THE USE OF DIETARY CATION ANION DIFFERENCE FOR THE REDUCTION OF URINE PH IN GOATS

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THE USE OF DIETARY CATION ANION DIFFERENCE FOR THE REDUCTION OF URINE PH IN GOATS

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INTRODUCTION

Obstructive urolithiasis is considered to be the most economically significant urinary tract disease of food animals, affecting intact and castrated male ruminants, swine and camelids. Urolithiasis is a common disease of animals fed high grain rations, with the most common stone compositions in this setting being struvite (magnesium ammonium phosphate) and apatite (calcium phosphate).

Obstructive urolithiasis is reported to be the cause of morbidity in up to 20% of all feedlot wethers and was the fifth most common cause of death in two Colorado lamb feedlots. In the National Animal Health Monitoring System Sheep 2001 survey, 20% of all sheep operations reported at least one incident of urinary calculi in the previous three years and it was the fourth most reported disease entity. Additionally, affected animals are often breeding, show or pet animals with high monetary or emotional value.

The first chapter of this document reviews the anatomy unique to the male ruminant. The anatomy as it relates to the predisposition of these animals to obstructive urolithiasis, as well as its contribution to the challenge of case management, are also presented.

The second and third chapters present a review of the literature. The second chapter is a review of the pathophysiology and prevention of obstructive urolithiasis. The third chapter is a review of dietary cation anion difference (DCAD). The strong ion difference theory, the basis of DCAD, is presented, along with a definition and brief
review of the traditional use of DCAD. Reports involving use of DCAD in small ruminants with regards to acid-base balance and urine composition are then presented.

The remainder of the text describes a clinical investigation of the use of DCAD for the prevention certain risk factors of obstructive urolithiasis. Various levels of DCAD were fed in order to evaluate the response of goats to each level.

The purposes of this study were to:

- Determine existence of variability of DCAD values in commercially available small ruminant diets.
- Determine the correlation between DCAD level and urinary pH values in goats.
- Determine a level of DCAD which produces urine with a pH of between 6.0 – 6.5.
- Determine the urine dilution effect of various DCAD levels.
- Evaluate the effect of DCAD level on blood pH.
- Determine appropriate urine sampling times to monitor effectiveness of DCAD balancing.

Hypotheses:

- There is significant variability in DCAD level among prepared small ruminant feeds, which may contribute to difficulty in preventing urolithiasis with anionic salts.
- The DCAD of a ration is positively correlated with urine pH, urine specific gravity and blood pH produced in goats.
CHAPTER I

ANATOMY

The distal urinary tract of male ruminants differs significantly from that of males of other species. The penis is sigmoid in arrangement, with two major bends occurring between the urinary bladder and the distal glans penis. The most proximal of these flexures is a common site of urethral obstruction by uroliths.1

The glans penis of the small ruminant also has a vermiform appendage, or urethral process, which is an extension of the urethra 2-4 cm beyond the distal end of the penis.6 It has a narrowed diameter compared to the more proximal portions of the urethra and also serves as a common location for obstruction.1

The male ruminant has an additional structure in the distal urinary tract which does not contribute to urethral obstruction, but rather complicates treatment of affected animals. A urethral diverticulum is present distal to the ischial arch.6 When a urinary catheter is passed into the urethra in a retrograde manner from the glans in order to achieve a patent pathway for urine drainage and lavage of the urinary bladder, this diverticulum readily accepts the catheter, rather than allowing the catheter to proceed up the urethra into the urinary bladder.
CHAPTER II

REVIEW OF THE LITERATURE

UROLITHIASIS OF SMALL RUMINANTS

PATHOPHYSIOLOGY OF CALCULogenesis

Uroliths are solid crystalline formations in the urine which are composed of organic matrix and organic and inorganic crystalloids. They are highly organized structures, usually with a grossly apparent nucleus surrounded by laminations when viewed in cross-section. The most common stone compositions in ruminants include magnesium ammonium phosphate (struvite), calcium phosphate (apatite) and calcium carbonate. Formation of a urolith is not consistent or predictable, as a wide variety of environmental, dietary and physiologic factors are believed to contribute to their incidence.

Urolith formation occurs as a two-step process beginning with the formation of an organic matrix followed by deposition of minerals on that matrix. These two steps may occur in repeated sequence, resulting in lamellar formation within the calculus, representing alternating periods of growth and reflecting changes in the environment of the urolith.

The matrix is a highly insoluble complex of macromolecules with the ability to bind ions. It extends throughout the urolith, acting as the skeleton of the urolith, yet many times composes less than 5% of the total stone weight. In one study in sheep,
matrix averaged 13% of the total weight of uroliths. Stones which contain >65% matrix, and therefore little mineral content, are called matrix stones, and have been demonstrated to occur in sheep. It is therefore important to understand and prevent the formation of matrix as well as the mineral components.

The formation of matrix, and uroliths as a whole, results from super-saturation of the urine by calculogenic crystalloids. Whether or not matrix appears pre-formed in urine or is formed from solutes in urine is not known. Many factors affect the super-saturation status of urine including rate of renal excretion of crystalloids, negative water balance, urine pH and the presence or absence of crystallization inhibitors.

Matrix is isolated from uroliths by demineralization and attempts have been made to characterize its components. In sheep, isolated matrix has included nitrogen, reducing sugars, hexosamine, mucopolysaccharides, and tyrosine along with various other amino acids and sugars, erythrocytes, leukocytes and epithelial cells. An additional component of matrix are lipids, primarily cholesterol, which have composed 2.5-12.5% of uroliths in cattle. Suture, tissue debris, blood clots or bacteria may also serve as nuclear components initiating urolith formation. Urinary tract infection and metaplasia of uroepithelium as a result of vitamin A deficiency, may contribute cells and protein for nuclear formation. Infection, however, is considered to be a minor factor in urolith formation in ruminants. Sheep with urethral occlusion have negative urinary bladder cultures in most cases; while advanced cases may contain *Streptococcus* spp. or *Escherichia coli*. *Ureaplasma* spp. have been isolated from sheep with urolithiasis and isolates induce struvite sedimentation in cultures. In lambs with experimental ureaplasmal infections, there was not a difference in the incidence of calculi, but infection significantly increased the total mass of calculi recovered. *Ureaplasma* spp.
do increase urine pH, \(^3,^{18}\) a risk factor in urolith formation, but this did not increase incidence of urolithiasis in one study.\(^{18}\) Further, infection may cause renal damage, \(^{16}\) allowing macromolecules into be lost into urine.

Proteins in the form of mucoproteins and amino acids make up a portion of matrix and therefore play a significant role in stone formation. Urine peptides play a role in the formation of matrix and therefore the initiation of a calculus and have been shown to have a high affinity for organic ions, such as calcium and magnesium.\(^{13}\) The breakdown of normal urinary proteins is believed to be a contributor to matrix formation.\(^{14}\) Biocolloids are large macromolecules present in urine and incorporated into uroliths. They are nondialyzable and may be divided into serum macromolecules, ovine urinary mucoprotein (OUM) and urine polyelectrolytes (UPE).\(^9,^{19,20}\) It appears as though these mucoproteins originate in the renal tubules.\(^{14}\) This mucoprotein may not initiate crystal formation, but rather may act as a stabilizer for supersaturated solutions.\(^{21}\)

Urine polyelectrolytes are peptides which contain various quantities of inorganic ions.\(^8\) They are divided into small molecular weight UPE, which are peptides, and large molecular weight UPE, which are proteins and mucoproteins.\(^{13}\) Small molecular weight UPE have a higher binding capacity and can bind each other as well as larger molecular weight UPE when in solutions containing calcium and magnesium.\(^{13}\) Urine polyelectrolytes of animals on calculogenic diets have increased binding capacity for inorganic ions, with the relative order of decreasing affinity being calcium, magnesium, potassium and sodium.\(^{13}\) Elevated urine protein levels have been demonstrated in lambs which developed calculi compared to lambs which did not develop urinary calculi. Half of this protein was mucoprotein.\(^{14,22}\) Another study, however, showed the highest urine protein levels to occur in lambs with the lowest incidence of urolithiasis.\(^8\)
The exact function of matrix is yet to be fully understood. Proposed functions include provision of ion-binding groups allowing for epitaxial growth of crystals, serving as a template or binding agent for uroliths or may protect from further calculus growth. Epitaxial growth occurs when one type of crystal grows on the surface of a different type of crystal and is an important concept as many uroliths are of mixed composition.

In sheep, uroliths may appear as aggregates of microcalculi, containing phosphatic crystals and mucoprotein, cemented by an organic envelope, formed of mucoprotein. In the majority of cases of urolithiasis in feedlot sheep and cattle, phosphate crystals appear as packets of microcalculi. These microcalculi may serve as structural units for a single, larger stone. One study showed that matrix enclosed and cemented minerals and was therefore more important in cementing microcalculi together than in initiation of crystallization.

The presence of crystallization inhibitors are important to consider, as urine is normally a supersaturated solution. Crystalloids are consistently maintained in solution in urine at higher concentrations than they could be held in water. Normal urine is considered a metastable solution, whereby it is able to maintain more solutes in solution than would be predicted by the solubility of the solutes alone. Metastability is the status between under-saturation and super-saturation, where the solution is saturated, but precipitation does not occur. As the solution becomes increasingly saturated, it becomes unstable and the formation product precipitates out of solution and nucleation occurs. Thus, these protective substances allow super-saturation without precipitation. Organic acids, magnesium, inorganic pyrophosphate, urea, mucopolysaccharides, glycosaminoglycans, an RNA-like substance, and many unidentified substances are suspected to inhibit crystallization. The addition of calcium citrate to a calculogenic diet
reduced urine volume, but also reduced urine protein excretion, mucoprotein and tyrosine. These crystallization inhibitors have been identified as having a role in inhibition of calcium oxalate and calcium phosphate crystallization, but have not been definitively identified for struvite and silica uroliths.

Three main theories exist to attempt to explain calculogenesis. The first is the precipitation-crystallization theory which places highest importance on urine supersaturation and spontaneous precipitation by crystalloids, independent of matrix and crystallization inhibitors. It is believed that matrix is then incorporated into the growing calculus. The matrix nucleation theory sites pre-formed matrix presence in the urine as the culprit of calculogenesis. Matrix forms from a nucleus which then allows binding by crystalloids for stone growth. The crystallization-inhibition theory states that calculogenesis occurs when crystallization inhibitor substances are present in inadequate quantities, and therefore require lower levels of super-saturation by calculogenic crystalloids to produce a urolith.

Once the nucleus is formed, to become a urolith, it must remain within the urinary tract and its environment must maintain continued and sufficient crystalloid supersaturation. If this occurs, deposition of inorganic minerals on the matrix or nucleus may occur. Salts make up greater than 95% of a stone’s weight and there is an intimate relationship between the mineral and matrix of uroliths. The major inorganic minerals deposited are magnesium, calcium and phosphate. Potassium and chloride play secondary roles and their presence may be more involved in inhibition of urolithiasis.

An elevated level of phosphorus in the diet, with a calcium:phosphorus ratio less than 2:1 increases the excretion of phosphorus in the urine and provides an ion to bind to
organic matrix.\textsuperscript{1,3,11,12,25-28} Increasing the level of calcium in the diet markedly decreased the incidence of urolithiasis, probably due to competition with phosphorus for intestinal absorption and matrix binding.\textsuperscript{26} Ruminants excrete phosphorus primarily by saliva, where it is then swallowed and removed from the body in the feces. This protects from phosphate calculi, but excessive dietary levels of phosphorus may saturate the salivary pathway, causing the excess to be excreted in the urine.\textsuperscript{1,28} High phosphorus levels are present in the grains, particularly sorghum and wheat.\textsuperscript{1,3,29} Fresh urine samples of animals fed rations high in phosphates produce cloudy precipitates \textit{in vitro}\textsuperscript{9} and phosphorus has the capacity to damage renal tubular epithelium, causing an increase in urinary proteins.\textsuperscript{14,30}

Consumption of high dietary magnesium has been shown to increase the incidence of struvite and apatite formation.\textsuperscript{1,3,12,28,31,32} Magnesium may act as an inhibitor of calcium-containing uroliths by competitive attachment to binding sites, but may predispose to magnesium-containing stones such as struvite.\textsuperscript{7} The contribution of magnesium to calculogenesis is not easily predictable. Magnesium levels of 0.29\% and 0.86\% in rations showed no difference in the incidence of urolithiasis.\textsuperscript{33} The contribution of dietary magnesium to urolithiasis may be aided by phosphorus. The feeding of high magnesium, consistent with levels that would cause urolith formation, of 0.63\% did not induce urolithiasis until phosphorus was concurrently raised to 0.52\%.\textsuperscript{32} High urine and serum levels of magnesium and phosphorus and low serum calcium have been noted in wethers on a calculogenic diet. Wethers that did not form calculi had lower serum phosphorus and magnesium, excreted more phosphorus and magnesium via the fecal route and less excretion of these minerals occurred through the urine.\textsuperscript{22}
Chloride ions compete with magnesium and phosphate for binding sites on the matrix and decrease the incidence of urolith formation. They may therefore prevent nucleation, but have also shown characteristics of inhibition of crystal growth through an inability to bind UPE directly. The anionic components of uroliths tend to be larger molecules and are quite varied in their form and therefore may be more easily competed against for binding sites on matrix.

Potassium may have mixed effects on urolithiasis depending on level. Studies have shown an increased incidence of stones with potassium supplementation, decreased incidence, no effect, and no increase in incidence, but an increase in mass of stones recovered. This is due to the fact that potassium level relative to urolithiasis is curvilinear with highest levels of urolithiasis occurring at moderate levels of potassium in the diet. In one study, potassium supplementation did not change the incidence of calculi, but did increase the amount of calculi recovered in lambs. Potassium may compete with other ion binding sites on UPE in potassium-containing solutions.

A significant factor in availability of urolith components and their binding ability is urine pH, although studies have shown conflicting results in urolith formation with regards to urine pH. Urolith formation may occur at both acid and alkaline urine pH, as evidenced by a study feeding phosphoric acid or potassium carbonate to induce acid or alkaline urine, respectively. Animals under both conditions formed uroliths, but crystal growth rate is increased in alkaline conditions.

Struvite, apatite and calcium carbonate uroliths are known to precipitate in alkaline urine. Struvite crystallization occurs only at a pH range of 7.2 – 8.4 and dissolution occurs at a pH of less than 6.5. Struvite had a 75% increase in solubility when the urine pH was reduced from 7.4 to 6.8 and struvite stones were
found to dissolve 35% faster when the urine pH was decreased from 6.5 – 5.75. In sheep fed high levels of dietary phosphorus, the addition of the urinary acidifier ammonium chloride was found to prevent the increased incidence of calculi formation. Thus it was concluded that pH was of greater importance than dietary phosphorus level in the development of urinary calculi. An additional study showed that changes in the cations, sodium and potassium, and an anion, chloride, of a diet without corresponding changes in urine pH do not play a role in the prevention of urolithiasis. Some sources state that cation anion balance which induces pH changes is likely to be more important than the pH itself. Anion source in the urine is very important as phosphate contributes to aggregation, while chloride reduces aggregation.

Matrix response to pH is not well understood. Conflicting information exists as to whether UPE binds ions more easily at acid or alkaline pH, based on folding and availability of binding sites. Ovine urinary mucoprotein of matrix, in an alkaline environment, may lose its terminal polysaccharide moiety, increasing the ability for ion binding.

Total body water balance plays an important role in calculogenesis by its effects on urine volume. Increased urine volume has two potential effects on the components of calculi: decreasing the level of saturation and precipitation of crystalloids and providing repeated removal of nuclear components. Water deprivation and a negative body water balance contribute to the super-saturation and precipitation of crystalloids in urine. However, a decrease in water intake by 20% under normal voluntary intake did not alter incidence of urolithiasis.
Time plays an important role in urolith formation and aggregation of calculi components occurs as urine composition changes.\textsuperscript{9} In dogs, uroliths have been experimentally formed within 2 weeks and dissolved in a similar time frame.\textsuperscript{7} In sheep, marked decreases in urine pH, increases in urine concentration and increases in urine calcium concentration occurred one hour after the initiation of feeding.\textsuperscript{46} Therefore, urine alterations of any time length may have a significant impact on urolith formation as well as destruction.

**DIETARY PREVENTATIVE MEASURES**

Due to the important role of metabolic by-products and minerals in the pathophysiology of urolithiasis, diet serves as a large contributor to urolithiasis and is therefore the primary focus of disease prevention. Risk factors addressed in preventative strategies include high dietary phosphorus and magnesium, low dietary calcium, low fiber content of rations, low urine output and an alkaline urine pH.

A reduction of phosphorus availability in the diet may be achieved by the avoidance of feeds with known high phosphorus content and the supplementation of calcium to reduce phosphorus availability. Phosphorus should comprise greater than 0.6\% of the total ration\textsuperscript{28} and it is recommended that a 2:1 calcium:phosphorus ratio be achieved, by the use of calcium salts, if necessary\textsuperscript{1,3,12,15,25}. Calcium oversupplementation should be avoided as increased calcium excretion in the urine may contribute to calcium-containing uroliths.\textsuperscript{1} Cereal grains, such as corn, milo and oats have increased levels of phosphorus in the presence of decreased calcium levels and should therefore be avoided.\textsuperscript{12} Grains also result in increased magnesium, phosphorus and peptides in the urine.\textsuperscript{1,3,29}
A reduction in phosphorus excretion into the urine is also desirable. Urine phosphorus excretion is greater in animals fed pelleted rations as compared to meal-type rations.\textsuperscript{36} This is due to a decrease in saliva production, and therefore a pathway for excess phosphorus excretion. Increases in the roughage component of diets are important from this standpoint as they increase the amount of saliva that must be produced for proper mastication.\textsuperscript{28}

Particularly in the case of struvite stones, an increase in magnesium excretion into the urine is contributory to crystallization. It is recommended that magnesium make up less than 0.6\% of the total ration of ruminants.\textsuperscript{12} Magnesium is more available and absorbed more efficiently from concentrate rations than from roughage diets.\textsuperscript{28}

Increasing water intake and urine volume is an important preventive measure for urolithiasis. Sources recommend the provision of adequate palatable water at desirable temperatures according to the ambient environment.\textsuperscript{1,3,12,15,28} Additionally, a reduction in urine output has been noted when sheep were changed from an alfalfa hay diet to a concentrate pellet,\textsuperscript{19} demonstrating a reduction in water intake for grain feeding over roughage feeding\textsuperscript{28}. Additionally, the feeding of intermittent meals may cause shunting of body water into the rumen due to increased osmotic pull from generated volatile fatty acids, resulting in a decrease in urine output. This has led to the recommendation that ruminants be fed \textit{ad libitum} to maintain urine output.\textsuperscript{1,28}

Due to an ability to alter acid-base balance and body water balance, salts have been widely used and recommended for the prevention of urolithiasis. Anionic salts containing primarily chlorides have been popular and used extensively for the prevention of urolithiasis, as they induce acidic urine and an increase in water intake and diuresis.\textsuperscript{1,12,25,28} Chloride ion also prevents the binding of phosphates to the mucoprotein
matrix of the struvite structure. Chlorides with various cation attachments, namely NaCl, KCl, CaCl$_2$ and NH$_4$Cl, have traditionally been used to prevent calculi formation. Chloride content itself does not appear to prevent calculi formation alone, as there is variability with calculi incidence with the feeding of chloride salts. In one study, ammonium chloride and calcium chloride were associated with reduced incidence of urolithiasis, while sodium chloride and potassium chloride increased the incidence in sheep. However, chloride excretion in the urine was found to be the only correlation of difference in incidence of urinary calculi on various salts. Limiting absorption of phosphate while increasing urine chloride excretion are considered important factors in urolith prevention.

Many studies have compared various salts against each other as well as various levels of different salts. Sodium chloride (NaCl) has been shown to increase urine volume at levels as low as 0.5% and 1.5%. Water intake, however, cannot be easily predicted as water intake did not change when NaCl levels were changed from 0.5% to 3% in goats. NaCl at a level of 4% increased urine volume by nearly double other chloride salts, but these changes in urine volume did not correlate with a decreased incidence in calculi formation. NaCl did increase urinary phosphorus excretion, which was significant even at the higher urine volumes. This may have led to the lack of decreased incidence of urolithiasis. On the other hand, NaCl at a level of 10% of the dry matter resulted in a 0% incidence of urolithiasis in another study while unsupplemented controls experienced a greater than 60% incidence. This level also increased water turnover, reduced serum protein and albumin, greatly increased urine volume and decreased urine pH in addition to significantly reducing urinary calculi formation. The primary difference in this incidence was found to be dilution of matrix components by
diuresis, although ionic action of the chloride may have also played a role.\textsuperscript{37} With the increase in urine volume with NaCl, there was also a decrease in urine hexosamine and protein.\textsuperscript{50} NaCl has been associated with decreased average daily gain and decreased ration palatability at levels as low as 4\%\textsuperscript{29,35} and therefore, most sources recommend that it be added only at a rate of 1-4\% to diets\textsuperscript{1,3,12,15}.

Potassium salts have shown potential to be more effective than sodium salts in the prevention of calculi.\textsuperscript{48} Potassium chloride, however, has been associated with increased incidence of urinary calculi and lowered average daily gain.\textsuperscript{35} Experimental rations designed to be calculogenic, containing high concentrate and K\textsubscript{2}HPO\textsubscript{4} have been shown to increase serum mucoprotein levels in sheep.\textsuperscript{17} Potassium salts, therefore, are quite unpredictable and not typically recommended for urolithiasis prevention.

Calcium chloride (CaCl\textsubscript{2}), fed at 1\% of the diet increased urine volume by nearly double other chloride salts, but these changes in urine volume did not correlate with a decreased incidence in calculi formation.\textsuperscript{35} In another study, 1\% reduced the incidence of urinary calculi and was the only calcium-containing salt which increased calcium excretion into the urine.\textsuperscript{35} When fed at a rate of 1.5\%, CaCl\textsubscript{2} reduced urinary calculi and urine pH, while 0.5\% had no effect.\textsuperscript{39} Calcium carbonate at 2\% of the diet, did not show a significant reduction in urinary calculi.\textsuperscript{35} Calcium salts, therefore, may be of value only to decrease phosphorus availability.

Ammonium chloride (NH\textsubscript{4}Cl) has been administered to horses\textsuperscript{52}, cats\textsuperscript{53-55}, and ruminants\textsuperscript{39,48,49,56}, with varied success at reducing urine pH and the rate of urolithiasis. NH\textsubscript{4}Cl may be preferred over other salts for growing animals as the ammonium may be used for metabolic processes, leaving chloride available to exert its urinary actions.\textsuperscript{9} NH\textsubscript{4}Cl at 0.5\% increased water intake, but also decreased feed consumption in sheep\textsuperscript{57}, a
negative effect also noted at 1%\textsuperscript{35}. Conversely, 0.5% in another study significantly decreased incidence of urolithiasis, while it increased feed efficiency.\textsuperscript{48} NH\textsubscript{4}Cl as 1% of the ration resulted in a significant reduction in the incidence of urinary calculi, increased urine calcium, showed a significant decrease in urine pH, but also lowered average daily gain.\textsuperscript{35} In one study, a significant decrease in urine pH to the acidic range was only achieved when NH\textsubscript{4}Cl was 1.5% of the diet.\textsuperscript{39} Historically, NH\textsubscript{4}Cl has been recommended and used in rations as a source of anion at a rate of 0.5 – 2% of the ration\textsuperscript{1,3,11,12,15,25,28,29} for at-risk ruminants.

The role of urine pH in urolithiasis is well documented and various sources recommend urine pH goals of 5.5 to 6.0\textsuperscript{29}, <6.5\textsuperscript{28} and <6.8\textsuperscript{12,27}, based on the solubilities of the common stone compositions. This protective change in urine pH by grain may, however, be outweighed by the risk of their association with increased urine mucoprotein and phosphorus\textsuperscript{1,3,11}. Anionic salts, therefore, are added to alter urine pH, but show significant inconsistencies in their abilities to reduce urine pH, urine output and, ultimately, to prevent urolithiasis. The traditional addition of ammonium chloride or any other anionic feed additive as a simple percentage of the diet without consideration for the components of the total ration may lead to inconsistent and unsuccessful maintenance of low urinary pH. The concept of DCAD states that with increased cations in the diet, alkalotic tendencies will occur. Conversely, increased anions in the diet have acidifying potential. Different commercial diets are commonly formulated using various commodities and these commodities are interchanged regularly in feed preparation based on availability. If a feedstuff of a particular brand or batch of feed is higher in cations, or anionic salts are fed in conjunction with a high potassium forage,\textsuperscript{12,58,59} the DCAD of the diet will be raised and urinary acidification may not occur, despite the addition of the
standard dose of anions. This one-dose-fits-all method may be the major cause of sporadic urolith formation in animals being fed anionic salts. The use of DCAD balancing for goats and urolithiasis is mentioned as a recommendation in some sources,\textsuperscript{1,12,60} and it is recommended that high cation-containing feedstuffs such as alfalfa and molasses should be avoided,\textsuperscript{12} but few controlled studies and no target DCAD levels currently exist. In one study of goats on DCAD balanced diets, a DCAD level of 70 mEq/kg resulted in urine pH of around 5.5, a diet of 458 mEq/kg established a urine pH of 7.5-8.0 and a diet of 900 mEq/kg resulted in a urine pH of around 8.2.\textsuperscript{61} Another study which fed a DCAD level of 0 mEq/100g resulted in urine pH levels from 5.75 to 5.85.\textsuperscript{60} Roughage diets typically produce urine with a pH of 7.8-8.5, while grain diets produce more acidic urine, between 5.2-7.0.\textsuperscript{28}
CHAPTER III
REVIEW OF THE LITERATURE
DIETARY CATION ANION DIFFERENCE

STRONG ION DIFFERENCE THEORY

The strong ion difference is a non-traditional approach to acid-base balance analysis formulated by Dr. Peter Stewart in the early 1980s.\textsuperscript{62,63} It counters the traditional approach, based on the Henderson-Hasselbach equation, by taking into account many factors which contribute to acid-base balance, rather than simply pH, $P_{CO_2}$ and $HCO_3^-$.\textsuperscript{62-64} It is quite complicated in its original form and involves seven equations that can be combined as a single, 3\textsuperscript{rd} order quadratic equation that can be solved for $[H^+]$.\textsuperscript{64} This is much more complex than is required in the clinical setting and for a basic understanding of the physiology.

In a biological system, electroneutrality must be maintained and there is conservation of mass.\textsuperscript{62} Therefore, the number of moles of cations equals the number of moles of anions\textsuperscript{62} and $[H^+] \times [OH^-] = 1 \times 10^{-14}$.\textsuperscript{65} Therefore, the body regulates acid and base such that when there are increased cations added to plasma, there is a compensatory increase in $OH^-$ and a decrease in $H^+$. Conversely, when there is an increase in anions added to plasma, there is a compensatory decrease in $OH^-$ and an increase in $H^+$.\textsuperscript{65}

The strong ion difference theory states that acid base balance is determined by three independent variables and two dependent variables. The independent variables are
strong ion difference [SID], PCO2 and total weak acids [ATOT]. The dependent variables, which do not determine acid-base balance directly, are pH and bicarbonate. This is in contrast to the traditional approach.62-64

The P CO2 is analogous to the respiratory component of the traditional approach and with increases in P CO2 there is respiratory acidosis and with decreases, a respiratory alkalosis.62-64

The A TOT represents the sum of the activity of the non-volatile weak acids in solution. This includes albumin, globulin and phosphate, with albumin the being the primary contributor. Increases in albumin result in decreases in bicarbonate and increases in H+. Decreases in albumin result in increasing bicarbonate and decreasing H+.62-64

The strong ions are primarily Na+, K+, Ca++, Mg++, Cl-, S=, P---.64,65 They only alter the SID if they are absorbed into the systemic circulation and therefore their relative bioavailability must be considered when analyzing each ion and its effect on acid base balance. They primarily enter the gastrointestinal system, therefore making diet the primary determinant of SID. These ions are then regulated by the kidneys, being excreted and resorbed to maintain electroneutrality and conserve mass. The organic acids, such as lactate, ketoacids and volatile fatty acids, are undissociated and are quickly metabolized by the liver, resulting in a small effect on pH.65 The formula for the SID is, based on measured plasma levels, (Na+ + K+) – (Cl+ lactic).62,63 Additional constituents are generally not considered as they are frequently not measured or are typically inconsequential in the clinical setting. When the SID increases, there is a compensatory increase in bicarbonate and metabolic alkalosis occurs. With decreases in SID, a metabolic acidosis is created.
DCAD DEFINED AND TRADITIONAL USE

Dietary cation anion difference is defined as the difference between the summation of the major biologic cations and anions of a diet. It is traditionally illustrated as \([(Na+K)-(Cl+S)]\), expressed in mEq/kg, mEq/lb or mEq/100g of feed. Additional formulas have been proposed, including \((Na + K + 0.15 Ca + 0.15 Mg) – (Cl + 0.20 S + 0.30 P)\), which accounts for additional ions and their relative bioavailability.

The DCAD is primarily controlled by adding physiologic anions, generally chlorides and sulfates, to a ration. As stated in the strong ion difference theory, this addition of anions will result in an increase in extracellular hydrogen ions and induction of a metabolic acidosis. The higher the DCAD, or excess of cations, the more alkalogenic the diet. This method of ration formulation is primarily utilized for transition dairy cows as a means of prevention for milk fever. Milk fever is a clinical condition where dairy cows become hypocalcemic immediately post-partum as a result of increased calcium demand at the initiation of milk production. Metabolic acidosis increases the available extracellular pool of calcium by improving the activity of parathyroid hormone and vitamin D. This results in an increase in intestinal absorption of calcium and an increase in calcium resorption from the bone. The appropriate level of metabolic acidosis is achieved when cattle consume a diet of DCAD level \(-150 \text{ mEq/kg} \) to \(-50 \text{ mEq/kg}\), which is associated with significantly decreased incidence of milk fever.

One of the problems associated with DCAD balancing is that different commercial diets are commonly formulated using various commodities and these commodities are interchanged regularly in feed preparation based on cost and availability. If a particular brand or batch of feed is higher in cations, or fed in conjunction with a high potassium forage, the DCAD of the diet will be altered and
will not induce metabolic acidosis to the desired degree, rendering the preventive measure ineffective. Feed analysis for every batch is not practical from an economic or time standpoint. Physiologically, the excess H⁺ in the extracellular fluid as a result of lower DCAD is excreted by the kidney to maintain electroneutrality, producing urine of a lower pH.¹²,⁶⁵ Therefore, measuring urine pH is the most suitable assessment of DCAD effectiveness⁷⁰, due to its simplicity and reliability⁵⁹. For milk fever prevention, it is recommended that Holsteins should have a urine pH of 6.0-7.0, with Jersey cattle having a urine pH of 5.5-6.0, for ideal acidogenic potential of the diet.⁶⁶ The relationship of DCAD and urine pH in dairy cattle is represented by a hyperbolic curve where a DCAD of −150 to −50 mEq/kg produces urine pH between 5.5 – 6.0 and DCAD above +200mEq/kg produces a urine pH of 8.0 – 8.5 in a plateau effect.⁶⁶

DCAD USE IN SMALL RUMINANTS

ACID-BASE BALANCE

In small ruminants as well as other species, with decreasing DCAD of a diet, and therefore an increase in anions, a systemic hyperchloremic, hyponatremic metabolic acidosis is produced.⁶¹,⁷¹-⁷³ Higher DCAD levels result in subclinical hypernatremic, hypochloremic metabolic alkalosis, while the lower DCAD levels resulted in a subclinical hyperchloremic, hyponatremic metabolic acidosis.⁶¹,⁷³

In periparturient ewes fed various DCAD levels, -88.5 mEq/kg resulted in a lower blood pH the two days prior to and after lambing, as compared with +164.5 and +272.6 mEq/kg, but this difference was only significant on the day of parturition. Blood bicarbonate and pCO₂ were unaffected by treatment, although anion gap was higher for the anionic group two weeks after initiation of diet. Blood pH, although lowered,
remained within the normal range for sheep and increased quickly after removal of anions.\textsuperscript{71}

Peripartum ewes fed diets of DCAD -4.1, +29.1 and +82.3 mEq/100g, showed no significant difference in blood pH during lactation.\textsuperscript{72} This lack of change in blood pH was likely due to the minimal differences in the range of DCAD levels fed in this study. Increasing the range led to significantly increased blood pH in 3 month old lambs as DCAD increased from 0 to 600 mEq/kg.\textsuperscript{74} In lambs, blood pH increased with increasing DCAD from +100 to +700 mEq/kg. DCAD levels of +100, +300, +500 and +700 mEq/kg corresponded to blood pH levels of 7.39, 7.45, 7.43 and 7.44. The P\textsubscript{CO2} was increased for the +100 mEq/kg group and blood bicarbonate and base excess increased with increasing DCAD.\textsuperscript{75} Anionic supplement fed with oat or grass hay at a level of 0 mEq/100g significantly decreased blood pH, bicarbonate and base deficit 12-13 and 27-28 days after supplementation.\textsuperscript{60}

In pregnant and lactating does fed diets of +0.7, +45.8 and +90 mEq/100g, rumen fluid H+ was increased by the +0.7 mEq/100g diet. The rumen fluid pH of pregnancy and lactation periods for +0.7 mEq/100g were 6.1 and 6.3, for +45.8 mEq/100g rumen fluid pH were 6.6 and 6.6 and for +90 mEq/100g values were 6.7 and 6.6.\textsuperscript{61}

There is no question that blood pH trends can be predicted with changes in DCAD level. It appears, although, that a relatively wide range of DCAD levels must be fed in order to achieve statistically significant changes in blood pH. This demonstrates the ability and priority of the renal and respiratory systems to compensate for alterations in systemic pH.
URINE PARAMETERS

URINE PH

Changes in acid base balance of the body are often directly reflected with changes in urine pH. As with blood pH, urine pH tends to positively correlate with DCAD level. It has been suggested that, with regards to urolithiasis, the DCAD of the total diet may influence urine pH, altering the efficacy of NH₄Cl in the management of ruminant urolithiasis.¹ Several studies using DCAD in small ruminants have reported urine pH as a secondary finding.

High calcium diets were fed with DCAD levels of -27, +61 and +284 mEq/kg and +63, +218 and +343 mEq/kg with normal calcium levels. Urine pH for -27 mEq/kg was significantly lower (7.69) than that for the higher DCAD levels (8.85, 8.57) for high calcium diets. For normal calcium diets, no differences were noted between urine pH of various DCAD levels (8.75, 8.57, 8.35).⁷⁶

In intact bucks fed a commercial anion supplement on grass or oat hay to achieve 0 mEq/100g, the supplement significantly decreased urine pH from control period, 8.03, to 5.75 on days 12-13 and to 5.85 on days 27-28 of supplementation. This demonstrates a potential trend for anionic supplementation to allow urine pH to trend upward with time. Three of eight goats on grass hay increased urine pH by >0.4 units between the 12-13 and 27-28 day samplings, while 3 of 8 on oat hay decreased their urine pH during the same sampling periods.⁶⁰

In pregnant and lactating does fed diets of +0.7, +45.8 and +90 mEq/100g Urine pH values for +0.7 mEq/100g were 5.59 and 5.46, for +45.8 mEq/100g were 7.59 and 8.01 and for +90 mEq/100g were 8.11 and 8.28 for pregnancy and lactation sampling periods, respectively. The low DCAD group had reduced urine bicarbonate excretion,
increases in net acid excretion and the presence of titratable acids in small amounts in the urine.\textsuperscript{61}

In lambs, urine pH increased with increasing DCAD from +100 to +700 mEq/kg. DCAD levels of +100, +300, +500 and +700 mEq/kg corresponded to urine pH levels of 6.87, 8.45, 8.57 and 8.60.\textsuperscript{75} Urine pH was 1.3 units higher (8.9 versus 7.6) in animals fed +250 mEq/kg over a -100 mEq/kg diet 12-14 days after initiation. This difference increased to 2.0 pH units (8.2 versus 6.2) 17-19 days after diet initiation.\textsuperscript{77}

Peripartum ewes fed diets of DCAD -4.1, +29.1 and +82.3 mEq/100g respectively produced urine of pH 5.16, 8.23 and 8.63 during pregnancy and 4.98, 8.47 and 8.75 during lactation.\textsuperscript{72} A comparison of -12, +30, +76 and +133 mEq/kg diets fed to adult sheep had no significant effect on fecal or urinary pH.\textsuperscript{78}

Decreasing DCAD does decrease urine pH, although findings are often inconsistent across studies. Direct studies demonstrating urine pH as it relates to DCAD level, without confounding ration formulations or physiologic status, are lacking.

**URINE CONCENTRATION AND WATER INTAKE**

Some studies using DCAD formulation in small ruminants have evaluated urine dilution and water intake as a result of the diet. Water intake, urine volume and rumen fluid dilution in goats were increased by +90 mEq/100g diet over that produced by +0.7 and +45.8 mEq/100g, particularly during pregnancy.\textsuperscript{61} This is likely due to the increase of sodium availability via addition of sodium hydroxide\textsuperscript{61} or sodium bicarbonate to the rations to increase DCAD\textsuperscript{27}.

Anionic supplement fed to goats with oat or grass hay at a level of 0 mEq/100g significantly increased water intake and urine volume at the start of supplementation and
days 27-28 after initiation. This may be difficult to interpret as urine production of animals on oat hay was significantly higher on oat hay than on grass hay prior to anionic supplementation. Six of 8 goats decreased urine creatinine with anionic supplementation, suggesting urine dilution, although urine specific gravity was not determined.\textsuperscript{60}

These studies demonstrate that the addition of salts, either to raise or lower DCAD level, may result in the production of more voluminous and dilute urine. There are no studies which determine whether DCAD effect of urine volume correlates to a reduction in urolithiasis.

**URINE MINERAL CONTENT**

In a study of peripartum ewes fed diets with DCAD levels of -4.1, +29.1 and +82.3 mEq/100g, there were no differences noted in urine levels of phosphorus. The -4.1 mEq/100g group experienced higher urine excretion of calcium than did the higher two groups.\textsuperscript{72} Similarly, in 10 month old lambs fed diets of -100 and +250 mEq/kg, the lower diet resulted in seven times more loss of calcium in the urine, while phosphorus excretion was not affected.\textsuperscript{77}

In a simulated calcium loss study using infusion of ethylene glycolbis tetraacetic acid (EGTA), DCAD treatment levels of -127, +35 and +339 mEq/kg were fed during eucalcemia and -147, +68, and +429 mEq/kg during infusion. Both lower DCAD levels increased urinary excretion of isotope-labelled calcium during eucalcemia, with less difference during the calcium loss phase. Low DCAD levels increased urinary excretion of calcium with no changes in calcium balance.\textsuperscript{79}

Anionic supplement fed with oat or grass hay at a level of 0 mEq/100g altered urinary fractional excretion of macrominerals.\textsuperscript{60} Urine samples were taken prior to
anionic supplementation and 12-13 and 27-28 days after anionic supplementation was initiated. Fractional excretion of sodium was significantly higher in the middle collection period compared to the early and late samples. Fractional excretion of potassium was increased during the late collection time as compared to early and middle collection times. Fractional excretion of chloride and calcium were increased during the middle and late sampling periods. Increased chloride excretion may prove useful in the prevention of urolithiasis due to the role of chloride in the inhibition of calculi formation. There were no differences in iron or magnesium. Fractional excretion of phosphorus increased in late compared to the early sampling period and fractional excretion of sulfur was increased in the late sampling period.

Comparing DCAD levels of -2.14 and 71.35 mEq/100g in does with simulated calcium loss using EGTA, the anionic diet increased urinary excretion of calcium during the eucalcemic period. During the hypocalcemic period, calcium loss in urine was increased and calcium absorption was higher while on the anionic diet. Cation excess induced alkalosis which decreases calcium absorption and calcium excretion via urine.73

High calcium diets fed with DCAD levels of -27, +61 and +284 mEq/kg and +63, +218 and +343 mEq/kg were fed with normal calcium levels to sheep. Reducing DCAD reduced calcium retention by increasing excretion of urinary calcium. Urine excretion of phosphorus for +218 mEq/kg was significantly lower than for +343 mEq/kg. Urine excretion of calcium for +284 mEq/kg was significantly lower than other high calcium diets, and for +343 mEq/kg, urine calcium excretion was significantly lower than other normal calcium diets. Urine excretion of magnesium was significantly higher for -27 mEq/kg, while urine excretion of sodium was lower for -27 mEq/kg and for chloride was higher for +61 and +218 mEq/kg diets.76
The establishment of a target DCAD in goats could potentially increase effectiveness of urinary acidification as well as prevent over acidification of diets, which may be equally undesirable. In a study of Jersey cattle and another in lambs, animals fed ammonium salts were slower to consume their entire ration than those on a control ration, suggesting reduced palatability of the ammonium salts. In studies in small ruminants, there are varying effects of DCAD on feed intake. Some studies show that DMI increases with increasing DCAD, while others show no difference in feed intake by DCAD level. Ammonium chloride has resulted in reduced feed consumption and weight gain. There is also evidence of bone loss due to long-term ingestion of acidified diets. In humans, gastrointestinal irritation is a side effect of ammonium chloride administration. For these reasons, it would be advantageous to establish a target DCAD which effectively acidifies and dilutes the urine for prevention of urolithiasis, yet avoids harmful side effects of over-administration. The notation of temporal relationship from the start of DCAD treatment until optimal pH reduction occurs would provide additional assistance to those attempting to treat and prevent recurrence of clinical urolithiasis.
CHAPTER IV

METHODOLOGY

VARIOUS DCAD LEVELS OF SMALL RUMINANT FEEDS

Before the initiation of the clinical trial, a survey was taken of several small ruminant feeds to determine DCAD levels of the prepared feeds. Companies were contacted and asked to provide mineral analyses for feeds sold for small ruminant use. Responses were obtained from three companies on seven feeds. Two additional feeds were obtained locally and mineral analysis performed. Using the equation, \[ ((\text{Na} + \text{K}) - (\text{Cl} + \text{S})) \] in mEq/kg of feed, the DCAD level for each feed was calculated.

Table 1: DCAD Levels of Various Small Ruminant Feeds

<table>
<thead>
<tr>
<th>Feed Company</th>
<th>Feed</th>
<th>DCAD (mEq/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evergreen Mills™</td>
<td>Goat Feed</td>
<td>-125 to -165</td>
</tr>
<tr>
<td>Hubbard Feeds™</td>
<td>14% Lamb Finisher</td>
<td>-29.8</td>
</tr>
<tr>
<td>Hubbard Feeds™</td>
<td>17% Lamb Ration</td>
<td>-10.4</td>
</tr>
<tr>
<td>Stillwater A&amp;M™</td>
<td>Sheep and Goat Feed</td>
<td>12.16</td>
</tr>
<tr>
<td>Hubbard Feeds™</td>
<td>17% Sheep/Goat ShowFeed</td>
<td>14.2</td>
</tr>
<tr>
<td>Hubbard Feeds™</td>
<td>14% Goat Crunch</td>
<td>45.1</td>
</tr>
<tr>
<td>Hubbard Feeds™</td>
<td>20% Lamb Starter</td>
<td>47.4</td>
</tr>
<tr>
<td>Purina Mills, Inc.™</td>
<td>Goat Chow®</td>
<td>70.9</td>
</tr>
<tr>
<td>OSU Feed Mill</td>
<td>SH004</td>
<td>121.831</td>
</tr>
</tbody>
</table>
ANIMALS

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 utilized in a completely randomized design. The goats ranged in age from 17 to 18 months and body weights ranged 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, packed cell volume, total protein determination, blood urea nitrogen, and glucose level. Urinalyses on free-catch samples, including pH, dipstick analysis, including detection for the presence of glucose, ketones, protein and occult blood, urine specific gravity, and microscopic sediment examination after centrifugation were also normal for inclusion. Albendazole (10mg/kg, PO) was administered to each animal once included in the study.

Prior to the start of the study, goats were sedated with xylazine hydrochloride (0.1 mg/kg, IM) and restrained in lateral recumbency. The fiber around the preputial orifice was surgically clipped and cleaned. Medical specimen vial lids were prepared by drilling a 3.5 cm diameter circle in the center of the lid, with six small pairs of holes drilled around the circumference of the remaining lid. The lids were then sutured to the skin by the small paired holes around the preputial orifice, centering the large opening around the preputial orifice, with the threads in a ventral position. Fiber over the ventral midcervical region overlying the jugular veins was clipped to facilitate venipuncture and nylon collars were fitted to each goat.
Goats were randomly assigned using a randomization software program\(^k\) to four treatment groups, corresponding to DCAD levels -150 mEq/kg, -75 mEq/kg, 0 mEq/kg and +75 mEq/kg of feed, each consisting of six goats.

**FACILITIES**

Goats were randomly assigned to four indoor, concrete stalls, with one goat from each treatment group in each stall. The stalls measured 2.46m by 3.51m and were bedded with pine shavings. A five 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 one large tub which provided *ad libitum* access to fresh water.

**FEEDING**

A basal ration was formulated using pelleted feed\(^l\) and ground prairie hay. During the treatment phase, feed-grade ammonium chloride\(^m\) was administered to goats at the time of feeding to attain DCAD levels of the total ration 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% decoquinate, mixed and pelleted as a single batch. Prairie hay was ground to a fiber length of 2.5 cm. National Research Council (NRC) requirements\(^83\) for metabolizable energy (ME) and crude protein (CP) 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 seventy five percent of the energy requirement daily, divided into two feedings, based on individual weight. Twenty-five
percent of the daily NRC ME requirement was met by the hay, with the remaining 50% met by the pelleted feed. Based on published values, the estimated ME of the pelleted feed was 2.796 Mcal/kg with a CP of 152 g/kg. The hay had an estimated ME of 1.91 Mcal/kg and a CP of 6.6%. The goats received an average of 0.108 kg hay and 0.147 kg pellets per 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 nitric acid digestion, followed by simultaneous determination of mineral analytes using optical emission spectrometry as specified in previous publications. The DCAD levels of the pellets and ground hay, using the equation [(Na+K)-(Cl+S)], were determined to be +119.14 mEq/kg and +139.13 mEq/kg, respectively, resulting in a total basal ration DCAD of +125.68 mEq/kg. The mineral analyses for the feeds were entered into a ration formulation software program and the proportion of hay, pellet, and ammonium chloride determined to formulate the total ration necessary to achieve treatment DCAD level for each goat.

Table 2: Mineral Analysis of Basal Ration

<table>
<thead>
<tr>
<th>Feed</th>
<th>P (%)</th>
<th>Ca (%)</th>
<th>K (%)</th>
<th>Mg (%)</th>
<th>Na (%)</th>
<th>S (%)</th>
<th>Cl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>0.058</td>
<td>0.414</td>
<td>0.701</td>
<td>0.22</td>
<td>0.002</td>
<td>0.065</td>
<td>0.00224</td>
</tr>
<tr>
<td>Pellets</td>
<td>0.283</td>
<td>0.589</td>
<td>0.870</td>
<td>0.144</td>
<td>0.430</td>
<td>0.172</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Table 3: Ration Composition Percentage As-Fed by Treatment Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Hay</th>
<th>Pelleted Feed</th>
<th>NH₄Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>-150 mEq/kg</td>
<td>41.80%</td>
<td>56.73%</td>
<td>1.47%</td>
</tr>
<tr>
<td>-75 mEq/kg</td>
<td>41.97%</td>
<td>56.96%</td>
<td>1.07%</td>
</tr>
<tr>
<td>0 mEq/kg</td>
<td>42.14%</td>
<td>57.19%</td>
<td>0.68%</td>
</tr>
<tr>
<td>+75 mEq/kg</td>
<td>42.31%</td>
<td>57.42%</td>
<td>0.29%</td>
</tr>
</tbody>
</table>
Table 4: Mean Ammonium Chloride Dose per Feeding by Treatment Group

<table>
<thead>
<tr>
<th>Group</th>
<th>NH₄Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>-150 mEq/kg</td>
<td>3.69 g</td>
</tr>
<tr>
<td>-75 mEq/kg</td>
<td>2.75 g</td>
</tr>
<tr>
<td>0 mEq/kg</td>
<td>1.74 g</td>
</tr>
<tr>
<td>+75 mEq/kg</td>
<td>0.70 g</td>
</tr>
</tbody>
</table>

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

On days 1-7, the treatment phase of the study period, goats were fed as during the acclimation period. As each goat completed his 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.

SAMPLING

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 jugular venipuncture and analyses of the urine and blood performed as previously for inclusion in the study. Additionally, electrolyte analysis was performed on blood on a handheld blood analyzer. Goats continued into the study period based on normal findings from this examination.

On days 1-7 of the study period, urine samples were collected during five three-hour sampling periods. Sampling periods were hours -3 to -1, 1-3, 5-7, 9-11 and 13-15
relative to the morning feeding. These five sampling periods were denoted 1-5, 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, 5-7 hours after the morning feeding. These samples were placed in tubes containing lithium heparin and situated in an icebath.

Urine samples were analyzed within 20 minutes of collection and were analyzed for pH via pH meter and urine specific gravity via refractometer. Heparinized venous blood samples were analyzed within one hour of collection for determination of blood pH using a handheld chemistry analyzer. The final urine sample collected on day 7 was also subjected to dipstick examination to assess continued urinary tract health. 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 using SAS statistical software. The experiment was a one-way treatment structure with four levels, corresponding to DCAD levels -150 mEq/kg, -75 mEq/kg, 0 mEq/kg and +75 mEq/kg of feed, and repeated measures taken at the beginning of the study and every day for the following seven days. Within each day after the start of the experiments, there were repeated measures taken at five times during the day, -3 to -1, 1-3, 5-7, 9-11 and 13-15 hours relative to the morning feeding, corresponding to sampling periods labeled 1-5. Ananysis of covariance (ANCOVA) methods were used in the analysis of urine pH, urine specific gravity 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 a compound symmetry covariance
structure. Additionally, a baseline measurement (day 0) of the three response variables
for each subject was incorporated into the analysis as a covariate, and unequal slopes
models were adopted for each of the three response variables.

Comparisons of DCAD levels, days and time within days were performed at
specified values of the covariate in each of the three analyses. For urine pH, the
comparisons were done at the baseline covariate of 8.0. For urine specific gravity,
baseline covariates of 1.000 to 1.060 were selected. For blood pH, comparisons were
made using the baseline covariate values of 7.40 and 7.45.
CHAPTER V

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 negative health effects were noted 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 in the 1-3 hour sampling period on day six. The other goat was in treatment group -75 mEq/kg with no sample obtained during the 9-11 hour sampling period of day 7. These samples represent missing data points.

Four urine samples were obtained after the three hour sampling period had ended. These included a goat in the +75 mEq/kg group during the 9-11 hour sampling period on day 3, a goat in the -150 mEq/kg group during the 1-3 hour sampling period on day 5, a goat in the +75 mEq/kg group in the 1-3 hour sampling period of day 6, and one goat in treatment group 0 mEq/kg during the 9-11 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.

All blood samples were obtained and successfully analyzed.

**URINE PH**

There was a significant three-way interaction of group, day and time for urine pH (p=0.0137). There was no significant group effect on day 1 for time periods 1-4 (p>0.4810). Starting sampling period 5 of day 1 (p=0.0064) and continuing through day 7, at all time periods (p<0.0001), significant differences existed among groups. This initial difference was due to a difference between the -150 mEq/kg and +75 mEq/kg groups (p=0.0005).

The urine pH from each sampling period 1-5 was compared with the daily mean of each treatment group in order to determine which time frame after feeding urine samples should be obtained in order to monitor overall effectiveness of DCAD level. 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 of the sampling periods. The 95% confidence intervals (CI) of sampling periods 1 and 5 contained the daily mean 82.14% and 64.28% of the time. Sampling period 2 contained the daily mean 92.86% of the time. Sampling period 3, 5-7 hours after feeding and ammonium chloride administration, captured the value of the daily mean 100% of the time, more frequently than the remainder of the time periods.

Time period effects were not equal among groups and as the days on the DCAD balanced ration progressed, the urine pH values across time periods within the day became less significantly different. Also, with increasing DCAD level, more days were
required for sampling period differences to become non-significant. At a baseline pH of 8.0, for DCAD level -150 mEq/kg, urine pH values were significantly different among time periods on days 1 (p<0.0001) and 2 (p<0.0001). On days 3-7, there were not significant differences across sampling times (p>0.2399). For DCAD level -75 mEq/kg, differences were only significant until day 3, for DCAD level 0 mEq/kg, differences were significant until day 5. For +75 mEq/kg, sampling time was not significant for days 1 and 3 of the study, but differences were significant for the remainder of the study.

Table 5: Variation of Urine pH Across Sampling Periods Day 1
Tests were performed to determine if urine pH values leveled off for DCAD during the study. Post-hoc analysis of the means showed that urine pH had not leveled off by day 3 (p=0.0079). On day 4, at time period 3, all groups leveled off (p=0.0999). For days 4-7, there is no significant fluctuation in the urine pH response (p=0.0999). Not all treatment levels leveled off at the same pH, each group maintained its own plateau.

Ninety five percent confidence intervals were calculated for each group’s daily mean using a baseline urine pH of 8.0 to determine achievement of the target urine pH of 6.0 to 6.5. In the table below, the daily mean upper confidence limit (UCL) and lower confidence limit (LCL) values are reported.
Table 7: 95% Confidence Interval Values for Daily Mean Urine pH by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>LCL &lt;6.0 UCL &lt;6.0</th>
<th>LCL &lt;6.0 UCL 6.0-6.5</th>
<th>LCL &lt;6.0 UCL &gt;6.5</th>
<th>LCL 6.0-6.5 UCL &gt;6.5</th>
<th>LCL &gt;6.5 UCL &gt;6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>-150 mEq/kg</td>
<td>Days 3-7</td>
<td>Day 2</td>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-75 mEq/kg</td>
<td>Days 6-7</td>
<td>Days 4-5</td>
<td>Days 2-3</td>
<td>Day 1</td>
<td></td>
</tr>
<tr>
<td>0 mEq/kg</td>
<td></td>
<td>Days 5-7</td>
<td>Day 4</td>
<td>Days 1-3</td>
<td></td>
</tr>
<tr>
<td>+75 mEq/kg</td>
<td></td>
<td></td>
<td></td>
<td>Days 1-7</td>
<td></td>
</tr>
</tbody>
</table>

LCL – lower confidence limit; UCL – upper confidence limit

The +75 mEq/kg group never achieved urine pH levels within the target range. The 0 mEq/kg group had measured values of days 1-3 that remained above the target range, with day 4 falling around the upper limit. Days 5-7, the CI for this group fully included the target range. For the -75 mEq/kg, day 1 was fully above the target range, days 2-3 fully contained the target range, days 4-5 fell around the lower limit of the target and the remaining 2 days fell fully below the target range. For -150 mEq/kg, the mean of day 1 fell above the target range, day 2 fully encompassed the target range and days 3-7 fell fully below 6.0.

At DCAD levels of -150 mEq/kg and -75mEq/kg, a urine pH of 6.0 – 6.5 was achieved two days after initiation of the treatment diet at the time of the 5-7 hour urine sampling. DCAD level 0 mEq/kg resulted in urine pH levels between 6.0 – 6.5 on day 5 of the treatment period, while urine pH levels at DCAD level +75 mEq/kg remained above 6.5 during the seven day trial period. By the end of the trial period, treatment levels -150 mEq/kg and -75 mEq/kg resulted in urine pH levels below the target range.
Using ANCOVA with baseline USG used as covariates in the analysis, there was a significant difference in group response based on baseline USG (p<0.0001). There was also significant interaction between day and time period, and analyses using time period C were performed, as performed for urine pH. At baseline USG of 1.000 for time 3, no significant difference among days were found (p=0.2844). There were no significant differences between the four treatment levels in USG produced from baseline USG levels of 1.000 to 1.059. At a baseline USG of 1.060, a significant difference occur between the -150 mEq/kg and -75 mEq/kg group (p=0.05). At this baseline, -150 mEq/kg had an estimated USG of 1.0346, while -75 mEq/kg had an estimate of 1.0502. The 0 mEq/kg and +75 mEq/kg USG were estimated at 1.0421 and 1.0422, respectively.

Urine Specific Gravity
Blood pH

ANCOVA analysis of blood pH was performed using the day 0 blood pH as a covariate. Blood pH was significantly linearly related to the baseline blood pH (p=0.0062). The influence of baseline blood pH is the same across all groups and all days. There is no significant interaction between group and day (p=0.5754) and no significant day effect (p=0.6212). There is, however a significant group effect (p<0.0001), which would remain the same at any baseline blood pH that falls within the normal range. When group means were calculated using other baseline blood pH levels of 7.40 and 7.45, the group differences remained the same. The following table indicates the blood pH means for each group in response to baseline pH of 7.40 and 7.45.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline Blood pH</th>
<th>Estimated Group Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>-150 mEq/kg</td>
<td>7.40</td>
<td>7.3582&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>-75 mEq/kg</td>
<td>7.40</td>
<td>7.4057&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 mEq/kg</td>
<td>7.40</td>
<td>7.4187&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>+75 mEq/kg</td>
<td>7.40</td>
<td>7.4233&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>-150 mEq/kg</td>
<td>7.45</td>
<td>7.3761&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>-75 mEq/kg</td>
<td>7.45</td>
<td>7.4237&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 mEq/kg</td>
<td>7.45</td>
<td>7.4367&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>+75 mEq/kg</td>
<td>7.45</td>
<td>7.4412&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values within the same baseline pH with the same superscript are not significantly different (α=0.05)
The -75 mEq/kg, 0 mEq/kg and +75 mEq/kg DCAD levels experienced similar blood pH responses and were not significantly different from each other. The -150 mEq/kg DCAD group had significantly lower blood pH levels than did the remaining treatment levels.

Table 10: Group Mean Blood pH Values by Group and Day
CHAPTER VI

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 is typical feeding practice of small ruminants in our practice area. The percentage of ammonium chloride used to lower the basal ration used in this study fell within the recommended 0.5-2%⁵,¹⁳,¹¹,¹⁵,²⁵,²⁸,²⁹ for three of the four levels. The three levels, -75, 0 and -150 mEq/kg, where the percentage was within this range, 0.68 - 1.47%, achieved urine pH levels in and around the target range. These percentages, therefore, are appropriate for use in rations which have a basal DCAD similar to this one of +126.68 mEq/kg. Rations with DCAD levels significantly above or below this ration, similar to those shown in Table 1, in combination with equal proportions of ammonium chloride, may result in under- or over-acidification.

Samples were taken five times daily at three hour intervals in order to determine a time interval for urine sampling would be a reliable predictor of DCAD effectiveness. It is not known for the prevention of urolithiasis if urine pH must remain consistently below a threshold for the majority of the day, if the mean throughout the day should fall below a threshold or if a single point low nadir is desirable. Analysis for this study was performed to determine which sampling period best represented the mean throughout the day, as a representation of the urine pH activity throughout the day. Time period 3, 5-7
hours after the initial feeding and salt administration was found to best represent the daily mean. Time period 2, 1-3 hours after feeding, represented the mean 92.86% of the time, 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 5-7 hours or 1-3 hours after feeding to monitor acidification.

The data indicates that after a number of days on a DCAD balanced ration, sampling time becomes less important as there is less difference between values achieved at different intervals. For -150 mEq/kg, day 3, sampling at any sampling interval would reveal a value that was not significantly different than values obtained at any other of the sampling intervals. This was also true for -75 mEq/kg beginning day 4 and for 0 mEq/kg beginning day 6. For +75 mEq/kg, the latter days of the study remained significantly different across sampling times. Therefore, urine pH sampling at 5-7 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 latter days.

In animals clinically affected by urolithiasis, once the acute obstruction is relieved, additional stones or mineral residue may remain in the urinary bladder. These patients are frequently treated with anionic salts or other acidifiers as part of their initial treatment to dissolve this residue. It may seem that there would be reason to start such animals out at a very low DCAD to achieve acidification quickly, then increase the DCAD to a more biologically sound level. The data in this experiment indicates that acidification occurs very quickly, the second day for -75 mEq/kg and the fifth day for 0 mEq/kg. These represent reasonable time frames to urine acidification to the target range
of 6.0 to 6.5 for clinical cases as well as the remainder of the herd or flock from which the animal originates.

The DCAD levels tested here, relative to each other, induced predictable responses in urine pH values. They are, however, somewhat different than those obtained from other studies, although these specific DCAD levels have not been directly tested in this class of animals. The +75 mEq/kg DCAD level produced urine pH levels which never achieved a urine pH between 6.0 to 6.5 throughout the course of the study. The 0 mEq/kg achieved a 95% CI which included the upper portion of the target range on day 4 and encompassed the target pH range for the final three days of the study. The -75 mEq/kg encompassed the target range on days 2 and 3 of the study, included the lower portion of the target range on days 4 and 5, but fell entirely below 6.0 for the final 2 days of the study. The -150 mEq/kg group encompassed the target range on day 2 of the study, but fell below 6.0 for the remainder of the study. Maintaining a urine pH below 6.0 consistently over time may represent significant alterations in acid-base balance, as in this study at the -150 mEq/kg level, with significantly low blood pH.

For the majority of USG baselines, there were no significant differences noted in values obtained among the groups. Only at a baseline of 1.060 was a difference noted between -150 mEq/kg and -75 mEq/kg. This counters the widely-held theory that increased salt in the diet significantly decreases urine concentration. While no studies specifically measure USG with relation to DCAD, one study showed that 6 of 8 goats significantly decreased their urine creatinine at a 0 mEq/100g level as compared to a forage diet only. The two groups with the highest salt intake group (-150 mEq/kg and -75 mEq/kg) were significantly different from each other, but neither were significantly different than the remaining two groups with the lowest salt intake. The -75
mEq/kg actually trended higher than the levels which required a lower salt mass, towards the end of the study. This likely reflects the large variability among individuals within the group (data not shown) and a non-linear response of USG to salt intake. Urine dilution may not be a predictable effect of various levels of DCAD.

Blood pH was significantly lower for the -150 mEq/kg group than for other groups. It also fell below published normal values for the goat of 7.42 to 7.46. Acidemia associated with this level of feeding for a prolonged period of time may induce harmful effects, such as decreased feed intake and weight gain or bone loss. The remaining groups produced blood pH levels that were the same and within the normal range, indicating that they produce responses in acid-base balance that are biologically sound and do not overwhelm compensatory mechanisms.

A significantly educed blood pH and over-acidified urine make the -150 mEq/kg DCAD level inappropriate for use in goats. A DCAD of -75 mEq/kg also resulted in over-acidified urine and +75 mEq/kg inadequately acidified the urine of goats. The 0 mEq/kg resulted in achievement of the target urine pH without significantly reduced blood pH. The range of values where adequate acidification without over-acidification occurs falls between -75 mEq/kg and +75 mEq/kg of feed. Based on the DCAD levels tested here, 0 mEq/kg appears to be the most appropriate target DCAD for reduction of urine pH in goats for the prevention of urolithiasis.
Footnotes

a. i-STAT® EC8+, Abbott Laboratories, Abbott Park, IL.

b. Fisherbrand® Capillary Tubes, Fisher Scientific, Pittsburgh, PA.


d. Azostix® Bayer Corporation, Elkhart, IN.

e. Medisense® Precision QID, Abbott Laboratories, Bedford, MA.

f. Cardy Twin® pH Meter, Spectrum Technologies, Plainfield, IL.

g. Multistix® Bayer Corporation, Elkhart, IN.

h. Valbazen® Suspension, 11.36% albendazole, Exton, PA.

i. X-Ject SA®, 20mg/mL xylazine, Vetus, Farmers Branch, TX.


l. Formula SH010. Oklahoma State University Feed Mill, Stillwater, OK.

m. Ammonium chloride, Prince Agri Products, Inc., Quincy, IL.

n. Spectro Ciros ICP, Spectro Analytical Instruments GmbH and Co. KG, Kleve, Germany.


References


64. Constable PD. Clinical assessment of acid-base status: Strong ion difference theory. 


VITA

Meredyth Lynn Jones

Candidate for the Degree of

Master of Science

Thesis: THE USE OF DIETARY CATION ANION DIFFERENCE FOR THE REDUCTION OF URINE PH IN GOATS

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Title of Study: THE USE OF DIETARY CATION ANION DIFFERENCE FOR THE REDUCTION OF URINE PH IN GOATS

Abstract:

Goat wethers were fed a pelleted and hay ration with ammonium chloride to achieve DCAD levels of -150, -75, 0 or +75 mEq/kg. Urine, for pH and specific gravity, was obtained hours -3 to -1, 1-3, 5-7, 9-11 and 13-15 relative to the morning feeding. Blood pH was determined on alternate days of the study period.

Levels of -150 and -75mEq/kg produced urine pH of 6.0–6.5 two days after initiation, and fell below this range by the end of the trial. 0 mEq/kg resulted in urine pH levels between 6.0 – 6.5 on day 5, while urine pH levels at DCAD level +75 mEq/kg remained above 6.5 during the trial period. Urine specific gravity differed only between the -150 mEq/kg and the -75 mEq/kg groups. Blood pH for the -150 mEq/kg level was significantly lower than that for the other treatment levels.