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An experimental model for calcium carbonate urolithiasis in goats

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Background: Calcium carbonate is a common urolith type in small ruminants with no high-yield experimental model to evaluate animal susceptibility or preventative measure response.

Hypothesis: That novel plastic winged implants would allow accumulation and quantification of calcium carbonate calculus formation in goats on a high-calcium diet and identify individual variation between goats in the mass of calculi produced.

Animals: Eight nonpregnant 3- and 4-year-old Boer-cross does, weighing 22.3–39.5 kg, determined to be healthy based on physical examination, were used in these experiments.

Methods: Prospective cohort study for in vivo experimental model development. Implants were placed into the urinary bladder lumen in 8 goats over 2 evaluation periods. The alfalfa-based ration had a total ration Ca : P of 3.29 and 3.84 : 1, respectively. Urine was collected at 0, 28, 56, and 84 days in the 1st experiment; blood and urine at those timepoints in the 2nd experiment. For each evaluation period, the implants were removed 84 days after implantation and weighed. Accumulated calculi mass was calculated and compared between goats and was analyzed for composition.

Results: Implant retention was 100% and 86% in the 2 studies. All goats with retained implants accumulated calcium carbonate at a mean implant gain per day across studies ranging from 0.44 to 57.45 mg. Two goats accumulated (0.44–7.65 mg/day and 33.64 & 57.45 mg/day) significantly more urolith material than the cohort across both studies ($P = .047$). No routine analytes on blood or urine were found to be explanatory for the difference observed.

Conclusions and Clinical Importance: These findings form a basis for implant and diet selection for use in future studies of urolithiasis development and for studies regarding individual susceptibility to urolithiasis.

KEYWORDS

3D printing, calculogenesis, urinary calculi, urolith

Abbreviations: IUD, intrauterine device; OD, outside diameter; ID, inside diameter.

This work was performed at the Texas A&M University College of Veterinary Medicine, College Station, TX, USA.

This work has not been presented at any meetings or conferences.

1 | INTRODUCTION

Small ruminant urolithiasis affects all types of small ruminant enterprises, including commercial production, exhibition, and pet keeping. It negatively influences welfare and longevity and causes important economic losses. Challenges in treating urolithiasis in ruminants require that prevention be the focus of disease management. Struvite,[1–3] calcium carbonate,[1,2,4–7] calcium phosphate (apatite),[2,8] and amorphous magnesium calcium phosphate (AMCP)[5,7] are cited as common urolith types in small ruminants and therefore serve as the focus of most research efforts. Shared characteristics of these urolith types are formation in supersaturated and alkaline urine and, as such, studies have focused on diluting and acidifying urine through dietary modification.[9–12] These projects have not evaluated these interventions on the formation or dissolution of uroliths or urolith components. Further, these preventative measures have generally been directed at these nonspecific, shared characteristics, rather than toward a specific urolith type. This is problematic because preventative measures for 1 urolith type could actually predispose to another. For example, urine acidification using anionic salts aimed at preventing phosphatic urolith types can predispose to calcium-containing uroliths by increasing urinary excretion of calcium.[4,9]

There is a need to evaluate preventative measures in light of urolith formation rather than simply urine biochemical characteristics, and to develop preventative strategies for specific urolith types. Experimental models of this sporadic disease provide an opportunity to obtain sufficient power for such studies while minimizing animal use. An experimental model of AMCP urolithiasis has been developed in female goats, using zinc washers as a nidus for urolith formation.[13] The model was successful in inducing urolith formation in all goats, but there were problems with implant retention, with 25%-50% of animals losing their implants across 4 studies.[13]

Recent studies have shown calcium carbonate to be the most common urolith type in some settings,[6,7] particularly in small ruminants identified as pets.[7] The development of a predictable and consistent model of calcium carbonate urolithiasis in goats is a critical need in the study of dietary causes and management of this disease. The 1st objective of these studies was to develop or identify a novel implant design with a high (>75%) rate of retention in the urinary bladder and ability to serve as a nidus for urolith accumulation. The 2nd objective was to determine if an alfalfa-based ration with an increased Ca : P ratio would induce calcium carbonate urolith formation on that intraluminal urinary bladder implant. The additional objective of Experiment 2 was to determine if there was individual variation in urolith mass accumulation among goats on a high Ca : P diet. Our hypotheses were that novel plastic winged implants would be retained at a rate greater than 75%, accumulate calcium carbonate uroliths on the high-calcium diet, and that individual variation exists between goats in urolith mass produced.

2 | MATERIALS AND METHODS

Procedures used in this study were approved by the Institutional Animal Care and Use Committee at Texas A&M University.

TABLE 1 Selected variables from dietary analysis of the total ration used in each experiment

Analyte	Experiment 1 diet	Experiment 2 diet
Net energy maintenance	0.29 Mcal/lb	0.30 Mcal/lb
Crude protein	17.25%	14.80%
Phosphorus	0.42%	0.37%
Calcium	1.38%	1.42%
Ca : P	3.29 : 1	3.84 : 1
Potassium	1.67%	2.09%
Magnesium	0.29%	0.32%
Sulfur	0.22%	0.21%
Sodium	0.21%	0.26%
Chloride	0.95%	1.23%
Dietary Cation Anion Difference (DCAD) (calc.)	113.69 mEq/kg	154.88 mEq/kg

2.1 | Animals and housing

Eight nonpregnant 3- and 4-year-old Boer-cross does, determined to be healthy based on physical examination, were used in these experiments. Initial body weights ranged from 22.3 to 39.5 kg. In Experiment 1, goats were group housed in 3 indoor stalls (3 goats in each of 2 stalls, 2 goats in the remaining stall) with wood shaving bedding. In Experiment 2, goats were individually housed in indoor stalls with wood shaving bedding.

2.2 | Feed

The diet for both studies consisted of chopped alfalfa hay and a pelleted ration. The pelleted ration base was corn, soybean hulls, wheat midds, alfalfa, soybean meal, rice bran, and included ammonium chloride. A partial dietary analysis including parameters of interest is shown in Table 1. In Experiment 1, the total diet was fed at a rate of 4% of body weight, 1 : 1 alfalfa : pellets by weight in a group-fed situation. In Experiment 2, the total diet was fed at a rate of 3% of body weight, 1 : 1 alfalfa : pellets by weight and each goat was fed individually. No additional forage or mineral sources were available during the experimental periods and municipal water was available ad libitum. Goats were acclimated to the diet in each experiment over the course of 14 days before the start of the study. The studies were performed 5.5 months apart.

2.3 | Implants

For Experiment 1, intrauterine contraceptive devices (Paragard, Duramed Pharmaceutical, Inc, Pomona, New York, 10970) designed for human use measuring 32 mm × 35 mm were prepared by removal of the copper wire wrap on the shaft and replacement with a 30-throw Chinese finger trap suture pattern using #1 catgut (Catgut Chrom, B Braun Aesculap, Center Valley, Pennsylvania, 18034). For Experiment 2, T-shaped devices were printed in plastic (Ninjaflex Semiflex, Ninjatek, Manheim, Pennsylvania, 17545) measuring 36 mm × 37 mm,

FIGURE 1 Printed implant from Experiment 2. A 30-throw Chinese finger trap suture pattern of #1 catgut has been placed on the vertical portion to enhance urolith adherence

with #1 catgut (Catgut Chrom, B Braun Aesculap, Center Valley, Pennsylvania, 18034) suture placed in a 30-throw Chinese finger trap suture pattern on the shaft (Figure 1). In both experiments, the prepared implants were weighed and stored in isopropyl alcohol before implantation.

2.4 | Implant procedure and maintenance

Before scheduled implant placement, feed was withheld for 12 hours with water available at all times. Xylazine (0.1 mg/kg, intravenous) was administered to the goats and anesthesia was induced with 3% sevoflurane in oxygen via facemask. Goats were maintained at a surgical plane of anesthesia using sevoflurane via facemask. Each animal was placed in sternal recumbency with hip extension. The vulva was cleaned with 2% chlorhexidine scrub and 99% isopropyl alcohol. The operator inserted a lubricated, gloved index finger into the vulva and identified the external urethral orifice. The implant was loaded into an insertion device, introduced into the urethra, and deployed into the bladder using a plunger. The insertion device for Experiment 1 was a 20 French red rubber catheter with the closed end removed and a 4 cm lengthwise slit created. In Experiment 2, the insertion device was rubber tubing with an outside diameter of 8 mm, inside diameter of 4 mm, and measuring 19.5 cm in length with a 4 cm lengthwise slit. For both, the plunger from the contraceptive kit was used to deploy the implant from the insertion device. Proper placement of the implant was confirmed by transurethral visualization using a 5 mm × 55 cm flexible endoscope. Goats were recovered from anesthesia and returned to their home pen and continued on the study diet. After implantation, goats were monitored at least twice daily for attitude, appetite, and any changes in micturition.

Goats underwent transurethral endoscopy of the urinary bladder every 4 weeks to determine the presence and appearance of the implant and assess urinary bladder health. Implants were removed after endoscopic visualization 84 days after placement using 5 mm × 43 cm laparoscopic atraumatic grasping forceps used in tandem with the endoscope, weighed, and stored in vials until analysis.

2.5 | Blood and urine analysis

During the group-fed study, urine was collected via the endoscope on days 0, 28, 56, and 84. During the individually fed study, urine collection and analysis were performed as for the group-fed study and blood was collected from the jugular vein at the same timepoints.

Urinalysis included dipstick examination (Multistix 10 SG, Siemens Healthcare Diagnostic, Inc, Tarrytown, New York, 10591), urine specific gravity via refractometer, and urine pH using a benchtop pH meter. Packed cell volume and total plasma protein were determined in blood and biochemistry analysis was performed including pH, total and ionized calcium, blood urea nitrogen, creatinine, and bicarbonate.

2.6 | Urolith analysis

After removal, the implant and associated urolith material was submitted to the G.V. Ling Urinary Stone Analysis Laboratory in Davis, California, where uroliths were screened using optical crystallography, followed by infrared spectroscopic confirmation of composition.

2.7 | Statistical analysis

Urolith accumulation was calculated by subtraction of the weight of each implant before implantation from the weight after implantation. Urolith mass accumulated per day was calculated for each implant and the mean determined for each goat across studies. One-way analysis of variance analysis was used to determine if there was a difference in the mean implant gain per day by goat across both studies. A 2 sample *t* test was then performed to determine if there was a difference in urolith accumulation between studies (group feeding versus individual feeding). Logistic regression was then used to compare blood and urine analytes of high and low urolith formers for explanatory variables.

3 | RESULTS

3.1 | Experiment 1

All implants were retained in the urinary bladder of all 8 goats for the duration of the study. All implants accumulated urolith material during the study. Implant gain per day for all goats ranged from 0.44 mg to 47.20 mg (median = 5.16 mg). Two goats accumulated high amounts of urolith material with an implant gain of 13.29–47.20 mg/day (median = 30.25 mg/day). The remaining 6 goats accumulated smaller amounts of urolith material, ranging from 0.44 to 7.40 mg/day (median = 3.77 mg/day). Accumulated urolith material was analyzed to be 100% calcium carbonate in all animals. Urine dipstick analysis

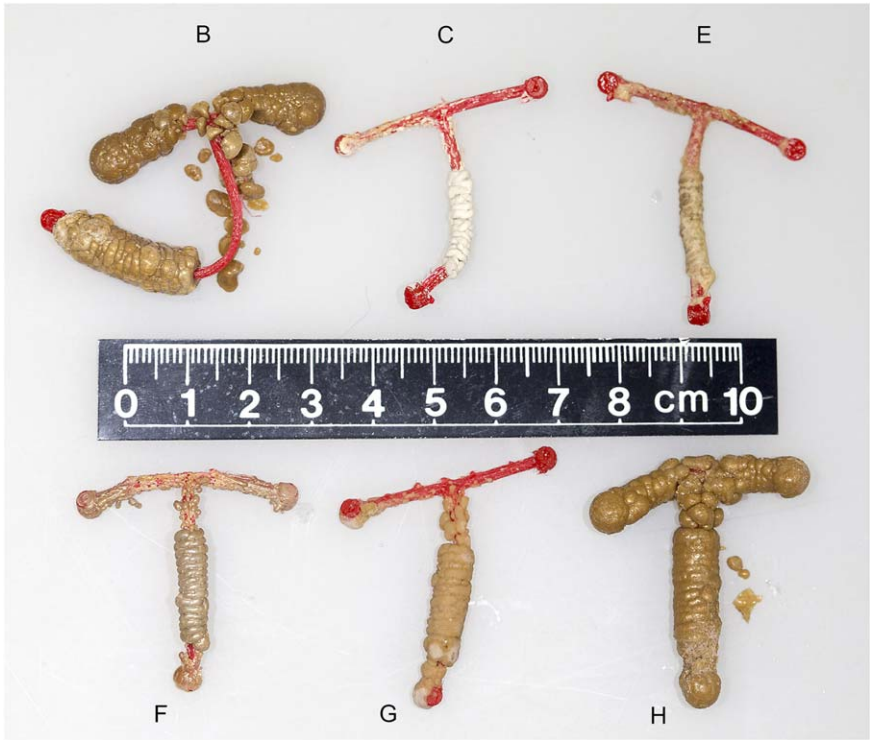

FIGURE 2 Printed implants from the 6 goats completing Experiment 2, goats B, C, E, F, G, H. Note the urolith mass accumulated by goats B and H, which was significantly greater than the other goats ($P = .047$)

revealed no notable abnormalities throughout the study. Mean urine pH throughout the study was 8.15 and mean urine specific gravity was 1.022 (reference range: 1.015–1.045).

3.2 | Experiment 2

Six goats completed Experiment 2 with the implant retained in the urinary bladder. One goat from Experiment 1 was excluded from Experiment 2 due to problems with implant placement and 1 additional goat had lost the implant by the time of endoscopy performed 4 weeks after implantation (retention rate: 86%). All retained implants accumulated urolith material during the study (Figure 2). Implant gain per day for all goats ranged from 2.64 mg to 67.71 mg (median = 9.22 mg). Two goats accumulated high amounts of urolith material with an implant gain of 53.98–67.71 mg/day (median = 60.85 mg/day). The remaining 4 goats accumulated smaller amounts of urolith material, ranging from 2.64 to 9.22 mg/day (median = 6.88 mg/day). Accumulated urolith material was analyzed to be 100% calcium carbonate in 5 animals, while urolith material for the remaining goat was 90%-95% calcium carbonate and 5%-10% struvite. Urine dipstick analysis revealed no notable abnormalities throughout the study. Mean urine pH throughout the study was 8.06 and mean urine specific gravity was 1.019 (reference range: 1.015-1.045). All blood analytes were within reference ranges throughout the study for all goats.

There was no significant difference between the 2 studies (group versus individual feeding) in implant weight gain per day ($P = .25$).

Mean implant weight per day for each goat across both studies was calculated. Mean implant gain per day was significantly different between goats, with 2 goats producing more urolith material than the remaining 4 ($P = .047$). The high urolith accumulation goats (n = 2) had an implant gain of 33.64–57.45 mg/day (median = 45.55 mg/day), while the low urolith accumulation goats had an implant gain of 0.44–7.65 mg/day (median = 4.70 mg/day).

Logistic regression comparison of groups (large mass formers versus small mass formers) found no explanatory variables from blood or urine analysis to be significantly different.

4 | DISCUSSION

Plastic winged implants used in this study were retained in greater than 75% of implanted goats. Goats consuming an alfalfa-based ration produced calcium carbonate urolith material and there were statistically significant differences between goats in urolith mass accumulated.

The 1st objective of the study was to determine if a novel winged implant would be retained in the urinary bladder of does for the duration of the study in greater than 75% of animals. Four previous experiments using zinc washers in goats achieved only 50%–75% retention rates for the duration of the studies.[13] The winged design of the implants in this current study appear to be an improvement on this model, with retention rates of 100% and 85.7%. Intrauterine devices designed for human use are not accessible to veterinary researchers due to a median device cost of $778,[14] so the 3D printed implant was designed. It can be printed for less than $0.25 USD in materials cost and appears to have the proper flexibility and rigidity to be retained, and tolerance for the environment of the urinary bladder, for sufficient

time to carry out experiments. The implant of Goat B, as seen in Figure 2, demonstrates the tensile strength of the material when urolith accumulation is excessive. It is clear that, in animals who are high urolith accumulators, the catgut suture modification may not be necessary, as urolith accumulation occurred on bare portions of the implant. In future studies where dietary interventions can be studied using animals along the spectrum of urolith accumulation potential, this suture modification appears to facilitate urolith component adhesion.

The experimental diet of a pelleted ration and chopped alfalfa, group-fed at 4% of body weight and individually fed at 3% of body weight, induced calcium carbonate urolithiasis in all goats. This confirms traditional ideas that legume-based rations predispose to calcium carbonate urolithiasis. It also raises concerns about general recommendations to feed high-calcium feeds based on the evidence suggesting that Ca : P ratios of rations above 2 : 1 prevent urolithiasis.[15,16] This recommendation can prevent phosphatic calculi such as struvite, but is likely to increase the risk of calcium-based uroliths. In addition, the common inclusion of ammonium chloride in these diets induces an acidemia, not demonstrated in this study, further increasing calcium excretion into the urine,[4,9] making it available for incorporation into the urolith matrix.

For various urolith types, a genetic predisposition to being a "stone former" has been suggested or confirmed in humans,[17] dogs,[18] and cats.[19] In small ruminants, individual susceptibility has been discussed but not documented. Often, there is a superimposition of risk factors. For example, it may be the impression of a clinician that Pygmy goats are predisposed to obstructive urolithiasis. However, Pygmy goats are more often kept as pets, an animal use type which is at increased odds of developing calcium carbonate urolithiasis,[7] although the mechanism for this is undetermined. At the end of Experiment 1, the differences in urolith mass formation of goats B and H became clear and we developed 2 hypotheses regarding this. First, there could be inherent characteristics, perhaps genetic or metabolic, which result in some individuals producing significantly more urolith mass than other animals on the same diet ($P = .047$). Second, given that animals were group-fed in the 1st experiment, these animals could have simply consumed more feed, making more mineral available for incorporation onto the implant. Experiment 2 controlled for possible differences in dry matter intake and we were therefore able to demonstrate that individuals of the same breed makeup as their cohort, when fed a diet identical to the cohort, including dry matter intake, developed significantly more urolith mass than the remainder of the cohort. This finding raises questions regarding the expected long-term outcomes for animals that have presented for calcium carbonate urolith obstruction.

We were unable to demonstrate a possible mechanism for the excess urolith formation by these 2 individuals by routine blood and urine analyses. In a case series of cats with calcium-containing uroliths (calcium oxalate), all had increased ionized and total calcium levels,[20] which was not seen in the individuals in our studies. Additional studies comparing the composition of the urine of stone formers to the general population of goats and other metabolic studies will need to be performed to elucidate the basis for the difference noted in these studies. A defined mechanism could form the basis for screening of individuals, particularly pets or breeding males, for predisposition to this disease.

In conclusion, a novel plastic winged implant was retained at a satisfactory rate in the urinary bladder of goats and served as a nidus for urolith accumulation. An alfalfa-based diet, including a commercial pelleted ration, resulted in calcium carbonate urolith formation in all goats on the study diet and there was a statistically significant difference in the mass of urolith accumulated across goats. These findings provide a basis for implant and diet selection for use in future studies of urolithiasis development, as well as a basis for client education regarding diet and management of individual goats in the clinical setting.

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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.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Procedures used in this study were approved by the IACUC at Texas A&M University.

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