THE EFFECT OF CONTINUOUS AND PULSE DOSE AMMONIUM CHLORIDE REGIMENS ON THE URINE PH OF GOATS

A Thesis

by

PHILIPPA MAY SPRAKE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2012

Major Subject: Biomedical Sciences

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ABSTRACT

The Effect of Continuous and Pulse Dose Ammonium Chloride Regimens on the Urine pH of Goats. (August 2012)

Philippa May Sprake, B.Sc., University of Reading; B.Vet.Med., University of London

Chair of Advisory Committee: Dr. Wesley Bissett

Ammonium chloride (NH_4Cl) has been the primary preventive modality for struvite urolithiasis in goats. This study investigated the effect of continuous and pulse dose NH_4Cl therapeutic regimens on urine pH in ten goats.

The initial regimen (feed additive) consisted of 0.007% NH_4Cl as a feed additive. Following this week long regime, the two treatment regimens were designed as a standard ten goat cross-over design. The first treatment regimen (continuous) consisted of daily administration of a titrated dosage of NH_4Cl for ten days, followed by four days without treatment. The third treatment regimen (pulse) used daily administration of a titrated dose of NH_4Cl for three consecutive days followed by four days without treatment for three treatment periods. Ammonium chloride dosages were titrated to result in a urine pH of < 6.5 (target level) prior to commencing treatment regimens. Urine pH was evaluated once daily during feed additive regimen and twice daily during the treatment regimens.

A Bayesian methodology was used to determine the daily odds ratios for production of target urine pH during treatment regimens. The odds ratios were also calculated

between pulse dosages during the pulse regimen. The feed additive regimen did not result in target urine pH within 7 days. Treatment with the continuous regimen resulted in target pH, however, pH returned to >6.5 within five days, (odds ratio 0.23-1.56 at Treatment Time 10). The odds ratios for each pulse period of the pulse dose regimen were 2.20-7.45, 0.41-1.68 and 1.59-5.62 respectively. The results of this study indicate that variability in response to therapy warrants titrating individual dosages of NH_4Cl, continuous therapy results in a loss of effectiveness, and pulse dosage is effective in repeatedly producing a urine pH of <6.5.

DEDICATION

I would like to dedicate this thesis to my family for all their love and encouragement in completing this piece of work, and throughout my master's degree.

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I would like to thank my committee chair, Dr. Bissett, and my committee members, Dr. Roussel, Dr. Stewart, and Dr. Nabity, for their guidance and support throughout the course of this research.

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Finally, thanks to my family for their encouragement.

NOMENCLATURE

DCAD	Dietary Cation Anion Difference
HCO_3^-	Bicarbonate ion
H^+	Hydrogen ion
Ca^{++}	Calcium ion
Mg^{++}	Magnesium ion
Na^+	Sodium ion
Cl^-	Chloride ion
NH_3	Ammonium
NH_4^+	Ammonium ion
NaCl	Sodium chloride
KCl	Potassium chloride
[SID]	Strong Ion Difference
$[A_{TOT}]$	Weak non-volatile buffers
DMI	Dry Matter Intake
CI	Confidence Interval
EBVM	Evidence Based Veterinary Medicine

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1. INTRODUCTION

1.1 Introduction

Struvite urolithiasis is one of the leading causes of morbidity and mortality in castrated and intact male show goats. Risk factors for development of struvite uroliths include excess minerals in the urine (particularly phosphates), concentrated urine and an alkaline urine pH. Feeding and management practices associated with these animals place them at a high risk for development of this potentially fatal disease. The cornerstone of preventive and therapeutic efforts has been dissolution of the uroliths through acidification of the urine by ammonium chloride administration. Our clinical perspective is that long-term continuous ammonium chloride treatment is ineffective due to a presumed physiological response returning the urine pH towards alkaline. Pulse dose regimens have been employed to counteract this proposed effect.

This thesis follows the style of the Journal of Veterinary Internal Medicine.

1.2 Literature Review

1.2.1 Incidence of urolithiasis

Urolithiasis has been reported in multiple species including ruminants worldwide.[1] While obstructive urolithiasis cases are typically treated as individual animal cases, urolithiasis has been shown to be a significant disease at the flock level in sheep.[2-4] The incidence of urolithiasis in show goats however, has not been reported. Many urolith types have been identified in small ruminants.[1] Phosphatic type and calcium carbonate uroliths have been obtained most often from studies of clinical cases of urolithiasis in small ruminants in the USA[5,6] and in cases of experimentally induced urolithiasis.[7-11] The most frequent urolith recovered from clinical cases in Central Texas has been struvite.[5]

The typical signalment in obstructive urolithiasis cases is the castrated male.[5,6,12] Although both males and females can develop uroliths at equal rates, males typically become obstructed due to their anatomically longer and narrower urethra,[13] or as a result of lower testosterone levels restricting urethral development following early castration.[14] Typical age of presentation is approximately 12 months,[5,6,12,15] and the two most common breeds represented in the American literature are Boer[5] and Pygmy.[15,16] This signalment does not necessarily represent a breed predisposition, rather the common management of these breeds. Male pygmy goats were thought more likely to be castrated, kept as pets and fed unbalanced diets thereby increasing the risk for developing urolithiasis.[16] Similarly, the higher incidence of urolithiasis in Boer goats is presumed to be as a result of typical show goat diets containing high levels of grain and/or mineral imbalances, and also as a result of early castration.

1.2.2 Formation of uroliths

Uroliths are organized crystal aggregates with a complex internal structure.[17] The formation of a urolith is thought to be initiated by a crystal nidus. The exact process of nidus initiation is undetermined. However, the precipitation-crystallization theory[17] is most applicable to struvite type uroliths, as a result of oversaturation of the urine with urolith forming components, specifically ammonium, magnesium and phosphate.[18] Spontaneous crystal formation occurs when the critical limit of saturation is exceeded.[19] Factors favoring crystallization, such as alkaline pH and concentrated urine, also influence crystal aggregation and urolith development.[17] Uroliths may contain a nucleus consisting of organic debris (blood, bacteria or tissue debris), and foreign or crystalline material;[17,20] and an organic matrix on which crystals can organize.[17] However, the presence of a nucleus and matrix is not essential for urolith formation.[17] Following formation of the crystal nidus, the urolith expands by continued aggregation of the same or another crystal type (epitaxial growth).[17]

1.2.3 Etiology of phosphatic uroliths

Struvite stones contain ammonium, magnesium and phosphate, but can also contain smaller quantities of calcium apatite, carbonate apatite and ammonium acid urate.[17] Canids and human beings typically have struvite stones associated with a urinary tract infection, however there are no reports to date of infection-related struvite stones in ruminants. The most accepted etiology of struvite stones not occurring as a result of

infection is high urinary solutes and alkaline pH resulting from dietary mineral imbalances and high mineral and carbohydrate content.[19]

Studies have shown that urolithiasis in small ruminants was associated with high dietary minerals, resulting in high urinary mineral concentrations.[21-24] The ratio of calcium to phosphorus in the diet was shown to have the most significant effect, with ratios of greater than 2:1 decreasing urolith incidence.[10,25] Certain feed types[26] or diets[27] have been associated with development of uroliths, however the effect of feed may be related to the level of mineral in the feed, rather than the feed itself.

1.2.4 *Phosphorus metabolism*

Phosphorus is absorbed by a sodium-dependent mechanism and has been documented to occur actively in all segments of the small intestine, the proximal colon and in the fore-stomachs.[28] The primary mechanism of phosphorus excretion in ruminants is in the feces, as a result of salivary recycling of phosphorus,[29] with a smaller amount excreted via the renal route compared to monogastrics. This is evident in the low reported urinary fractional excretion of phosphorus in cattle as 0.36-1.14%,[30] whereas the fractional excretion of phosphorus in horses is higher, at greater than 4%.[31] Individual variation has been demonstrated in urinary phosphorus excretion,[29,32] potentially indicating a genetic influence. This may explain the sporadic nature of urolithiasis cases in a group of animals on the same diet. The salivary excretion route has been reported to have a threshold of 2mmol/l.[29] Therefore, if increased consumption of phosphorus or

decreased saliva production as a result of pelleted and low roughage diets occurs, urinary phosphorus concentrations may increase.

Supersaturation occurs in concentrated urine. Increased incidence of urolithiasis has been reported at both extreme hot and cold environmental temperatures as a result of low water intakes,[13] and in high-concentrate, intermittent-feeding practices.[29]

1.2.5 *Treatment and prevention of urolithiasis*

Prevention of urolithiasis is best obtained by dietary manipulation to address mineral imbalances, increased forage and decreased concentrate feed levels in the diet, and by maximizing water intake. [33] However, these alterations are not always acceptable in animals where high growth rates are desirable. Treatment has typically involved immediate relief of obstruction by amputation of the urethral process,[16] direct urinary acidification by Walpole's solution,[5] or surgical intervention.[16] Following relief of obstruction, acidification of the urine to dissolve remaining uroliths is indicated.

Urinary acidification is most commonly achieved via dietary modification based on the Dietary Cation Anion Difference method (DCAD) that was developed for prevention of hypocalcemia in transition dairy cows.[34]

1.2.6 *Acid-base homeostasis*

The acid base balance of the body is tightly regulated as severe alteration to blood pH affects essential enzymatic reactions. Blood pH is regulated by 1) body fluid buffer systems, primarily via extracellular bicarbonate ions and intracellular proteins, 2) the

respiratory system, and 3) the renal system.[35,36] The kidneys balance pH by reabsorption of filtered bicarbonate ions (HCO_3^-), excretion of hydrogen ions (H^+) and production of new HCO_3^-. Ammonia (NH_3) is an important tubular fluid buffer that is formed from the amino acid glutamine. The glutamine is metabolized in the proximal tubules to NH_3 which is excreted into the urine filtrate, and to HCO_3^- ions which are reabsorbed. In the collecting ducts, NH_3 combines with H^+ ions to form ammonium ions (NH_4^+) which cannot be resorbed.[35] Increased extracellular H^+ ions stimulate renal glutamine metabolism, by increased activity of renal glutaminase enzyme, which is the most important mechanism of acid excretion in chronic acidosis.[36-38] This increase can be as much as 10 fold and is important in the compensation response to chronic acidosis.[35,36]

Acid base status can be determined by two methods: the traditional Henderson-Hasselbach equation and the Strong Ion Difference [SID] theory. The DCAD method of urinary acidification is based on the strong ion acid-base model. This model, devised by Stewart[39] and simplified by Constable,[40] explains that plasma pH can be decreased by decreasing [SID], increasing temperature, decreasing the weak non-volatile buffers (albumin, globulin and phosphate) [A_{TOT}] and increasing pCO_2. The major cations are sodium (Na^+), potassium (K^+), magnesium (Mg^{++}), calcium (Ca^{++}) and NH_4^+ and the major anions are chloride (Cl^-) and sulfate (SO_4^{--}). [34] The rationale behind DCAD to induce a metabolic acidosis is to feed salts of the strong anions, or acids of the cations.[34] Multiple equations to calculate the DCAD of the diet have been proposed: in a meta-analysis by Charbonneau, Pellerin and Oetzel.[41] The equation $(Na^{++} + K^+) – (Cl^- + 0.6S^{--})$ was most strongly correlated with urinary pH. In ruminants consuming a forage-based

diet, the pH of the urine is naturally alkaline as a result of high intakes of cations.[35] Urine pH is the most frequent parameter used to monitor the effectiveness of the DCAD diet.

1.2.7 *Urinary acidification*

Administration of DCAD diets have been associated with reduced plasma pH, plasma HCO_3^-, and urine pH and an increased plasma H^+ indicating metabolic acidosis was induced.[41-43] The underlying drive for these changes was the decrease in [SID]. The effect on pCO_2 has been shown to be variable.[42,43] Alteration in $[A_{tot}]$ has not been demonstrated confirming that it is not a major contributing factor to DCAD acid-base disturbances.[42]

The addition of anionic salts to the diet has been the most frequently used method for both treatment and prevention of urolithiasis, and it has been evaluated in multiple species.[42,44-49] In studies investigating the relative acidifying effect of anionic salts on lowering urine pH, chloride salts were more effective than sulfate salts.[50,51] Ammonium chloride results in decreased [SID] due to greater gastrointestinal absorption of Cl^- over NH_4^+.[52] Sodium chloride (NaCl) and potassium chloride (KCl) do not affect the [SID] as Na^+ and K^+ are absorbed with similar efficacy to Cl^-. [34] In support of this, NaCl has been shown to be ineffective in reducing urolithiasis cases in goats.[9] In urolithiasis studies, both ammonium chloride and ammonium sulfate reduced clinical cases in sheep fed a calculogenic diets.[9,11] Anionic salt supplementation also can result in increased water intake that may result in formation of dilute urine, decreasing the likelihood of urine supersaturation and thereby preventing urolithiasis. In goats, although a trend towards

higher urine volumes was seen in animals supplemented with 1.5 % NaCl, no reduction in urolith incidence was observed.[9] High level concentrations of NaCl (4% and 7%) did reduce urolith incidence in goats.[53] More recent studies indicated that water consumption and urine volume was increased at 27-28 days of a low DCAD diet, although the effect of this on the ability to prevent urolithiasis was not studied.[46] When urine specific gravity was used as a measure for urinary dilution, the results were inconsistent.[47,48]

1.2.8 *Acidification dynamics*

The DCAD level required to achieve desired results has been extensively investigated for milk fever prevention. A curvi-linear relationship between DCAD and urine pH has been demonstrated, with DCAD having minimal influence on urine pH until DCAD <20mEq/100g DM.[34] No similar studies on the relationship between DCAD and urine pH have been evaluated in goats, for the prevention or treatment of urolithiasis. In both in vivo and in vitro experiments, a decrease in urine pH to <6.5 resulted in struvite-stone dissolution.[54-56] Target ranges for urine pH have therefore been set at pH 6.5-7 in various urolithiasis studies.[46-48] The length of time required for struvite stones to dissolve in goat urine has not been investigated. Ammonium chloride dosages of 5-200mg/kg have been recommended, with variation in the dosage required to lower urine pH to the desired acidic range.[9,16,33,48] Likewise, the DCAD level used has had variable effect on the production of acidic urine pH.[46,48] The variation in effective dosages may be a result of base diet variation altering the total DCAD of the diet, or individual variation. Diets based on alfalfa are associated with alkalinization in dairy cattle, and therefore higher

doses of anionic salt or lower DCAD levels may be required to lower urine pH.[50,57] Oat and grass hay fed to goats, however, did not result in a significant difference in urine pH between the 2 groups.[46] Variation in protein levels in feed has also not affected the acid-base status in cattle.[58]

Show goat diets commonly have ammonium chloride added as a preventive for urolithiasis, and levels are typically 0.01% of the ration. No studies have evaluated the effects of this low dosage on prevention or treatment of urolithiasis; however, previous studies suggest that it is likely to be ineffective at lowering urine pH to below the target range.

The time to urinary acidification following administration of ammonium chloride or feeding of a low DCAD level varies by level of dosing, where higher doses result in shorter time to attainment of target urine pH.[47,48] A pattern of acid-base change following administration of anionic salt has been described by Las et al.[42] in sheep where a drop in plasma pH after feeding occurred, followed by a gradual decrease to a minimum point, and then an increase or maintenance of the nadir until the next dose. Jones et al.[48] investigated appropriate time to measure urine pH post ammonium chloride administration. Urine samples collected 5-7 hours after treatment and feeding best represented the daily mean urinary pH.[48] In cattle, administration of anionic salts once a day resulted in variability of pH at different times; however, this effect was not evident when treatment was performed twice a day.[59]

1.2.9 *Complications associated with dietary acidification*

1.2.9.1 Uncompensated metabolic acidosis and decreased dry matter intakes

High levels of ammonium chloride have been reported to result in detrimental effects, including decreased feed intakes as a result of induction of a metabolic acidosis or poor palatability of high levels of anionic salts added to the ration.[9] Decreased DMI was observed when urine pH decreased to <7[41], or <5.5[60] and was suggested to be as a result of uncompensated metabolic acidosis. Experimental clinical studies have minimized the effect of decreased intakes by administering the anionic salt dissolved in water directly by mouth,[47,48] or mixed with sugar and topped dressed on the feed.[46]

1.2.9.2 Bone resorption

Long term administration of anionic salts has also raised concerns regarding bone resorption that may result in decreased bone density and development of osteoporosis, osteopenia and fractures.[46] In goats, there was increased resorption rate of bone pre-partum, as measured by the bone resorption marker carboxyterminal telopeptide of Type I collagen.[61] There have been no clinical reports in the literature of bone pathology associated with renal acidification except one report in a human with renal tubular acidosis.[62]

1.2.9.3 Increased excretion of calcium

Urinary acidification as achieved by DCAD, results in increased renal excretion of calcium [46,47,57,58,61,63,64] and phosphorus.[46,56] This increase in urinary calcium may

predispose the animal to formation of calcium containing uroliths. However, calcium carbonate formation is also reduced in acidic urine thereby potentially negating this effect.[46] A trend in increased incidence of calcium containing stones has, however, been observed in cats, and is thought to be a result of dietary modification and acidification as a preventative for struvite urolithiasis.[65]

1.2.10 *Continuous urinary acidification*

Continuous treatment with anionic salt therapy to acidify the urine has been associated with an increase of urine pH following production of an acidic urine pH. This re-alkalinization is thought to be a physiological response to chronic acidification.[37] This increase of urine pH to above that at which struvite stones dissolve and formation is prevented suggests that continuous ammonium chloride treatment may become ineffective in the prevention and treatment of struvite urolithiasis. In ruminants, a trend towards re-alkalinization has been observed in beef cattle,[66] sheep,[61] and goats.[46] In all 3 studies however, the urine pH remained below pH 6.5. The most significant effect of re-alkalinization was observed in male rats receiving ammonium chloride.[37] The urine pH nadir was achieved in 2 days, after which the pH increased, returning close to control values by 7 days. A similar effect has been observed in humans.[67] Glutaminase activity and urinary ammonia excretion increased during the initial phase of induced acidosis, indicating that buffering by ammonia was occurring.[37] It was this mechanism that was thought to result in the physiological adaptation as manifested by return of blood acid base parameters and an increase in urine pH.[37] Goff,[64] however, suggested that in cattle

fed anionic salts longer than 6 weeks, the urine pH of freshening cows will have risen as a result of the bone buffering the acidity. An alternative hypothesis is the alteration of intestinal electrolyte transport in response to alterations in acid-base.[68,69] The effect of re-alkalinization is variable: long term treatment of male cats with an acidifiying diet appeared to be effective for up to 11 months [49] and urine pH remained acidified in rats receiving ammonium chloride for 52 days.[56] No studies have specifically investigated the occurrence of re-alkalinization in a clinical trial in goats.

1.3 Bayesian Statistical Concepts

Use of evidence based approaches for treatment and diagnostic decisions is critical for making valid and robust clinical decisions for diagnosis, treatment, and prevention.[70] Evidence Based Veterinary Medicine (EBVM) in essence requires critical appraisal of scientific evidence along with clinical expertise and knowledge of each case.[70] Bayesian statistics uses prior information and the sample data in order to formulate the posterior distribution as does EBVM. Therefore, EBVM employs the Bayesian concept. Prior data that is precise and informative influences the posterior distribution to a greater effect than prior information that is vague. Bayesian statistics examine the probability distribution of the true value given the data which is fixed, and the parameter of interest, which is considered a random variable. In comparison, traditional frequentist methods determine the true value to be fixed, with the sample data being variable.[71] Bayesian methodology therefore considers the data from the trial to be real with the population mean an abstraction.[72] This allows the Bayesian statistician to

draw direct conclusions from the data regarding the study population: Bayesians can state that there is a 95% probability that the reported confidence interval contains the mean.[72] Confidence intervals which are greater than 1 indicate a high probability for achieving the desired result. Frequentists however, can only accept or reject the null hypothesis; the results cannot support another parameter value. For example, for a patient receiving a drug, if the 95% confidence interval of the odd ratios is 3-6 for achieving the desired effect, one can state using Bayesian methodology that the likelihood for this drug achieving the desired effect is between 3 and 6 times more likely. A Frequentist would be required to state that if the study was to be repeated many times there is only a 5% chance that more extreme results would be obtained. Other advantages of Bayesian methods include the ability to generate distributions and evaluate results in small sample sizes with confidence without violating distribution assumptions and the use of complex data sets.[71]

1.4 Objectives and Aims

The objective of this study was to investigate the effect of continuous ammonium chloride therapy on urine pH in show goats and compare continuous and pulse dosing regimens.

The three specific aims were to: 1) estimate the effectiveness of low dose ammonium chloride as a feed additive as a urinary acidifier; 2) estimate the duration of urinary acidification with continuous dose therapy, and 3) to estimate the effectiveness of pulse therapy in intermittently achieving the target urinary pH during periods of treatment.

1.5 Hypothesis

Based on the literature reviewed and data from previous studies, the following working hypotheses were used: 1) low dose ammonium chloride in the feed does not result in urine pH below the target level (<6.5) within 7 days of feeding; 2) continuous dosing of ammonium chloride does not result in persistence of the target urine pH; 3) and pulse therapy results in repeated attainment of the target urine pH during treatment periods.

2. MATERIALS AND METHODS

2.1 Animals

Ten castrated yearling goats were obtained from the Department of Large Animal Clinical Sciences Research herd at Texas A&M University. All goats were in good body condition at commencement of the study, with no history of urolithiasis. Physical examination and serum biochemistry was performed on each goat prior to inclusion in the study to identify goats with any prior disease. Goats were individually housed during the study period. Goats were weighed weekly and their individual ammonium chloride dosage recalculated as necessary. Animal use in this study was approved by the Texas A&M Institutional Animal Care and Use Committee (IACUC). AUP #2011-179.

2.2 Experimental Design

All goats underwent a 7 day feed additive period where no additional ammonium chloride treatment was administered. The feed additive period was followed by the continuous or pulse dose treatment regimens in a 2-group cross over design. Five goats were allocated to each group. Group 1 goats were allocated to the 14-day continuous treatment regime first, followed by a 7 day washout period then the 21-day pulse dosing treatment regime. The second group underwent the pulse dose regime followed by a 7-day washout period and then the continuous treatment regimen. See Appendix A for the study timeline.

2.3 Urine Collection and Analysis

During the feed additive period, urine was collected once a day at approximately 1200 hours. During the continuous and pulse dose treatment regimens, urine was collected twice a day before feeding and/or treatment, at approximately 0700 and 1700. To collect urine, a 5oz plastic specimen cup was placed under the prepuce and secured around the abdomen with brown gauze. The collection cups were left in place until a urine sample was obtained or 1 hour post placement, which ever was the soonest. No urine was collected during the washout periods. The urine pH that was collected at one collection time was presumed to correspond to the response to the ammonium chloride treatment 12 hours previously. No urine was collected during the 7 day washout periods.

Urine pH was measured within 1 hour after urine collection using a static laboratory pH meter.[1] The pH meter was calibrated weekly as per manufacturer recommendations.

2.4 Treatment Protocol

Feed-grade ammonium chloride was administered twice a day at 0700 and 1700 on treatment days of the continuous and pulse dose treatment regimens. The ammonium chloride dose was mixed with corn syrup and administered by mouth before feeding. The dose of ammonium chloride was calculated individually based on body weight (kg).

[1] Mettler-Toledo InLab® Expert Pro pH Meter. Columbus, OH

2.4.1 Dose titration

In order for the goats to produce a urine with pH <6.5 within 3 days (6 collection times), it was necessary to titrate the dose for individual goats. The titration of dose occurred during the first treatment period where all goats received an initial dose of 200mg/kg ammonium chloride twice a day. Urine pH was measured twice a day as described above. Goats that did not produce urine with a pH below the target range (pH <6.5) within 6 treatments (3 days) received no treatment for the following 4 days. The dose was then increased to 250mg/kg and administered for 6 treatments. Again, goats that did not produce urine below the target pH range within this time, received no treatment for 4 days. Finally the dose was increased to 300mg/kg twice a day. The first 5 goats to achieve their effective dose were allocated to Group 1 (continuous dose followed by pulse dose) and the remaining 5 goats in Group 2 (pulse dose followed by continuous dose).

Once a dose that resulted in attainment of a urine pH <6.5 within 6 collection times was determined, this became the first 6 collection times of the continuous treatment regimen for goats in Group 1, and the first pulse dose period for goats in Group 2.

2.4.2 Continuous treatment regimen

Ammonium chloride treatment was administered twice a day for the first 10 days of the continuous treatment regimen resulting in 20 treatment times times (Collection Times 1-20) with corresponding urine pH measurements. Following this, no ammonium

chloride treatment was administered for 4 days, resulting in 7 non-treatment times with corresponding urine pH measurements (Collection Times 21-27).

2.4.3 Pulse dose treatment regimen

Ammonium chloride was administered twice a day for 3 days followed by 4 days of no treatment. The 7 day cycle was repeated 3 times, resulting in 6 treatment times each for Pulse 1 (Collection Times 1-6); Pulse 2 (Collection Times 15-20); and Pulse 3 (Collection Times 29-34). In total there were 23 non-treatment times during the pulse dose treatment regimen (Collection Times 7-14; 21-28; and 34-41).

2.5 Feeding Protocol

Prior to entering the study goats received coastal Bermuda hay and commercially available goat feed[2] with no added ammonium chloride. Goats were introduced to the study diet 1 week before the feed additive period commenced. The study diet consisted of a commercially available goat feed[3] containing 0.007% ammonium chloride and alfalfa hay. The ration was designed using a feed analysis program[4] to provide 70% concentrate, 30% forage, with a dry matter intake of 2.5% body weight. The ration was divided into 2 equal daily feedings. Fresh water was available at all times. See Appendices B-D for feed analysis, ration design and DCAD levels of diet.

[2] NatureWise Goat Feed. Nutrena. Cargill. MN
[3] Producers Show Goat Feed. Producers Co-op. Bryan, TX
[4] Aries Feed Ration Analysis. UC-Davis, CA

2.6 Statistical Analysis

Descriptive statistics were used to plot the median, interquartile range, first and third quartiles, and total range of the urine pH as box and whisker plots for all goats during the feed additive period and continuous and pulse dose treatment regimens.

Bayesian statistical methods were employed in this study to generate odds ratios in order to test the hypothesis that ammonium chloride treatment will result in production of urine with a pH <6.5. In the Bayesian model the parameters were estimated using a Monte Carlo Markov Chain (MCMC) method using OpenBugs version 3.2.1software.[73] The likelihood that the pH was less than 6.5 was modeled as a Bernoulli distribution. The logit of the Bernoulli parameter was modeled as a linear function of an intercept, a random effect for goat, treatment effect and, to control for possible order effects in the analysis, the effect of order. Vague priors were used for the model coefficients. Specifically, the intercept was a flat (-infinity, +infinity) prior. The random effect of goat was modeled as a vague normal distribution with a zero mean and a Gamma (0.01,0.01) precision parameter. For the effect of continuous treatment, the so-called random walk prior was used.[74] The effect of each pulse treatment was assigned an independent Normal prior with zero mean and precision of 0.001. A burn-in of 5,000 iterations and a sampling of 10,000 iterations were used for the MCMC simulation. The Bayesian estimate was taken as the posterior median of the parameter. The Bayesian Confidence Interval, often also called the Credibility Interval was the 2.5 and 97.5 percentiles taken directly from the posterior distribution. If the 95% confidence interval excluded odds ratios of <1, we defined this as statistical significance. Odds ratios (median and 95%

confidence interval) for achieving target urine pH (<6.5) during the continuous treatment period were calculated against urine pH obtained during the non-treatment period (Collection Times 21-27 of the continuous treatment period and Collection Times 7-14; 21-28; and 35-41 of the pulse treatment period). In the pulse treatment period, odds ratios for achieving urine pH <6.5 during the treatment times for each pulse period compared to urine pH obtained during both continuous and pulse non-treatment times were calculated. The odds ratios for achieving urine pH <6.5 for each pulse treatment period were compared to each other in order to identify differences between the pulse periods.

3. RESULTS*

The mean goat body weight at enrollment of the study was 65lbs (29.5kg). During the experimental period, the weight gain ranged from 0-16lbs. Goats tolerated the oral administration of ammonium chloride mixed with corn syrup well and no goats were excluded during the study. Of 750 urine pH data points, 69 were reported missing (9.2%) due to inability to collect urine within 1 hour. Mean age of goats at enrollment in the study was 15.7 months.

3.1 Effectiveness of Ammonium Chloride as a Feed Additive for Urine Acidification

Figure 3-1 shows the median urine pH of goats over the feed additive period. Appendix E shows the tabulated data. The percentage of goats achieving the target urine pH during the 7 days was 0. There was no difference between goats in Group 1 and 2.

*Part of the data in this section is reprinted with permission from "The effect of ammonium chloride as a long term preventative approach for urolithiasis in goats and a comparison of continuous and pulse dosing regimens" by Sprake, P. Roussel, AJ. Stewart, R. Bissett, WT. 2012. Journal of Veterinary Internal Medicine. *In press.* Copyright 2012 by Journal of Veterinary Internal Medicine.

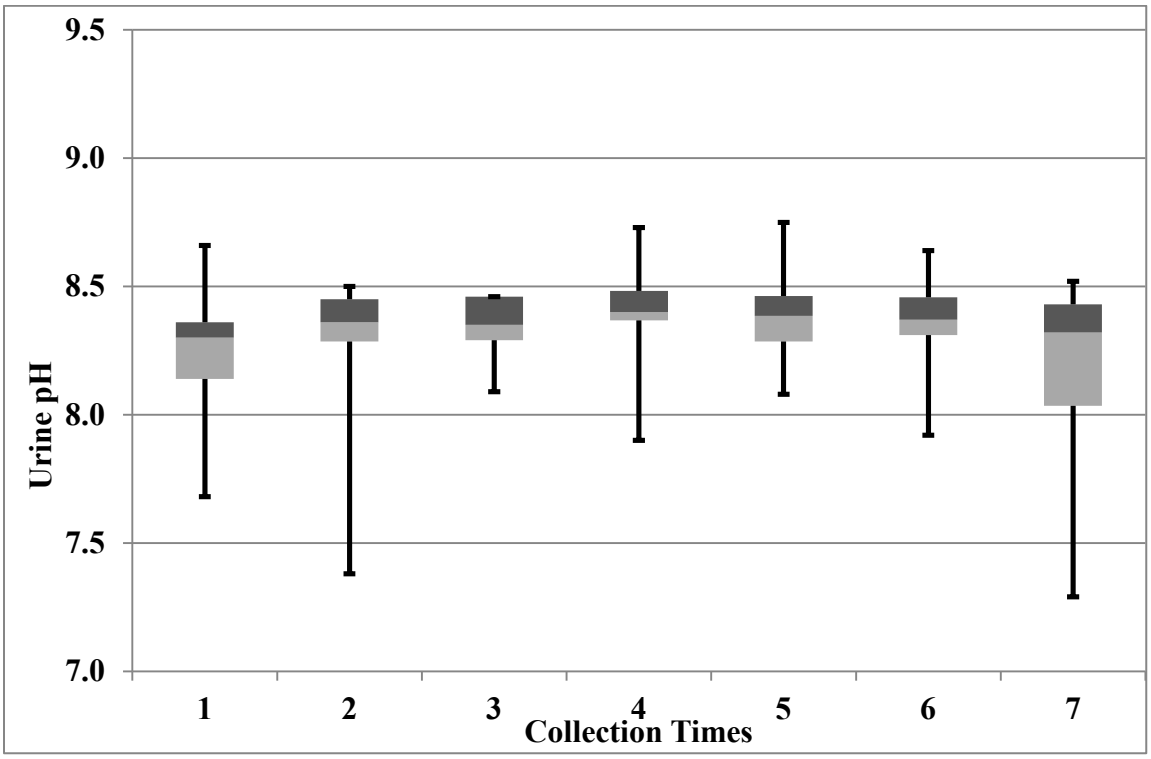

Figure 3-1. Box and whisker plot of urine pH for all goats during the feed additive period. Median, first (light grey) and third (dark grey) quartiles, interquartile range, minimum and maximum are presented. N=10.

3.2 Titration of Ammonium Chloride Dose

Five of 10 goats produced a urine pH <6.5 within 3 days of treatment with ammonium chloride at 200mg/kg twice a day. Of the 5 remaining goats, 4 produced a urine pH <6.5 at a dose of 250mg/kg twice a day, with the fifth goat requiring a dose of 300mg/kg twice a day to achieve target urine pH within 3 days. Data is presented in Appendix F.

3.3 Pre-treatment Urine pH

The median urine pH for all goats at the start of each treatment regimen (before any treatment was administered or following the washout period), was pH 8.42 with a range of 7.95-8.99.

3.4 Effectiveness of Continuous Treatment Regimen for Urine Acidification

Figure 3-2 shows the box and whisker plots for all goats during the continuous treatment regimen. Table 3-1 shows the daily median and 95% confidence (CI) intervals off the odds ratio for achieving target urine pH at each collection time. The tabulated data is presented in Appendix G. The median urine pH for all goats was below the target level of pH 6.5 by the second collection time (24 hours after the first dose of continuous treatment) with 89% of goats producing urine with pH <6.5. Only 1 goat (in Group 2) did not produce urine below target pH level within 3 days. In this goat, a urine pH<6.5 occurred at Collection Time 9. The lowest urine pH achieved during the continuous treatment regimen by any goat was pH 4.59 at Collection Time 2. Following production of median urine <6.5, the median pH remained below the target level for a further 7 collection times with median odds ratios >1. However, odds ratios (95% CI) were only >1 for Collection Times 3-6. Therefore, it was at these times only, that there was a significant effect of the treatment to result in production of urine with pH below the target range. At Collection Time 10 (5 days) after initiating continuous treatment, the median urine pH was above the target range at 7.05 (odds ratio 95% CI 0.23-1.56), with only 30% of goats maintaining production of urine pH <6.5.One goat maintained urine

pH <6.5 for the duration of the treatment period (Collection Times 1-20). Median urine pH remained above the target level for a further 6 collection times. At Collection Times 13-15, odds ratios (95% CI) were <1 indicating that there was a significant inability of the treatment to produce urine pH below the target level. Fifty percent of goats produced urine with pH >6.5 at any point during Collection Times 13-15. This effect of re-alkalinization was evident until Collection Time 17 when the urine appeared to re-acidify (with a median urine pH <6.5 and median odds ratios >1). At Collection Time 17, 67% of goats had urine pH <6.5. Following discontinuation of the ammonium chloride treatment at Collection Time 20, median urine pH increased to alkaline 24 hours later, with 71% goats producing urine pH >6.5 at Collection Time 21. Sixty hours after discontinuation of treatment, all goats had an alkaline urine pH within the pre-treatment range (pH 7.95-8.99). Figure 3-2 shows that during treatment times, there was a wide interquartile and total range for urine pH, compared to non-treatment times, thus indicating there is variability both within and between goats in the response to treatment with ammonium chloride. Figure 3-3 shows the median urine pH for Groups 1 and 2 showing the diverging median urine pH between the 2 groups from Treatment Time 7.

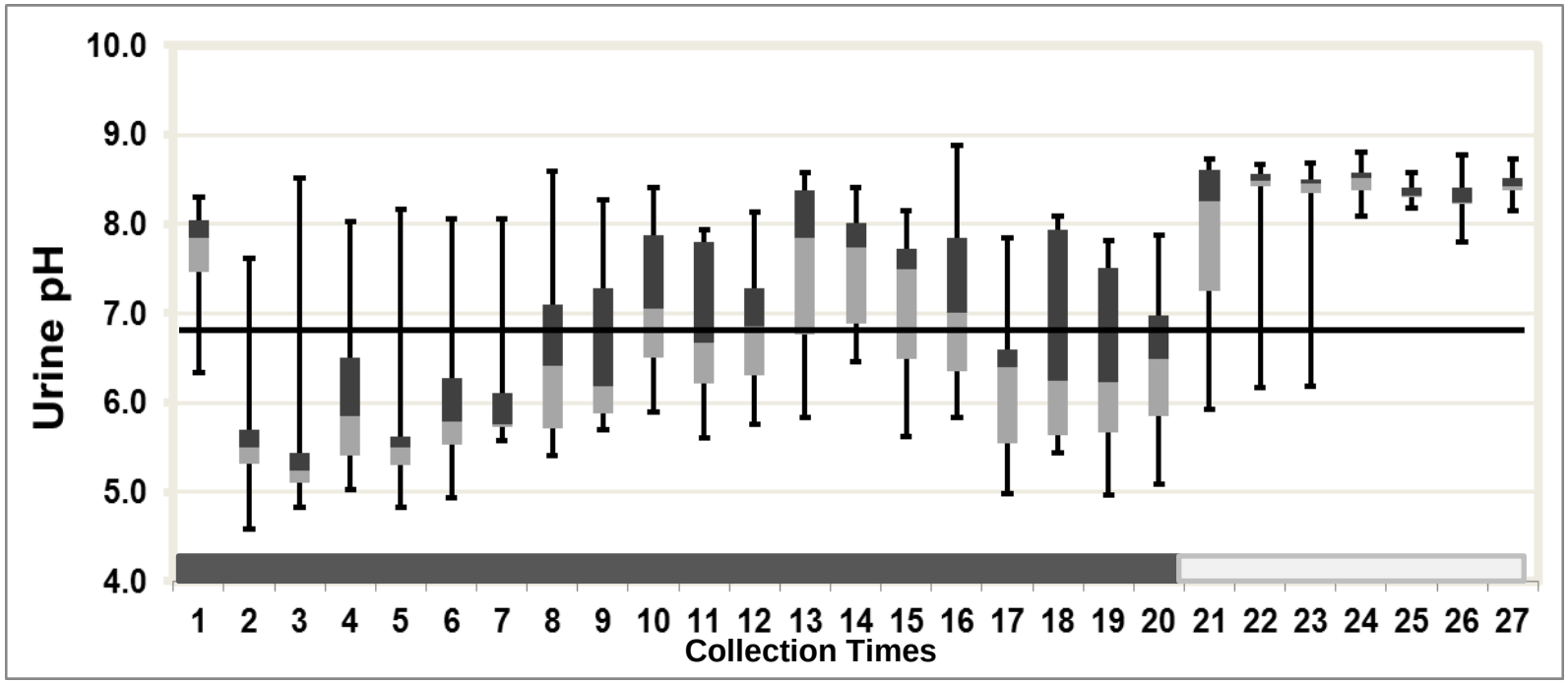

Figure 3-2. Box and whisker plot of urine pH for all goats during the continuous treatment regimen. The median, interquartile range, first (light grey) and third (dark grey) quartiles, minimum and maximum are presented. The darker horizontal line represents the target urine pH level (pH 6.5). The grey box indicates the treatment times, the white box the non-treatment times. N= 10).

Table 3-1. Continuous treatment regimen odds ratios. Median odds ratios and 95% CI for all goats in achieving urine pH below the target (<pH 6.5) during the 10 treatment times of the continuous treatment regimen.

Collection time	Median Odds Ratio	95% CI
1	0.59	0.12 - 1.80
2	2.20	0.83 - 6.68
3	2.72	1.08 - 7.90
4	2.86	1.20 - 8.00
5	3.89	1.55 - 12.79
6	3.11	1.27 - 8.62
7	1.85	0.62 - 5.51
8	1.25	0.46 - 3.21
9	1.33	0.56 - 3.40
10	0.64	0.23 - 1.56
11	0.62	0.24 - 1.71
12	0.45	0.18 - 1.33
13	0.24	0.08 - 0.64
14	0.19	0.05 - 0.52
15	0.31	0.10 - 0.81
16	0.47	0.18 - 1.27
17	0.83	0.34 - 2.13
18	1.06	0.44 - 3.00
19	1.48	0.56 - 3.90
20	1.92	0.56 - 5.68

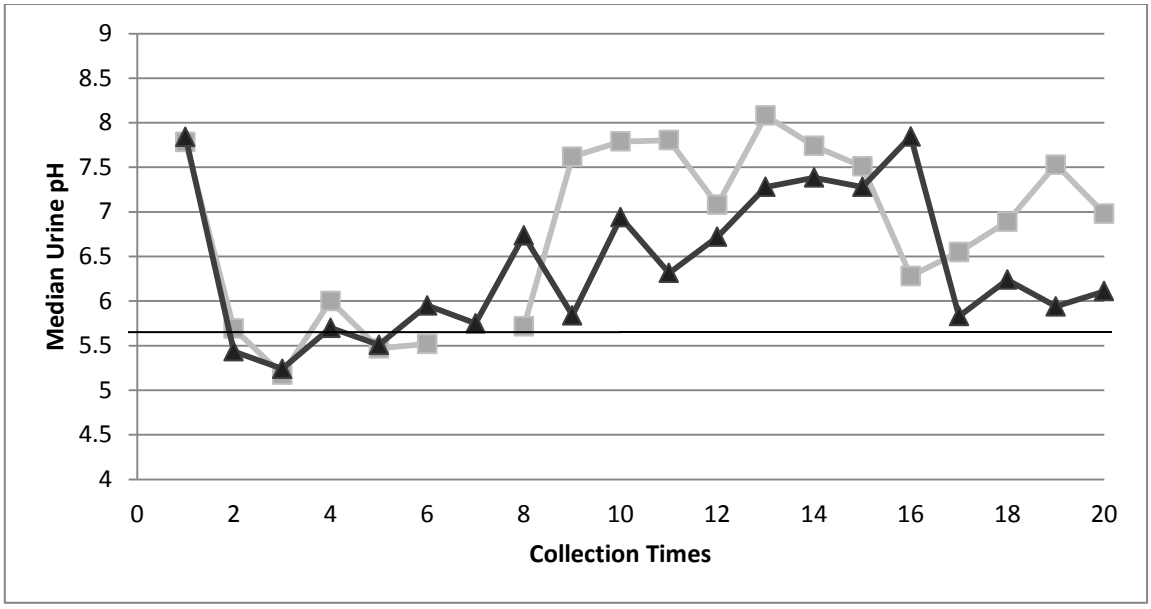

Figure 3-3. Median urine pH for Group 1 and Group 2 goats (n = 5 per group) during the continuous treatment regimen. Group 1 (squares) and Group 2 (triangles) correspond to each collection time for the 20 treatment times. The darker horizontal line represents the target urine pH (pH <6.5).

Table 3-2. Median odds ratio and 95% CI for each pulse treatment period. Odds ratios for achieving urine pH below the target (pH<6.5) during treatment times compared to urine pH obtained during the non-treatment times of the pulse and continuous treatment regimens.

Odds Ratios	Median	95% CI
Pulse 1* vs. no treatment	4.10	2.20-7.45
Pulse 2† vs. no treatment	0.89	0.41-1.68
Pulse 3‡ vs. no treatment	3.09	1.59-5.62

- Pulse 1 = collection times 1-6
† Pulse 2 = collection times 15-20
‡ Pulse 3 = collection times 29-34

3.5 Effectiveness of Pulse Dose Treatment Regimen for Urine Acidification

Figure 3-4 displays the median urine pH for the duration of the pulse treatment regimen for goats in Groups 1 and 2. Appendix H shows the tabulated data. Median urine pH <6.5 was achieved by 36 hours in Pulse Treatment Periods 1 and 3. Seventy % and 60% of goats in Pulse Treatment Periods 1 and 3 achieved urine pH <6.5 by 36 hours respectively. Median urine pH <6.5 was not achieved during Pulse Treatment Period 2 at any time. Table 3-2 shows the odds ratios for the titrated ammonium chloride dose in achieving urine pH below the target for the duration of each pulse dose period. The odds ratios indicate that the titrated dose was significant (95% CI of the odds ratio >1) in producing target urine pH for Pulse Treatment pPriods 1 and 3, but not 2.

Table 3-3 also shows that Pulse Treatments 1 and 3 had higher odds ratios for achieving target urine pH compared to Pulse Treatment 2. Figure 3-5 shows the median pH plotted for Group 1 and Group 2 goats. Only 40% of goats produced urine below the target at any time during all 3 pulse treatment periods, and all were from Group 2. The lowest pH achieved for Pulse Treatment Times 1-3 were 4.87, 5.27 and 5.37 respectively.

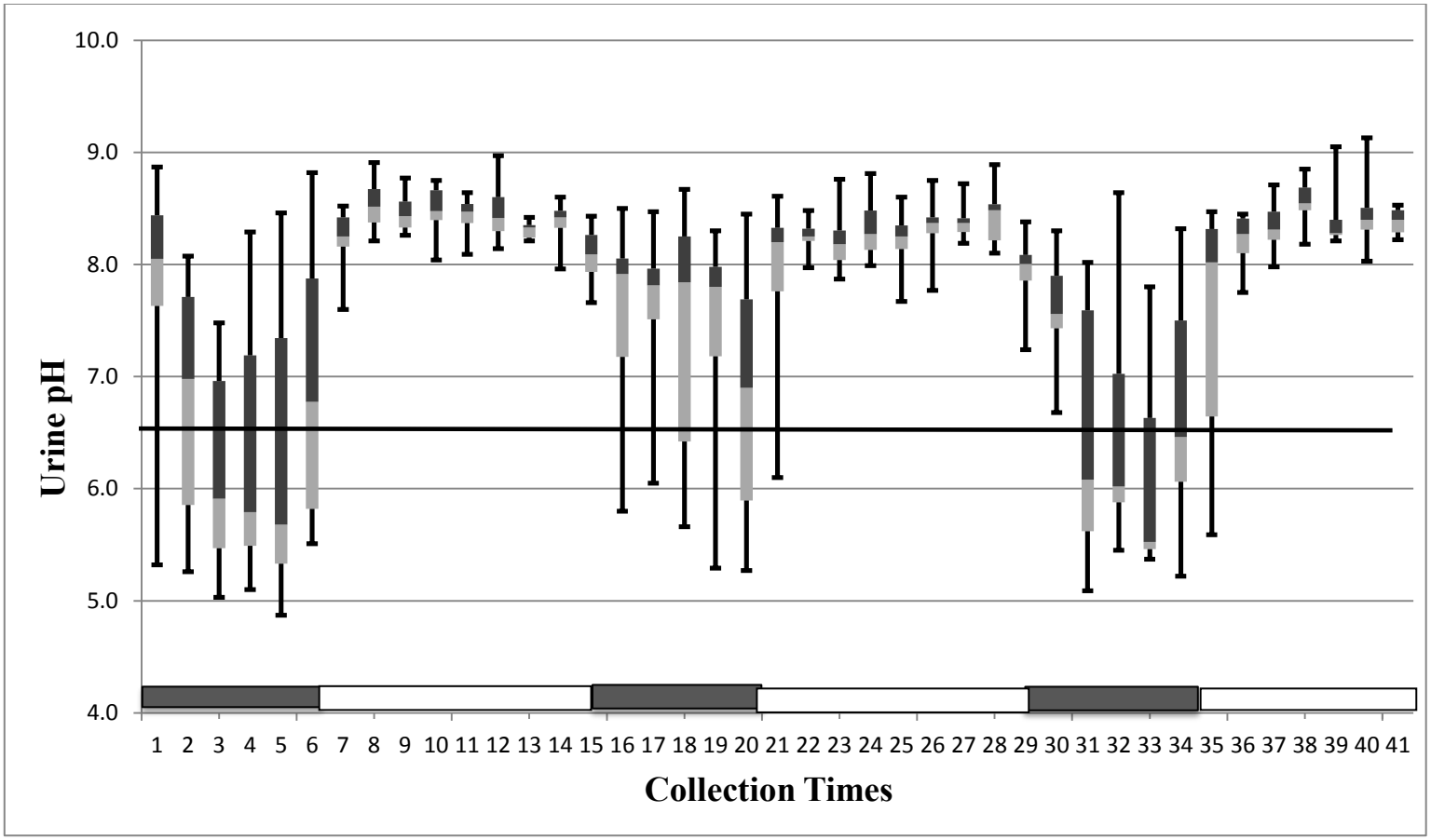

Figure 3-4. Box and Whisker plot of urine pH for all goats during the pulse dose treatment regimen. The median, interquartile range, and maximum and minimum urine pH are presented. The darker horizontal line represents the target urine pH level (<6.5). The grey boxes on the x axis represent treatment times, white boxes represent non-treatment times. N=10.

Table 3-3. Median odds ratio and 95% CI for comparison of all 3 pulse treatment regimens.

Odds Ratios	Median	95% CI
Pulse 1* vs. Pulse 2†	4.66	2.14-11.41
Pulse 1* vs. Pulse 3‡	1.33	0.63-2.90
Pulse 3‡ vs. Pulse 2†	3.44	1.51-8.48

* Pulse 1 = collection times 1-6
† Pulse 2 = collection times 15-20
‡ Pulse 3 = collection times 29-34

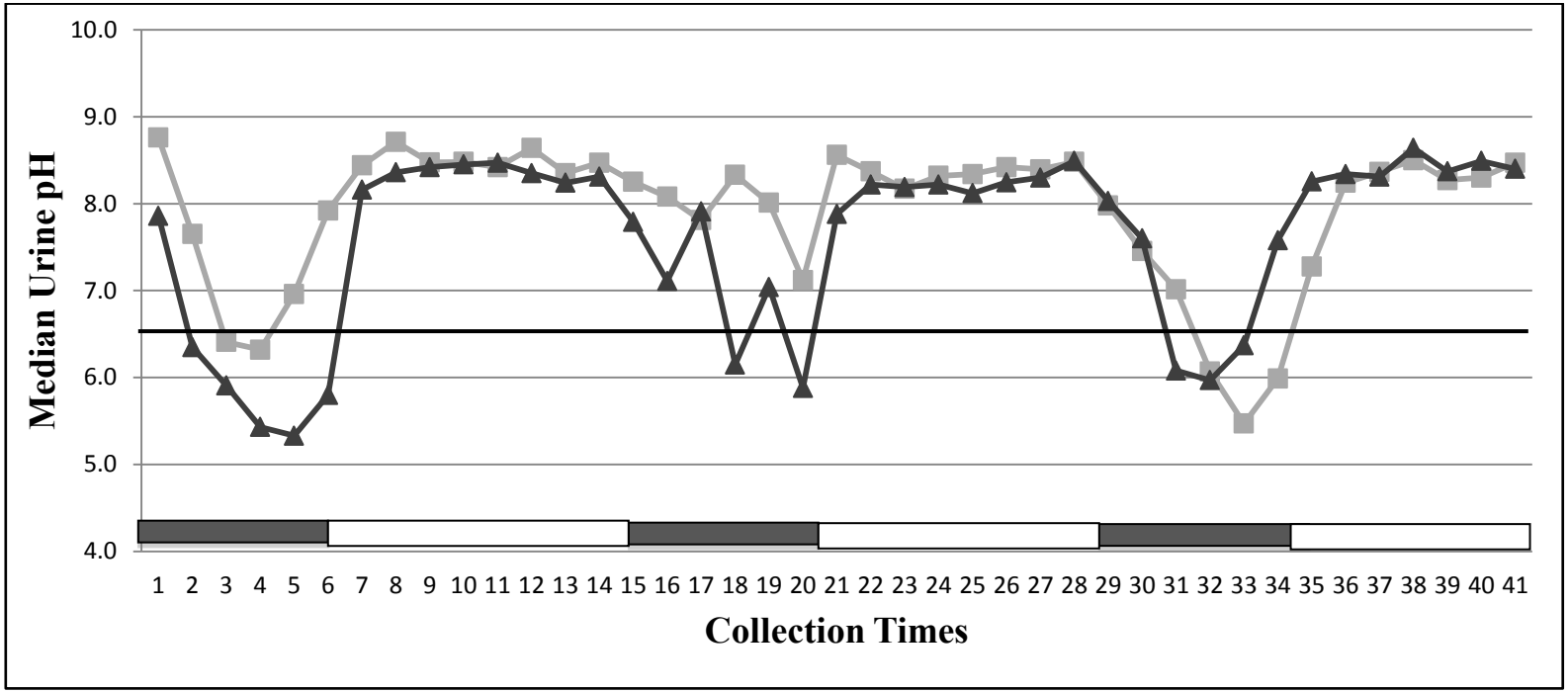

Figure 3-5. Median urine pH for each treatment time for goats (n= 5 per group) in Group 1(grey squares) and Group 2 (black triangles) during the pulse dose regimen. The darker horizontal line represents the target urine pH (<6.5). The grey boxes on the x axis represent treatment times, white boxes represent non-treatment times.

4. DISCUSSION

Low levels of ammonium chloride are routinely added to commercially available goat feed at low levels as a preventative against urolithiasis. As discussed earlier, the proposed preventative mechanism is through urine acidification. The results of this study showed that a urine pH <6.5 was not produced during 7 days of feeding a concentrate feed with 0.007% ammonium chloride added. Struvite stones have been shown to dissolve at a pH <6.5;[54-56] therefore, the inability of low dose ammonium chloride in feed to produce a pH below this level indicates that it would be ineffective in preventing struvite urolith formation by lowering the urine pH. Another preventative mechanism is increased water consumption, and production of dilute urine, to reduce urinary mineral supersaturation.[9,46,53] Water intake and urine production were not measured in this study.

In this study, titration of an ammonium chloride dose to produce a urine pH <6.5 by 3 days (6 collection times) was necessary. The target time of 3 days for the urine to acidify <6.5 was chosen for clinical reasons. In the treatment of urolithiasis, a rapid production of an acidic pH below which struvite stones dissolve is desirable to prevent re-obstruction. At the individual titrated dose, median urine pH for all goats was below the target level by 24 hours in the continuous treatment regimen, and 36 hours for Pulse Treatment Periods 1 and 3 in the pulse dose regimen.

Ammonium chloride mixed with corn syrup was administered by mouth to facilitate ease of administration and prevent palatability issues that have been recognized

with addition of ammonium chloride directly to feed.[9] Goats in this study tolerated oral doing, which differed from other studies.[47]

The necessity for titration of dosage, and the wide ranges of urine pH observed during treatment times, as compared to urine pH during non-treatment times, indicated that there was variability between goats in the individual response to ammonium chloride treatment. As all goats received the same diet in this study, the variability was unlikely to be a result of markedly differing basal DCAD.

The lowest urine pH produced during the continuous and pulse regimens was 4.59 and 4.87 respectively. Increased dissolution rates of struvite stones have been observed at lower pH.[54] Therefore production of low urine pH was considered clinically desirable. Production of low urine pH has been suggested to indicate uncompensated metabolic acidosis, as observed by decreased blood pH, which may result in lowered feed intakes.[41,48,60] Feed intakes and blood acid-base parameters were not measured in this study and therefore it is unknown whether an uncompensated metabolic acidosis was induced. In this study, pH <6.5 was only produced for short periods of time, especially during pulse dose treatment periods. The potential detrimental effects of decreased feed intakes were considered clinically insignificant.

Within 7 days, continuous ammonium chloride treatment was shown to be significantly ineffective in maintaining production of a urine pH <6.5, as the odds ratio for producing urine <6.5 was <1 during this time period. These results indicate that, similar to other studies in goats, there was a response of re-alkalinization during continuous treatment.[46,48] This effect of re-alkalinization of urine indicates that within 7

days, ammonium chloride may no longer effective in treatment of urolithiasis by dissolution of calculogenic material, or in prevention by creating an acidic environment in which struvite stones cannot form. The mechanism for re-alkalinization is thought to be a physiological response to the metabolic acidosis. Theories have included production of ammonia via metabolism of glutamine[37], bone metabolism[64] or alteration of intestinal electrolyte transport.[68,69] This study did not investigate the physiology underlying this re-alkalinization effect.

Following the re-alkalinization, the urine appeared to re-acidify with median urine pH decreasing to <6.5 at Collection Time 17. This effect has not previously been recognized, but may suggest that there is a cyclical nature of urine pH in response to continuous ammonium chloride treatment. Continuous treatment was not continued beyond 20 treatments. The effect of long-term effect of ammonium chloride treatment on urine pH is unknown and is an area that warrants further study.

After discontinuation of treatment during both continuous and pulse regimens, median urine pH was above the target range by 24 hours after the last treatment was administered. This shows that ammonium chloride did not have a persistent effect on urine pH.

In order to prevent the re-alkalinization that has been recognized clinically with continuous ammonium chloride treatment, pulse dosing has been used. This study showed that in 2 of 3 sequential pulse dose treatment periods production of urine below the target level occurred. Pulse Treatment Period 2 did not result in a statistically significant reduction in urine pH to below the target level. The only goats to produce

urine <6.5 in all 3 pulse periods were in Group 2. These goats received the pulse dose ammonium chloride regimen first. This finding suggests that there was an effect of group on urine pH. External factors, such as changes in feed or forage batch, ambient temperature or personnel may have resulted in variation of base DCAD or differences in the effectiveness of ammonium chloride administration. Alternatively, there may be an effect of treatment order, suggesting the washout period was not long enough to eliminate any physiological compensation that occurred. Goats in Group 2 also received higher concentrations of ammonium chloride, therefore individual variation and ammonium chloride dosage may have resulted in the variability observed between groups. Pulsed administration of the titrated dosages resulted in urine pH of <6.5 in 40% of the goats. This indicates that the pulse dose treatment regimen can be clinically effective for the prevention and long-term treatment of urolithiasis.

Struvite stones have been shown to dissolve in acidic urine, both in vitro and *in vivo* in rats.[54-56] The length of time necessary to dissolve struvite stones in goats, using acidified urine and naturally obtained uroliths, is unknown. Clinically, pulse dosing is used in obstructive urolithiasis cases following relief of the acute obstruction. It is currently unknown whether lowering urine pH to <6.5 for short periods of time during the pulse dosing periods, would be effective in dissolving remaining stones. This is an area that warrants further study.

Long term administration of ammonium chloride has been associated with bone reabsorption and metabolism,[61] which has raised concerns with decreased bone density predisposing to fractures, osteopenia and osteoporosis.[46] Ammonium chloride also

results in increased renal excretion of calcium[46,47] which may increase the risk for calcium carbonate type urolith formation.[65] Pulse dose treatment regimens that only results in short term urinary acidification may be of use in preventing these effects clinically in goats.

Urine was collected up to 1 hour after placement of the cup under the prepuce. The large numbers (9.2%) of missing data points were largely due to failure to collect urine within this timeframe and was a limitation of this study. Alternative collection methods or extending the duration of collection may prevent this in future studies. Urine was collected twice a day, approximately 12 hours after feeding and treatment. Treatment was given twice a day in order to administer the high dosage of ammonium chloride required to produce urine with a pH <6.5 within 3 days, and this is a common regimen used clinically. It has previously been shown that urine collection 5-7 hours after treatment best represented the daily mean urine pH.[48] Due to the twice daily treatment administration, this sampling time was not feasible in this study. Another limitation of this study was the small sample size which influenced the variability in urine pH during treatment with ammonium chloride.

Bayesian statistical methods were used for this study due to the author's opinion that Bayesian results are easily integrated into the clinical decision making process. Clinical decision making is typically based on the prior beliefs and experiences of the clinician and the data presented by the patient under consideration. When presented with a urolithiasis patient whose signalment and nutritional history is similar to this study population, the clinician will be able to use the results of this study to directly

update their prior beliefs on the use and effectiveness of ammonium chloride and arrive at a clinical decision more likely to produce the desired result. Bayesian methods were also applicable to this study as they have advantages for use in complex models and small sample sizes.

5. SUMMARY AND CONCLUSIONS

There are several clinically relevant conclusions that can be drawn from this study. First, this study showed that individual titration of dosage is necessary to produce a urine pH <6.5. This study also showed that re-alkalinization of urine pH does occur in goats receiving continuous ammonium chloride treatment. In this study, continual ammonium chloride treatment was not effective beyond Day 6.5. The pulse dose regimen was effective in producing a urine pH below the target range for short periods. Therefore, pulse dose regimen of ammonium chloride can maintain effectiveness at production of acidic urine pH <6.5 for long term treatment or prevention.

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APPENDIX A

STUDY DESIGN

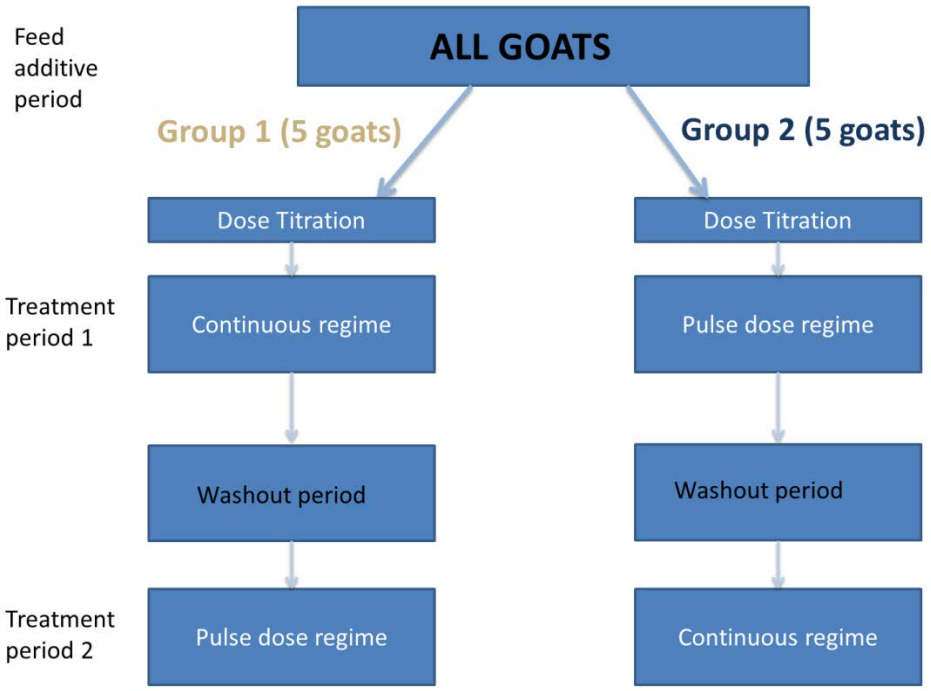

Figure A-1. Study design flow chart.

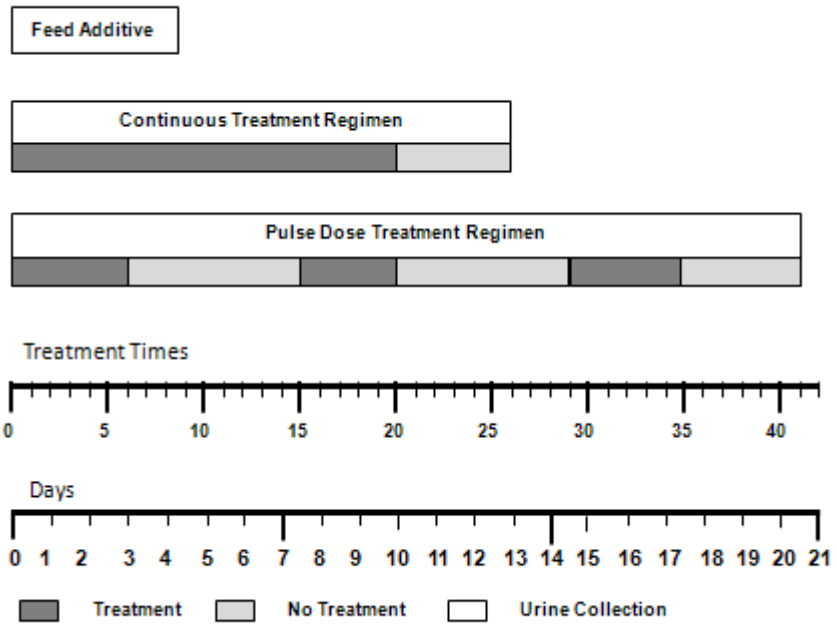

Figure A-2. Timeline for each treatment regimen.

APPENDIX B

FEED ANALYSIS

Table B-1. Alfalfa hay analysis (wet chemistry method).[5]

Component	Unit	As Fed	Dry Matter
Moisture	%	7.8	
Dry Matter	%	92.2	
Crude Protein	%	11.4	12.3
Adjusted Crude Protein	%	11.4	12.3
Acid Detergent Fiber	%	34.1	37
Neutral Detergent Fiber	%	46.6	50.5
NFC	%	27.4	29.7
TDN	%	55	60
NEL	Mcal/kg	1.19	1.26
NEM	Mcal/kg	1.13	1.23
NEG	Mcal/kg	0.61	0.66
Calcium	%	1.56	1.11
Phosphorus	%	0.19	0.17
Magnesium	%	0.43	0.2
Potassium	%	1.54	0.47
Sodium	%	0.179	1.67
Iron	Ppm	151	0.19
Zinc	Ppm	23	163
Copper	Ppm	9	25
Manganese	Ppm	39	10
Molybdenum	Ppm	1.2	43
Sulfur	%	0.26	1.3
Chloride	%	0.93	0.28

[5] Dairy One. Cornell University. Ithaca, NY

Table B-2. Concentrate feed analysis (wet chemistry method).[6]

Component	Unit	As Fed	DM
Moisture	%	10.6	
Dry Matter	%	89.4	
Crude Protein	%	13.6	18.5
Adjusted Crude Protein	%	16.6	18.5
Acid Detergent Fiber	%	15.1	16.9
Neutral Detergent Fiber	%	28.8	32.2
TDN	%	69	77
NEL	Mcal/kg	1.62	1.81
NEM	Mcal/kg	1.67	1.87
NEG	Mcal/kg	1.1	1.23
Calcium	%	1.04	1.17
Phosphorus	%	0.4	0.45
Magnesium	%	0.23	0.26
Potassium	%	1.1	1.23
Sodium	%	0.437	0.489
Iron	ppm	3.9	346
Zinc	ppm	129	144
Copper	ppm	30	33
Manganese	ppm	93	104
Molybdenum	ppm	1.2	1.3
Sulfur	%	0.21	0.24
Chloride	%	1.06	1.19

Calcium: phosphorus ratio = 2.5:1

[6] Dairy One. Cornell University. Ithaca, NY

Table B-3. Feed label guaranteed analysis.

	Minimum	**Maximum**
Crude Protein	16%	
Crude Fat	3%	
Crude Fiber		14%
Calcium	0.6%	0.9%
Phosphorus	0.45%	
Salt	0.8%	1.1%
Copper	15ppm	20ppm
Selenium	0.3ppm	
Vitamin A	12,000 IU/lb	
Monensin	20g/ton	
Ammonium chloride	14lb/ton	

APPENDIX C

DIETARY CATION ANION DIFFERENCE OF RATION

DCAD equations used:

$[(Na^{+} + K^{+}) – (Cl^{-} + S^{--})]$

Using conversion factor from % to mEq/kg

[(sodium x 435)+(potassium x 256)] - [(chloride x 282)+(sulfur x 624)]

As fed 70% dry matter concentrate, 30% dry matter alfalfa.

Table C-1. DCAD of feed.[7]

	Na (%)	**K (%)**	**Cl (%)**	**S (%)**	**DCAD of Feed (mEq/kg DM)**	**% of Diet DM**	**DCAD of Diet (mEq/kg DM)**
Alfalfa	0.194	1.67	1.01	0.28	52.37	30	15.71
Concentrate	0.489	1.23	1.19	0.24	41.96	70	29.36

Total DCAD of Diet 45 mEq/kg DM

[7] Arm & Hammer Animal Nutrition. DCAD Calculator. http://ahdairy.com/our-products/dcad-balancers/dcad-calculator.aspx . Accessed on 03/22/12.

APPENDIX D

RATION ANALYSIS

Mean goat weight used 85 lbs. Ration designed to provide 2.5% body weight in dry matter. Ration balance 70% concentrate, 30% forage.[8]

Table D-1. Ration analysis.

Feed	Lb/day	% in diet
Producers Show Goat Feed	2lb	70
Alfalfa Hay	0.85 lb	30

[8] Aries Feed Ration Analysis. UC-Davis, CA

Table D-2. Ration analysis of nutritional content

Nutrient	Amount
DM	90.1% / 2.55lb
TDN	69.97% / 1.78lb
DE	1.44 Mcal/lb
ME	1.26 Mcal/lb
NEG	0.77 Mcal/lb
CP	17.68% / 0.45 lb
Ca	1.60% / 0.041 lb
P	0.36% / 0.009 lb
DIP	22.00 % / 0.56 lb
ADF	22.95% / 0.59 lb
NDF	35.53 % / 0.91 lb
CF	24.39 % / 0.62 lb
LIGN	2.78% / 0.071 lb
ASH	3.47% / 0.089 lb
Cl	0.39% / 0.01 lb
Mg	0.166% / 0.004 lb
Na	0.34 % / 0.009 lb
K	1.11% / 0.028 lb
S	0.064% / 0.002 lb
Cu	22.91%
Fe	240.27 %

APPENDIX E

AMMONIUM CHLORIDE AS A FEED ADDITIVE

Table E-1. Daily urine pH and median pH for individual goats during feed additive period.

	COLLECTION TIME	**1**	**2**	**3**	**4**	**5**	**6**	**7**
GOAT	GROUP							
1	1	8.14		8.31	8.41	8.42	8.45	
2	1	8.30		8.35	8.45	8.44	8.64	
3	1		8.36	8.46		8.28	8.31	8.34
4	1	7.68	7.38	8.09	7.90	8.08	7.92	7.79
5	1	8.08	8.29	8.27		8.47	8.14	
6	2	8.66		8.46	8.58	8.53	8.5	8.52
7	2	8.36	8.50		8.39	8.23	8.32	8.28
8	2	8.21	8.46	8.46	8.39	8.75	8.31	8.32
9	2	8.33	8.28	8.4	8.30	8.35	8.42	8.52
10	2	8.43	8.44	8.29	8.73	8.30	8.46	7.29

APPENDIX F

TITRATION OF AMMONIUM CHLORIDE DOSE

Week 1: all goats in Group 2 received 200mg/kg ammonium chloride twice a day.

Week 2: all goats in Group 2 received 250mg/kg ammonium chloride twice a day. Pulse period 1 for Goats 6-9.

Week 3: Goats 6-9 received 250mg/kg ammonium chloride twice a day, Goat 10 received 300mg/kg ammonium chloride twice a day. Pulse Period 2 for goats 6-9), Pulse Period 1 for Goat 10.

Week 4: Goats 6-9 received 250mg/kg ammonium chloride twice a day, Goat 10 received 300mg/kg ammonium chloride twice a day. Pulse Period 3 for Goats 6-9, Pulse Period 2 for Goat 10.

Week 5: Goats 6-9 received no treatment (washout period). Pulse Period 3 for Goat 10

Table F-1. Titration of dosage for goats in Group 2 during Treatment Period 1 (pulse dose regimen).

			Goat	6	7	8	9	10
Week	**Day**	**Collection Time of Day**	**Dose mg/kg**					
1	1	1	200	8.39	8.08	8.33	8.10	8.21
		2	200	7.98	7.67	7.87	7.49	7.90
	2	1	200	7.88	7.66	7.65	7.29	7.09
		2	200	7.69	8.02	7.44	7.45	7.72
	3	1	200	8.20		8.08		7.84
		2	200		8.23	8.30	7.25	8.19
	4	1		7.63	7.73	8.00		6.97
		2						
	5	1			8.14	8.12	8.53	8.41
		2		8.41	8.34	8.21	8.38	8.48
	6	1		8.07	8.78	8.23	8.31	8.51
		2		8.40	8.28	8.23		8.36
	7	1			8.39	8.33		8.34
		2				8.48	8.40	8.30
2	8	1	250	8.74	8.33	8.40		8.76
		2	250		5.32	8.05	8.12	7.86
	9	1	250	5.69	5.48	7.26	6.35	7.12
		2	250	5.47	5.03	5.91	6.8	
	10	1	250	5.41	5.10	5.43	5.75	8.15
		2	250	5.33	4.87	5.33	5.36	7.87
	11	1		5.76	5.80	6.05	7.50	7.14
		2		7.60	8.25	8.18	8.16	8.52
	12	1			8.61	8.39	8.33	8.31
		2		8.56	8.77	8.33	8.26	
	13	1		8.75	8.74	8.45	8.35	7.04
		2		8.64	8.54	8.37	8.47	8.39
	14	1		8.27	8.43	8.35	8.40	8.33
		2			8.21	8.27	8.33	8.32

Table F-1 continued.

			Goat	6	7	8	9	10
Week	**Day**	**Collection Time of Day**	**Dose mg/kg**					
3	15	1	250/300	8.26	8.31	8.54	7.96	8.51
		2	250/300		7.98	7.79	7.66	7.67
	16	1	250/300	5.80	7.37	7.9	7.01	7.73
		2	250/300	7.98	7.42	7.91	8.23	7.48
	17	1	250/300	6.02	7.76	6.15	7.92	8.29
		2	250/300	6.25	7.04	7.61	7.87	7.47
	18	1		5.27	5.54	5.94	7.88	5.51
		2		7.40	6.10	8.22	8.18	8.02
	19	1		8.26	8.25	8.22	8.19	8.21
		2		8.19	8.34	7.87	8.76	8.42
	20	1		8.22	8.81	7.99	8.22	8.04
		2		8.30	8.12	7.67	7.92	8.09
	21	1			7.77	8.16	8.37	8.14
		2		8.37	8.23		8.19	8.21
4	22	1	250/300	8.49	8.69	8.10	8.53	8.40
		2	250/300	8.38	7.85	8.10	8.03	
	23	1	250/300	7.90	8.30	7.60	7.56	7.11
		2	250/300	5.62	7.59	6.24	6.08	6.05
	24	1	250/300	5.87	8.64	5.77	6.08	5.66
		2	250/300		7.80		6.37	5.29
	25	1		7.58	8.32	7.96	6.28	5.88
		2			8.31	8.20	8.34	7.88
	26	1			8.27	8.43	8.41	8.21
		2		8.58	8.37	8.31	8.22	8.10
	27	1		8.85	8.70	8.53	8.64	8.50
		2		9.05	8.41	8.37	8.24	8.35
	28	1		8.51	9.13	8.49	8.40	8.33
		2		8.40	8.50	8.40	8.31	8.41

Table F-1 continued.

.

			Goat	6	7	8	9	10
Week	**Day**	**Collection Time of Day**	**Dose mg/kg**					
5	29	1	300	-	-	-	-	8.15
		2	300	-	-	-	-	7.24
	30	1	300	-	-	-	-	6.68
		2	300	-	-	-	-	5.09
	31	1	300	-	-	-	-	5.97
		2	300	-	-	-	-	5.48
	32	1		-	-	-	-	6.41
		2		-	-	-	-	6.45
	32	1		-	-	-	-	7.75
		2		-	-	-	-	7.98
	34	1		-	-	-	-	8.30
		2		-	-	-	-	8.21
	35	1		-	-	-	-	8.34
		2		-	-	-	-	8.24

APPENDIX G

CONTINUOUS TREATMENT REGIMEN DATA

Table G-1. Individual urine pH for continuous treatment regimen.

GOAT	1	2	3	4	5	6	7	8	9	10	Median	Q 1	Q 3	Min	Max
GROUP	1	1	1	1	1	2	2	2	2	2					
COLLECTION TIME															
1		8.13	6.33	8.3	7.44		8.02	7.74	7.47	7.94	7.84	7.46	8.05	6.33	8.30
2	7.61	4.59	5.95	5.69	4.96	5.38		5.32	5.54	5.49	5.49	5.32	5.69	4.59	7.61
3		8.02	4.82	5.44	4.91	5.12	8.51	5.10	5.36	5.24	5.24	5.10	5.44	4.82	8.51
4	7.70	5.47	6.64	6.00	5.02	5.38	8.03	5.19	5.7	6.1	5.85	5.4	6.51	5.02	8.03
5	4.82	5.47	5.74	5.65	4.86	5.25	8.17	5.46	5.51	5.53	5.49	5.3	5.62	4.82	8.17
6	4.94	6.53	6.37	5.49	5.52	5.56	8.05	5.61	5.98	5.95	5.78	5.53	6.27	4.94	8.05
7						6.11	8.06	5.75	5.73	5.57	5.75	5.73	6.11	5.57	8.06
8	5.41	5.72	8.59	5.71		6.26	7.63	6.56	6.91		6.41	5.72	7.09	5.41	8.59
9	6.02	7.62	8.27	6.12	8.11	5.84	6.27	5.75	6.24	5.69	6.18	5.89	7.28	5.69	8.27
10	7.79	7.94	7.9	5.89	6.85	6.38	8.40	6.94	5.97	7.16	7.05	6.5	7.87	5.89	8.40
11		7.93	7.78	5.61	7.83	6.93	6.20	6.41	6.22		6.67	6.21	7.8	5.61	7.93
12	7.08	7.96	7.35	5.89	6.49	6.25	8.14	6.99	6.72	5.75	6.90	6.31	7.28	5.75	8.14
13	8.53	8.47	8.08	5.84	7.74	6.37	7.94	6.59	8.57	7.28	7.84	6.76	8.37	5.84	8.57
14	7.56	8.38	8.01	6.45	7.74	6.88	8.41	6.70	7.89		7.74	6.88	8.01	6.45	8.41
15	7.51	7.48	7.75	5.63	7.65	7.28	8.15	6.22	8.01	5.62	7.50	6.49	7.73	5.62	8.15

Table G-1 continued.

GOAT	1	2	3	4	5	6	7	8	9	10	Median	Q 1	Q 3	Min	Max
GROUP	1	1	1	1	1	2	2	2	2	2					
COLLECTION TIME															
19	5.35	7.53	7.78	6.11	7.81	5.94	4.97	6.36	5.58	7.45	6.24	5.67	7.51	4.97	7.81
20	6.72	6.98	7.88	5.76	7.86	6.37	5.09	6.49	5.85		6.49	5.85	6.98	5.09	7.88
21	8.49	8.25	8.73	6.85	8.71			7.64	5.92		8.25	7.25	8.6	5.92	8.73
22		8.64	8.48	8.47	8.67	8.49		8.36	6.17		8.48	8.42	8.57	6.17	8.67
23	8.50	8.67	8.68	8.36	8.34		8.49	8.46	6.18	8.08	8.46	8.34	8.5	6.18	8.68
24	8.59	8.80	8.51	8.43	8.14	8.54	8.38	8.57	8.08		8.51	8.38	8.57	8.08	8.80
25	8.44	8.32	8.31	8.58	8.30					8.18	8.32	8.3	8.41	8.18	8.58
26	8.23	8.41	8.22	7.80	8.14	8.77	8.50	8.24	8.35		8.24	8.22	8.41	7.80	8.77
27	8.52	8.42	8.73	8.15	8.61	8.38	8.44	8.38			8.42	8.38	8.52	8.15	8.73

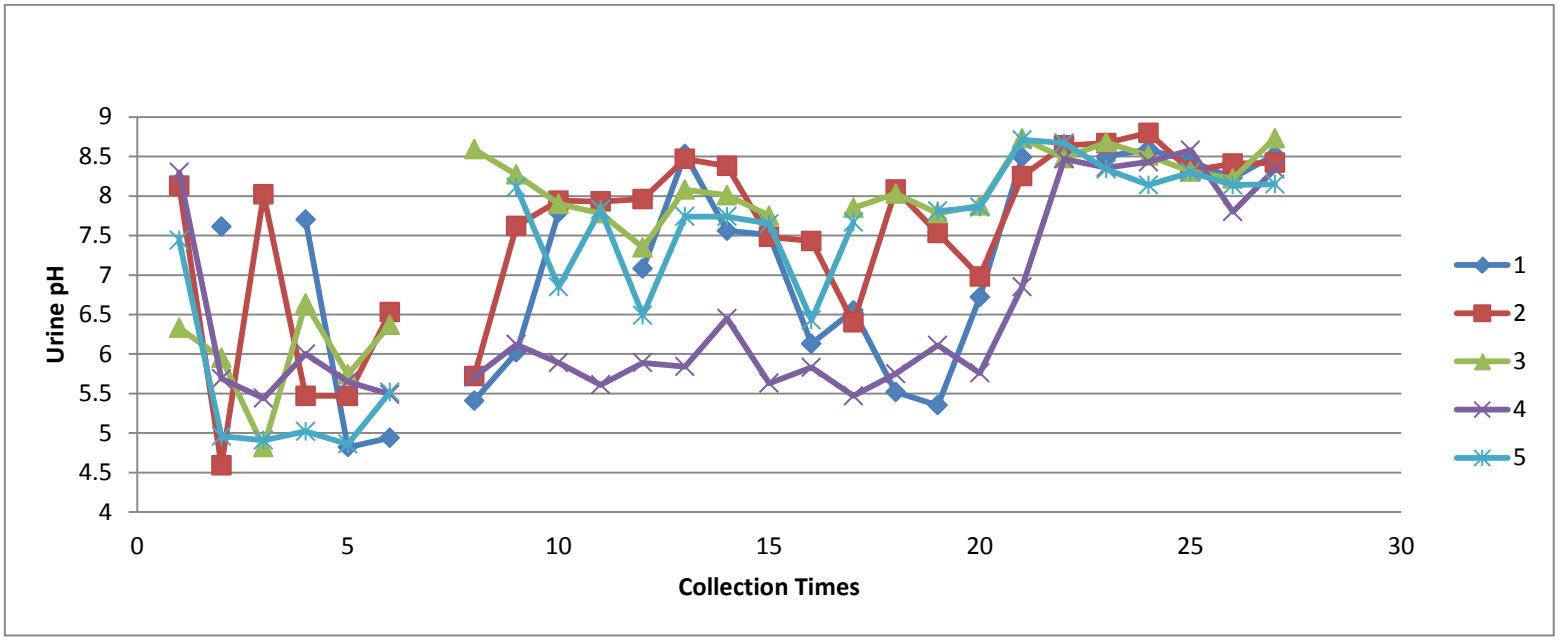

Figure G-1. Individual urine pH for goats in Group 1 during the continuous treatment regimen. Individual goats are indicated in the key.

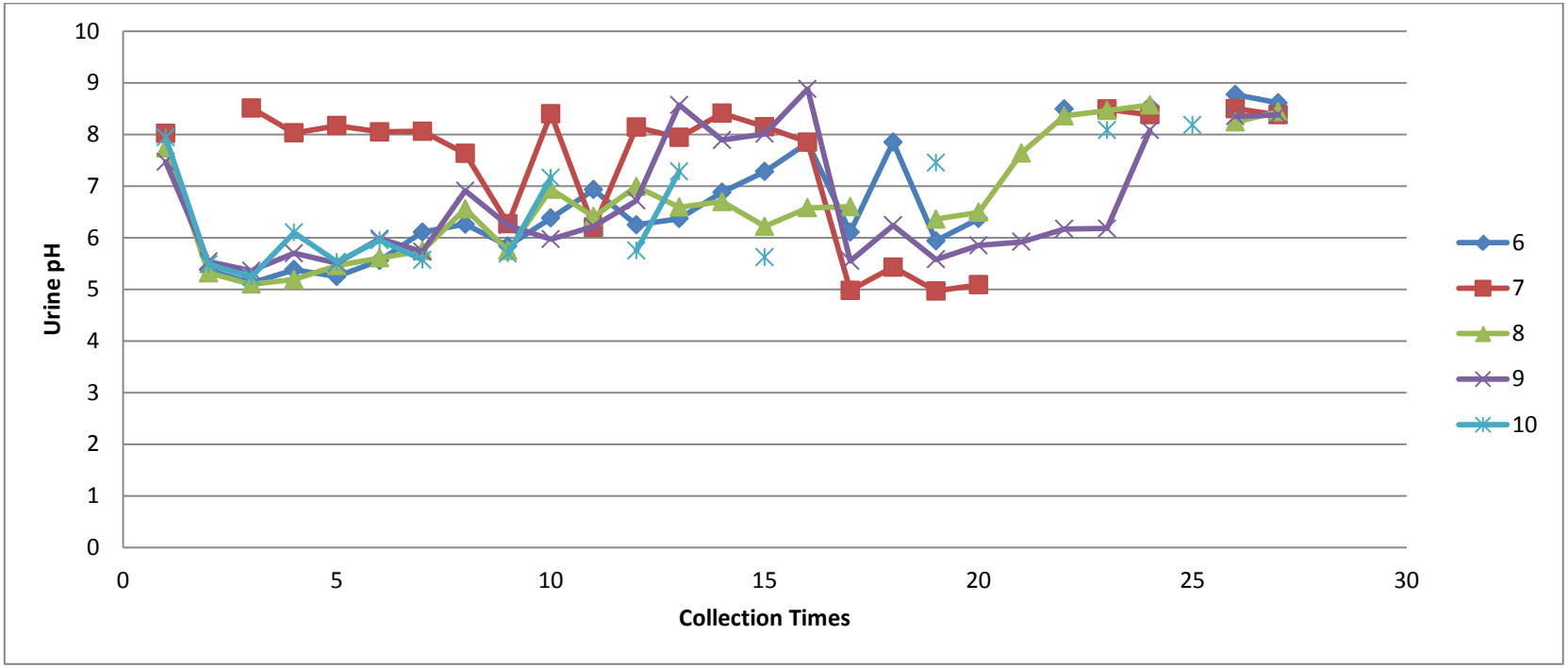

Figure G-2. Individual urine pH for goats in Group 2 during the continuous treatment regimen. Individual goats are indicated in the key.

APPENDIX H

PULSE DOSE TREATMENT REGIMEN DATA

Table H-1. Individual pH during pulse dose treatment regimen

Goat	1	2	3	4	5	6	7	8	9	10	Median	Q1	Q3	Min	Max
Group															
Collection Tine	1	1	1	1	1	2	2	2	2	2					
1			8.76	8.87	7.59		5.32	8.05	8.12	7.67	8.05	7.63	8.44	5.32	8.87
2	5.26	6.70	7.96	8.07	7.65	5.69	5.48	7.26	6.35	7.73	6.98	5.86	7.71	5.26	8.07
3	5.05		5.86	7.31	6.96	5.47	5.03	5.91	6.80	7.48	5.91	5.47	6.96	5.03	7.48
4	5.83	5.67	7.83	6.32	7.48	5.41	5.10	5.43	5.75	8.29	5.79	5.49	7.19	5.10	8.29
5	5.20	6.96	8.46	6.00	8.01	5.33	4.87	5.33	5.36	7.47	5.68	5.33	7.34	4.87	8.46
6	5.88	7.92	8.16	8.82	7.74	5.76	5.8	6.05	7.50	5.51	6.78	5.82	7.88	5.51	8.82
7	8.36		8.52	8.46	8.42	7.60	8.25	8.18	8.16	8.02	8.25	8.16	8.42	7.60	8.52
8	8.91	8.47	8.86	8.56			8.61	8.39	8.33	8.21	8.52	8.38	8.67	8.21	8.91
9		8.76	8.28	8.52	8.43	8.56	8.77	8.33	8.26	8.42	8.43	8.33	8.56	8.26	8.77
10	8.48	8.49	8.72	8.47	8.38	8.75	8.74	8.45	8.35	8.04	8.48	8.4	8.66	8.04	8.75
11	8.37		8.54	8.47	8.24	8.64	8.54	8.37	8.47	8.09	8.47	8.37	8.54	8.09	8.64
12	8.48	8.73	8.64	8.97	8.28	8.27	8.43	8.35	8.4	8.14	8.42	8.30	8.6	8.14	8.97
13	8.35	8.42	8.24	8.35	8.35		8.21	8.27	8.33	8.21	8.33	8.24	8.35	8.21	8.42
14	8.47	8.60	8.48	8.44	8.38	8.26	8.31	8.54	7.96	8.40	8.42	8.33	8.48	7.96	8.60
15	8.03	8.15	8.30	8.25	8.43		7.98	7.79	7.66		8.09	7.93	8.26	7.66	8.43

Table H-1 continued.

Goat	1	2	3	4	5	6	7	8	9	10	Median	Q1	Q2	Min	Max
Group	1	1	1	1	1	2	2	2	2	2					
Collection Time															
16	7.93	8.12	8.50	8.08	7.97	5.80	7.37	7.90	7.01	7.11	7.92	7.18	8.05	5.80	8.50
17	6.96	7.82	8.47	7.81	7.78	7.98	7.42	7.91	8.23	6.05	7.82	7.51	7.96	6.05	8.47
18	8.33	8.44	8.67	7.23	8.01	6.02	7.76	6.15	7.92	5.66	7.84	6.42	8.25	5.66	8.67
19	7.73	8.01	8.30	8.18	7.88	6.25	7.04	7.61	7.87	5.29	7.80	7.18	8.00	5.29	8.30
20	7.12	6.94	8.36	8.45	6.86	5.27	5.54	5.94	7.88	5.88	6.90	5.90	7.69	5.27	8.45
21			8.56	8.61	8.25	7.40	6.10	8.22	8.18	7.88	8.20	7.76	8.33	6.10	8.61
22	8.32		8.48	8.42	7.97	8.26	8.25	8.22	8.19	8.21	8.25	8.21	8.32	7.97	8.48
23	8.19	8.17	8.02	8.57	8.01	8.19	8.34	7.87	8.76	8.10	8.18	8.04	8.30	7.87	8.76
24	8.32	8.48	8.08	8.48	8.10	8.22	8.81	7.99	8.22	8.50	8.27	8.13	8.48	7.99	8.81
25	8.34	8.19	8.60	8.35	8.20	8.30	8.12	7.67	7.92	8.35	8.25	8.14	8.35	7.67	8.60
26	8.50	8.75	8.42	8.40	8.28		7.77	8.16	8.37	8.33	8.37	8.28	8.42	7.77	8.75
27		8.42	8.72	8.37	8.31	8.37	8.23		8.19	8.41	8.37	8.29	8.41	8.19	8.72
28	8.54	8.41	8.89	8.48	8.15	8.49	8.69	8.10	8.53	8.15	8.49	8.22	8.54	8.10	8.89
29	7.73	7.98	8.29	7.88	8.04	8.38	7.85	8.10	8.03	7.24	8.01	7.86	8.09	7.24	8.38
30		7.43	8.02	7.22	7.48	7.90	8.30	7.60	7.56	6.68	7.56	7.43	7.90	6.68	8.30
31	5.17		8.02	6.02	8.01	5.62	7.59	6.24	6.08	5.09	6.08	5.62	7.59	5.09	8.02

Table H-1 continued.

Goat	1	2	3	4	5	6	7	8	9	10	Median	Q1	Q2	Min	Max
Group	1	1	1	1	1	2	2	2	2	2					
Collection Time															
32	5.91	5.45	8.05	6.07	7.34	5.87	8.64	5.77	6.08	5.97	6.02	5.88	7.03	5.45	8.64
33	5.44	5.37	7.41	5.57	5.47		7.80		6.37	5.48	5.53	5.46	6.63	5.37	7.80
34	5.98	5.22	7.26	5.99	6.51	7.58	8.32	7.96	6.28	6.41	6.46	6.06	7.50	5.22	8.32
35		7.84	8.47	6.71	5.59		8.31	8.20	8.34	6.45	8.02	6.65	8.32	5.59	8.47
36	8.02	8.45	8.24	8.41	8.10		8.27	8.43	8.41	7.75	8.27	8.10	8.41	7.75	8.45
37		8.47	8.71	8.26	8.15	8.58	8.37	8.31	8.22	7.98	8.31	8.22	8.47	7.98	8.71
38	8.18	8.56	8.7	8.48	8.5	8.85	8.70	8.53	8.64	8.30	8.55	8.49	8.69	8.18	8.85
39	8.41	8.27	8.26	8.29	8.27	9.05	8.41	8.37	8.24	8.21	8.28	8.26	8.40	8.21	9.05
40	8.30	8.51	8.03	8.40	8.3	8.51	9.13	8.49	8.40	8.34	8.40	8.31	8.51	8.03	9.13
41	8.22	8.47	8.28	8.53	8.49	8.40	8.50	8.40	8.31	8.24	8.40	8.29	8.49	8.22	8.53

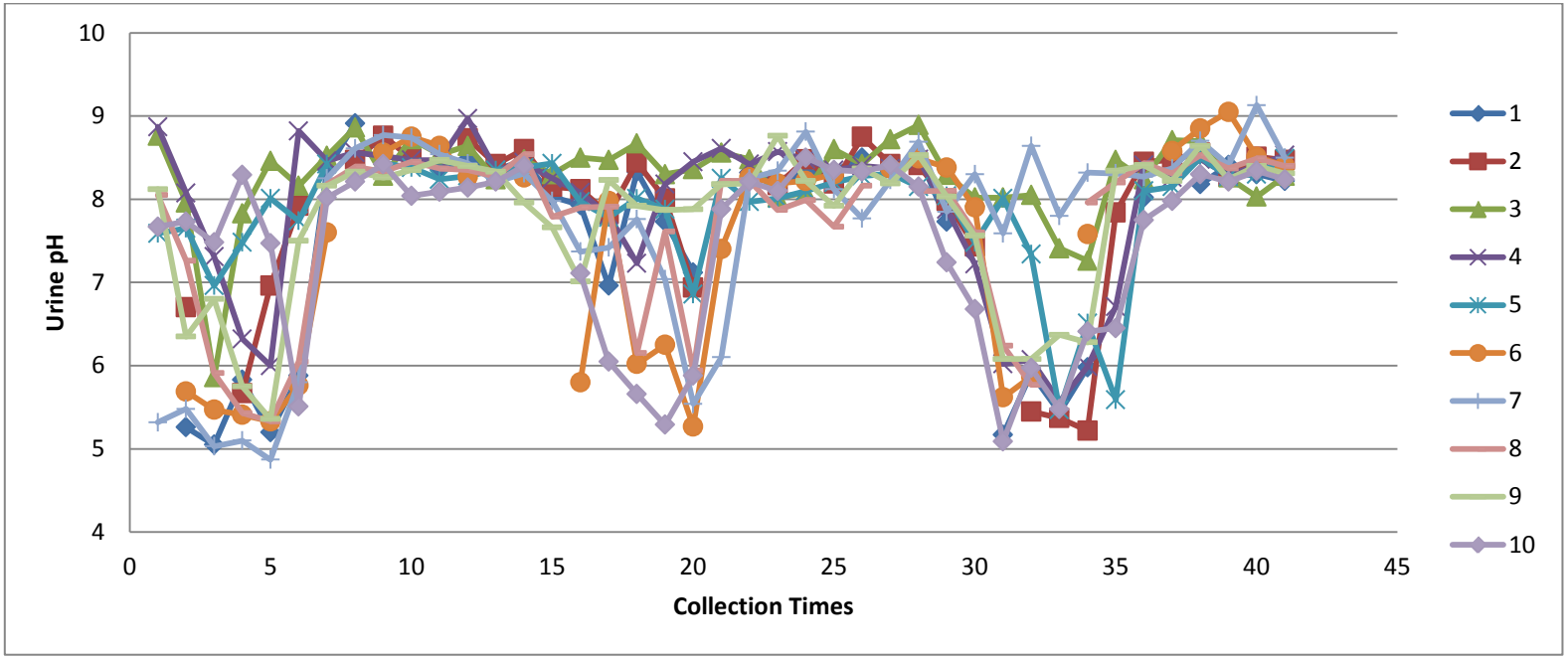

Figure I-1. Individual urine pH for goats during pulse dose treatment regimen. Individual goats are indicated in the key (n=10).

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