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Research Article

Diyetin Üre İdrartaşlı Dalmaçyaların İdrar Yapısına Etkileri

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Özet: Bu araştırma, Dalmaçya ırkı köpeklerinde üre idrar taşlarının yönetilmesinde, modifiye edilmiş idrar taşı önleme diyetinin orijinal idrar taşı önleme diyeti kadar etkisini tespit etmek amacıyla; altı yetişkin, kastre edilmiş, erkek, üre idrar taş geçmişli köpekte yapılmıştır. Her bir köpekte, 1- orijinal idrar taşı önleme diyeti, 2- Yüksek miktarda protein ve sodium ve düşük miktarda yağ içeren modifiye idrar taşı önleme diyeti, ve 3- yetişkin koruyucu diyeti olmak üzere üç konserve diyet değerlendirilmiştir. Diyet uygulama sırası rast gele düzenlenmiş, iki haftalık her bir tedavi sonucunda, yirmi dört saat süre ile idrar örnekleri toplanmıştır. İdrar miktarı, pH'sı, kreatinin, sodyum, potasyum, klor, kalsiyum, magnezyum, fosfor, okzalik asit, sitrik asit ve urik asit düzeyleri belirlenmiştir. Bu değerler, amonyum üre aktiviti ürünlerinin hesaplanmasında kullanılmıştır. Verilerin geldiği her bir köpek, bloke edilerek idrar değerleri arasındaki istatistiksel farklılıkları değerlendirmek için Kovariyans analizine tabii tutulmuştur.

Araştırmanın sonucunda, modifiye idrar taşı önleme diyeti tüketen köpeklerde, üre idrar taşı tekrarlamasının önlemesini mümkün kılan bir çok değişiklik tespit edilmiştir. Modifiye ve orijinal idrar taşı önleme diyetleri tüketimine bağlı idrar urik asit konsantrasyon ve atılım değerleri benzer olup, bu sonuçlar yetişkin koruyucu diyeti tüketimindeki sonuçlardan daha düşük bulunmuştur. Modifiye ve orijinal İdrar taşı önleme diyetleri mukayese edildiğinde, modifiye diyet tüketiminde beklenmedik bir şekilde, idrar pH'sının önemli derecede düşük ve idrar amonyum konsantrasyonu ve amonyum üre aktiviti ürünlerinin önemli derecede yüksek olduğu tespit edilmiştir.

Sonuç olarak, idrar amonyum konsantrasyonunu azaltan ve amonyum çözünürlüğünü artıran yeterli derecede yüksek pH'lı idrar atılımını artırmak için modifiye idrar taşı önleme diyeti daha da değiştirilerek diyetin etkisinin sağlanabileceği ortaya konulmuştur.

Anahtar sözcükler: Amonyum üre, dalmaçya, diyet, ürik asit, idrar taşı

Effects of Diets on Urine Composition of Dalmatians with Urate Urolithiasis*

Summary : Objective— To determine if a modified urolith prevention diet is as effective as a urolith prevention diet in the management of urate uroliths in Dalmatian dogs.

Design— Prospective crossover study.
Sample Population— Six adult neutered male Dalmatians with a history of urate urolithiasis.

Procedure— Three canned diets were evaluated in each dog; 1) the original urolith prevention diet, 2) a modified urolith prevention diet containing higher quantities of protein and sodium, and lower fat, and 3) an adult maintenance diet. The order of the treatments was randomly assigned. At the end of each 2-week treatment period, 24-hour urine samples were collected. Urine volume and pH, and the concentrations of creatinine, sodium, potassium, chloride, calcium, magnesium, phosphorus, oxalic acid, citric acid, and uric acid were determined. These values were used to calculate activity products for ammonium urate in the urine of each dog for each diet. An analysis of covariance with data from each dog as blocks was used to evaluate statistical differences between urine values.

Results— When dogs consumed the modified urolith prevention diet, several changes likely to prevent urate urolith recurrence were maintained. Urine uric acid concentration and excretion associated with consumption of the modified urolith prevention diet were similar to results during consumption of the original urolith prevention diet and lower than results during consumption of the maintenance diet. Unexpectedly, urine pH was substantially lower and urine ammonium concentration and activity product for ammonium urate during consumption of the modified diet were substantially higher than values observed during consumption of the urolith prevention diet.

Conclusions and Clinical Relevance— To maintain diet efficacy, the modified urolith prevention diet should be further altered to promote excretion of urine with sufficiently higher pH such that urinary ammonium concentration is reduced and ammonium urate solubility is increased.

Key Words: Ammonium urate, dalmatian, diet, uric acid, urolith.

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Introduction

Uroliths primarily composed of urate are the most common type retrieved from Dalmatian dogs. This is not unexpected, since Dalmatians have a unique pathway for metabolizing purines. Compared to most other breeds of dogs in which the end product of purine metabolism is allantoin, the principal end production of purine metabolism in Dalmatians is uric acid. Because this metabolic defect is inherited, surgical removal or medical dissolution of urate stones provides only a short-term cure.

To minimize urate urolith recurrence in Dalmatians, a commercially available, urolith prevention diet[a] is available. Although this diet was primarily designed to prevent calcium oxalate uroliths, several features also reduce the risk of urate urolith formation. Compared to maintenance diets, the protein content of the urolith prevention diet is approximately 50% lower. This is important because most protein sources provide additional purines that are ultimately metabolized to uric acid. Therefore, reducing dietary protein reduces the quantity of uric acid available for urolith formation (1,2). In addition to lower protein; this diet was supplemented with potassium citrate to promote formation of alkaline urine. Alkaline urine increases uric acid solubility and reduces ammonium excretion (the cation associated with most urate uroliths in Dalmatians) (3). In a prospective study, the urolith prevention diet was 37% more effective in preventing urolith recurrence than another commercially available diet providing nutritional maintenance requirements for the adult dog.[b] In this study, the urolith prevention diet significantly decreased urine concentration and excretion of uric acid. When dogs consumed the urolith prevention diet, urine activity product for ammonium urate was decreased, and urine volume and urine pH were increased.

Although the urolith prevention diet helped minimize urate urolith recurrence in Dalmatians, the safety associated with its long-term consumption had been questioned. Results of a retrospective case series of 9 Dalmatians with dilated cardiomyopathy recognized that 8 Dalmatians had consumed the urolith prevention diet for an average of 33 months prior to the diagnosis of heart disease (4). Although a cause and effect relationship was not established in this study, reduced dietary protein was considered as a risk factor. A related study evaluating urolith prevention diet consumption in healthy beagles detected a significant decrease in whole blood taurine after 12 months (5). In addition; we have observed pica and aggressive behavior toward food acquisition in several Dalmatians fed the urolith prevention diet. In several breeds of dogs that commonly form calcium oxalate uroliths, the high fat content of the diet was considered inappropriate because it may exacerbate obesity, diabetes mellitus, pancreatitis, hyperadrenocorticism and hyperlipidemia.[c]

In an attempt to improve safety and yet maintain efficacy, the diet was modified. Protein was increased by 22% and fat was decreased by 8%. Because this new formulation would also be used to prevent calcium oxalate uroliths, the levels of calcium, magnesium, and phosphorus were increased.

We hypothesize that these nutritional changes will minimally affect the diet's efficacy in the management of urate uroliths. To test this hypothesis, we compared urine uric acid excretion and activity products for ammonium urate during consumption of the modified urolith prevention (MUP) diet[d], the original urolith prevention (UP) diet,[a] and an adult maintenance (MN) diet.[e] Because this was a pilot study we only evaluated the diets in 6 Dalmatians with a history of urate uroliths understanding that this low number of dogs may be insufficient to obtain statistical significance.

Materials and Methods

Dog- On the basis of quantitative evaluation of uroliths submitted to the Minnesota Urolith Center by practitioners in the Minneapolis-St. Paul metropolitan area, 6 Dalmatian dogs with a history of ammonium urate urolithiasis were evaluated. Only dogs with uroliths composed of 100% urate were included (Table1). All dogs were neutered males. Dogs were 3.6 to 9.2 years old and ranged in weight from 48.0 to 74.0 lb (21.8 to 33.6 kg). At the time of study, complete blood counts (CBC), serum biochemical analysis, urinalysis, bacteriologic culture of urine, and double contrast cystography were performed. Neither renal failure, urinary tract infections, nor uroliths were evident in any dog.

Experimental design- To minimize variability attributable to individual dogs, a cross over design was used to evaluate the effects of three diets on urine activity products for ammonium urate (6). The design of this study allowed evaluation of all

Table 1. Quantitative Mineral Analysis of Uroliths of 6 Dalmatian Dogs

Dogs	Urolith Types
1	100% Ammonium urate
2	100% Sodium urate
3	100% Ammonium urate
4	100% Ammonium urate
5	100% Sodium urate
6	100% Ammonium urate

three diets in each dog. The order in which the diets were fed to each dog was randomly assigned. The amount of food given to each dog was calculated on the basis of daily caloric requirements determined by body weight (140 kcal/d/kg$^{0.73}$) (7). Each diet was fed for 14 days.

Treatment diets- Three diets were evaluated (Table 2). The primary test diet identified in this report as modified urolith prevention diet contained the following features: high moisture, reduced protein (chicken), and moderate fat. The diet was also designed to promote formation of alkaline urine with a low urine specific gravity. A urate urolith prevention diet with less protein (chicken), and more fat served as a negative control. The control diet is consisted of high moisture adult maintenance diet formulated to nutritionally maintain the health of adult dogs. This diet contained more protein and less fat than the other two diets.

Animal housing- Dogs lived with and were fed by their owners except during periods of 24-hour urine collections. Dogs were housed in metabolism cages at the Veterinary Medical Center, University of Minnesota during the 24-hour urine collection.

Urine collection and analysis- At the end of each two-week feeding trial, dogs were returned to the veterinary hospital for evaluation. At the beginning of the 24-hour urine collection, urinary bladders of dogs were emptied via transurethral catheterization. An aliquot of this urine was saved for urinalysis. To minimize catheter-induced bacterial urinary tract infection, ampicillin[f] was initiated (25mg/kg[11.4mg/ld], PO, q 8 h) and continued for 2 days. Once urinary bladders were emptied, dogs were fed half of their daily food allotment and placed in metabolism cages. They were fed the remaining half of their daily food allotment 12 hours later. Water was accessible during the 24-hour urine collection period. Because these dogs were trained not to urinate in their owner's house, they did not urinate while in the metabolism cages. Therefore, urine samples were collected every 8 hours by transurethral catheterization and stored in capped receptacles at 4^{o}C. At the end of 24-hour urine collection, urine samples were pooled.

Urine concentrations of creatinine, sodium, potassium, and chloride were determined with an automated serum chemistry analyzer.[g] Calcium concentrations of urine samples acidified with 10% hydrochloric acid and diluted with 5% lanthanum[h] were measured by use of atomic absorption spectrophotometry.[i] Urine concentration of phosphorus and magnesium were also measured by atomic absorption spectrophotometry. To minimize precipitation of uric acid, 0.5 ml aliquots of pooled 24-hr urine samples were mixed with 4.5ml of distilled water (8). Urine uric acid concentrations were determined by high-pressure liquid chromatography.[k] One half milliliter aliquots of pooled 24 hr urine samples were acidified with 0.75 ml of 10% hydrochloric acid for determination of urine oxalic acid by ion chromatography.[l] Urine concentration of ammonia was determined by ion-selective electrode.[m] Urine pH was measured with a pH meter.[n]

Calculation of activity products- Concentrations of urinary analytes were entered into a microcomputer-based program for calculation of activity products for ammonium urate (APau**)** (9). The activity product (AP) was calculated as follow:[1]

$$APau = [urate^{2-}] \times f2 \times [H^{+}] \times f1 \times [NH_4^{+}] \times f1$$

where:

- $[urate^{2-}]$ = ionic concentration (mol/L) of urate ion
- $[H^{+}]$ = ionic concentration (mol/L) of hydrogen ion
- $[NH_4^{+}]$ = ionic concentration (mol/L) of ammonium ion
- f1 = activity coefficient for a single-charged ion
- f2 = activity coefficient for a double-charged ion.

Blood sample collection and analysis- Blood samples were collected from the jugular vein of each dog at the midpoint of 24-hour urine collections. Serum was harvested from samples within 30 minutes of collection by standard procedures and stored for approximately 12 hours at 4^{o}C until analyzed.[g] Aliquots of serum for determination of uric acid were stored for approximately 60 days before analysis. Serum iochemical profiles were evaluated to determine changes associated with diet consumption.

Table 2. Key Nutrient Content* of Diets Fed to 6 Dalmatians with a History of Urate Uroliths

Component	Diets		
	MUP[d]	UP[a]	MN[e]
Moisture	75.0	72.0	73.0
Protein	14.0	11.47	22.83
Fat	25.0	27.24	18.12
Carbohydrate	49.0	57.35	52.68
Fiber	5.7	1.43	2.17
Calcium	0.6	0.29	0.69
Phosphorus	0.7	0.14	0.54
Sodium	0.5	0.25	0.22
Potassium	1.1	0.39	0.51
Magnesium	0.13	0.072	0.087
Chloride	1.26	0.39	0.3
Potassium citrate	0.55	0.80	0.0
Caloric density	108	132	113

* Moisture is expressed as percent of diet as fed. Caloric density is expressed as kcal / 100 grams of food. Other components are expressed as percent dry matter.

Double contrast cystography- Contrast radiography was performed to ensure that dogs remained stone free during the study. This procedure was performed by passing a catheter through the urethra into urinary bladder. After removing all urine, the bladder lumen was distended by injecting air (3 ml/lb body weight). Radiopaque contrast solution[q] (approximately 4 -5 ml) was then injected through the transurethral catheter into the bladder lumen. A formal technique chart was used to establish the kilovolt (peak) (kV[p]) and milliamp-seconds (mAs) settings for all exposures according to thickness of mid abdomen. With the patient in right lateral recumbency, radiographs of the abdomen were obtained. After the exposure, air and contrast agent remaining in the bladder lumen were aspirated through the catheter into a syringe.

Statistical Analysis- Means and standard deviations were used to compare the results of blood and urine analyte measurements in three diet groups. An analysis of covariance with data from each dog as blocks was used to evaluate statistical differences in values of body weight, urinalysis, urine and serum analytes, 24-hour urine volume, and AP's between the three dietary treatments (10). Assumption of normality was tested by use of residual plot analysis. Data were corrected with log transformation. A paired t-test was used to determine differences between the groups. Analyses were performed with statistical software.[s] *P* values < 0.05 were considered significant.

Results

At the time of initial diet assignment, there was no difference in hematological or serum biochemical characteristics of the dogs. Furthermore, values for CBC and serum biochemical analytes were within the normal reference range (11).

Table 3. Effects of Diets on Concentration of Urine Analytes from 6 Dalmatian Dogs with History of Urate Uroliths

Analytes	Diets			P
	MUP[d]	UP[a]	MN[e]	
Calcium (ppm)	9.83±8.29	9.42±7.94	10.26±8.88	0.9
Calcium (uM/kg/24 hr)	5.60±4.23	5.79±4.58	5.54±6.15	0.9
Creatinine (mg/dl)	126.93±32.98	128.83±57.84	166.50±94.94	0.5
Uric acid (mg/dl)	74.06±62.92	68.17±75.21	120.78±102.42	0.2
Uric acid (uM/kg/24 hr)	109.12±77.83	89.68±91.58	123.55±89.12	0.5
Phosphorous (mM)	36.66±13.12[†]	11.52±4.14[*]	42.25±23.83[†]	0.02
Phosphorous (mM/kg/24 hr)	0.92±0.23[†]	0.31±0.14[*]	0.80±0.13[†]	0.002
Magnesium (ppm)	76.21±35.72	47.13±24.94	79.65±35.12	0.08
Magnesium (uM/kg/24 hr)	74.48±17.26	49.09±24.80	64.57±31.86	0.09
Chloride (mm/L)	183.33±35.18[†]	54.50±19.31[*]	44.50±26.50[*]	< 0.001
Chloride (mM/kg/24 hr)	4.84±1.62[†]	1.46±0.66[*]	0.98±0.60[*]	< 0.001
Sodium (mm/L)	101.50±27.94[†]	55.50±22.60[*]	44.25±27.68[‡]	< 0.001
Sodium (mM/kg/24 hr)	2.66±0.93[†]	1.44±0.61[*]	0.72±0.63[*]	< 0.001
Potassium (mm/L)	125.20±29.45[†]	52.40±20.32[*]	70.37±18.92[*]	< 0.001
Potassium (mM/kg/24 hr)	3.25±0.95[†]	1.31±0.43[*]	1.51±0.58[*]	< 0.001
NH3 (mM/L)	56.15±14.90[†]	17.30±4.66[*]	58.17±51.82[†*]	0.04
NH3 (mM/kg/24 hr)	1.43±0.33[†]	0.46±0.18[*]	0.99±0.32[‡]	< 0.001
Oxalate (uM/L)	375.25±126.07	349.58±114.23	649.70±442.52	0.0.8
Oxalate (uM/kg/24 hr)	9.40±199[†]	8.63±1.17[†]	11.79±1.83[*]	< 0.001
Citrate (uM/L)	88.58±92.60	266.45±357.84	101.30±121.49	0.07
Citrate (uM/kg/24 hr)	2.16±1.89	6.69±8.49	2.33±2.66	0.06
Urine pH	5.87±0.36[†]	7.01±0.19[*]	6.36±0.26[‡]	< 0.001
Urine Volume (ml/24hr)	784±258.6	807±324.2	669±293.6	0.70
Urine Specific Gravity	1.020±0.006	1.013±0.005	1.023±0.01	0.2

Values with different superscript are significantly different

Table 4. Effect of Diets on Urine Activity Products for Ammonium Urate in 6 Dalmatians with History of Urate Uroliths

Dogs	Activity Product (moles/L x 10^{-15})		
	Diets		
	MUP[d]	**UP[a]**	**MN[e]**
1	0.22	0.06	0.36
2	0.17	0.07	0.41
3	14.3	0.33	9.82
4	2.69	2.18	7.58
5	8.13	1.33	22.2
6	1.77	2.41	40.5
Mean	**4.5[†]**	**1.06[*]**	**13.5[†]**
SD	**5.6**	**1.06**	**15.5**
Values with different superscript are significantly different			

Table 5. Effects of Diets on Body Weights (kg) from 6 Dalmatians with History of Urate Uroliths

Dogs	Diets		
	MUP[d]	**UP[a]**	**MN[e]**
1	31.1	31.5	31.5
2	32.3	33.6	33.5
3	31.0	30.4	30.2
4	27.2	27.8	28.3
5	32.3	32.5	31.0
6	23.3	22.0	21.8
Mean	**29.5[†]**	**29.6[†]**	**29.4[†]**
SD	**3.6**	**4.2**	**4.1**
Values with different superscript are significantly different			

Table 6. Effects of Diets on the Concentration of Serum Analytes from 6 Dalmatian Dogs with History of Urate Urolithiasis

Serum	Baseline	Diets			***P***	Reference Range
		MUP[d]	UP[a]	MA[e]		
Albumin (g/dl)	3.5 ±0.2[†]	3.5±0.3[†*]	3.4±0.3[†]	3.6±0.3[*]	0.04	**3.2 – 4.7**
Total Protein (g/dl)	6.2±0.3[†]	6.1±0.3[†*]	5.9±0.1[*]	6.2±0.2[†*]	0.04	5.3 - 7.6
Urea Nitrogen (mg/dl)	10.0±7.4[†‡]	6.8±4.2[†*]	5.3±1.9[*]	11.0±3.7[‡]	0.03	5.9 - 27.2
Total Biluribin (mg/dl)	0.4±0.4[†]	0.2±0.2[*]	0.3±0.3[†*]	0.2±0.2[*]	0.02	01 - 0.6
Cholesterol (mg/dl)	303.7±66.1	300.3±86.7	317.0±94.6	277.2±75.1	0.4	106.0 - 367.4
Glucose (mg/dl)	110.8±5.8	110.3±10.8	106.0±10.2	112.7±11.6	0.7	53 - 117
ALP (U/L)	122.8±121.4	109.0±141.1	126.5±161.1	100.8±129.3	0.4	0 - 200
ALT (U/L)	35.8±9.2	42.0±8.2	42.8±14.2	40.3±17.3	0.7	10 - 94
Amylase (U/L)	724.2±434.3	564.7±101.4	634.8±83.7	654.7±69.8	0.7	371 - 1503
AST (U/L)	23.2±4.4	27.0±6.9	25.2±2.8	24.2±4.4	0.2	10 - 50
GGT (U/L)	3.7±1.4	4.0±2.1	3.3±1.0	4.0±2.1	0.6	1 - 6
TCO_2 (mmol/L)	21.9±0.8[†]	21.9±2.1[†]	23.6±2.3[†*]	24.0±0.3[*]	0.03	16.9 – 26.9
Sodium (mmol/L)	148.5±1.8	147.7±2.7	147.8±1.9	147.5±2.0	0.9	146 - 156
Chloride (mmol/L)	116.8±2.1	118.3±1.0	116.0±2.4	116.0±1.4	0.09	113 - 123
Potassium (mmol/L)	4.4±0.4	4.9±0.9	4.4±0.2	4.4±0.3	0.2	3.9 – 5.5
Creatinine (mg/dl)	1.2±0.1	1.1±0.1	1.1±0.1	1.1±0.1	0.9	0.5 – 1.4
Magnesium (mg/dl)	2.1±0.2	2.2±0.2	2.2±0.3	2.2±0.2	0.2	1.36 – 2.09
Calcium (mg/dL)	10.1±0.2	10.0±0.3	10.1±0.3	10.1±0.3	0.9	9.0 – 11.9
Phosphorus (mg/dl)	3.9±0.8[†]	4.8±0.7[*]	4.5±0.2[†*]	4.9±0.4[*]	< 0.001	1.9 – 7.9

ALP = Alkaline phosphatase,
ALT = Alanine aminotransferase,
AST = Asparate aminotransferase,
GGT = Gamma-glutamyl transferase,
TCO_2 = Total carbon dioxide,

Values with different superscript are significantly different.

Effects of diets on urine- Mean urine concentrations of uric acid were decreased when dogs were fed the MUP or UP diets compared to when they were fed the MN diet (Table 3). However, mean urine uric acid concentration was not significantly different among the three diet groups. Likewise, **Effects of diets on urine-** Mean urine concentrations of uric acid were decreased when dogs were fed the MUP or UP diets compared to when they were fed the MN diet (Table 3). However, mean urine uric acid concentration was not significantly different among the three diet groups. Likewise, urine excretion of uric acid was not significantly different among the three diet groups. Urine concentration of NH_3 was significantly (p = 0.002) higher when dogs were fed the MUP diet compared to when they were fed the UP diet. Urine excretion of NH_3 was significantly (p < 0.001) higher when dogs were fed the MUP diet compared to when they were fed the UP or MN diet. Consumption of UP diet was associated with the lowest urine excretion of NH_3.

Unexpectedly, mean urine pH was lowest when dogs consumed MUP diet compared to when they were fed the UP or MN diet (p value < 0.001). Urine pH was highest when dogs consumed UP diet. Significant differences in mean urine volumes were not observed when dogs consumed MUP, UP or MN diets.

Effects of diets on urine activity products- Activity product for ammonium urate in 24-hour urine samples was significantly (p = 0.005 & 0.002, respectively) lower when dogs were fed the UP diet compared to when they were fed the MN or MUP diets (Table 4). Activity product for ammonium urate in 24-hour urine samples was also lower when dogs were fed the MUP diet compared to when they were fed the MN diet. This difference approaches statistical significance (p = *0*.055).

Effects of diets on body weight- At the end of 2-week study intervals, the body weights of dogs fed MUP diet was higher compared to the body weight of dogs fed UP or maintenance diet; however, the differences were not significant (p = 0.9; Table 5).

Effects of diets on serum and blood analytes- At the end of 2-week study intervals, serum analytes including amylase and cholesterol were with in the reference range except mean serum concentration of urea nitrogen when dogs were fed the UP diet that was lower than reference range (Table 6). Mean serum urea nitrogen concentrations were significantly (p = 0.02) lower when dogs were fed the UP diet (5.3 ± 1.9 mg/dl) compared to when they were fed the MUP diet (6.1 ± 0.3 mg/dl) or MN diet (11.0 ± 3.7 mg/dl).

Discussion

Factors that reduce the risk of ammonium urate urolithiasis by fostering formation of urine that is undersaturated with uric acid include reduction in renal excretion and urine concentration of uric acid, reduction in renal excretion and urine concentration of ammonium, and formation of neutral to alkaline urine. In this type of environment, urate uroliths will dissolve and new stones will not form. Results of this study indicate that modifications of the original urolith prevention diet to improve safety preserved some of the aforementioned features considered to maintain its efficacy. For example, when Dalmatians consumed the modified diet, urine uric acid excretion and concentration were similar to values obtained when the original urolith prevention diet was consumed. By comparison, during consumption of the maintenance diet, urine uric acid excretion was approximately 20% higher and urine uric acid concentration was approximately 40% higher. In addition, Dalmatians consuming the MUP diet sustained a high 24-hour urine volume similar in magnitude to that observed when UP diet was fed.

Although Dalmatians consuming the MUP diet maintained low urine uric acid concentration and high urine volumes, this modified diet did not sustain the desired features of increased urine pH and reduced urine NH_3 concentration. When Dalmatians consumed the MUP diet, urine pH was significantly lower than when they consumed either the UP or MN diets. At least 2 factors may have contributed to formation of more acidic urine. One is related to the fact that animal-source protein ingredients contain sulfur-containing amino acids that promote formation of acidic urine. Therefore, increasing the protein content of the diet increased the production of and subsequent excretion of acidic metabolites. In addition, the quantity of potassium citrate, an alkalinizing ingredient, was lower in MUP diet (0.55% DM) than the UP diet (0.8% DM).

Formation of acidic urine during consumption of the MUP diet may have also contributed to increased formation of NH_3 by renal tubules. The renal tubules generate ammonium from amino acid metabolism (principally glutamine). Ammonium enters the tubular lumen via a carrier-mediated step. Interstitial ammonia diffuses into the tubular lumen more distally. By combining with hydrogen ions in the tubular fluid, ammonia becomes ionized, losing its ability to diffuse back into the cell. During acidosis and

aciduria, ammonia production and excretion by both of these processes increases.

The solubility of uric acid and the salts of urate in urine is also pH-dependent. Uric acid has two dissociable protons. In human urine, the first proton has a dissociation constant (pKa) of 5.35 at 37^{o}C (12). A pKa of 5.35 implies that at a urine pH of 5.35, 50% of uric acid exists as mono-hydrogen form. As urine pH becomes more acidic the concentration of hydrogen urate increases and vice versa. The concentration of hydrogen urate is important because it forms salts with monovalent cations (i.e. ammonium). Although we are unaware of similar studies evaluating uric acid solubility in urine of dogs, we presume that the physiochemical influence of pH on urine uric acid solubility would be similar. In addition to increasing the concentration of hydrogen urate, a low urine pH also increases the quantity of ammonia excreted in urine. For these reasons higher urine pH values are considered important for the dissolution and prevention of urate uroliths.

On the basis of these findings, we recognize that formation of a neutral to more alkaline urine would be beneficial. Therefore, to improve efficacy and maintain safety, we recommend that this diet be modified further to promote formation of urine with higher pH. Ingredients traditionally added to alkalinize canine diets include calcium carbonate, potassium citrate and magnesium oxide (13). Because this diet will also be used to manage patients with calcium oxalate uroliths, additional potassium may prove more advantageous than other alkalinizers.

Our study had several limitations. Because commercially manufactured diets were used, it was not possible to determine effects of any single ingredient on urinary values. Thus, our recommendation to modify the diet using specific ingredients may not predict how they will be influenced through potential interactions among the other various dietary components. In addition, the feeding period before evaluating each diet was 2 weeks. Changes in the urine following long-term consumption may not be similar. We also emphasize that although specifically formulated diets can reduce uric acid supersaturation, appropriate studies evaluating rates of urolith dissolution and recurrence are needed to confirm the clinical relevancy and effectiveness of dietary therapy.

In context of our inability to demonstrate statistically significant differences in many urinary analytes between the three treatments, our study lacked sufficient statistical power to validate potentially significant clinical effects. This was not unintentional. Statistical power increases with increasing sample size and effect size, and declines with increasing variance. Our pilot study was designed to only evaluate a small number of urolith-forming Dalmatians with the goal of performing a larger clinical study if preliminary results were favorable. When statistical power was calculated retrospectively using data derived from this study (i.e.AP of MUP diet=4.5x10^{-15}, AP of MN diet = 13.5x10^{-15}, sample size = 6, and a = 0.05), it became apparent that there was only a 41% chance of validating the research hypothesis. Increasing the statistical power to 80% would have required sampling 19 Dalmatians instead of 6 to detect the differences in Apau (14). Similarly, when statistical power was calculated retrospectively using data of mean urine uric acid concentration; it became apparent that there were only 36% chances of validating the research hypothesis. Increasing the statistical power to 80% would have required sampling 23 Dalmatians instead of 6 to detect the differences of uric acid concentration (14).

Footnotes

a. Prescription Diet Canine canned u/d (T0421101 5540), Hill's Pet Nutrition Inc, Topeka,Kan.
b. Albasan H, Lulich JP, Osborne CA, et al. Effects of diet on urolith recurrence and urine composition of Dalmatians with urate urolithiasis. Ph.D. Thesis, University of Minnesota. 2003.
c. Leckharoensuk C, Lulich JP, Osborne CA, et al. Is canine pancreatitis associated with urolithiasis? In, *Proceedings.* UC Davis Urological Colloquium 2000, Davis California, May 4-6, 2000.
d. Modified Urolith Prevention canned diet (1130012 0436607), Hill's Pet Nutrition Inc, Topeka, Kan.
e. Science Diet canned Canine Maintenance (T2221025 6660), Hill's Pet Nutrition Inc, Topeka, Kan.
f. Ampicillin capsules, Warner Chilcott Labs, Morris Plains, NJ.
g. Synchron Clinical System CX-7 Delta, Beckman Instruments Inc, Brea, Calif.
h. Lanthanum, Sigma-Aldrich Com., St. Louis, Missouri
i. SpectrAA Systems, Varian Techtron PTY. Limited, Victoria, Australia.
k. Model 1090a High Pressure Liquid Chromatography, Hewlett-Packard, Avondale, Penn.
l. Dionex series 4500i, Dionex Corp, Sunnyvale, Calif.
m. Orion Model SA-720 pH/ISE Meter and Orion Model 95-12 Ammonia Electrode, Orion Research Inc., Boston, Massachsetts.
n. pH Meter 245, Corning Glass Works Science Products Division, Corning, New York. Model TS-66722 AX-1, Precision Scientific, Inc., Chicago, Illiniois.

p. Micron Separations, Inc., Wetboro Massachusetts.
q. Hypaque 50, Winthrop Laboratories, New York, New York.
r. Lewis C. Herring Co, Orlando, Florida.
s. Arc, Version 1.00, Cook, RD and Weisberg, S (1999), John Wiley & Sons Inc, New York.

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14. Brant R, 2003. Inference for Means: Comparing Two Independent Samples. Available at: http://www.health.ucalgary.ca/~rollin/stats/ssize/n2.html Accessed October 22.

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