Use of dietary cation anion difference for control of urolithiasis risk factors in goats

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Objective—To determine correlations between dietary cation anion difference (DCAD) and urine pH, urine specific gravity, and blood pH in goats.

Animals—24 crossbred goat wethers.

Procedures—Goats were randomly assigned to 1 of 4 DCAD groups (–150, –75, 0, or +75 mEq/kg of feed) and fed pelleted feed and ground hay for 7 days. The diet was then supplemented with ammonium chloride to achieve the assigned DCAD of each group for 7 days. Urine was obtained for pH and specific gravity measurements at hours –3 to –1, 1 to 3, 5 to 7, 9 to 11, and 13 to 15 relative to the morning feeding. Blood pH was determined on alternate days of the study period.

Results—Goats in the –150 and –75 mEq/kg groups had a urine pH of 6.0 to 6.5 two days after initiation of administration of ammonium chloride, and urine pH decreased to < 6.0 by day 7. Goats in the 0 mEq/kg group had a urine pH from 6.0 to 6.5 on day 5, whereas urine pH in goats in the +75 mEq/kg group remained > 6.5 throughout the trial. Urine specific gravity differed only between the –150 mEq/kg and the –75 mEq/kg groups. Blood pH in the –150 mEq/kg group was significantly lower than that in the other groups.

Conclusions and Clinical Relevance—Goats in the 0 mEq/kg DCAD group had a urine pH of 6.0 to 6.5 five days after initiation of feeding the diet, and that pH was maintained through day 7, without significant reduction in blood pH. This may serve as a target for diet formulation for the prevention of urolithiasis. (Am J Vet Res 2009;70:149–155)

Urolithiasis is a common disease of males of small ruminant species, with the most common urolith compositions in grain-fed animals being struvite (magnesium ammonium phosphate) and apatite (calcium phosphate).1–2 Urolith formation and subsequent obstruction have a multifactorial pathogenesis, with major factors being urine pH and urine concentration.1–3 Struvite crystallization occurs only at a pH range of 7.2 to 8.8, and dissolution occurs at pH < 6.3.4,6 Apatite uroliths develop at a urine pH of 6.6 to 7.8.4,7 Urine supersaturation by calculogenic materials, including electrolytes, minerals, and mucoproteins, allows precipitation of urolith components.5,8–10 Because of the large role of urine pH and urine concentration of urolith components in calculogenesis, considerable prophylactic and therapeutic focus has been placed on the production of acidic urine and inducing diuresis to promote dilution of urine.

Anionic salts, primarily ammonium chloride, have been used extensively for prevention of urolithiasis because they induce acidic urine and cause an increase in water intake and diuresis.1,3–9 Their use, however, has led to inconsistent results in lowering urine pH and the rate of urolithiasis.8,10–12 The cause of these inconsistencies is unknown; however, the most likely explanations involve the multifactorial nature of urolith production and high-potassium diets that interfere with urine acidification in animals fed anionic salts.5,13

Dietary cation anion difference is defined as the difference between the summation of the major biological cations and anions of a diet. It is traditionally illustrated as ([Na+K] – [Cl+S]), expressed in milliequivalents per kilogram of feed.14 The addition of anions to a ration induces metabolic acidosis from a compensatory increase in extracellular hydrogen ions. The excess H+ is excreted by the kidney to maintain electroneutrality, producing urine of a lower pH.15,16 A chart correlating DCAD and urine pH exists for dairy cattle,9 but no such estimation exists for goats.

The establishment of a target DCAD in goats could potentially increase effectiveness of urinary acidification as well as prevent overacidification of diets, which may be equally undesirable. Negative effects of anion administration include reduced palatability16,12,17 and bone loss attributable to long-term ingestion.18–22 A For these reasons, it would be advantageous to establish a

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<th>DCAD</th>
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target DCAD that effectively acidifies and dilutes the urine for prevention of urolithiasis, yet avoids harmful adverse effects of overadministration. Determination of the time required from the start of DCAD treatment until optimal pH reduction occurs would provide additional assistance to those attempting to treat and prevent recurrence of urolithiasis and monitor herd response to preventive measures.

The purposes of the study reported here were to determine correlations between DCAD and urine pH, USG, and blood pH in goats and determine a DCAD that induces production of urine with a pH from 6.0 to 6.5. We sought to determine appropriate urine sampling times to monitor effectiveness of DCAD balancing. Our hypothesis was that the DCAD of a ration is positively correlated with urine pH, USG, and blood pH in goats.

**Materials and Methods**

**Experimental design—**Animals and procedures used in this study were approved by the Oklahoma State University Institutional Animal Care and Use Committee. Twenty-four adult Boer-Spanish crossbred goat wethers were used in a completely randomized design. The goats ranged in age from 17 to 18 months and in body weight from 36.5 kg to 44.5 kg, with a mean body weight of 42.9 kg.

Inclusion in the study was based on normal results from complete physical examination, venous blood gas analysis, total PCV, total protein concentration, BUN concentration, and blood glucose concentration. Results of urinalyses on free-catch samples, including pH, dipstick analysis (glucose, ketones, protein, and occult blood), USG, and microscopic sediment examination after centrifugation were also required to be within reference ranges for inclusion. Goats were determined to be free of clinical or microscopic evidence of urolithiasis. Albendazole (10 mg/kg, PO) was administered once to each goat as an anthelminthic.

Prior to the study, goats were sedated with xylazine hydrochloride (0.1 mg/kg, IM, once) and restrained in lateral recumbency. A 10-cm² area of hair centered around the prepuce, including the prepuce, hairs, was surgically clipped and cleaned. Medical speci-imen vial lids were prepared by drilling a 3.5-cm-diameter hole in the center of the lid, with 6 small pairs of holes drilled around the circumference of the remaining lid. The lids were sutured to the skin by use of the small paired holes around the preputial orifice, centering the large opening around the preputial orifice, with the threads in a ventral position. Hair over the ventral midcervical region overlying the jugular veins was clipped to facilitate venipuncture, and nylon collars used for restraint were fitted to each goat.

Goats were randomly assigned by use of a randomization software program to 4 treatment groups, corresponding to DCAD concentrations of –150, –75, 0, and +75 mEq/kg of feed, each consisting of 6 goats. The goats received a diet with the specified DCAD throughout the 7-day study period. Goats were randomly assigned to 6 indoor, concrete stalls, with 1 goat from each treatment group in each stall. A 5-gallon bucket with a cutout in the side was placed in each corner of all stalls and had a small rope with a clip affixed to provide individual restraint for goats at feeding times. Each stall contained 1 large tub, which provided ad libitum access to fresh water.

A basal ration was formulated with pelleted feed and ground native prairie grass hay. During the treatment phase, feed-grade ammonium chloride was administered to goats at the time of feeding to attain DCAD of the total diet appropriate for the assigned treatment group.

The pelleted ration consisted of 60% ground corn, 10% soybean meal, 25% ground alfalfa, 1% sodium chloride, 5% molasses, 1% limestone, and 0.1% deconinurate and was mixed and pelleted as a single batch. Prairie hay was ground to a fiber length of 2.5 cm. National Research Council requirements for metabolizable energy and crude protein were determined for this class of goats to be 0.04 Mcal/kg and 1.6 g/kg, respectively. The goats were limit-fed to meet 75% of the energy requirement daily, divided into 2 feedings, on the basis of individual weight. Limited feeding was used to ensure full intake of the ration, corresponding to the dose of ammonium chloride. Twenty-five percent of the daily National Research Council metabolizable energy requirement was met by the hay, and 50% was met by the pelleted feed. On the basis of published values, the estimated metabolizable energy of the pelleted feed was 2.796 Mcal/kg, with a crude protein of 152 g/kg. The hay had an estimated metabolizable energy of 1.91 Mcal/kg and a crude protein of 6.6%. The goats received a mean of 0.108 kg of hay and 0.147 kg of pellets/feeding, equivalent to a dry-matter intake of 1.20% of body weight daily.

The pelleted feed and a composite sample of the hay were analyzed for mineral content by use of nitric acid digestion, followed by simultaneous determination of mineral analytes via optical emission spectrometry as specified in previous publications. The DCAD of the pellets and ground hay, determined by use of an equation ([(Na+K) – (Cl+S)]) were determined to be +119.14 mEq/kg and +139.13 mEq/kg, respectively. The pellets represented 66% of the ration, and the hay represented 33% of the total ration. The respective DCADs of the feeds were combined by use of these percentages, resulting in a total basal ration DCAD of +123.68 mEq/kg. The mineral analyses for the feeds were entered into a ration formulation software program, and the proportions of hay, pellet, and ammonium chloride were determined to formulate the total ration necessary to achieve the treatment DCAD for each goat. Ammonium chloride percentage of the diet was 1.47% for the −150 mEq/kg group, 1.07% for the −75 mEq/kg group, 0.68% for the 0 mEq/kg group, and 0.29% for the +75 mEq/kg group.

On days –6 to 0, the acclimation period of the study, goats were fed divided, individual basal rations twice daily, 12 hours apart. This was accomplished by restraining each goat with a collar to a feed bucket in the home stall, providing the individual ration, and releasing the goats after all goats had completely consumed their ration.

On days 1 to 7, the treatment phase of the study period, goats were fed as during the acclimation period.
As each goat completed eating its ration, a dose of ammonium chloride was dissolved in 50 mL of deionized water and administered via esophageal feeder to ensure full intake. No additional sources of salt or mineral were provided.

On day 0, urine was collected by placing a 120-mL specimen vial into the specimen cup lid situated around the prepuce. Venous blood was obtained by use of jugular venipuncture, and analyses of the urine and blood were performed as previously for inclusion in the study. Additionally, electrolyte analysis was performed on blood by use of a handheld blood analyzer. Goats continued into the study period on the basis of findings from this examination that were within reference ranges.

On days 1 to 7 of the study period, urine samples were collected during five 3-hour sampling periods. Sampling periods were hours –3 to –1, 1 to 3, 5 to 7, 9 to 11, and 13 to 15 relative to the morning feeding. These 5 sampling periods were denoted 1 to 5, respectively. These samples were obtained by specimen cup, and at the time of voluntary urination, cups were collected and sealed. Venous blood samples were obtained by jugular venipuncture at the time of voluntary urination on days 1, 3, 5, and 7 during sampling period 3, 5 to 7 hours after the morning feeding. These samples were placed in tubes containing lithium heparin and situated in an ice bath.

Urine samples were analyzed within 20 minutes of collection for pH via pH meter and USG via refractometer. Heparinized venous blood samples were analyzed within 1 hour of collection for determination of blood pH by use of a handheld biochemical analyzer. The final urine sample collected on day 7 was also analyzed via dipstick examination to monitor urine variables, including urine glucose, ketones, protein, occult blood, and specific gravity. All instrumentation was calibrated according to manufacturer’s instructions immediately prior to analysis of each batch of samples.

Statistical analysis—All statistical analyses were performed by use of statistical software. Preliminary estimates of the variances indicated that critical differences of 0.2 for urine pH, 0.015 for USG, and 0.05 for blood pH could be detected with a power of at least 0.8 for all 3 response variables when a sample size ≥ 6 was chosen for each DCAD group.

The experiment was a 1-way treatment structure with 4 levels, corresponding to DCAD groups –150, –75, 0, and +75 mEq/kg of feed, and repeated measures were taken at the beginning of the study and every day for the following 7 days. Within each day after the start of the experiments, repeated measures were taken at 5 times during the day (~3 to –1, 1 to 3, 5 to 7, 9 to 11, and 13 to 15 hours relative to the morning feeding), corresponding to sampling periods labeled 1 to 5. The ANCOVA methods were used in the analysis of urine pH, USG, and blood pH. A repeated-measures analysis was performed to examine the correlation structures among days and times within days. For all response variables, the times within days were adequately modeled by use of a compound-symmetry covariance structure. Additionally, a baseline measurement (day 0) of the 3 response variables for each subject was incorporated into the analysis as a covariate, and unequal slope models were adopted for each of the 3 response variables.

Comparisons of DCAD, days, and time within days were performed at specified values of the covariate in each of the 3 analyses. For urine pH, the comparisons were made at the baseline covariate of 8.0. For USG, baseline covariates of 1.000 to 1.060 were selected. For blood pH, comparisons were made by use of the baseline covariate values of 7.40 and 7.45. For all comparisons, P < 0.05 was considered significant.

Results

All goats originally included in the study met health criteria for continuance in the study at the end of the acclimation period. Each goat fully consumed the individual ration daily in the acclimation and study periods and received the full dosage of ammonium chloride during the study period. No adverse health effects were detected in any goat during the trial, and urine dipstick analysis at the end of the treatment phase indicated no adverse effects on the urinary tract as a result of the study.

All blood samples were obtained and successfully analyzed. Eight hundred thirty-eight urine samples were obtained during the trial phase. Two goats each failed to urinate once during an allotted time period. One goat was in treatment group 0 mEq/kg, and no sample was obtained during the 1- to 3-hour sampling period on day 6. The other goat was in treatment group –75 mEq/kg, and no sample was obtained during the 9- to 11-hour sampling period of day 7. These samples represented missing data points.

Four urine samples were obtained after the 3-hour sampling period had ended. These included a goat in the +75 mEq/kg group during the 9- to 11-hour sampling period on day 3, a goat in the –150 mEq/kg group during the 1- to 3-hour sampling period on day 3, a goat in the +75 mEq/kg group in the 1- to 3-hour sampling period of day 6, and 1 goat in treatment group 0 mEq/kg during the 9- to 11-hour sampling period on day 6. Each of these samples were obtained within 15 minutes after the sampling period had ended and were therefore analyzed and included as data.

Urine pH—There was a significant 3-way interaction of group, day, and time for urine pH (P = 0.014). There was no significant group effect on day 1 for time periods 1 to 4 (P = 0.481). During starting sampling period 5 on day 1 (P = 0.006) and continuing through day 7 (P < 0.001), significant differences existed among groups at all time periods. This initial difference was attributable to a difference between the –150 mEq/kg and +75 mEq/kg groups (P < 0.001).

The urine pH from each sampling period (periods 1 to 5) was compared with the daily mean of each treatment group to determine which time frame after feeding that urine samples should be obtained to monitor overall effectiveness of DCAD. Frequency testing of the 28 intervals for each time period was performed to detect the presence of the daily mean of a group being within the confidence intervals of each sampling period. The 95% confidence intervals of sampling periods 1 and 5 contained the daily mean 82.14% and 64.28% of the time. Sampling period 2
5 were around the lower limit of the target range, and was greater than the target range, values on day 2 to the target range. For the –75 mEq/kg group, day 1 value remained greater than the target range, values on days 2 to 3 that remained greater than the target range, and values on days 3 to 7 were < 6.0.

At DCADs of –150 and –75 mEq/kg, a urine pH of 6.0 to 6.5 was achieved 2 days after initiation of the treatment diet at the time of the 5- to 7-hour urine sampling. The DCAD of 0 mEq/kg resulted in an increase of urine pH from 6.0 to 6.5 on day 5 of the treatment period, whereas urine pH at DCAD +75 mEq/kg remained > 6.5 during the 7-day trial period. By the end of the trial period, groups –150 mEq/kg and –75 mEq/kg had urine pH less than the target range (Figure 1).

USG—As analyzed by use of ANCOVA with baseline USG used as covariates in the analysis, there was a significant (P < 0.001) difference in group response. There was also significant interaction between day and time period, and analyses that used time period C were performed, as for urine pH. At baseline USG of 1.00, for time 3, no significant (P = 0.284) differences among days were found. There were no significant differences among the 4 treatments in USG produced from a baseline USG of 1.00 to 1.05. At a baseline USG of 1.00, a significant difference occurred between the –150 mEq/kg and –75 mEq/kg groups. At this baseline, group –150 mEq/kg had an estimated USG of 1.035, whereas group –75 mEq/kg had an estimated USG of 1.050. The 0 mEq/kg and +75 mEq/kg groups had USG estimated at 1.0421 and 1.0422, respectively.

Blood pH—The ANCOVA analysis of blood pH was performed by use of the day 0 blood pH as a covariate. Blood pH was significantly (P = 0.006) linearly related to the baseline blood pH. The influence of baseline blood pH was the same across all groups and all days. There was no significant interaction between group and day (P = 0.575) and no significant day effect (P = 0.621). There was, however, a significant (P < 0.001) group effect, which would remain the same at any baseline blood pH within the reference range. When group means were calculated by use of other baseline blood pH concentrations of 7.40 and 7.45, the group differences remained the same.

The –75 mEq/kg, 0 mEq/kg, and +75 mEq/kg groups had similar blood pH responses and were not significantly different from each other. The –150 mEq/kg group had significantly lower blood pH than did the other groups.

On the basis of these results, the hypothesis that the DCAD of a ration is positively correlated with urine pH, USG, and blood pH in goats was accepted for urine pH and blood pH, although only the blood pH of the –150 mEq/kg group differed significantly from the other groups. For USG, the hypothesis was rejected.

**Discussion**

The use of a pelleted feed with roughage comprising a minority percentage of the total ration is both a risk factor for calculogenesis and a typical feeding practice of small ruminants in our practice area. The percentage of ammonium chloride used to lower the basal ration in this study was within the recommended 0.5% to 2%1–3.

![Figure 1—Mean urine pH values at time period 3 on days 1 to 7 of a study of use of DCAD for control of urolithiasis risk factors in goats. The DCAD groups received –150 mEq/kg of feed (asterisks), –75 mEq/kg (squares), 0 mEq/kg (triangles), or +75 mEq/kg (diamonds).](image)
for 3 of the 4 concentrations. Use of the 3 concentrations, −150, −75, and 0 mEq/kg in which the percentage was within the range of 0.68% to 1.47%, achieved urine pH in and around the target range. These percentages, therefore, are appropriate for use in rations that have a basal DCAD similar to that of +126.68 mEq/kg. Rations with DCAD substantially greater than or less than this ration, in combination with equal proportions of ammonium chloride, may result in under- or overacidification.

Samples were taken 5 times daily at 3-hour intervals to determine a time interval for urine sampling that would be a reliable predictor of DCAD effectiveness. It is not known, for the prevention of urolithiasis, whether urine pH must remain consistently less than a threshold value for most of the day, whether the mean throughout the day should be less than a threshold value, or whether a single-point nadir is desirable. Analysis for this study was performed to determine which sampling period best represented the mean urine pH throughout the day. Time period 3, 5 to 7 hours after the initial feeding and salt administration, was found to best represent the daily mean. Time period 2, 1 to 3 hours after feeding, represented the mean 92.86% of the time, which was only slightly less than did time 3 at 100%, and may be used for sampling if it is more convenient or improves producer compliance. It is advisable that goats consuming DCAD-balanced rations be sampled either 1 to 3 hours or 5 to 7 hours after feeding to monitor acidification.

The data indicated that after a number of days on a DCAD-balanced ration, sampling time became less important because there was less difference among values achieved at different intervals. For a DCAD of −150 mEq/kg beginning on day 3, sampling at any interval revealed a value that was not significantly different than values obtained at any other sampling intervals. This was also true for a DCAD of −75 mEq/kg beginning on day 4 and for a DCAD of 0 mEq/kg beginning on day 6. For a DCAD of +75 mEq/kg, significant differences existed across sampling times on all days except days 1 and 3. Therefore, urine pH sampling at 3 to 7 hours after the first feeding of the day may only be required for a few days after initiation of a DCAD-balanced diet, followed by less stringent time requirements for sampling in later days. This may assist in achieving client compliance with DCAD monitoring.

In animals clinically affected by urolithiasis, once the acute obstruction is relieved, additional uroliths may result in compensation for ammonium chloride–induced acidosis. It has been reported that physiologic renal adaptation occurs after the feeding of ammonium chloride, resulting in correction of acid-base balance and reduction of the urine-acidifying effect of the salt beyond 5 to 6 days.34 This has been documented in humans and rats to occur as early as 2 to 3 days.35,36 In a study36 of rats, regulation of glutamine metabolism in the renal cortex during ammonium chloride-induced acidosis was observed, and the major pathway of acid-base correction was via accumulation of bicarbonate ions, which enter the circulation, providing systemic alkalization.37 The production of ammonium ions also provides a route of excretion for acids because the major pathway of acid elimination is via renal excretion as an ammonium salt.38 Additional effects of glutamate metabolism include the production of basic ammonia, glutamate, and glucose, which neutralize the urine upon excretion.39 The net effect of these processes is to correct systemic acidosis and aciduria.

Some clinical studies, however, have failed to detect a compensation for ammonium chloride–induced aciduria. Two studies in cats, however, revealed that urine acidification continued after 16 days38 and 11 months39 of ammonium chloride feeding, whereas in another study,40 urine pH increased as early as 10 weeks after initiation of a diet containing ammonium chloride, although the urine remained acidified.

In sexually intact bucks fed a commercial anion supplement with grass or oat hay to achieve a DCAD

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of 0 mEq/100 g, the supplement significantly decreased urine pH from 8.03 during the control period to 7.75 on days 12 to 13 and to 5.85 on days 27 to 28 of supplementation, indicating the potential for anionic supplementation to result in increased urine pH with time. Three of 8 goats that ate grass hay had increased urine pH by >0.4 units between the 12- to 13-day and 27- to 28-day samplings, whereas 3 of 8 that received oats hay had decreased urine pH during the same sampling periods. Pregnant and lactating ewes maintained acidified urine after 16 days (mean, 5.16) and 44 days (mean, 4.98), whereas lambs had continued reduction in urine pH at 12 to 14 days (mean ± SD, 7.6 ± 0.41) and 17 to 19 days (mean ± SD, 6.2 ± 0.50) after initiation of diets balanced for low DCAD by use of ammonium chloride.

The present study established a target DCAD for dissolution of urolith components and the time required to achieve that target urine pH. Further studies are indicated to determine the duration of effect of these DCADs. The present results indicate that there is a statistical plateau for urine pH for each treatment group, beginning on day 4 of the treatment phase, with no significant fluctuation in urine pH in any group through day 7. Although urine pH leveled off statistically through day 7, it is unknown whether urine acidification would be maintained by use of ammonium chloride beyond this time frame. The possibility of renal adaptation to ammonium chloride emphasizes the importance of monitoring urine pH in animals consuming diets designed to prevent urolithiasis.

For most of the USG baseline values, there were no significant differences among the groups. Only at a baseline of 1.060 was a difference detected between the −150 mEq/kg and −75 mEq/kg groups. This counters the widely held theory that increased ammonium salt in the diet substantially decreases urine concentration. Although no studies specifically measured USG with relation to DCAD, 1 study found that 6 of 8 goats had significantly decreased urine creatinine concentration at a DCAD of 0 mEq/100 g, compared with a forage diet only. The 2 groups with the highest salt intake (−150 mEq/kg and −75 mEq/kg) had significantly different urine pH, but neither had significantly different urine pH, compared with the 2 groups with the lowest salt intake.

Blood pH was significantly lower for the −150 mEq/kg group than for other groups. It also decreased to less than published reference values for goats of 7.42 to 7.46. Acidity associated with this level of feeding for a prolonged period may induce harmful effects, such as decreased feed intake and weight gain or bone loss from long-term, daily usage. The remaining groups had blood pH values that were the same and within the reference range, indicating that they had responses in acid-base balance that were biologically sound and did not overwhelm compensatory mechanisms.

A significantly reduced blood pH and overacidified urine make the −150 mEq/kg DCAD inappropriate for long-term use in goats. A DCAD of −75 mEq/kg also resulted in overacidified urine, and a DCAD of +75 mEq/kg inadequately acidified the urine of goats. The −150 mEq/kg DCAD resulted in achievement of the target urine pH without significantly reducing blood pH. The range of values at which adequate acidification without overacidification occurred was between −75 and +75 mEq/kg of feed. On the basis of the DCADs tested here, 0 mEq/kg appears to be the most appropriate DCAD for reduction of urine pH in goats from 6.0 to 6.5. This provides a target DCAD for ration formulation and a framework for additional studies on the long-term effects of these diets for the prevention of urolithiasis.

References