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Mineral Composition of Uroliths Obtained from Sheep and Goats with Obstructive Urolithiasis

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Background: Knowledge of the mineral composition of the causative urolith is important to develop preventative strategies. Advances in analytic techniques have led to detection of urolith components not previously recognized.

Hypothesis/Objectives: The objectives of this study were to characterize uroliths in sheep and goats structurally and clinically. We hypothesized that amorphous magnesium calcium phosphate (AMCP) would be a naturally occurring urolith type in sheep and goats.

Animals: Forty-nine sheep and goats presenting for obstructive urolithiasis from June 15, 2014 through June 14, 2016 were reviewed along with the demographic data of all small ruminants admitted during that same period.

Methods: Medical records were reviewed for demographic and historical data, and 36 uroliths obtained from these cases during diagnostic or therapeutic procedures were analyzed by infrared spectroscopy to determine chemical composition.

Results: AMCP is a naturally occurring urolith type in obstructed male sheep and goats and was the most common urolith type in this study, where it occurred as a majority component with struvite (39% of uroliths) or as a pure component (11%). Pure struvite was found in 1 urolith (2%). Calcium carbonate was the second most frequent urolith with 31% of uroliths being pure calcium carbonate.

Conclusions and Clinical Importance: This study demonstrates that uroliths, which appear consistent with struvite, could actually be calcium-containing AMCP. Urolith analysis is critical in determining mineral content of uroliths to guide dietary recommendations for prevention.

Key words: Amorphous magnesium calcium phosphate; Calcium carbonate; Struvite; Urinary calculi.

Urolithiasis is a common and costly problem for many goat and sheep owners. In sources readily available to veterinary practitioners, including text-books, review articles, and the primary literature, different authors report different types of uroliths to be the most common, including struvite (magnesium ammonium phosphate), ¹⁻³ calcium carbonate, ^{1,2,4-6} and calcium phosphate (apatite). ^{2,7} Several factors might be involved in the variability of reported urolith types, including geographic origin of cases and type of diet.

Veterinarians attending obstructive urolithiasis cases must know the mineral composition of the causative urolith in order to develop preventative strategies to limit the impact of the disease on the herd or flock. Similarly, researchers in the field need to know the mineral composition of naturally occurring uroliths in

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Abbreviation:

AMCP

amorphous magnesium calcium phosphate

order to test and refine hypotheses for preventative measures. It could not be possible to develop a single strategy, which addresses all of the most common urolith types. Several investigators have recently published investigations of dietary modification as a means of achieving urinary acidification, 8,9,a,b which is believed to be most effective at preventing struvite urolithiasis. Increasing Ca:P ratio in diets is also believed to assist in the prevention of phosphatic uroliths.10 Urine acidification using anionic salts and increasing calcium in diets could, however, predispose to calcium-containing uroliths by increasing urinary excretion of calcium.^{4,8} Furthermore, in potbellied pigs, infrared spectroscopy of uroliths previously analyzed as struvite was determined to be AMCP.11 It has been proposed that AMCP is a transitional urolith type of struvite. 11 It is important to know whether AMCP is an important component of naturally occurring, obstructive sheep and goat uroliths in addition to or instead of struvite. Research projects and preventative recommendations would need to be developed to target this calcium-containing struvite derivative.

We hypothesize that AMCP is a naturally occurring urolith type in sheep and goats and is the most common type of urolith found in the population of goats and sheep presenting to our hospital for obstructive urolithiasis. The first objective of this study was to characterize the clinical features of urolithiasis cases in obstructed commercial, exhibition, and pet goats and sheep. The second objective was to determine the types of uroliths found in these cases.

Materials and Methods

This study was a retrospective review of medical records with analysis of urolith samples collected in the course of case management.

Uroliths

Uroliths obtained as part of the treatment or necropsy examination of all sheep and goats presenting for obstructive urolithiasis to the Texas A&M University Veterinary Medical Teaching Hospital (TAMU-VMTH) from June 15, 2014 through June 14, 2016 were collected. Recovered uroliths were submitted within 2 weeks of collection to the G.V. Ling Urinary Stone Analysis Laboratory in Davis, California, where uroliths were screened by optical crystallography, followed with infrared spectroscopic confirmation of composition. Uroliths were then quantitatively classified as pure (100% of one component), compound ($\geq 70\%$ to < 100% of one component), and mixed (< 70% of each component) by a modified classification system. 12

Dietary and Related History

A retrospective medical record analysis of all sheep and goats presenting for obstructive urolithiasis during the study period was performed, and the dietary and health histories, collected as part of initial veterinary evaluation of the animals, were reviewed. Diets were categorized as containing alfalfa, grain, pellet, non-alfalfa forage (coastal Bermuda hay or pasture), and mineral, alone or in combination. Date and season of presentation, species, breed, sex, age, intended purpose, previous history of obstructive urolithiasis, previous or concurrent illnesses, and gross and radiographic appearance of the recovered urolith(s) were also analyzed.

Radiopacity

The uroliths from one sheep, a Hampshire wether, were radiographed ex vivo before being analyzed for urolith composition. The gross appearance of the uroliths suggested that their composition would be AMCP.

Statistical Analysis

Data from medical records were uploaded into a spreadsheet^c by case number with breeds listed as a categorical variable and age as a continuous variable. All other information was entered as binary indicator variables for species, castrated, dietary components: alfalfa hay, coastal hay, grain, pellets, or mineral, season(s) presented: winter, spring, summer, fall, uses: show, pet, fiber, breeding, or not indicated, and urolith type: AMCP, struvite, calcium carbonate, apatite, silicate, or calcium oxalate. Statistical analysis was performed in STATA 11.2 SE.d Because of the close clinical relationship between AMCP and struvite uroliths, the indicator variables for each of those types were combined into one indicator variable. Logistic regression was performed by AMCP/ struvite (coding 1) and non-AMCP/struvite (coding 0), calcium carbonate (coding 1), and noncalcium carbonate (coding 0) and each variable or like group of variable (i.e, season) analyzed. For all analyses, P < .05 was considered significant. The final model was assessed with likelihood ratios to find the best fit.

Results

A total of 49 animals (sheep: n = 6; goats: n = 43) were admitted with obstructive urolithiasis during the study period, with six of these animals presenting twice

during the study period for obstruction. During these 55 obstructive episodes, 36 urolith samples were retrieved during treatment for analysis. In the remaining 19 episodes, no mineralization was found, owners declined treatment or necropsy that may have resulted in urolith retrieval, or medical management resulted in resolution of the obstruction without urolith recovery.

Urolith Type and Classification

Urolith compositions and quantitative classifications are summarized in Table 1.

Of the 13 compound AMCP/struvite uroliths, AMCP was the major component of 11, with 2 having struvite as the major component of some stones within the sample.

Signalment

All 49 obstructed animals were male, with 16 being intact (33%) and 33 (67%) castrated. Univariable logistic regression found that sex status was not significant for presentation with obstructive urolithiasis. Intact animals had a 2.5 greater odds (95% CI (0.78,7.97), P=.12) of developing AMCP/struvite uroliths as compared with castrated males, whereas castrated males had a 4.5 greater odds (95% CI (0.90, 22.8), P=.068) of developing calcium carbonate uroliths when compared with intact males.

The age range for urolithiasis cases was 2–133 months (mean = 23 months; median = 12 months). The data were skewed to the right, and thus, age was categorized into "Juvenile," "Adult," and "Mature" at 0–4 months, 5–36 months, and 37+ months, respectively. Analyzed urolith type by age at presentation is presented in Table 2.

Table 1. Urolith compositions and quantitative classifications

Urolith Components	Cases	Classification
AMCP/struvite combination	13	Compound
	1	Mixed
AMCP	4	Pure
Struvite	1	Pure
Calcium carbonate	11	Pure
Struvite/apatite	2	Compound
Calcium carbonate /amorphous silica	1	Compound
	1	Pure and compound ^a
AMCP, struvite, calcium carbonate	1	Mixed
Calcium oxalate/ amorphous silica/ calcium carbonate	1	Pure and compound ^b

Urolith type, number of cases of each type or combination, and classification based upon component percentages.

^aThis sample included 6 uroliths, 3 of which were pure calcium carbonate, 3 were 99-100% amorphous silica.

^bThis sample contained 2 separate urolith types: 95% calcium oxalate, 5% amorphous silica and 100% calcium carbonate.

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Urolith Type	Age Juvenile ≤4 m	Adult 5–36 m	Mature ≥37 m	Age not reported
AMCP/struvite	3	11	0	1
AMCP	0	2	1	0
Struvite	0	1	0	0
Calcium carbonate	0	2	9	0
Struvite/apatite	0	1	0	1
Calcium carbonate/amorphous silica	0	1	0	0
AMCP, struvite, calcium carbonate	0	1	0	0
Calcium oxalate/amorphous silica/calcium carbonate	0	1	0	0
Calcium carbonate/ovalate	0	0	0	1

Table 2. Analyzed Urolith Type by Age at Presentation

Number of cases of each urolith type for each age range at the time of presentation.

By AMCP/struvite as the dependent variable, the odds of developing obstructive urolithiasis were 1.25 (95% CI (0.24, 6.5), P = .79) for Adult animals and 0.36 (95% CI (0.05, 2.60), P = .31) for Mature animals compared to Juvenile. With calcium carbonate as the dependent variable, the odds of developing obstructive urolithiasis were 0.07 (95% CI (0.02, 0.32), P = .001) for Adult compared to Juvenile animals.

Goat breeds represented included: Boer (n = 30), Pygmy (n = 5), Nubian (n = 2), Nigerian Dwarf (n = 1), Pygora (n = 1), and crossbred (n = 4). Represented sheep breeds were Dorper (n = 2), Hampshire (n = 2), and crossbred (n = 2).

Risk of AMCP/struvite being detected in sheep was not different to that in goats (3.3, 0.55, 20.1, P = .19). Goats were the only species to present with calcium carbonate uroliths. There was no significant breed predisposition of animals presenting with urolithiasis (P-values >.05).

Purpose

Owner-reported purposes for animals presenting with obstructive urolithiasis were exhibition (n = 19), companion (n = 18), breeding (n = 7), fiber (n = 1), and purpose not listed (n = 4).

Pet animals had significantly higher odds (OR = 6.5, 95% CI (1.18, 36.3), P = .032) of presenting with calcium carbonate urolithiasis compared to exhibition animals (base). Nonsignificant interactions were seen for pet animals (OR = 1.0, 95% CI (0.08, 12.8), P = 1.0) compared with breeding animals and pet animals (OR = 2.0, 95% CI (0.14, 28.0), P = .61) compared with animals where the use was not indicated. For animals presenting with AMCP/struvite uroliths, there were no significant interactions by animal use. Using exhibition animals as the base, comparisons were made for pets (OR = 1.1, 95% CI (0.28, 4.12), P = .91), breeding (OR = 2.5, 95% CI (0.49, 12.9), P = .49), or had no use indicated (OR = 3.0, 95% CI (0.39, 23.1), P = .29).

Season of Presentation

Seasonal distribution of all urinary obstruction cases was winter (December-January-February) 9 cases,

spring (March–April–May) 11 cases, summer (June–July–August) 16 cases, and fall (September–October–November) 13 cases.

Seasonal associations were as follows: spring (OR = 3.3, 95% CI (0.78, 7.97), P = .19) compared to winter, summer (OR = 1.8, 95% CI (0.34, 9.69), P = .49) compared to winter, and fall (OR = 1.06, 95% CI (0.19, 5.90) P = .95) compared to winter. Odds ratios for detection of calcium carbonate uroliths were as follows: fall (OR = 2.4, 95% CI (0.38, 15.3), P = .35) compared to winter, summer (OR = 1.3, 95% CI (0.20, 9.08) P = .77) compared to winter, and spring (OR = 1.3, 95% CI (0.18, 10.1), P = .78) compared to winter.

Health History

Six animals (5 goats, 1 sheep) had reobstruction during the study period, with reobstruction occurring a range of 6 days to 8.5 months after the original episode. Four animals reobstructed at 31 days or less. One goat reobstructed twice during the study period after obstructing before the study period. Three animals which reobstructed had the same urolith type at the second episode; of the remaining 3, one had no urolith found during the second episode and two were medically managed with no urolith retrieval during either episode.

Eleven animals had current or recent comorbidities within the month preceding their obstructive urolithiasis episode. These included coccidiosis (4 cases), upper respiratory disease (3 cases), bronchopneumonia (2 cases), *Chorioptes* mange (1 case), bloat (1 case), arthritis (1 case), diarrhea (1 case), and subclinical gastrointestinal nematode infection (1 case). Two cases had more than 1 comorbidity with 1 case having both subclinical gastrointestinal nematode infection and coccidiosis and another with coccidiosis, pneumonia, and bloat.

Gross Appearance of Uroliths

Terms used in records to describe uroliths later determined to be AMCP/struvite uroliths included, "beige," "gritty," "irregular," and "packed white sand." One record listed the appearance of an AMCP/struvite urolith as "struvite." Pure or majority struvite uroliths were

described as "grit." Pure AMCP uroliths were described as "small and white" and "sand." Struvite/apatite uroliths were described as "grit." Calcium carbonate uroliths were described as "gold or brown beads," whereas the calcium carbonate/amorphous silica urolith was described as "gold beads with white grit." The calcium oxalate/amorphous silica/calcium carbonate uroliths were described as "brown and white round beads."

Radiography

Thirty-one cases had abdominal and pelvic radiography performed for diagnostic purposes, 10 of which did not have uroliths analyzed. Uroliths eventually analyzed from radiographed cases were calcium carbonate (n=12), AMCP/struvite (n=3), AMCP (n=2), struvite (n=1), calcium oxalate/amorphous silica/calcium carbonate (n=1), and calcium carbonate/amorphous silica (n=2).

Calculi were observed on radiographs of 11 of 12 confirmed calcium carbonate cases (see Fig 1) in both the urinary bladder and urethra (7 cases), the urinary bladder only (1 case), the urethra only (2 cases), and the prepuce (1 case). In the five cases of AMCP/struvite and pure AMCP, radiology reports stated that no calculi were observed in the urinary bladder or urethra in 3 of 5 cases, whereas one case had suspected urinary bladder sediment. The final case had notation of a small amount of mineralized sediment in the urinary bladder and penile urethra. The single pure struvite case had no calculi detected on radiography. The calcium oxalate/amorphous silica/calcium carbonate case had reported no definitive calculi observed, whereas the three calcium carbonate/amorphous silica cases all had linear mineral opaque material or radiopaque round uroliths observed in the urethra and urinary bladder. The single AMCP/ calcium carbonate/struvite case had two small round radiopaque structures noted in the prepuce.

The uroliths from the Hampshire wether, which were radiographed ex vivo before analysis, were radiopaque



Fig 1. Caudal abdominal and pelvic radiograph of a Pygmy goat showing calculi, analyzed to be 100% calcium carbonate, in the gravity-dependent portion of the urinary bladder, trigone, and pelvic urethra.

(see Fig 2). The analysis of the uroliths showed that their composition ranged from 40–100% AMCP and 0–60% struvite.

Dietary History

Previous to the episode of urolithiasis, the diet of obstructed animals contained forage (n = 39), grain (n = 31), pellet (n = 31), alfalfa (n = 15), and mineral (n = 9). A model was generated that included reported dietary components of alfalfa, grain, pelleted diet, hay or pasture, and trace mineral access.

The odds ratios and associated *P*-values for developing AMCP/struvite uroliths are reported in Table 3 for reported rations of animals with obstructive urolithiasis. There was no significant difference in OR for different diet types.

Four animals were on hay or pasture only with no supplemental grain or pelleted feed. Three of these animals had pure calcium carbonate uroliths, and one had a calcium carbonate/amorphous silica compound uroliths.



Fig 2. Ex vivo radiograph of uroliths obtained from a single case, each analyzed to be 40–100% AMCP and 0–60% struvite.

Table 3. Odds ratios for developing AMCP/struvite uroliths by ration composition

Ration	Odds Ratio	95% CI	P-value
P+A	1.7	0.07, 37.7	.748
G+A	5.0	0.15, 166.6	.368
F+P	3.8	0.27, 51.4	.322
F+P+M	5.0	0.27, 91.5	.278
F+G	6.25	0.50, 77.5	.154
F+G+A	10.0	0.40, 250.4	.161
F+G+M	5.0	0.15, 166.6	.368
F+G+P	1.0		Base

Odds ratio for the development of AMCP/struvite urolithiasis based on diet combination.

A, alfalfa; G, grain; P, pellet; M, mineral; F, forage (coastal Bermuda hay, pasture).

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Discussion

This study population was obstructed with uroliths containing combinations of seven different components, the most common of which was AMCP in combination with struvite, confirming our hypothesis that AMCP is naturally occurring and the most common urolith component in the sheep and goat population presenting to our hospital. Record analysis indicated that pure and compound AMCP uroliths are common in animals less than 3 years of age, are visually indistinguishable from struvite uroliths, and are not visible radiographically. The presence of calcium in uroliths clinically expected to be magnesium ammonium phosphate is an important development in the study of urolith pathophysiology in sheep and goats.

Consistent with previous reports, our population of obstructed animals was entirely male, with 67% being castrated. Although it has been stated that castrated males are at increased risk of obstructive urolithiasis, ¹³ we found the difference to be nonsignificant compared to intact males in this study population. Other sources¹⁴ have indicated an increased incidence in Pygmy goats, but our data across breeds indicated no significant overrepresentation by breed for obstructive urolithiasis. Odds for development of obstructive urolithiasis with AMCP/struvite uroliths were nonsignificant by age; however, the odds of developing calcium carbonate obstructive urolithiasis significantly increase with age. Similarly, age greater than 1 year has been previously shown to be a risk factor for calcium carbonate urolithiasis.6 Other studies have not assessed animal use directly for risk, but our data indicate that small ruminants raised as pets have a significantly increased risk of developing calcium carbonate urolithiasis.

The highest prevalence of urolithiasis in ruminants has historically been considered to occur in late fall and winter, 15 theoretically due to decreased water intake and increased urine concentration. Others have indicated increased prevalence of urolithiasis in the spring and summer,² whereas one study indicates that calcium carbonate is found least often in spring.⁶ The seasonal distribution in our study showed no significant increased odds by season of the year. Previous statements regarding increased incidence in wintertime may reflect the geographic and environmental risk factors associated with large-scale production not applicable to the small-scale producers in our geographic area. Breeding in sheep and goats is largely seasonal, with lambs and kids being born in the spring, so age and season are linked. The presence of AMCP/struvite in young, growing animals and in the spring and summer could be considered a reflection of high grain rations being delivered to young animals in preparation for exhibition, although analysis by animal purpose did not support this.

Struvite has been widely reported to be the most common cause of obstructive urolithiasis in male small ruminants consuming grain-based diets. 16-21 Due to high diet variability and small case number per diet combination, our study was unable to find significant

correlations between urolith type and diet. However, in 31 cases of urolithiasis in animals consuming grain, we found that AMCP was a much more prevalent urolith component than struvite. Only one urolith was pure struvite, and only two compound AMCP/struvite uroliths did struvite comprise the majority of some uroliths within the sample. AMCP is a urolith type recently reported to occur in potbellied pigs¹¹ and has been previously reported in wild and domestic ruminants. Advanced analytic methods for urolith analysis have improved differentiation of urolith types, and infrared spectroscopy reveals that AMCP produces a similar pattern to that of calcium phosphate and struvite. For this reason, it is believed that AMCP could be an intermediate form in the transition from amorphous phosphate to struvite. 11 Calcium carbonate has been recently and previously reported to be the most common urolith type from sheep and goats at a single national service laboratory which accepts samples from a wide variety of animal species.^{5,6} Our study indicates that pure calcium carbonate comprised 11 of 36 (31%) uroliths and was a minority component in three uroliths.

Radiography can be a valuable tool in the initial evaluation of urolith cases, with both calcium carbonate and struvite uroliths classified as radiopaque. 11,22 Consistent with this, 91.6% of the radiographed calcium carbonate cases had visible uroliths. However, no AMCP/struvite, struvite, or AMCP uroliths were visible on radiography in our study. A study of radiography and small ruminant urolithiasis similarly found that calcium carbonate uroliths were radiographically visible, whereas a single struvite case had no visible uroliths.²³ Ex vivo radiography was performed on a urolith subsequently analyzed to be AMCP/struvite where radiopacity was confirmed. It is believed that the inability to view AMCP/struvite uroliths on pelvic and abdominal radiography of sheep and goats could be due to small urolith size (often in the form of sand), that the radiographic technique required to penetrate the abdomen might result in loss of detail of these moderately radiopaque uroliths,²² or that tissue superimposition might obscure these uroliths. Radiography cannot be relied upon for the detection of AMCP and struvite uroliths, and a negative radiographic study does not rule out obstructive urolithiasis by these urolith types. For cases where calcium carbonate is suspected, radiography can be useful in confirming the presence of the uroliths and their location, as well as suggesting their composition by their characteristic morphology. In the authors' experience, calcium carbonate uroliths are refractory to chemolysis and are not well managed with techniques relying on chemolysis, such as tube cystotomy, in which uroliths are present within the urethra or remain in the urinary bladder. Radiography can therefore be a valuable tool in guiding therapeutic decisions for calcium carbonate cases.

Diet remains a focus of urolithiasis prevention but we were unfortunately unable to demonstrate significant correlation of diet to urolith type due to high diet variability and low case numbers, resulting in low power per diet combination. Further, we were unable to

analyze ingredients in grain and pelleted diet components retrospectively and we did not have information regarding the inclusion of dietary acidifiers, including ammonium chloride, in rations. References state that struvite and apatite crystals may be commonly seen in animals consuming a high grain diet, whereas calcium carbonate uroliths are more likely found in animals consuming large amounts of legumes. 16-21 Pelleted diets have been hypothesized to increase risk of phosphatic uroliths.²⁴ Feeding rations that are high in phosphorus such as the diets in feedlots that are grain-based can result in struvite uroliths.^{24–27} Recently, an experimental model of AMCP/struvite urolithiasis was created using a small grain-based diet with a 1:1.5 Ca:P ratio, indicating that excess phosphorus is capable of inducing the urolith type seen most commonly in this study.²⁸ For these reasons, nonalfalfa, forage-only diets are frequently recommended by clinicians in animals at risk for urolithiasis. It is interesting to note that in this study, four animals were on forage-only (nonalfalfa) diets and all were obstructed with pure calcium carbonate uroliths, accounting for 3 of 11 (27.2%) of the pure calcium carbonate uroliths and one case of compound calcium carbonate/silica. The lack of statistical correlation in this study and the lack of other studies which definitively link diet type to urolith type indicate that significant gaps remain in our understanding of the pathophysiology of this disease.

This study highlights the importance of performing urolith analysis by infrared spectroscopy on every case to better make recommendations for individual treatment and herd management. Most uroliths in this study were of mixed or compound composition (20 of 36 uroliths), and knowledge of the contributors to each components is important for dietary control. Visual inspection of uroliths as an indicator of urolith type is inadequate as 18 of 20 (86%) of the uroliths in this study that were classified as having an appearance consistent with struvite by the clinician contained AMCP, most of which contained a majority of AMCP. Dietary control recommendations should be reconsidered in light of the fact that what we previously considered to be struvite uroliths in fact contain calcium. It is unknown if this urolith type is a recent development or if struvite has always contained AMCP and analytical methods have improved and allowed for the detection of this urolith component.

Our data indicate that with increasing age, the odds of developing calcium carbonate urolithiasis increase. Further, pet animals are at a significantly increased risk of developing calcium carbonate urolithiasis and, as such, older pet animals should be carefully monitored for obstruction. This study also indicates that uroliths visually identified by clinicians as being suggestive of struvite (white to beige, gritty) could in fact be pure or combination stones of calcium-containing AMCP with or without struvite. Further, it was determined that AMCP and struvite combination uroliths are radiopaque, but are not reliably identified on survey radiography indicating that negative findings should not result in the conclusion that uroliths are

not present. Calcium carbonate uroliths, however, are readily identified on survey radiography and, for animals at increased risk of this urolith type (pet, increased age), survey abdominal radiography upon presentation may suggest urolith type and guide therapeutic decisions. Unfortunately, this study was unable to elucidate dietary factors that may be involved in urolith development and this, along with other individual and environmental risk factors, remains an area requiring further investigation.

Footnotes

- ^a Sprake P, Roussel AJ, Stewart R, Bissett WT. The effect of ammonium chloride treatment as a long-term preventative approach for urolithiasis in goats and a comparison of continuous and pulse dosing regimes. *J Vet Intern Med* 2012;26:760
- ^b Grissett G, Fleming S, Neizman K. Evaluation of orally supplemented D, L-methionine as a urine acidifier for small ruminants. *J Vet Intern Med* 2012;26:760
- ^c Microsoft Excel®2013. Microsoft Corporation, Remond, WA
- ^d Stata 11.2 SE. StataCorp, College Station, TX

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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