

Effect of Adopting a Timothy Hay–based Diet at Weaning or in Adulthood on Urinary Tract Parameters in Strain 13/N Guinea Pigs (*Cavia porcellus*)

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Type of feed is an important consideration in herbivore colony management, yet limited studies report on the effects of diet on common conditions such as urolithiasis in guinea pigs. Urolithiasis is a well-documented cause of lower urinary tract disease in guinea pigs, with calcium carbonate uroliths reported as the predominant calculi formed in the guinea pig urinary tract. A calcium-rich diet has been suggested as a risk factor for urolithiasis, with numerous commercially available guinea pig diets formulated for adults avoiding ingredients that are higher in calcium. Due to the high incidence of urolithiasis in our strain 13/N guinea pig colony, we conducted a prospective control study following the implementation of dietary changes aimed at improving overall urinary tract health and reducing risk factors for urolithiasis, thus improving colony welfare. A control group was kept on the original ad libitum alfalfa hay–based pellet diet with restricted loose timothy hay (control diet, 14 juveniles and 24 adults). An experimental group was placed on a portioned, 1 oz daily, timothy hay–based pellet diet with ad libitum loose timothy hay (experimental diet, 21 juveniles and 23 adults). Juveniles and adults were followed for a total of 14 and 26 wk, respectively. Longitudinal blood and urine samples were collected to evaluate blood chemistry and urinary parameters, along with weight and body condition scores to assess general health. Overall, dietary changes did not improve parameters associated with improved urinary tract health or reduced risk of urolithiasis; feeding strategy was not found to meaningfully affect calcium crystalluria, urine protein, urine specific gravity, or renal values. These data support alfalfa hay–based pellet or timothy hay–based pellet, when fed with loose timothy hay, as viable options and suggest that practices aimed at reducing dietary calcium by reducing pelleted diet portions are insufficient to mitigate risk factors for urolithiasis in guinea pigs.

Abbreviations and Acronyms: AHP, alfalfa hay–based pellet; ALB, albumin; CRE, creatinine; GLU, glucose; NRC, National Research Council; THP, timothy hay–based pellet, TBIL, total bilirubin; TP, total protein; USG, urine specific gravity.

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Introduction

Inbred strain 13/N guinea pigs are an important animal model of viral hemorrhagic fever diseases, such as arenaviruses,^{6,43} as well as noninfectious diseases such as osteoarthritis and autoimmune thyroiditis.⁸ They are not commercially available and are maintained in a limited number of small breeding colonies throughout the United States. While laboratory animals living under rigorous husbandry practices benefit from reduced risk of illness caused by poor husbandry and infectious diseases, guinea pigs often experience illness of idiopathic or ill-defined origin⁴¹ particularly when they are inbred. In our breeding colony of approximately 200 adult strain 13/N guinea pigs, renal disease and urolithiasis represent a significant percentage

of their overall disease burden contributing to morbidity and mortality. Over an 8-y span (2015 to 2023) 32 cases of confirmed urolithiasis were diagnosed via radiographic imaging or at necropsy; however, this may be an underrepresentation, as not all animals that were found dead or euthanized were necropsied during this timeframe. Urolithiasis is a well-documented cause of lower urinary tract disease of guinea pigs, particularly in adult animals, that can increase morbidity and mortality depending on anatomic location and clinical status of the affected animal.^{3,13,30} The clinical signs of urolithiasis in guinea pigs range from subclinical to life-threatening. When stones are located in the lower urinary tract, clinical signs can include dysuria, stranguria, and hematuria; when stones are located in the upper urinary tract, clinical signs can include weight loss, hyporexia, and decreased activity level. Complete urinary obstruction is noted in the most severe cases.¹³ Medical management and prevention strategies are often unrewarding.^{3,13} Surgical urolith removal is possible but can be accompanied by complications and recurrence of uroliths. Recent reports indicate that calcium carbonate uroliths are the predominant urinary

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calculi formed in the guinea pig^{13,20}; historically, calcium oxalate uroliths were reported as the predominate concretion.^{34,44} The uroliths that we have seen in our colony have been predominantly 100% calcium carbonate, with the occasional mixture of calcium carbonate with a small percentage of struvite.

Development of urolithiasis is thought to be multifactorial, but the physiologic and anatomic etiologies of this condition have not been definitively elucidated. While crystalluria does not always lead to urolithiasis, supersaturation of urine with urolith-forming components can lead to the development of concretions in susceptible animals.²⁶ Calcium-rich diets have been suggested to put guinea pigs at increased risk of urolithiasis development, and numerous commercially available pet guinea pig diets formulated for adults avoid high calcium ingredients (alfalfa meal) in favor of other sources of fiber (ground timothy hay).^{3,13,41,47} Calcium requirement has not been specifically documented, but rather the importance of the relationship of calcium to other cations (phosphorus, magnesium, and potassium) has been reported, specifically to prevent calcium deficiency and protect against metastatic calcification in growing animals.^{21,27,31–33} Although diet and water intake have been suggested as contributing factors for the development of urolithiasis, no clear preventative measures have been established.^{3,13,30,48}

Historically, female guinea pigs have been reported to have higher prevalence of urolithiasis than males; however, some reports have found equal distribution among males and females.^{3,20,38} Male guinea pigs are at increased risk for obstructive urolithiasis due to the narrower diameter of the male urethra.^{19,41} Retrospective analyses of risk factors for urolithiasis have been published,¹³ but no prospective studies are investigating the effect of diet composition on these physiologic parameters in guinea pigs to our knowledge.

In this study, we investigated whether changing from a control diet consisting of ad libitum alfalfa hay–based pellet (AHP) with restricted loose timothy hay to an experimental diet of a portioned timothy hay–based pellet (THP) diet with ad libitum loose timothy hay in strain 13/N guinea pigs reduces the urinary crystal load while maintaining healthy weight, body condition, blood chemistry, and urinalysis parameters. Our aim was to investigate this diet plan in a way that would have practical application for a typical husbandry setting without the need for specialized housing, such as metabolic caging. We hypothesized that a portioned THP diet in conjunction with ad libitum loose timothy hay will reduce systemic calcium levels and correspondingly reduce the urine calcium crystal burden as compared with an ad libitum AHP with restricted loose timothy hay while maintaining normal weight and body condition.

Materials and Methods

Animals. All animal procedures were approved by the Centers for Disease Control and Prevention IACUC and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* at an AAALAC international-accredited facility. Healthy strain 13/N guinea pigs were selected from the Centers for Disease Control and Prevention breeding colony. Health monitoring of the colony includes serology (guinea pig basic Opti-Spot, IDEXX BioResearch, Columbia, MO), fecal examination, and bloodwork, including CBC and clinical chemistry analyses. All study animals were free of *Clostridium piliforme*, guinea pig parainfluenza virus 3, lymphocytic choriomeningitis virus, murine pneumonia virus, Sendai virus, and *Encephalitozoon cuniculi*, as determined by using serologic screening tests. Adult animals selected for

study had no history of preexisting conditions (e.g., dental malocclusion, previous cystotomy, previous surgical mass removal, recurrent cystitis).

One 88-d-old female in the juvenile experimental group was delayed starting the study after developing circling behavior and reduced weight compared with conspecifics at the first timepoint. She was removed from study and treated empirically for otitis media/interna by our clinical veterinary staff with sulfamethoxazole and trimethoprim (Pharmaceutical Associates, Greenville, SC) 20 mg/kg PO twice daily for 18 d, and Critical Care Herbivore (Oxbow Animal Health, Omaha, NE) nutritional supplement PO twice daily for 18 d, and clinical signs resolved. After she recovered, she was reassigned to the control juvenile group since there were only 4 females available from the contemporary litters for this group. Otherwise, physical examinations and clinical chemistry were within normal limits for all study animals at baseline.

A total of 82 guinea pigs were divided by sex into cohorts of juveniles and adults: 37 females and 45 males; 35 juveniles and 47 adults (Table 1). Animals were socially housed in sex-segregated floor pens with paper (Techboard and Poly Pads, Shepherd Specialty Papers, Watertown, TN) and paper nesting material (EnviroDri, Fibercore, Cleveland, OH). Guinea pigs were provided unrestricted filtered municipal water via bottle with sipper tubes. Environmental enrichment was provided in the form of plastic huts (guinea pig hut, Bio-Serv, Flemington, NJ) and wood materials for gnawing (manzanita sticks and wood blocks, Bio-Serv). Environmental parameters were maintained within a temperature range of 68 to 79 °F (20.0 to 26.1 °C), 30% to 70% relative humidity, and a 12:12 h light:dark cycle. Age group guidelines at baseline were defined as 0- to 199-d-old for juveniles and 200- to 900-d-old for adults, adapted from previously established guidelines.¹⁵ Animals older than 900 d were considered geriatric. All animals were of intact reproductive status.

Adult animals were randomly selected groups of socially compatible animals. Juveniles were randomized by assigning animals to study as the next available litters were weaned. One juvenile assigned to the male cohort was diagnosed with a disorder of sexual development after the cessation of the study. Data from this animal were included in the male juvenile data set after no outlier analytes were identified. The first phase of the study was performed on the adult cohort and the second phase included procedures on the juvenile cohort and a 26-wk timepoint for the adult cohort. To facilitate blinding of investigators, all animals were assigned a unique study ID that did not indicate age, sex, or diet.

The juvenile cohort consisted of animals aged 88 to 197 d (median, 102 d) weighing 430 to 834 g (median, 633 g) at baseline. Juveniles were followed for a total of 14 wk, after which point some animals were bred. The adult cohort consisted of animals aged 254 to 865 d (median, 447 d) weighing 730 to 1,215 g (median, 962 g) at baseline. Adult animals were followed for a total of 26 wk, except for one male from the experimental group that was euthanized prior to week 26 for clinical reasons

Table 1. Animal groups

Age	Sex	Control diet	Experimental diet
Juvenile	M	9	13 ^a
	F	5	8
Adult	M	12	11 ^b
	F	12	12

^aIncluded intersex male.

^bOne animal was euthanized prior to the last timepoint.

determined to be unrelated to this study. Longitudinal weight, body condition scores, blood (plasma chemistry), and urine (urinalysis) samples were collected at baseline and 4, 14, and 26 wk. Two males reached geriatric age (> 900 d) by week 14, and 3 males were geriatric by week 26. No females were geriatric by week 26 of study.

Diet. Two closed-formula diets were chosen for adherence to guidelines published by the National Research Council (NRC), commercial availability, specific formulation for guinea pigs, and difference in primary ingredient, but otherwise had similar nutrient composition. The control group was maintained on the colony diet: AHP diet ad libitum (guinea pig diet 5025, LabDiet) and a daily allotment of ½ flake of loose timothy hay (sourced from Michigan) per 12 animals. The experimental group was fed 1 oz daily of a THP diet (timothy-based guinea pig diet, formula number 5664, Mazuri) with ad libitum loose timothy hay replenished throughout the day and given a second ½ flake per 12 animals in the afternoon. The ad libitum loose timothy hay was given to increase available roughage, and correspondingly decrease the overall calcium by providing an ad libitum food option that is lower in calcium than pelleted feed. Timothy hay calcium content can range from 0.32% to 0.46% depending on when in the bloom cycle it is harvested.¹⁶ Edible enrichment was provided in the same manner as described below for the control diet. Animals in experimental groups were transitioned from the control diet to the experimental diet incrementally during a period of 7 d. Both pellet diets have calcium contractions (1.10%) and phosphorus (0.6%) above what is recommended by the NRC for growing animals (0.8% and 0.4%, respectively). The NRC guidelines recommend magnesium concentrations of 0.1%; however, the experimental diet contains 0.4% and the control diet contains 0.35% magnesium. Finally, the NRC recommend 0.5% potassium, and the experimental diet and control diet contain 1.20% and 1.42%, respectively.³¹ These concentrations are calculated by the manufacturer based on most recent nutrient analysis information. Manufacturer guaranteed analysis and selected nutrient/chemical composition are provided (Tables 2 and 3).^{24,28}

Prior to the study, colony management practices included providing edible enrichment in the form of fresh vegetables 3 times a week; these vegetables were selected for vitamin C content, palatability, and availability. During the first phase of the study, the enrichment items given to the adult animals

were restricted to carrots, Swiss chard, and bell peppers (red, yellow, or green). This combination of items was selected based on historic availability from vendor, historic animal preference, low calcium content (1% to 2% daily value based on human daily value requirements), or high vitamin C content, especially from bell pepper (190% daily value) and Swiss chard (18% daily value). In the second phase (juvenile animals), edible enrichment was not restricted by item to reduce the risk of development of food-related neophobia.^{7,12} Enrichment selection was also widened for the adult cohort during weeks 15 to 26 to capture effects on broader enrichment selection in this group. The selection of enrichment items during this phase included carrots and bell peppers, given most often; kale, celery, mustard greens and snow peas, given 2 to 5 times during this period; and other

Table 2. Manufacturer guaranteed analysis of control and experimental pelleted diets

Guaranteed analysis	Control diet (LabDiet)	Experimental diet (Mazuri)
Crude protein not less than (%)	18.0	18.0
Crude fat not less than (%)	4.0	4.0
Crude fiber not less than (%)	NR	14.0
Crude fiber not more than (%)	16.0	18.0
Moisture not more than (%)	12.0	12.0
Ash not more than (%)	8.0	9.0
Vitamin E not less than (IU/kg)	NR	75.0
Ascorbic acid not less than (mg/kg)	NR	1,000.0
Total micororganisms, min (cfu/g)	NR	27,0000.0

NR, not reported.

Table 3. Selected manufacturer nutrient/chemical composition analysis of control and experimental pelleted diets

Nutrient/chemical composition	Control diet (LabDiet)	Experimental diet (Mazuri)
Protein (%)	19.30	18.00
Fat (ether extract) (%)	4.30	4.00
Fat (acid hydrolysis) (%)	5.60	NR
Linoleic acid (%)	1.05	1.50
Linolenic acid (%)	0.20	0.77
Omega-3 fatty acids (%)	0.30	0.77
Omega-6 fatty acids (%)	NR	1.50
Cholesterol (ppm)	30.00	NR
Fiber (crude) (%)	14.60	13.60
NDF (%)	29.90	30.00
ADF (%)	19.40	17.00
Nitrogen-free extract (by difference) (%)	44.00	NR
Starch (%)	14.80	NR
Sucrose (%)	2.04	NR
Calcium (%)	1.10	1.10
Chloride (%)	0.67	0.51
Magnesium (%)	0.35	0.40
Phosphorus (%)	0.60	0.60
Phosphorus (nonphytate) (%)	0.37	0.40
Potassium (%)	1.42	1.20
Sodium (%)	0.35	0.35
Sulfur (%)	0.25	0.23
Copper (ppm)	13.00	16.00
Iodine (ppm)	0.87	1.00
Iron (ppm)	360.00	284.00
Manganese (ppm)	89.00	113.00
Selenium (added) (ppm)	0.55	0.20
Zinc (ppm)	74.00	100.00
Fluorine (%)	18.00	NR
Cobalt (%)	3.50	NR
Chromium (added) (ppm)	0.01	NR
Ascorbic acid	0.50 mg/g	1,283.00 ppm
Biotin (ppm)	0.30	0.20
Vitamin D ₃ (added) (IU/kg)	3,100.00	2,921.00
Choline (ppm)	1,530.00	1,900.00
Beta-carotene (ppm)	NR	4.10
Carotene	13.0	NR

ADF, acid detergent fiber; NDF, neutral detergent fiber; NR, not reported.

items such as broccoli, parsley, lettuce, Swiss chard, Brussels sprouts, turnip greens, bok choy, spinach, and cilantro, only given once to a given group during this period. The trends of mixed enrichment were consistent across all groups, represent the routine selection of enrichment for our colony, and let us evaluate the impact of the diet modifications in the context of our routine practice.

Weight, body condition, blood collection, and plasma chemistry. Weights were collected using a pediatric scale (Scale-Tronix 4802, Welch Allyn) calibrated annually and checked for accuracy prior to each group of weight collections with a calibrated weight. Body condition scores were assessed by one investigator at all timepoints and based on a modification of previously published scoring systems (Table 4).^{36,46} Weights were collected weekly for the first 4 wk of the adult cohort. When no problems with weight maintenance were seen, weights were collected again at weeks 14 and 26. For the juvenile cohort weights were collected at weeks 0, 4, and 14.

Up to 1 mL of blood was collected from the cranial vena cava under general anesthesia (3% to 4% isoflurane via induction box, then nose cone) into a 1.3-mL lithium heparin Microtainer tube (Sarstedt, Nümbrecht, Germany). Samples were placed on ice, then plasma was separated and frozen at -80 °C in 2-mL microtubes (PCR-PT, Sarstedt, Nümbrecht, Germany) for later analyses. Plasma chemistry was analyzed using the comprehensive metabolic panel on the Piccolo Xpress analyzer (Abaxis). Samples were coded at time of collection and stored frozen 4 to 66 d prior to analysis.

Urine collection. Urine was collected from each animal by natural, conscious urination or urination induced by anesthesia. If a sample was not collected at a scheduled timepoint, the sample was collected up to 10 d after. All urine samples were collected from a sanitized transport box (urination while

conscious), anesthesia induction chamber, or free catch with stainless steel collection cup while under general anesthesia. Prior to sampling, each animal was individually held in a sanitized clear filter top static cage. Animals were monitored and urine was collected as soon as it was seen in the cage by aspirating it into a 3-mL syringe (Henke-Sass, Wolf, Tuttlingen, Germany); urine was then placed in a 2-mL microtube (PCR-PT, Sarstedt, Nümbrecht, Germany) and immediately placed on ice.

Urination was often stimulated with the induction of anesthesia and collected from the induction box. When urine samples were collected during induction of anesthesia, the induction box was cleaned with Peroxigard (Virox Technologies, Oakville, ON, Canada) between each animal and dried with a paper towel. Animals were monitored during induction, and, when produced, urine was immediately aspirated into a 3-mL syringe (Henke-Sass, Wolf, Tuttlingen, Germany), then placed in a 2-mL microtube (PCR-PT, Sarstedt, Nümbrecht, Germany) and immediately placed on ice.

After the first adult baseline sampling, a collection modification was adopted: use of a disposable Elizabethan-style collar to prevent contamination of the urine sample with lacrimal and/or salivary secretions. An Elizabethan-style collar was developed using an 8-in.-diameter paper coffee filter (WinCo Foods, Boise, ID). A 3-cm 'X' was cut in the center of the filter and slipped over the head and ears for use during anesthesia to catch secretions. Each disposable filter was used once per animal.

If an animal did not urinate in either the transport box or the induction box, a stainless-steel cup was positioned near the prepuce or vulva to catch urine if the animal urinated during the blood draw procedure. Only 3 urine samples were collected this way. Of the 292 urine collections, 205 occurred in the induction box (70%), 84 occurred in the transport boxes (28%), and 3 were collected in the stainless-steel cup (1%). Males and juveniles were more likely to produce a urine sample at anesthesia induction than while conscious in a transport box.

Urinalysis. Urine samples were evaluated for turbidity, urine specific gravity (USG), chemistry, and sediment within 2 h of collection or placed in refrigerator (4 °C) and analyzed within 24 h of collection. Methods and workflow are summarized in Figure 1. Most samples were read within 6 h of collection. Refrigerated samples were allowed to equilibrate to room temperature for at least 20 min before analysis. To assess turbidity, settled sediment was resuspended using a VWR VM-3000 mini vortexer (Henry Troemner).

Some sources state that an accurate urinalysis should be run on uncentrifuged urine samples and then repeated if blood or turbidity impedes the results.⁵ Thus, during the first timepoint, USG, urinalysis test strips, and sediment were read on samples both before and after centrifugation to determine the method that would prove reliable and most time efficient. While chemistry test strip results were generally consistent between precentrifuged urine and postcentrifuged supernatant, notable differences were observed in the USG and sediment analyses. For USG determination, urine samples that had moderate to marked turbidity blurred the shadow line, making interpretation difficult. Spinning down the sample and reading USG from the supernatant consistently corrected this issue. Sediment analysis was also impacted, as centrifugation concentrates the material for microscopic examination. We elected to always read the sediment after centrifugation to capture all crystals in a sample, rather than reading part of the urinalysis on precentrifuged urine and part on postcentrifuged urine.

Urine samples were coded at the time of collection, urinalysis strips performed by 5 investigators, and turbidity and sediment

Table 4. Body condition scoring for strain 13/N guinea pigs

Score	Characteristics
1—Very thin	<ul style="list-style-type: none"> • Individual ribs are palpable • No fat stores under chin and over neck (crest) • Thorax and abdomen follow a straight line when viewed from the top • Pelvis is visible with sharp points
2—Thin	<ul style="list-style-type: none"> • Individual ribs are palpable • Reduced fat stores under chin and over neck (crest) • Slight pear shape (thorax to abdomen) when viewed from the top • Pelvis is visible with sharp points
3—Ideal	<ul style="list-style-type: none"> • Ribs are palpable but not distinct • Fat stores under chin and over neck (crest) • Pear shape (thorax to abdomen) when viewed from the top • Pelvis is visible but does not have sharp points
4—Overweight	<ul style="list-style-type: none"> • Ribs are barely palpable • Excessive fat stores under chin and over neck (crest) • Gradual slant from thorax to abdomen with no obvious waist • Pelvis is not visible but palpable with mild pressure
5—Obese	<ul style="list-style-type: none"> • Ribs are not palpable • Excessive fat stores under chin and over neck (crest) • No demarcation between thorax to abdomen • Pelvis is not visible but palpable with mild pressure

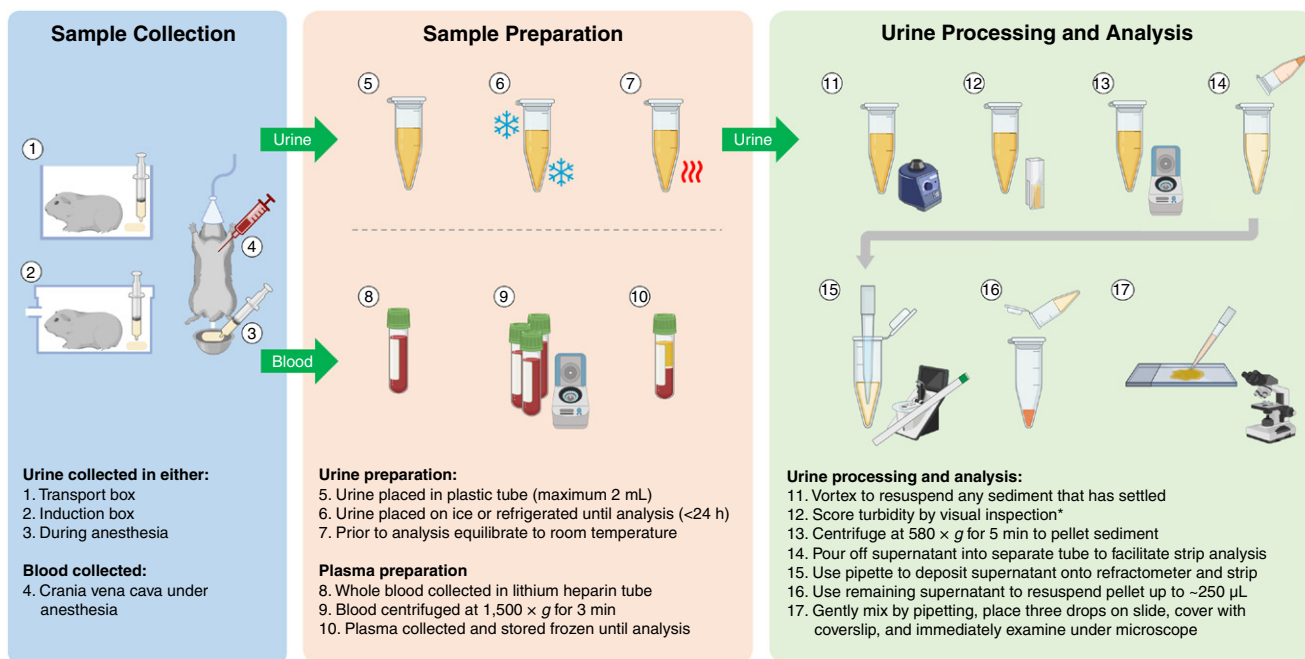


Figure 1. Blood and urine sample collection, preparation, processing, and analysis. Samples were collected as depicted below at baseline and 4, 14, and 26 wk (adults) or at baseline and 4 and 14 wk (juveniles). *Urine turbidity was assessed using a scale of 0 to 2, developed by investigators: 0, can read black text on white background perfectly through sample; 1, can see text but it may be blurred or difficult to read; 2, cannot see text at all through sample (completely opaque).

were evaluated by one investigator. Urine turbidity was noted first (Figure 1), after which samples were centrifuged ($5810 \times g$, Eppendorf) at $580 \times g$ for 5 min. After centrifugation, supernatants were poured off into a separate tube and the sediment was set aside. Supernatants were used for USG and chemistry readings. USG was read by a handheld refractometer (Schuco 5711 to 2020). Calibration was checked with distilled water each day that urinalyses were run. If the shadow line did not read 1.000 with distilled water, the calibration was adjusted so that the shadow line matched the 1.000 line. Urine chemistry was performed with VetStix 11 test strips (Vet One, Boise, ID) by placing a drop of urine on each test pad using a $200\text{-}\mu\text{L}$ pipette and waiting the recommended time interval before reading (30 to 60 s for bilirubin, urobilinogen, ketone, protein, nitrite, glucose [GLU], pH, occult blood, USG, and ascorbic acid; 90 to 120 s for leukocytes). For sediment analysis, supernatant was either removed or added so that the total volume in the sediment tube equaled 0.25 mL . The sediment was then resuspended by gently stirring the puck of material with the pipette tip until a homogeneous mixture was obtained. Three drops of resuspended sediment were placed on a glass slide, covered with a glass coverslip, and viewed with light microscopy at $20\times$ at the center of the coverslip. All sediment samples were read by one investigator.

A crystal load scoring system was developed to categorize the findings on sediment analysis. Crystal load was graded as 1 to 4 (Figure 2): 1) thin layer with ample negative space between crystals and no large clumps of crystals; 2) moderate amount of crystals with reduced negative space, or frequent large clumps of crystals; 3) thick layer with no negative space visible between crystals or large areas of clumped crystals, but crystal morphology can be readily determined; and 4) transillumination obscured, creating a dark and blurry field, and crystal morphology could only be determined by looking at the margin of the coverslip where crystal accumulation was thinnest. Red blood cells were also noted as present or not present.

Statistical methods. We performed initial data analysis for the 25 analytes to include overall mean and SD across all inputs. ANOVA was conducted for each of the 25 analytes, using sex, diet, age, and sample timepoint as the independent variables. To statistically evaluate effects of diet, all parameters were consolidated independently of sample timepoint to determine whether the input variables produced statistically significant models based on time, sex, age, and diet. We evaluated the residuals from the models and found the ANOVA model appropriate. We used the Benjamini–Hochberg technique of controlling the false discovery rate to balance the power and type-1 error of our analysis with an $\alpha = 0.05$.¹⁵ Further analysis was performed on the 19 analytes found to be statistically significant to identify interactions between diet with sex and age, including analysis of first-order interactions between diet and age (adult \times diet) and diet and sex (sex \times diet); this allowed us to determine the effects of diet on subpopulations (age and/or sex). Stepwise regression was used for each significant model to find the best model for each analyte, excluding insignificant variables at $\alpha = 0.05$. To confirm adequate group sizes, a power analysis was conducted based on the aim to assess differences between the 2 diets, males and females, and adults and juveniles with the following parameters: $\alpha = 0.05$, $\beta = 0.1$, and 1 SD distinction; results indicated that for each combination of diet/sex/age there should be a minimum of 5 animals.

Results

Body weight, body condition, and blood chemistry. Twenty-five analytes were selected for ANOVA using sex, diet, and sample timepoint as the independent variables. In this input variable testing, we found 19 analytes that were statistically significant overall (Table 5): weight, ALP, sediment calcium oxalate, plasma potassium, plasma calcium, overall sediment crystal burden, plasma albumin (ALB), ALT, GLU, urine turbidity, urine protein, urine USG, urine pH, plasma total protein (TP), plasma chloride, plasma BUN, body condition score, plasma total bilirubin (TBIL), and plasma creatinine (CRE). In addition,

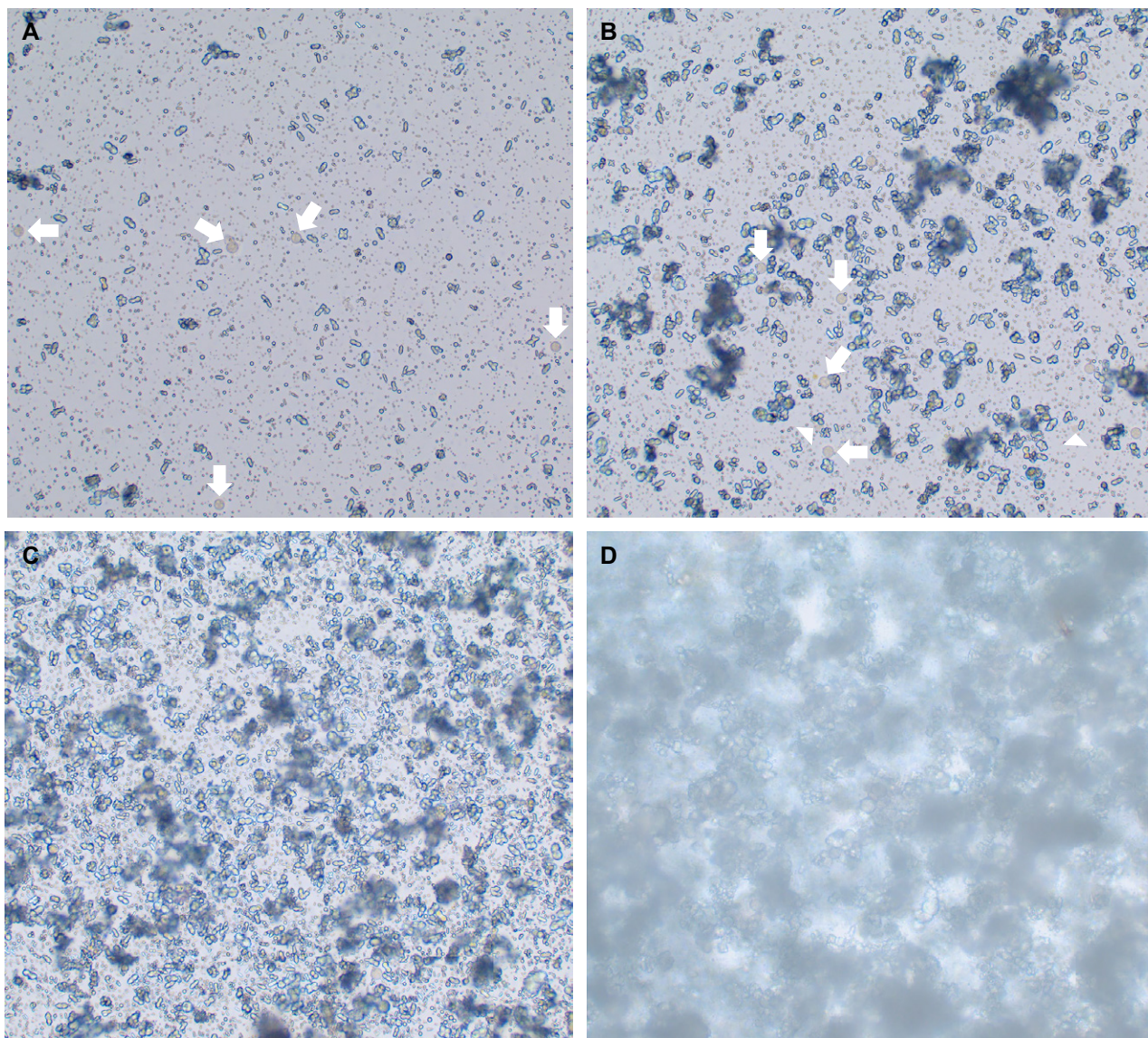


Figure 2. Representative images of crystal load grading scale, from 1 to 4. (A) Grade 1, thin layer with ample negative space between crystals and no large clumps of crystals. White blood cells are visible (arrows). (B) Grade 2, moderate amount of crystals with reduced negative space, or frequent large clumps of crystals. White blood cells (arrows) and red blood cells (arrow heads) are visible. (C) Grade 3, thick layer with no negative space visible between crystals or large areas of clumped crystals, but crystal morphology can be readily determined. (D) Grade 4, transillumination obscured, creating a dark and blurry field, and crystal morphology could only be determined by looking at the margin of the coverslip where crystal accumulation was thinnest. Original magnification, 20 \times .

AST and sediment struvite had *P* values below 0.05 but were not significant under the criteria for multiple comparisons.

Of the 11 analytes found to be significant by feed (Table 6), 5 were unaffected by either sex or age (potassium, ALB, ALT, GLU, BUN). Four were unaffected by sex (ALB, GLU, chloride, TBIL), whereas 7 were found to have interactions with sex (ALP, potassium, calcium, ALT, TP, BUN, CRE). Similarly, 2 were unaffected by age (GLU, CRE), and 9 were found to have interactions with age (ALP, potassium, calcium, ALB, ALT, TP, CL, BUN, TBIL). The experimental diet had no impact on chloride, TBIL, and CRE; overall, values for study animals remained within published ranges.^{9,37,45} The experimental diet affected 8 of the analytes. It reduced potassium (average difference of 0.6 mmol/L; in adults only), calcium (average difference of 0.43 mg/dL; males only), and ALT (average difference of 2.8 U/L) and GLU (average difference of 8.4mg/dL), both independent of sex or age. The experimental diet increased ALP (average difference of 18.8 U/L) and ALB

(average difference of 0.34 g/dL) in juveniles only, BUN (average difference of 2.4 mg/dL for males and average difference of 4.0 mg/dL for juveniles), and TP (average difference of 0.14 g/dL, independently of sex or age).

Weights were significantly different between diet groups for the male cohort and juvenile cohort. The males fed the experimental diet had an average weight of 0.891 kg compared with the males fed the control diet with an average weight of 0.992 kg (101 g difference between averages). The juveniles fed the experimental diet had an average weight of 0.701 kg compared with the control diet with an average weight of 0.781 kg (80 g difference between averages). These differences were mirrored by significant reductions in body condition score for males and juveniles fed the experimental diet; however, these changes were clinically minimal, equaling a fraction of a point on the body condition scale with all animals between ideal weight and overweight, but not obese. Despite male and overall juvenile weights being lower, there

Table 5. Statistical results of input variable testing

Variable	pi	E[pi]	E[pi]-pi
Significant overall			
Weight	6.334E-69	0.002	0.002
Alkaline phosphatase	2.246E-62	0.004	0.004
Calcium oxalate crystals on sediment	7.480E-19	0.006	0.006
Potassium	1.837E-17	0.008	0.008
Calcium	9.878E-17	0.010	0.010
Total crystal estimate on sediment	1.912E-14	0.012	0.012
Albumin	3.072E-11	0.014	0.014
ALT	1.167E-09	0.016	0.016
Glucose	7.418E-08	0.018	0.018
Urine turbidity	3.005E-07	0.020	0.020
Urine protein	5.033E-06	0.022	0.022
Urine specific gravity	1.621E-05	0.024	0.024
Urine pH	1.687E-05	0.026	0.026
Total protein	1.767E-05	0.028	0.028
Chloride	6.769E-05	0.030	0.030
BUN	1.004E-04	0.032	0.032
Body condition score	1.820E-03	0.034	0.032
Total bilirubin	5.591E-03	0.036	0.030
Creatinine	5.917E-03	0.038	0.032
Not significant under multiple comparisons			
AST	0.0432	0.040	-0.003
Struvite crystals on sediment	0.0475	0.042	-0.006
Not significant			
Calcium carbonate crystals on sediment	0.0608	0.044	-0.017
Calcium phosphate crystals on sediment	0.0840	0.046	-0.038
Total carbon dioxide	0.0959	0.048	-0.048
Sodium	0.835	0.050	-0.785

Variables that were significant overall (indicated by a pi value < 0.05) are listed, followed by variables that had pi values < 0.05, but that were not significant under the criteria for multiple comparisons. Variables were not found to be significant (pi > 0.05). pi: the P value of the *i*th order statistic. E[pi], expected value of the *i*th order statistic under the null hypothesis that all the P values are significant with the overall $\alpha = 0.05$. Under the null hypothesis, the order statistics will be uniformly distributed 0 to 0.05. E[pi]-pi: the difference between the actual P value and the expected P value. Negative differences (that is, P value > expected P value) serve as the cutoff for significance.

was not a significant difference in female weight or body condition scores between diet groups.

Urinary parameters. After urinalysis results were reviewed, we elected to include 9 urine parameters in statistical models based on suspected relevance to in vivo urolith formation: sediment crystal burden of sample, occurrence of calcium oxalate crystals, occurrence of struvite crystals, occurrence of calcium carbonate crystals, occurrence of calcium phosphate crystals, urine turbidity, urine protein, USG, and urine pH. Six analytes were statistically significant using sex, diet, and sample time as the independent variables (sediment crystal burden, occurrence of calcium oxalate crystals, urine turbidity, urine protein, USG, and urine pH). The experimental diet affected only 2 of the urine analytes (Table 7): it reduced urine turbidity (in females only) and increased USG (in adult males only). Overall, the experimental

diet had no impact on total crystal estimate in sediment, calcium oxalate crystals in sediment, or urine protein. Urine pH was observed to significantly decrease over time with no significant interactions with age, sex, or diet. Sediment crystal burden, occurrence of calcium oxalate crystals, urine protein, and average USG interacted with age, but overall, males showed on average more concentrated urine on the experimental diet compared with the control (average of 1.024 and 1.019, respectively). Some changes were observed independently of diet. Males had a higher crystal load than females; juveniles had a higher crystal load than adults; and calcium oxalate crystals increased with age among males. Four types of crystals were seen on sediment analysis: calcium carbonate, calcium oxalate monohydrate, calcium phosphate, and struvite. Overall, 34% percent of juveniles (12 of 35) had only one type of crystal at all time points (calcium carbonate). All adults and 51% of juveniles (18 of 35) had calcium oxalate crystals at one or more time points. Calcium oxalate crystals are subclassified as monohydrate, dihydrate, or trihydrate; the morphology most consistently seen in these animals was monohydrate. Calcium phosphate and struvite crystals were noted less frequently. Only 11% of adults (5 of 47) and 29% of juveniles (10 of 35) had struvite crystals observed at one or more timepoints. Eleven percent of juveniles (4 of 35) had calcium phosphate crystals at least once, but none of the adults had this crystal type. A statistical analysis was performed to assess the impact of different urine collection methods, on the sediment crystal burden. Between the most frequent methods used, we found urine collected from the induction box (mean = 3.17, SD = 0.73, n = 205) compared to the transport box (mean = 2.21, SD = 0.79, n = 84) had a 0.96 higher sediment crystal burden grade (p-value = 1.85 E-19).

Discussion

Urinary tract stones and kidney disease are common issues in our strain 13/N guinea pig colony. As most uroliths in our experience were calcium based, we looked at the effects of transitioning to an experimental timothy hay pelleted diet and reducing overall dietary calcium by shifting our feeding approach: from ad libitum AHP feed with restricted loose timothy hay (roughly ½ flake per 12 animals for the day) to portioned THP feed (1 oz per animal) with ad libitum loose timothy hay (½ flake per 12 animals in the morning, and an additional ½ flake per 12 animals at afternoon health checks). We chose an experimental diet with a similar total calcium content that eliminated alfalfa and reduced overall calcium intake without altering the calcium/phosphorus ratio by decreasing the pellet/hay ratio in the diet.^{21,31-33} Guinea pig care resources^{10,17,41,49} recommend that alfalfa hay not be fed to adult guinea pigs, since alfalfa hay is higher in calcium than other roughages.¹⁶ Despite these guidelines, many guinea pig pelleted diets, particularly those made for growing and lactating animals, are formulated with alfalfa hay meal. A benefit of using pelleted diets is the ability to control a specific nutrient chemical balance regardless of the primary ingredient, as long as they are used in balance with other components. However, many of these standard diets are closed, fixed formula or constant nutrition formula, meaning that the nutrient chemical composition is reported based on calculations from intermittent ingredient analysis.⁴² This creates the potential that there can still be variation in composition from what is on the label. With many commercial diets available that are formulated with timothy hay instead of alfalfa, we wanted to see whether this would be a better option for colony management. Retrospective and case reports have suggested that eliminating alfalfa did not change outcomes for animals that developed urolithiasis.¹³ This study

Table 6. Results of final model analysis to evaluate interactions between body condition score or plasma analyte variables and age, sex, or diet

Final model		Variable					
		Adult	Sex	Diet	Sample week	Adult × diet ^a	Sex × diet ^a
BCS	Estimate	3.13E-01	2.72E-01	2.20E-01	–	–	–2.98E-01
	Estimate error	9.20E-02	8.50E-02	1.22E-01			1.17E-01
	P value	7.72E-04	1.54E-03	7.30E-02	–	–	1.10E-02
ALP	Estimate	–3.97E+01	–2.53E+00	1.86E+01	–1.11E+00	–2.43E+01	–
	Estimate error	4.17E+00	2.61E+00	4.39E+00	1.47E-01	5.45E+00	
	P value	7.71E-19	3.34E-01	2.91E-05	5.55E-13	1.13E-05	–
Potassium	Estimate	8.89E-01	5.39E-01	–2.51E-01	–1.94E-02	–4.58E-01	–
	Estimate error	1.57E-01	1.44E-01	2.06E-01	5.66E-03	2.08E-01	
	P value	4.00E-08	2.27E-04	2.25E-01	7.10E-04	2.85E-02	–
Calcium	Estimate	1.07E+00	2.28E-01	4.46E-01	–1.43E-02	–	–5.91E-01
	Estimate error	9.54E-02	1.28E-01	1.28E-01	4.98E-03		1.75E-01
	P value	4.07E-13	7.64E-02	1.60E-02	4.24E-03	–	8.95E-04
ALB	Estimate	2.25E-01	–	3.43E-01	–5.53E-03	–3.02E-01	–
	Estimate error	3.40E-02		3.59E-02	1.21E-03	4.45E-02	
	P value	1.69E-10	–	5.46E-19	6.72E-06	6.66E-11	–
ALT	Estimate	–3.39E+00	6.54E+00	–2.80E+00	1.51E-01	–	–
	Estimate error	1.24E+00	1.15E+00	1.15E+00	6.50E-02		
	P value	6.83E-03	3.30E-08	1.51E-02	2.08E-02	–	–
GLU	Estimate	–	–	–8.42E+00	–7.63E-01	–	–
	Estimate error			1.44E-01	2.55E+00		
	P value	–	–	1.07E-03	2.48E-07	–	–
TP	Estimate	1.37E-01	–1.40E-01	1.39E-01	–	–	–
	Estimate error	4.87E-02	4.50E-02	4.49E-02			
	P value	5.25E-03	2.09E-03	2.17E-03	–	–	–
Chloride	Estimate	2.19E+00	–	–	–1.16E-01	–	–
	Estimate error	5.56E-01			2.91E-02		
	P value	1.07E-04	–	–	8.00E-05	–	–
BUN	Estimate	1.30E+00	–2.05E-01	2.79E+00	–	–4.17E+00	1.99E+00
	Estimate error	6.07E-01	5.63E-01	8.04E-01		8.07E-01	7.69E-01
	P value	3.37E-02	7.16E-01	6.04E-04	–	4.50E-07	1.02E-02
TBIL	Estimate	–5.23E-02	–	–	1.86E-03	–	–
	Estimate error	1.47E-02			7.80E-04		
	P value	4.60E-04	–	–	1.78E-02	–	–
CRE	Estimate	–	3.49E-02	–	2.27E-03	–	–
	Estimate error		1.76E-02		9.93E-04		
	P value	–	4.84E-02	–	2.32E-02	–	–

– indicates variable with which no significant interactions were found ($P > 0.05$). ALB, albumin; BCS, body condition score; CRE, creatinine; GLU, glucose; TBIL, total bilirubin; TP, total protein.

^aVariables investigated for significance of interactions with diet.

serves as a prospective look at whether eliminating alfalfa from the diet makes a difference in the parameters we tested.

After evaluating plasma and urine analytes in animals after the diet change, we found that excluding alfalfa as the primary feed ingredient, reducing the amount of pelleted feed available, and increasing the amount of available timothy hay did not reduce the amount of calcium crystals formed in urine overall but did alter a subset of urinalysis parameters and blood chemistry analytes. In addition, the dietary changes did not meaningfully alter clinical parameters or analytes to support overarching beneficial effects of this management approach. However, we recognize that a limitation in this study is that we did not quantify calcium consumed from diet and water sources and renal calcium excretion. Additional studies evaluating these parameters in inbred animals are warranted.

Functionally, both diets are viable options and suggest that practices aimed at reducing dietary calcium by reducing pelleted diet portions is insufficient to mitigate risk factors for urolithiasis in guinea pigs.

Comprehensive urolith reports indicate calcium carbonate and mixed/compound stones as the most common stones found in guinea pigs, with calcium oxalate, calcium phosphate, and struvite less commonly reported.^{35,38} Notably, we found no reduction in crystal burden in animals receiving the experimental diet plan. All adults, regardless of diet, had at least 2 types of crystals at one or more timepoints; most frequently observed crystal types included calcium carbonate and calcium oxalate monohydrate. These findings are consistent with other reports that list calcium carbonate, calcium oxalate, amorphous phosphate, and struvite as common crystal types^{20,21} and

Table 7. Results of final model analysis to evaluate interactions between urine analyte variables and age, sex, or diet

Final model		Variable					
		Adult	Sex	Diet	Sample week	Adult × diet	Sex × diet
Calcium oxalate crystals	Estimate	4.55E-01	-1.52E-01	-	-	-	-
	Estimate error	5.49E-02	5.09E-02	-	-	-	-
	<i>P</i> value	3.73E-08	4.23E-02	-	-	-	-
Total crystal estimate	Estimate	-7.09E-01	2.76E-01	-	-	-	-
	Estimate error	9.86E-02	9.13E-02	-	-	-	-
	<i>P</i> value	5.45E-12	2.70E-03	-	-	-	-
Urine turbidity	Estimate	-	1.58E-01	-1.79E-01	-7.37E-03	-	2.38E-01
	Estimate error	-	7.97E-02	8.03E-02	3.02E-03	-	1.09E-01
	<i>P</i> value	-	4.86E-02	2.67E-02	1.51E-02	-	3.05E-02
Urine protein	Estimate	-7.74E+01	4.81E+01	-	-3.99E+00	-	-
	Estimate error	2.59E+01	2.40E+01	-	1.36E+00	-	-
	<i>P</i> value	3.04E-03	4.57E-02	-	3.60E-03	-	-
USG	Estimate	-1.67E-02	-4.40E-03	-1.35E-02	-	8.75E-03	1.20E-02
	Estimate error	3.43E-03	3.18E-03	4.56E-03	-	4.36E-03	4.58E-03
	<i>P</i> value	1.80E-06	1.67E-01	3.27E-03	-	5.71E-02	6.23E-03
Urine pH	Estimate	-	-	-	-1.54E-02	-	-
	Estimate error	-	-	-	3.14E-03	-	-
	<i>P</i> value	-	-	-	1.46E-06	-	-

- indicates variable with which no significant interactions were found ($P > 0.05$). USG, urine specific gravity.

find that crystalluria often involves multiple types of crystals concurrently.³

An unexpected finding was juveniles having a higher average crystal load than adults by one grade (average 3.5 compared with 2.45, respectively). One explanation may be that the young animals are still developing behavioral patterns and may not spend as much time drinking as their older counterparts. This is supported by the higher USG also seen in juveniles as compared with adults. Another explanation could be that juveniles were more likely than adults (as a whole) to produce urine in the induction box rather than the transport box. If the bladder relaxation that occurs during the induction of anesthesia causes a more complete emptying of the bladder, this may explain why the juveniles had a higher crystal load than adults. This could in addition explain why males had a higher crystal load than females, as females were more like to urinate in the transport box prior to anesthesia than males.

Although no differences in crystal burden due to diet were noted in this study, some limitations to consider include potential effects of urine collection, storage, and processing approaches. Urine was collected by natural or induced voiding and was assumed to be full emptying of the bladder, but some samples may not be complete terminal voiding, which could falsely decrease the number of crystals seen in some sediment samples.

Interestingly, we found a significant difference in sediment crystal burden between the most frequent urine collection methods; urine collected from the induction box had a higher crystal burden than urine collected from the transport box. However, given that both experimental and control groups had similar proportions of urine collection methods, and that multiple time points were evaluated, we believe that potential bias was reduced, especially in the adult cohort that was followed for 6 mo. Other factors may include timing of sample collection and processing. We were not able to perform the urinalyses within 30 min of collection as some sources recommend.^{5,18} Although most samples were read at 2 to 8 h after collection, a small number were read within 24 h. To preserve the chemical

constituents of urine and prevent bacterial growth, the samples were refrigerated at 4°C and then allowed to equilibrate to room temperature for analysis. A study performed on urine crystal formation in dogs suggested storing one aliquot of urine at 4°C and another at room temperature, as the group found that storing samples at room temperature may slow formation of calcium oxalate crystals in standing samples.¹ In the current study, using multiple aliquots was not always practical due to the limited urine sample volume obtained from guinea pigs at any given time. Furthermore, the impact of refrigeration on crystal formation in guinea pig urine has not been evaluated.

It is also noteworthy that all urine samples evaluated in this study had some level of crystalluria. In one report, crystalluria was not evident in 14% of submitted sediment samples on urinalysis.²⁰ Unfortunately, the only details available in the report were the absence/presence of crystals and the type of crystals, if present. Also, differences in urine crystal type between adults and juveniles are not commonly reported, likely because urinalysis is usually conducted to investigate clinical problems such as urolithiasis that do not typically manifest until 3 to 4 y of life in pet guinea pigs.^{13,20} In our colony, clinical urolithiasis has been reported in animals as young as 151 d of age.

An interesting discovery made after the end of the study was that one juvenile assigned to the male cohort was diagnosed with disorder of sexual development after the cessation of the study that was characterized by ambiguous genitalia (micropenis and vagina), a uterus with marked cystic endometrial hyperplasia, testicular dysplasia, no discernable ovaries, a cervix, and seminiferous tubules. Karyotyping was not performed. No outliers were identified after reviewing this animal's data considering these findings, so they were included in final analysis to maintain the group sizes of the cohort.

Importantly, the etiology of urolith formation has not been elucidated in many species,² including guinea pigs, and we do not know what level of crystalluria, if any, is a direct cause of stone formation. Many sources agree that the presence of urinary crystals increases risk, supersaturation of urine with solutes precedes crystal formation, and crystal formation can

serve as a nidus for stone formation.^{2,26} In both juvenile and adult guinea pigs, calcium carbonate crystals were most frequently detected. Calcium carbonate is a food additive present in both pelleted diets used in this experiment; it is used to balance calcium and regulate pH of pelleted food, which is important because guinea pigs do not tolerate an acidic diet³³ and have a high requirement for cations due to their inability to conserve fixed bases with ammonia excretion.³² However, some commercially available guinea pig feeds do not contain this ingredient. The incidence of calcium carbonate uroliths is reported to be overrepresented in guinea pigs, chinchillas, domestic rabbits, domestic horses, and nonhuman primates. Calcium carbonate stones are also reported in capybaras, kangaroos, wallabies, elephants, and tapir, but sample sizes of these animals were small.³⁵ Interestingly, a study found that increases in urolith incidence in rats fed calcium carbonate appear to result from an alkalization of urine and a reduction of urine phosphorus concentration,⁴⁰ suggesting that a similar mechanism resulting from the inclusion of calcium carbonate additive in pelleted or extruded diets may be associated with the incidence of calcium carbonate uroliths in these animals.

Renal disease in guinea pigs is often seen in conjunction or secondarily to urolithiasis in our colony. Staging of renal disease is not well described in guinea pigs, and evaluation of urinary tract health in these animals must be extrapolated from other species. BUN and blood CRE remain key analytes for assessing renal function. Differentiation of prerenal, renal, or postrenal azotemia is largely determined by history and physical examination findings but can be aided by concurrent assessment of urine concentration and CBC findings. Electrolyte dyscrasias can also accompany renal impairment. In our study, we used BUN, CRE, potassium, and total calcium to look for trends that may be indicators of overall kidney function. USG was also measured to correlate with any indicators of early renal impairment that could be related to diet. Guidelines for identifying early azotemia and acceptable magnitudes of elevation in BUN and CRE have not been well described in guinea pigs. Similarly, what is considered normal USG is reported with wide reference ranges for this species.^{9,11,37} The experimental diet groups showed increased ALB in juveniles, increased BUN in males and juveniles, and increased TP independent of sex or age. Given that CRE levels were largely unchanged, the changes in BUN may suggest increased protein intake or improved assimilation rather than effects on renal function.²² In guinea pigs, CRE is a more reliable indicator of renal function,²² and in all cohorts, CRE remained stable or slightly decreased. Males and juveniles fed the experimental diet also had lower weights but maintained a normal body condition, which may suggest a lean body mass rather than fat. The changes in plasma proteins were unexpected, as the manufacturer of the THP lists the approximate nutrient composition of protein as 18%, very similar to the 19.3% listed for the AHP diet. One possibility is that the THP also contain prebiotics that may contribute to more efficient absorption of consumed nutrients.

Increased USG was observed in adult males fed the experimental diet although the increase was mild. It is possible that this finding simply reflects decreased water intake. Animals on the experimental diet had more access to loose timothy hay and may have spent more time grazing or masticating than drinking, a previously reported finding in guinea pigs fed more roughage in their diet compared with more pelleted feed.⁴ Frequency of drinking and water intake were not measured in this study. We also noted that urine pH across all animals decreased slightly

over time; however, all values were either 8 or 9, and both were considered normal for this species.^{9,14,45} Because this is a color reaction test pad, the gradual decrease over time may represent the users of this test adapting to the nuance between the 2 colors (teal and dark teal) representing the different pH values.

Animals fed the experimental diet had lower average GLU than did animals fed the control diet. This may be related to the amount of pelleted feed offered as well as the feed type, but it emphasizes that a diet composed of timothy hay (THP combined with ad libitum hay) may offer a healthier glycemic index than a diet with AHP. Cane molasses (glucose source) has similar placement on the ingredient list of both pellet formulations, but the AHP also includes corn, which may serve as an additional source of sugars. Average plasma total calcium was also reduced in males fed the experimental diet. This may have to do with the changes in plasma proteins, but the absence of a concurrent increase in ALB levels suggests that is not the principal cause. Hemolysis can falsely increase plasma calcium readings, and in some samples, calcium was >12.0 mg/dL, but only one sample of >16.0 mg/dL correlated to an increased hemolysis index.³⁹ Age differences in calcium levels are reported in dogs and humans; young animals have slightly higher calcium levels due to higher bone metabolism.^{25,39} However, a previous report on inbred strain 13/N guinea pigs describes a steady increase in total calcium with age while another report in Weiser-Maples guinea pigs did not find a significant correlation between age and serum calcium.^{15,23} In rabbits, blood calcium is lower in growing animals due to increased use.²⁹ Finally, adult animals fed the experimental diet had reduced average potassium, which may simply reflect direct diet composition; although actual content may vary due to natural ingredients, the guaranteed analysis of the THP lists slightly less potassium content than that of the AHP.

While the findings in this study were not reflective of either marked improvements in health or adverse findings that require immediate husbandry changes, the results do raise questions about optimal feed constituents for strain 13/N guinea pigs. Future studies using metabolic caging and diets that omit certain ingredients that have been historically used, such as calcium carbonate, may be useful. In addition, studies investigating possible genetic factors for urolithiasis development in strain 13/N guinea pigs are also warranted.

Conclusions

Our study provides important data suggesting that transitioning from an AHP diet to a THP diet with practices to reduce overall reduction in dietary calcium were not sufficient to mitigate risk factors for urolithiasis in guinea pigs. Husbandry practices that avoid alfalfa products are often recommended for keeping adult pet guinea pigs. Although the dietary changes investigated in this study did not significantly reduce crystal burden or result in other meaningful clinical effects supporting an advantage to this husbandry approach, the changes did not confer any detrimental effects, indicating that both THP and AHP diets are viable options for colony management. Future studies should continue to examine the role of other feed components, such as calcium carbonate, and conducting similar studies in additional guinea pig strains would further support these findings. Overall, our data suggest that urolith formation is a complex, multifactorial process requiring several mitigation approaches to significantly decrease incidence.

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Conflict of Interest

The authors have no competing interest to declare.

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