evidence suggests that this is a result of inhibited amino acid transport primary to the ornithine cycle (Boon et al., 1996). Renal ureagenesis presumably accounts for the increase in urea excretion with NH_4Cl - vs HCl -induced acidosis (Boon et al., 1996).

Little is known of the effect of organic acid load on urinary N composition. Telle and Preston (1971) induced acute acidosis in ewes by dosing with lactate (.225% of BW) 3 h after feeding a high-concentrate diet. Urinary NH_4^+ increased from approximately 8 mEq/L before infusion to 66 mEq/L by 10 h after infusion, whereas UUN was approximately 643, 500, 661, 714, 821, and 786 mM at 0, 2, 4, 6, 8, and 10 h, respectively. Lemieux et al. (1986) reported that acute lactic acidosis in dogs (arterial pH 7.09) increased urinary NH_4^+ excretion from 6 to 16 mEq/min during a 1-h infusion of lactate.

Serum Metabolites. Hematocrit was not measured in the present experiment. Therefore, the extent to which concentrations of serum constituents were influenced by decreased blood volume is uncertain. Cao et al. (1987) reported packed cell volumes of 29, 27, 26, and $29 \pm 5\%$ at 0, 4, 8, and 12 h, respectively, after dosing goats with 16 g of sucrose/kg of BW. Similarly, Krehbiel et al. (1995a) observed that ruminal glucose infusion at 0, 6, 12, or 18 g/kg of BW did not influence packed cell volume. Notwithstanding, our interest was that potential markers reflect or include possible changes in sampling compartment volume resulting from an organic acid insult.

Serum metabolites that responded to glucose infusion level with a treatment \times time interaction ($P < .10$) included glucose, urea N, creatinine, Na, Cl, K, P, anion gap, lactate dehydrogenase (**LDH**), glutamate-oxaloacetate transaminase (**GOT**) or aspartate transaminase, and glutamate-pyruvate transaminase (**GPT**) or alanine transaminase. Therefore, data for these constituents were analyzed within times after infusion.

Serum glucose increased linearly ($P = .06$) at 3 (79, 100, and 195 ± 22 mg/dL) and 6 h (79, 87, and 115 ± 12 mg/dL) and was numerically lower for the 10g/kg of BW treatment at 9 h (80, 90, and 68 ± 9 mg/dL). Serum urea N was unchanged ($P = .26$) with increasing glucose infusion. Creatinine concentration was not different through 6 h but increased linearly ($P = .003$) with glucose infusion level at 9 h (.93, .95, and $1.13 \pm .04$ mg/dL). At 12 h, ewes given 0 and 5 g of glucose/kg of BW had lower creatinine (1.03 and $.98 \pm .06$ mg/dL, respectively) than those given 10 g/kg of BW ($1.35 \pm .06$ mg/dL), resulting in a quadratic ($P = .02$) response. Cao et al. (1987) reported that plasma urea N concentration of sucrose-loaded goats decreased from 7.3 to 4.2 mM by 24 h, whereas plasma creatinine was unchanged (70 and 68 ± 10 μM at 0 and 24 h, respectively).

Serum Na (mEq/L) increased linearly ($P = .005$) at 3 and 9 h with increasing glucose infusion (Table 2). Serum Cl increased linearly ($P < .05$) at 3 h then decreased quadratically ($P = .09$) at 12 h such that animals treated

Table 2. Effect of ruminal glucose infusion (0, 5, or 10 g/kg of BW) on serum metabolites^a

Item	Time, h																			
	3				6				9				12							
	0	5	10	SE ^b	C ^c	0	5	10	SE ^b	C ^c	0	5	10	SE ^b	C ^c					
Sodium, mEq/L	145	151	153	1	L	147	153	152	4	—	145	153	152	1	L	146	146	148	2	—
Chloride, mEq/L	109	111	114	1	L	111	113	112	2	—	109	110	110	1	—	110	111	105	1	Q
Anion gap, mEq/L	13	18	18	1	Q	14	15	21	1	Q	14	16	23	1	Q	15	14	21	2	Q
Potassium, mEq/L ^d	4.6	3.8	4.1	.1	Q	4.8	4.1	3.6	.1	L	4.4	4.4	3.6	.2	Q	4.9	4.7	4.2	.2	L
Phosphorus, mEq/L	3.1	3.9	4.7	.4	L	3.1	3.7	4.3	.6	—	2.9	3.3	5.3	.6	L	2.8	2.8	6.3	.6	Q
LDH, Units/dL ^{de}	74	63	111	9	Q	80	68	129	12	Q	76	68	123	10	Q	81	65	106	12	Q
GOT, Units/L ^e	209	164	237	44	—	222	170	267	47	—	214	163	285	42	—	226	159	274	45	—
GPT, Units/L ^e	20	13	23	5	—	24	14	27	6	—	22	15	28	5	—	26	15	27	6	—

^aTreatment \times time ($P < .05$).

^bStandard error of the least squares mean, n = 4.

^cContrasts: L = linear effect ($P < .10$); Q = quadratic effect ($P < .10$).

^dThe 0-h value was used as a covariate.

^eLDH = lactate dehydrogenase; GOT = glutamate-oxaloacetate transaminase; GPT = glutamate-pyruvate transaminase.

with 10 g of glucose/kg of BW had lower Cl than those in the remaining treatments. Serum K decreased quadratically ($P = .04$) at 3 and 9 h and linearly ($P < .05$) with increasing glucose level at 6 and 12 h. Serum P increased linearly ($P = .02$) at 3 and 9 h and quadratically ($P = .06$) at 12 h. Anion gap increased quadratically ($P = .09$) at 3 through 12 h with increasing glucose infusion.

Patra et al. (1993) reported that serum Na increased from 141 mEq/L before offering feed-deprived ewes 90 g of wheat/kg of BW to 153 mEq/L at 12 h, whereas Cl was unchanged over time (103 and 106 mEq/L at 0 and 12 h, respectively). Irwin et al. (1979) induced acute acidosis in sheep by glucose infusion (dose not reported) and indicated that plasma Na increased slightly (5 mEq/L) by 14 h. Cao et al. (1987) observed plasma Na of 141, 151, 152, and 148 ± 3.5 mEq/L at 0, 4, 8, 12, and 24 h, respectively. Plasma Cl increased numerically after dosing (105, 108, 110, and 111 ± 4 mEq/L at 0, 4, 8, and 12 h, respectively). Serum K has generally been shown to decrease, whereas serum P is generally increased (Oster et al., 1978; Perez et al., 1980; Patra et al., 1993). Cao et al. (1987) reported that plasma K and P were unchanged by sucrose dosing.

Our serum Na, K, and P results generally agree with previous observations. Serum Na seems to be increased, whereas plasma K seems to be either unchanged or decreased in response to systemic lactic acidosis. However, a prompt rise (by 3 h) in plasma K concentration occurs with mineral acid-induced acute metabolic acidosis in dogs and sheep (Oster et al., 1978; Wood and Isa, 1991, respectively), presumably resulting from a shift of K from the intracellular to the extracellular compartment (Oster et al., 1978). In contrast, increased plasma P concentration seems reflective of lactic acidosis, whereas P concentrations are either unchanged or decreased by mineral acid acidosis (Oster et al., 1978). Hyperphosphatemia associated with lactic acidosis may arise from degradation of intracellular organic phosphate compounds followed by an extracellular flux of inorganic phosphate (Oster et al., 1978) and/or mobilization of skeletal stores through bone resorption (Bus-hinsky, 1989).

Anion gap is calculated as the difference between the sum of Na and K and the sum of Cl and bicarbonate (Carlson, 1997). Moreover, an increasing anion gap is indicative of metabolic acidosis caused by organic acids (Carlson, 1997). Present results indicate that the anion gap was greater for ewes given 5 or 10 g of glucose/kg of BW than for control ewes at 3 h (quadratic, $P = .06$). The quadratic trend continued at 6, 9, and 12 h; however, ewes given 0 or 5 g/kg of BW had a smaller anion gap than those given 10 g/kg of BW at these times. The general trend of serum D(-)-lactate (Figure 1) seemed consistent with that of the anion gap.

Serum GOT and GPT were not affected ($P > .10$) by increasing ruminal glucose infusion (Table 2). In contrast, LDH increased quadratically ($P < .10$) at 3 through 12 h. The trend was consistent at 3, 6, 9, and

12 h, in that animals given 10 g of glucose/kg of BW had higher LDH than those infused with 0 or 5 g/kg of BW. It should be noted that one control animal exhibited enzyme activities comparable to those of ewes given 10 g of glucose/kg of BW, which is reflected by the large standard errors for LDH and GOT. Omitting data from this animal decreased observed means of control animals for GOT (160, 156, 160, 158, and 157 U/L), GPT (14, 12, 14, 13, and 16 U/L), and LDH (584, 585, 607, 600, and 598 U/L) at 0, 3, 6, 9, and 12 h, respectively. Because urine pH and serum insulin were unchanged over time for this animal, we suspect that this ewe had experienced a recent episode of metabolic acidosis or some other condition that could increase these enzymes some time before the glucose challenge.

Present GOT results generally agree with those of Cao et al. (1987), who reported that plasma GOT was 31, 34, 35, and 31 ± 18 U/L at 0, 4, 8, and 12 h in sucrose-loaded goats. Actual values in the present experiment were approximately fivefold higher, which may be explained partly by the dramatically different diets (lucerne chaff vs 90% concentrate) fed before dosing (Mullen, 1973). Lal et al. (1991) reported that serum GOT increased from 35 to 56 ± 4 U/L by 12 h in goats deprived of feed for 24 h and ruminally dosed with 100 g of whole wheat/kg of BW. Lal et al. (1991) further indicated that LDH increased from 139 to 383 ± 32 U/L by 12 h. Increased concentration of serum GOT is considered a more specific indicator of hepatic damage than GPT, whereas muscle, liver, and red blood cells are the major sources of LDH (Turk and Casteel, 1997). Present results suggest muscle as the origin of LDH, because GOT, GPT, and total bilirubin (see below) were not affected ($P > .10$) by glucose infusion.

Concentrations of triacylglyceride, cholesterol, total bilirubin, and alkaline phosphatase were not altered ($P > .10$) by glucose infusion (data not shown). Serum Ca, albumin, globulin, and total protein responded similarly among treatments across time ($P > .10$). Serum Ca increased linearly ($P = .09$; 4.9, 4.9, and $5.2 \pm .1$ mEq/L) over 12 h with increasing glucose infusion. Irwin et al. (1979) reported that plasma Ca increased by 14 h, whereas Huntington and Britton (1979) found increased serum Ca of cattle 30 h after being abruptly switched from a high-forage to a high-concentrate diet. In contrast, Patra et al. (1993) reported decreased serum Ca in wheat-challenged sheep. In our study, albumin (4.0, 4.0, and $4.4 \pm .1$ g/dL) and total protein (6.8, 6.9, and $7.5 \pm .2$ g/dL) increased linearly ($P = .08$), whereas globulin (2.9, 2.7, and $3.1 \pm .1$ g/dL) responded quadratically ($P = .06$) as a result of the glucose challenge. Cao et al. (1987) indicated that total plasma protein was not altered by sucrose dosing of goats.

Serum Hormone Profiles. Serum prolactin (29.6, 28.2, and 29.0 ± 8.1 ng/mL) was not influenced ($P > .10$) by an increasing glucose infusion. Serum insulin concentration (Figure 3) increased linearly at 3 h ($P < .01$) and peaked at 5 h (quadratic, $P < .01$). Area under the insulin curve over the 12-h period increased quadratically

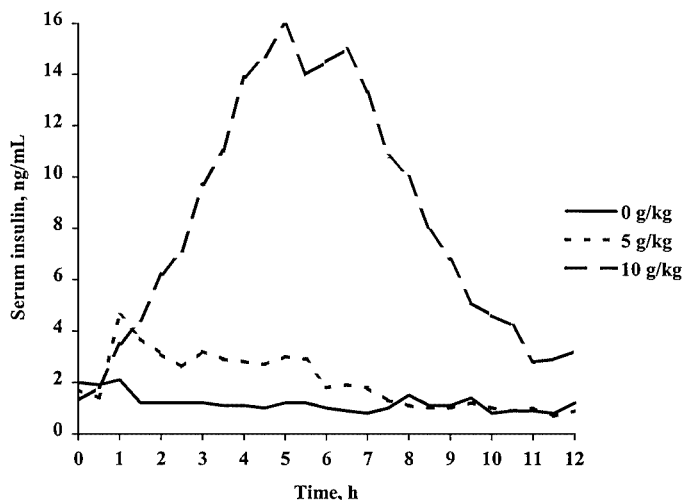


Figure 3. Effect of ruminal glucose infusion on serum insulin concentration. The treatment \times time interaction was significant ($P < .01$). Across time, the SEM = 1.5, with four observations per data point. Area under the curve ($14, 24,$ and 101 ± 6 units for 0, 5, and 10 g of glucose/kg of BW, respectively) increased quadratically ($P < .002$).

($P < .01$) with increasing glucose infusion ($14, 24,$ and 101 ± 6 units, respectively). Serum aldosterone did not differ with glucose infusion through 5 h. After 5 h, aldosterone generally increased quadratically ($P = .09$) with increasing glucose infusion (Figure 4), in agreement with our observed hypernatremia and hypokalemia with increasing glucose infusion. Serum aldosterone of one control ewe increased in a manner similar to that

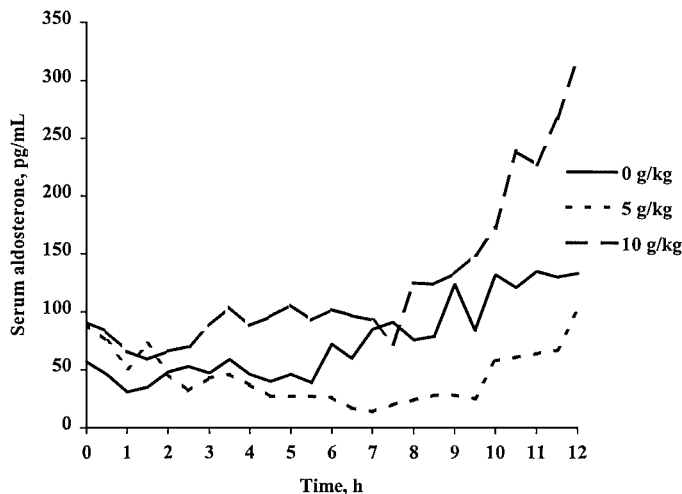


Figure 4. Effect of ruminal glucose infusion on serum aldosterone concentration. The treatment \times time interaction was significant ($P < .01$). Across time, the SEM = 45, with four observations per data point. Area under the curve ($885, 504,$ and $1,258 \pm 260$ units for 0, 5, and 10 g of glucose/kg of BW, respectively) increased quadratically ($P < .08$).

in ewes given 10 g of glucose/kg of BW. As mentioned previously, this same animal exhibited a decrease in urine pH similar to that of animals infused with 10 g of glucose/kg of BW.

Regardless of the type of acidosis, earlier studies have shown that metabolic acidosis is generally accompanied by increased plasma aldosterone concentration in dogs (Perez et al., 1980), humans (Perez et al., 1977), rats (Scandling and Ornt, 1987), and sheep (Cudd and Wood, 1994). The primary stimuli for aldosterone synthesis in adrenal glomerulosa cells are angiotensin II, ACTH, K, and Na (Funder, 1993). The physiological response of mammals is a decrease in urinary Na excretion and increased urinary K and H^+ excretion with inverse changes in plasma concentrations (Sharp and Leaf, 1966). As mentioned previously, mineral acids, but not lactate, seem to stimulate an extracellular shift of K. Moreover, NH_4Cl and NH_4^+ may increase aldosterone secretion independently (Muller, 1965; Blair-West et al., 1968).

Eiam-Ong et al. (1994) induced respiratory acidosis in rats subjected to hypercapnia. Following 6 h of exposure, blood pH decreased from 7.4 to 7.18 and plasma aldosterone concentration of acidotic rats was greater than that of controls (9.7 vs 3.3 pg/mL). Hypercapnia for 30 min in goats did not increase aldosterone concentration (Augustisson and Forslid, 1989). Hyperchloremic metabolic acidosis in goats induced by duodenal NH_4Cl infusion for 30 min increased plasma aldosterone from 80 to 270 pmol/L by the end of infusion, followed by a decrease, whereas blood pH decreased from 7.41 to 7.37 by 1.5 h after infusion (Augustisson and Forslid, 1989). Andersson et al. (1986) offered hay for 30 min to goats that had been deprived of feed for 12 h. Blood pH decreased from 7.38 to 7.32 by 30 min after feeding, whereas the mild acid load increased plasma aldosterone from 130 to 240 ± 25 pmol/L.

We speculated that circulating vasopressin concentrations might be increased in the present experiment as a result of potential blood osmolality changes (Robertson, 1987) and/or potential tissue hypoxia (Rose et al., 1984). However, serum vasopressin concentrations did not differ ($P = .29$) across time and were not influenced ($P = .53$) by increasing glucose infusion ($39.6, 29.6,$ and 30.8 ± 7.8 pg/mL). Increasing glucose infusion resulted in a linear decrease ($P = .08$) in serum GH ($1.8, 1.7,$ and $1.3 \pm .2$ ng/mL). This response may have been associated with the quadratic increase in insulin concentrations that we observed, which also has been noted by others (Bassett, 1974; Hornick et al., 1998).

Implications

Marked changes in serum metabolite, endocrine, and urine profiles accompany induction of metabolic acidosis in concentrate-adapted, mature ewes. Serum D(-)-lactate, lactate dehydrogenase, creatinine, Na, K, and P seem to exhibit the most utility as potential markers of metabolic acidosis under the conditions of the present

experiment, whereas urinary pH and NH_4^+ may be of limited use. Endocrine responses to glucose-induced metabolic acidosis seem to involve changes in aldosterone and insulin, but not vasopressin or prolactin. Further research is needed to determine the behavior of these potential markers in animals fed high-concentrate diets differing in rates of fermentation and to explore possible relationships with animal performance.

Literature Cited

- Andersson, B., H. Andersson, O. Augustsson, M. Forsgren, H. Holst, and H. Jonasson. 1986. Effects of hay-feeding on acid/base balance, renal sodium excretion, aldosterone and vasopressin secretion in the goat. *Acta. Physiol. Scand.* 126:9–14.
- AOAC. 1990. Official Methods of Analysis (15th Ed.) Association of Official Analytical Chemists, Arlington, VA.
- Augustsson, O., and A. Forslid. 1989. Aldosterone secretion during acute respiratory acidosis and NH_4Cl -induced metabolic acidosis in the goat. *Acta. Physiol. Scand.* 136:339–345.
- Basset, J. M. 1974. Diurnal patterns of plasma insulin, growth hormone, corticosteroid and metabolite concentrations in fed and fasted sheep. *Aust. J. Biol. Sci.* 27:167–181.
- Blair-West, J. R., J. P. Coghlan, D. A. Denton, J. R. Goding, M. Wintour, and R. D. Wright. 1968. The local action of ammonium, calcium and magnesium on adrenocortical secretion. *Aust. J. Exp. Biol. Med. Sci.* 46:371–374.
- Boon, L., P. E. Blommarrt, A. J. Meijer, W. H. Lamers, and A. C. Schoolwerth. 1996. Response of hepatic amino acid consumption to chronic metabolic acidosis. *Am. J. Physiol.* 271:F198–F202.
- Brandt, R. B., S. A. Siegel, M. G. Waters, and M. H. Bloch. 1980. Spectrophotometric assay for D(–)-lactate in plasma. *Anal. Biochem.* 102:39–46.
- Brent, B. E. 1976. Relationship of acidosis to other feedlot ailments. *J. Anim. Sci.* 43:930–935.
- Brink, D. R., S. R. Lowry, R. A. Stock, and J. C. Parrott. 1990. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. *J. Anim. Sci.* 68:1201–1207.
- Bushinsky, D. A. 1989. Internal exchanges of hydrogen ions: Bone. In: D. W. Seldin and G. Giebisch (Ed.) *The Regulation of Acid-Base Balance*. pp 69–88. Raven Press, New York.
- Cao, G. R., P. B. English, L. J. Filippich, and S. Inglis. 1987. Experimentally induced lactic acidosis in the goat. *Aust. Vet. J.* 64:367–370.
- Carlson, G. P. 1997. Fluid, electrolyte, and acid-base balance. In: J. J. Kaneko, J. W. Harvey, and M. L. Bruss (Ed.) *Clinical Biochemistry of Domestic Animals* (5th Ed.). pp 485–516. Academic Press, San Diego, CA.
- Chan, J.C.M. 1972. The rapid determination of urinary titratable acid and ammonium and evaluation of freezing as a method of preservation. *Clin. Biochem.* 5:94–98.
- Cudd, T. A., and C. E. Wood. 1994. Thromboxane A_2 receptor antagonism prevents hormone and cardiovascular responses to mineral acid infusion. *Am. J. Physiol.* 267:R1235–R1240.
- Eiam-Ong, S., M. E. Laski, N. A. Kurtzman, and S. Sabatini. 1994. Effect of respiratory acidosis and respiratory alkalosis on renal transport enzymes. *Am. J. Physiol.* 267:F390–F399.
- Elam, C. J. 1976. Acidosis in feedlot cattle: Practical observations. *J. Anim. Sci.* 43:898–901.
- Engel, P., and J. B. Jones. 1978. Causes and elimination of erratic blanks in enzymatic metabolite assays involving the use of NAD^+ in alkaline hydrazine buffers and improved conditions for the assay of L-glutamine, L-lactate, and other metabolites. *Anal. Biochem.* 88:475–484.
- Fulton, W. R., T. J. Klopfenstein, and R. A. Britton. 1979. Adaptation to high concentrate diets by beef cattle. I. Adaptation to corn and wheat diets. *J. Anim. Sci.* 49:775–784.
- Funder, J. W. 1993. Aldosterone action. *Ann. Rev. Physiol.* 55:115–130.
- Gawehn, K., and H. U. Bergmeyer. 1974. D(–)-lactate. In: H. Bergmeyer (Ed.) *Methods of Enzymatic Analysis*, Vol 3. Academic Press, New York.
- Gill, J. L., and H. D. Hafs. 1971. Analysis of repeated measurements of animals. *J. Anim. Sci.* 33:331–336.
- Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). *Agric. Handbook No. 379*. ARS, USDA, Washington, DC.
- Gutmann, I., and A. W. Wahlefeld. 1974. L(+)-lactate determination with lactate dehydrogenase and NAD. In: H. Bergmeyer (Ed.) *Methods of Enzymatic Analysis*, Vol. 3. Academic Press, New York.
- Heitmann, R. N., and E. N. Bergman. 1978. Glutamine metabolism, interorgan transport and acidosis in sheep. *Am. J. Physiol.* 234:E197–E203.
- Hoefer, W. C., and D. M. Hallford. 1987. Influence of suckling status and type of birth on serum hormone profiles and return to estrus in early-postpartum spring-lambing ewes. *Theriogenology* 27:887–895.
- Hornick, J. L., C. Van Eenaeme, M. Diez, V. Minet, and L. Istasse. 1998. Different periods of feed restriction before compensatory growth in Belgian Blue bulls: II. Plasma metabolites and hormones. *J. Anim. Sci.* 76:260–271.
- Huber, T. L. 1976. Physiological effects of acidosis on feedlot cattle. *J. Anim. Sci.* 43:902–909.
- Huntington, G. B., and R. A. Britton. 1979. Effect of dietary lactic acid on rumen lactate metabolism and blood acid-base status of lambs switched from low to high concentrate diets. *J. Anim. Sci.* 49:1569–1576.
- Irwin, L. N., G. E. Mitchell, Jr., R. E. Tucker, and G. T. Schelling. 1979. Histamine, tyramine, tryptamine and electrolytes during glucose induced lactic acidosis. *J. Anim. Sci.* 48:367–374.
- Krehbiel, C. R., R. A. Britton, D. L. Harmon, T. J. Wester, and R. A. Stock. 1995a. The effects of ruminal acidosis on volatile fatty acid absorption and plasma activities of pancreatic enzymes in lambs. *J. Anim. Sci.* 73:3111–3121.
- Krehbiel, C. R., R. A. Stock, D. W. Herold, D. H. Shain, G. A. Ham, and J. E. Carulla. 1995b. Feeding wet corn gluten feed to reduce subacute acidosis in cattle. *J. Anim. Sci.* 73:2931–2939.
- Lal, S. B., D. Swarup, S. K. Dwivedi, and M. C. Sharma. 1991. Biochemical alterations in serum and cerebrospinal fluid in experimental acidosis in goats. *Res. Vet. Sci.* 50:208–210.
- Lemieux, G., E. Junco, R. Perez, E. Allignet, C. Lemieux, M. R. Aranda, and F. V. Quintana. 1986. Renal metabolism during four types of lactic acidosis in the dog including anoxia. *Can. J. Physiol. Pharmacol.* 64:169–175.
- Montaño, M. F., W. Chai, T. E. Zinn-Ware, and R. A. Zinn. 1999. Influence of malic acid supplementation on ruminal pH, lactic acid utilization, and digestive function in steers fed high-concentrate finishing diets. *J. Anim. Sci.* 77:780–784.
- Mullen, P. A. 1973. Barley beef: Serum enzyme activity during barley feeding, and particularly during the introduction of the ration. *Br. Vet. J.* 129:439–447.
- Muller, J. 1965. Aldosterone stimulation in vitro. II. Stimulation of aldosterone production by monovalent cations. *Acta Endocrinol.* 50:301–309.
- Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: A review. *J. Anim. Sci.* 76:287–298.
- Oster, J. R., G. O. Perez, and C. A. Vaamonde. 1978. Relationship between blood pH and potassium and phosphorus during acute metabolic acidosis. *Am. J. Physiol.* 235:F345–F351.
- Owens, F. N., D. Secrist, J. Hill, and D. Gill. 1996. A new look at acidosis. In: *Proc. Southwest Nutrition and Management Conf.* pp 1–16. Univ. of Arizona, Tucson.
- Parry, D. M., and J. T. Brosnan. 1978. Glutamine metabolism in the kidney during induction of, and recovery from, metabolic acidosis in the rat. *Biochem. J.* 174:387–396.
- Patra, R. C., S. B. Lal, and D. Swarup. 1993. Physiochemical alterations in blood, cerebrospinal fluid, and urine in experimental lactic acidosis in sheep. *Res. Vet. Sci.* 54:217–220.

- Perez, G. O., D. C. Kem, J. R. Oster, and C. A. Vaamonde. 1980. Effect of acute metabolic acidosis on the renin-aldosterone system. *J. Lab. Clin. Med.* 96:371-378.
- Perez, G. O., J. R. Oster, C. A. Vaamonde, and F. H. Katz. 1977. Effect of NH_4Cl on plasma aldosterone, cortisol, and renin activity in supine man. *J. Clin. Endocrinol. & Metab.* 45:762-767.
- Robertson, G. L. 1987. Physiology of ADH secretion. *Kidney Int. Suppl.* 21:S20-S26.
- Rose, C. E., Jr., R. J. Anderson, and R. M. Carey. 1984. Antidiuresis and vasopressin release with hypoxemia and hypercapnia in conscious dogs. *Am. J. Physiol.* 247:R127-R134.
- SAS. 1985. SAS User's Guide: Statistics (Version 5 Ed.). SAS Inst., Inc., Cary, NC.
- Scandling, J. D., and D. B. Ornt. 1987. Mechanism of potassium depletion during chronic metabolic acidosis in the rat. *Am. J. Physiol.* 252:F122-F130.
- Senn, M., P. M. Maier, and W. Langhans. 1995. ACTH, cortisol, and glucose responses after administration of vasopressin in cattle and sheep. *J. Comp. Physiol. B.* 164:570-578.
- Sharp, G.W.G., and A. Leaf. 1966. Mechanisms of action of aldosterone. *Physiol. Rev.* 46:593-633.
- Slyter, L. L. 1976. Influence of acidosis on rumen function. *J. Anim. Sci.* 43:910-929.
- Spoon, R. A., and D. M. Hallford. 1989. Growth response, endocrine profiles, and reproductive performance of fine-wool ewe lambs treated with ovine prolactin before breeding. *Theriogenology* 32:45-53.
- Stock, R., T. Klopfenstein, and D. Shain. 1995. Feed intake variation. In: F. N. Owens (Ed.) *Symp. Proc.: Feed Intake by Beef Cattle*. Okla. Agric. Exp. Sta. MP-942:56-59. Oklahoma State University, Stillwater.
- Stock, R. A., M. H. Sindt, J. C. Parrott, and F. K. Goedeken. 1990. Effects of grain type, roughage level and monensin level on finishing cattle performance. *J. Anim. Sci.* 68:3441-3455.
- Telle, P. P., and R. L. Preston. 1971. Ovine lactic acidosis: Intraruminal and systemic. *J. Anim. Sci.* 33:698-705.
- Turk, J. R., and S. W. Casteel. 1997. Clinical biochemistry in toxicology. In: J. J. Kaneko, J. W. Harvey, and M. L. Bruss (Ed.) *Clinical Biochemistry of Domestic Animals* (5th Ed.). pp 829-843. Academic Press, San Diego, CA.
- Wang, C., and D. K. Beede. 1992. Effects of ammonium chloride and sulfate on acid-base status and calcium metabolism of dry Jersey cows. *J. Dairy Sci.* 75:820-828.
- Welbourne, T. C., D. Childress, and G. Givens. 1986. Renal regulation of interorgan glutamine flow in metabolic acidosis. *Am. J. Physiol.* 251:R858-R866.
- Wood, C. E., and A. Isa. 1991. Intravenous acid infusion stimulates ACTH secretion in sheep. *Am. J. Physiol.* 260:E154-161.
- Xiong, Y., S. J. Bartle, and R. L. Preston. 1990. Improved enzymatic method to measure processing effects and starch availability in sorghum grain. *J. Anim. Sci.* 68:3861-3870.