Effect of ammonium chloride supplementation on urine pH and urinary fractional excretion of electrolytes in goats

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Objective—To determine whether dietary supplementation with ammonium chloride would affect urine pH or urinary fractional excretion (FE) of electrolytes in goats fed grass hay.

Design—Clinical trial.

Animals—15 yearling castrated male goats.

Procedures—In the dose response study, 3 yearling goats fed orchard grass hay and water ad libitum were administered ammonium chloride at either 200, 400, or 500 mg/kg (91, 182, or 227 mg/lb), PO, every 24 hours. In the FE study, 8 goats fed orchard grass hay were randomly divided into either a treatment (n = 4) or a control group (4). In the treatment group, ammonium chloride was administered at 450 mg/kg (2.25% of dry matter intake [DMI]), PO, every 24 hours for 8 days. The FE of electrolytes was compared between groups; FE measurements were also determined for 4 client-owned goats fed alfalfa hay.

Results—Ammonium chloride administered at 450 mg/kg (2.25% of DMI) achieved and maintained urine pH < 6.5 for 24 hours. Goats fed orchard grass hay with ammonium chloride supplementation had significantly higher FE of calcium and chloride than did goats fed orchard grass hay without supplemental ammonium chloride.

Conclusions and Clinical Relevance—Dietary ammonium chloride supplementation at a dose of 450 mg/kg may be necessary to achieve a urine pH < 6.5 in goats. Further studies of ammonium chloride supplementation and urolithiasis in goats fed low-calcium diets are indicated. (J Am Vet Med Assoc 2010;237:1299–1304)

Obstructive urolithiasis in domestic animals has been widely documented.1–3 Male goats are at increased risk for this condition, compared with female goats, because of the length and curvature of the urethra and the presence of a urethral process with a narrow diameter in which calculi can become lodged.2,4 Whereas struvite and apatite uroliths are reportedly more common in other regions of North America,5 it has been our clinical experience that male goats admitted to the University of California-Davis Veterinary Medical Teaching Hospital because of obstructive urolithiasis most commonly have calcium carbonate uroliths.

Treatment options for goats with obstructive urolithiasis include medical management, surgery, or both, but medical management alone is often unrewarding.5 After urethral process amputation, smooth muscle relaxants can be administered,5 and recently, the use of drugs that can dissolve some types of urinary calculi following percutaneous placement of Foley catheters into the bladder has been reported.6–8 For male small ruminants, tube cystostomy appears to be the most satisfactory surgical treat-
ammonium chloride is unpalatable,17 with the result that owner compliance with daily ammonium chloride administration is often suboptimal.18 Also, changes in bone morphology and subsequent osteopenia associated with dietary-induced metabolic acidosis in sheep have been reported.19-21 Finally, ammonium chloride supplementation has been associated with increased urine calcium content in cats22 and dogs,23,24 and increased urine calcium content has been shown to be a risk factor for the formation of calcium oxalate uroliths in dogs25 and calcium phosphate nephroliths in rats.26 To our knowledge, no studies have examined urinary FE of electrolytes in goats fed grass hay with or without supplemental ammonium chloride. If dietary supplementation with ammonium chloride increases urinary calcium excretion, even in goats fed diets that are relatively low in calcium, to such an extent that it increases the risk of calcium carbonate urolithiasis, then the common recommendation to feed grass hay and supplemental ammonium chloride, although seemingly appropriate when considering urine pH alone, may in fact be inappropriate for decreasing the risk of urolithiasis. The purpose of the study reported here was to determine whether dietary supplementation with ammonium chloride would affect urine pH or urinary FE of electrolytes in goats fed grass hay. The specific objectives were to determine the dosage of ammonium chloride that would result in a urine pH < 6.5 for 24 hours in goats fed orchard grass (Dactylis glomerata L.) hay, to determine the effects of dietary ammonium chloride supplementation in male goats fed orchard grass hay on urinary FE of electrolytes, and to compare urinary FE of electrolytes between goats fed an orchard grass hay diet with or without supplemental ammonium chloride and goats fed an alfalfa hay diet.

Materials and Methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of California-Davis.

Dose response study—To determine the dosage of ammonium chloride that would result in a urine pH < 6.5 for 24 hours in goats fed orchard grass hay, a dose-response study was performed with 3 yearling castrated male Boer goats fed orchard grass hay and water ad libitum. Goats were administered ammonium chloride at a dosage of 200, 400, or 500 mg/kg (91, 182, or 227 mg/lb), PO, every 24 hours (1 goat/dosage), and urine pH was measured. The doses of ammonium chloride corresponded to 1%, 2%, and 2.5% of DMI (defined as 2% of body weight), respectively. Number of times individual goats were given ammonium chloride at each dosage varied depending on whether the target urine pH (< 6.5 for 24 hours) was achieved. Prior to the first administration of ammonium chloride and then at least every 4 hours until 72 hours after the first dose, urine pH was measured with 3 color indicator test strips that determined pH values from 5 through 10 in 0.5-pH unit increments. For the 200 mg/kg dosage, a total of 3 doses of ammonium chloride were administered; for both the 400 and 500 mg/kg dosages, a total of 2 doses were administered.

FE study—To determine the effects of dietary ammonium chloride supplementation on urinary FE of electrolytes, FE data were collected from 8 healthy castrated male Boer goats weighing between 16.5 and 25.5 kg (36 and 56 lb). Goats were randomly assigned to a treatment (n = 4) or control (4) group. Randomization was accomplished by assigning goats to a treatment or control group by order of body weight from the highest to the lowest. The heaviest goat was assigned to treatment or control by coin toss. The second heaviest goat was thus assigned to the opposite group and so on until all 8 goats were assigned to a group. Throughout the study, goats were housed together in their groups in 2 adjacent stalls and offered orchard grass hay and water ad libitum.

Goat were anesthetized and positioned in dorsal recumbency, and a Foley catheter was surgically placed into the bladder as described.7 Preoperative medications consisted of flunixin meglumine (1 mg/kg [0.45 mg/lb], IV) and ceftiofur sodium (2 mg/kg [0.91 mg/lb], SC). For anesthetic induction, goats were administered diazepam (0.25 mg/kg [0.11 mg/lb], IV) followed by ketamine hydrochloride (4 mg/kg [1.8 mg/lb], IV). Goats were intubated, and anesthesia was maintained with isoflurane delivered in oxygen. Postoperatively, a transdermal fentanyl patch (50 µg/h) was applied to the skin and secured with an adhesive elastic bandage.1 Fentanyl patches were removed 3 days after surgery. Goats received ceftiofur sodium (2 mg/kg, SC, q 24 h) while Foley catheters were in place. Goats were monitored for the development of complications with twice-daily determinations of rectal temperature, respiratory rate, and heart rate and twice-daily assessments of appetite, water intake, character and passage of fecal material, signs of pain associated with the surgical site, and patency of the Foley catheters.

Four days after placement of the Foley catheters (day 1), a urine sample (minimum, 5 mL) was collected from the Foley catheter and a venous blood sample was collected by means of jugular venipuncture from each goat for determination of baseline values. Samples were placed in serum separator tubes, and urine pH was determined immediately with a pH test strip. Blood samples were allowed to clot for 30 minutes, and serum was harvested by means of centrifugation at 1,877 × g for 10 minutes. Urine and serum samples were analyzed for sodium, potassium, chloride, calcium, phosphorus, magnesium, and creatinine concentrations with a commercial chemistry analyzer.6 Sodium, potassium, chloride, phosphorus, and magnesium concentrations were determined by use of an ion-selective electrode. Calcium concentrations were determined by use of a colorimetric assay with an endpoint determination method. Creatinine concentrations were measured with a kinetic colorimetric assay method.

On days 1 through 8, goats in the treatment group were treated with ammonium chloride (450 mg/kg [205 mg/lb], PO, q 24 h) corresponding to 2.25% of DMI. Each dose of ammonium chloride was mixed with 10 mL of corn syrup as a vehicle. Goats in the control group received 10 mL of the vehicle only. Twelve hours after each dose of ammonium chloride was administered, urine and serum samples were obtained from each goat and tested for sodium, potassium, chloride, calcium, phosphorus, magnesium, and creatinine concentrations. In addition, a urine sample was collected from each goat just prior to administration of the next dose of ammonium chloride and tested for urine pH.
Fractional excretion of electrolytes in urine was calculated by use of the following formula:

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FE(x) = \frac{\text{Urine}[x]}{\text{Serum}[x]} \times \frac{\text{Serum}[\text{Cr}]}{\text{Urine}[\text{Cr}]} \times 100
\]

where \(FE(x)\) represents FE of electrolyte \(x\), \([x]\) represents concentration of the electrolyte, and \([\text{Cr}]\) represents concentration of creatinine. Fractional excretion was expressed as a percentage.

Urine specific gravity was determined on days 7, 8, and 9 by use of a handheld refractometer. On days 8 and 9, samples of urine sediment were obtained by means of centrifugation of 5 mL of urine at 252 × g for 6 minutes. The sediment was analyzed qualitatively by means of phase-contrast microscopy at 400× magnification for type and presence of crystals. Number of crystals was qualitatively graded as none, rare, few, moderate, or many, according to established criteria. In brief, a grade of none was given if crystals were not seen in any microscopic field, a grade of rare was given if crystals were seen in some but not all microscopic fields, a grade of few was given if approximately 25% of each microscopic field was occupied by crystals, a grade of moderate was given if approximately 50% of each microscopic field was occupied by crystals, and a grade of many was given if approximately 100% of each microscopic field was occupied by crystals.

**Effect of diet on FE**—To determine the effects of feeding a diet containing a high calcium content without ammonium chloride supplementation on urinary FE of electrolytes, urine and blood samples were collected from 4 privately owned yearling castrated male Boer goats that were being maintained on an alfalfa hay diet with no supplementation. Urine and blood samples were obtained from these goats in their usual farm environment, after client consent was obtained. Free-catch urine samples were collected; venous blood samples were collected by means of jugular venipuncture immediately after urine collection. Urine and blood samples were stored in a refrigerated cooler until sample processing at the veterinary medical teaching hospital as described.

**Statistical analysis**—Analysis of variance for repeated measures was performed to identify differences between treatment groups at each time and within treatment groups over time. Days of the study (9 levels) were included in the analysis as the within-subjects factor, whereas group membership (treatment or control) was included as the between-subjects factor. The Tukey honestly significantly different test was used to determine mean differences for FE of calcium between days for the treatment group. A t test for independent means was used to compare FE data for client-owned animals fed alfalfa hay with data for the 2 experimental groups. Values of \(P < 0.05\) were considered significant. All statistical analyses were performed with a commercial software program.

**Results**

Throughout the study, ammonium chloride was poorly tolerated by goats in the treatment group and administration by mouth was difficult. In the dose response study, administration of ammonium chloride at a dosage of 200 mg/kg, PO, every 24 hours did not result in a urine pH < 6.5 in any of the goats. Administration at a dosage of 400 mg/kg, PO, every 24 hours resulted in a urine pH < 6.5 after administration of the second dose; however, a pH < 6.5 was not maintained during the succeeding 24 hours. Administration of ammonium chloride at a dosage of 500 mg/kg, PO, every 24 hours resulted in a urine pH < 6.5 that was maintained for ≥ 24 hours after administration of the second dose (Figure 1). On the basis of these results, an ammonium chloride dose of 450 mg/kg was chosen for the FE study.

In the FE study, 1 goat in the control group developed surgical complications and was excluded from data analyses. Baseline urine pH for the remaining goats was ≥ 7.5 (range, 7.5 to 8.5). Twelve hours after administration of the first dose of ammonium chloride at a dosage of 450 mg/kg, PO, every 24 hours, mean urine pH for goats in the treatment group was significantly (\(P < 0.05\)) decreased, compared with mean baseline urine pH, and mean urine pH for goats in the treatment group was < 6.25 until 48 hours after the last dose of ammonium chloride was given (Figure 2). In contrast, mean urine pH for control group goats ranged from 8.00 to 8.50 throughout the study period.
Mean FE of calcium on days 2 through 9 was significantly greater in the treatment group than the control group (Table 1). When compared with baseline values, FE of calcium was significantly ($P < 0.05$) higher in the treatment group from day 4 through day 9. Mean FE of chloride was significantly ($P < 0.001$) higher in the treatment group, compared with the control group; however, no significant differences over time were detected within either group (Table 2). For FE of potassium and magnesium, no significant within- or between-group differences were identified. For goats in both groups, FE's of sodium and phosphorus were below the limit of detection.

On days 7, 8, and 9 USG for goats in the treatment and control groups (Table 3) was within reference ranges. Crystals were present in the urine sediment from 1 goat in the treatment group and 2 goats in the control group. In 1 goat in the treatment group, few oxalate crystals were identified on days 8 and 9. In 1 control group goat, few triple phosphate crystals were identified on day 8; in another control group goat, moderate calcium phosphate crystals were identified on day 9.

When FE data for goats fed alfalfa hay were compared with values for goats fed orchard grass hay, with or without supplemental ammonium chloride, the FE of chloride was significantly ($P = 0.001$) higher for goats fed alfalfa hay than for either of the groups fed orchard grass hay (Table 4). The FE's of sodium and phosphorus could not be compared because mean concentration values for the experimental group could not be calculated as some of the measurements were below the detection limit of the chemistry analyzer. Whereas FE of calcium for the group fed alfalfa hay was significantly ($P = 0.001$) higher than FE of calcium ($0.86\%$) for the group fed orchard grass hay without supplemental ammonium chloride, it was not significantly different from FE of calcium for the group fed orchard grass hay with supplemental ammonium chloride. No significant differences were detected among groups with regard to FE of potassium or magnesium.

**Discussion**

Results of the present study suggest that in goats given supplemental ammonium chloride, administration at a high dosage (ie, $>400$ mg/kg, PO, q 24 h) was needed to maintain urine pH $<6.5$ for 24 hours. For goats fed an orchard grass diet, administration of ammonium chloride at this high dosage ($450$ mg/kg, PO, q 24 h) resulted in a significant increase in urinary FE of calcium, compared with baseline FE and FE in goats that did not receive supplemental ammonium chloride. Fractional excretion of calcium in goats fed orchard grass hay and supplemental ammonium chloride was similar to that for goats fed alfalfa hay. Together, these findings suggest that additional studies are needed on the effects of supplemental ammonium chloride administration in goats fed a diet with low calcium content.

In the present study, administration of ammonium chloride at a dosage of $430$ mg/kg, PO, every 24 hours resulted in formation of acidic urine ($pH < 6.5$) and increased the FE of calcium and chloride. Similar observations were made in a study$^{18}$ in which anionic salts or diets with a low DCAD were provided to goats. In the dose response portion of the present study, administration of ammonium chloride at a dosage of $200$ mg/kg, PO, every 24 hours did not produce acidic urine, whereas administration at a dosage of $400$ mg/kg, PO, every 24 hours resulted in acidic urine ($pH, 6$) for $<20$ hours. Administration at a dosage of $500$ mg/kg, PO, every 24 hours resulted in acidic urine for $\geq 30$ hours, but we were concerned that daily administration at this high a dosage would result in metabolic acidosis. In addition, transient loss of appetite was reported in a study$^{27}$ of goats given a $500$ mg/kg dose of ammonium
chloride. On the basis of our results, once-daily administration of ammonium chloride at a dosage of 450 mg/kg, PO, appeared adequate to achieve urine acidification, and we elected to use this dosage in the FE portion of our study. We currently recommend that urine pH be monitored if an ammonium chloride dosage this high is used.

Feeds containing a high concentration of calcium, such as alfalfa, clover, and kudzu, have been associated with formation of calcium oxalate and calcium carbonate uroliths and, therefore, are not recommended for male small ruminants.17 In the present study, goats fed an orchard grass hay diet without supplemental ammonium chloride had low values for FE of calcium. In contrast, goats fed an orchard grass hay with supplemental ammonium chloride had significantly higher values for FE of calcium, which approximated values for goats fed an alfalfa hay diet. In other species, urine acidification achieved by feeding ammonium chloride or low DCAD diets has been found to contribute to the formation of calcium-based uroliths.24,28–30 Calcium oxalate urolithiasis in dogs24,28,29 and cats30 and calcium phosphate nephrolithiasis in rats31 have been reported. The high values for FE of calcium in goats fed the orchard grass hay diet with supplemental ammonium chloride in the present study suggested that the risk for formation of calcium-based uroliths in these goats may have been similar to that for goats fed alfalfa hay. However, the acidic urine in goats fed supplemental ammonium chloride may have offset the additional risk of calcium-based urolith formation associated with the increase in FE of calcium. Struvite uroliths form at a urine pH range of 7.2 to 8.8, whereas calcium phosphate uroliths form at a urine pH range of 6.5 to 7.5.31 To reduce the risk of urolith formation, it has been recommended that urine pH be maintained between 5.5 and 6.5.31 Further long-term studies of urolithiasis in goats and the effects of diets with or without ammonium chloride supplementation are necessary.

Feeding diets supplemented with ammonium chloride to goats in the present study resulted in increased excretion of chloride in the urine. This has been documented in previous studies in which various chloride salts were fed to sheep33 or ammonium chloride was fed to cats.34 Chloride ions in the urine are reportedly directly protective against the formation and growth of calcium phosphate uroliths.17,33 It is possible that any presumed increase in the risk of calcium-based urolith formation associated with calculuria resulting from ammonium chloride supplementation in goats may be offset by the protective effect of increased urinary chloride excretion.

Encouraging production of more dilute urine has been suggested as a method to prevent urolithiasis in goats. This can be achieved through management changes that ensure access to clean water at all times, as well as by provision of dietary sodium chloride supplementation at 3% to 5% of DMI.30 In the present study, USG values were greater than the isosthenuric range for goats and were similar in the treatment and control groups. We speculate that consumption of water and subsequent urine output were similar between the treatment and control groups in our study; however, we did not measure water intake or urine output. In a previous study of goats,12 there was no correlation between the DCAD of a diet and USG.

In the present study, there was no association between ammonium chloride supplementation and detection of urine crystals on days 8 and 9, when urine sediment analyses were performed. We did, however, observe triple phosphate and calcium phosphate crystals in the urine of 2 control group goats. Oxalate crystals, which are more likely to form in acidic urine, were observed in urine from 1 goat given supplemental ammonium chloride. Importantly, we did not examine the urine sediment prior to ammonium chloride administration, and we do not know whether any crystals may have been present before the start of the study. Further studies to evaluate the relative risks of urolith formation in goats fed specific diets with and without supplemental ammonium chloride are necessary.

c. ColorHast Indicator Strips pH 5-10, EM Science, Gibbstown, NJ.
d. Foley Catheter, AMSINO International Inc, Pomona, Calif.
f. Elastikon, Johnson & Johnson, Skillman, NJ.
g. Hitachi 917 Chemistry analyzer, Diamond diagnostics, Holliston, Mass.
h. Leica VET 360 refractometer, Leica Microsystems Inc, Buffalo, NY.
i. SPSS, version 16.0, SPSS Inc, Chicago, Ill.

References