26052

Urolithiasis 2000;
Editors: A.L. Rodgers, B.E. Hibbert, B. Hess, S.R. Khan and G.M. Preminger

Urine pH and urinary relative supersaturation in healthy adult cats

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INTRODUCTION

Conditions involving precipitation of minerals are amongst the numerically important identifiable causes of lower urinary tract diseases in cats. In two studies, urolithiasis was present in approximately 11% of obstructed cats and 15 to 30% of non-obstructed cats.[1] Struvite (magnesium ammonium phosphate) has been the mineral found most commonly in feline uroliths but recent data suggest that calcium oxalate is of increasing importance.[2,3]

Dietary manipulation has been the mainstay for the management and prevention of struvite in cats for some years, primarily because of the influence of dietary ingredients on urine pH. Urine pH is a much more important determinant of struvite formation than is the magnesium content of the diet [4,5,6], because changes in pH have a proportionally greater effect on changing struvite activity product than can be achieved by changes in the concentrations of one or more crystalloid components of struvite. Reduction of urinary pH through dietary manipulation is the most reliable means of creating urine that is undersaturated for struvite.

The effect of urine pH on the risk of calcium oxalate (CaOx) formation is more controversial. Consequently, this study was conduced to assess the effect of dietary acid load on urinary pH and relative supersaturation (RSS) of CaOx and struvite.

MATERIALS AND METHODS

Six cats were randomly allocated to three feeding groups and fed a test diet solus (C), or supplemented with ammonium chloride (NH_4Cl), a urinary acidifier, (200 mg / kg BWT / day) or sodium bicarbonate ($NaHCO_3$), a urinary alkaliniser, (640 mg / kg BWT / day) for two weeks in a Latin square design. The cats were housed individually in two roomed lodges throughout each phase of the trial and exposed to a natural light cycle.[7] Housing conditions and procedures were within the requirements of the Animals (Scientific Procedures) Act 1986. The cats were trained to urinate into angled litter trays from which urine drained rapidly into glass U-tubes for assessment of urine pH, as has been described previously.[8] For 48 hours during each test the U-tubes were substituted by enclosed glass, dry ice-chilled containers, to ensure rapid freezing of collected urine. At the end of the 48 hour period the urine was defrosted and homogenised after which urine volume and pH were measured. The samples were then acidified to pH 2 with a 37% solution of hydrochloric acid and re-frozen at –20°C whilst awaiting analysis.

The urine samples were subsequently defrosted at 4°C overnight and sonicated[a] at room temperature for 5 minutes at 50 hertz. Samples were then filtered using a 10 ml syringe fitted with a 0.2 µm filter[a] and thereafter diluted with deionised water tenfold for the determination of calcium, magnesium, oxalate, citrate and pyrophosphate, and 100 fold for the determination of potassium, sodium, ammonium, chloride, sulphate and phosphate. The cations were analysed using a Dionex DX120 ion-exchange high performance liquid chromatograph. [b,9] Diluted samples (25 µl) were injected automatically using a AS3500 autosampler on a Dionex CS12A column fitted with a CG12 A guard column using a Dionex chemical suppressor CSRS-11 to reduce background conductivity detection.[10] The maximum run time for these samples was ten minutes. Urine samples were analysed for anions using a Dionex ion-exchange Chromatograph series 4500i.[b] Diluted samples (25 µl) were injected automatically using a AS3500 autosampler on a Dionex AS11 column fitted with an AG11 guard column using a Dionex chemical suppressor ASRS to reduce background conductivity.[11] Components were eluted using a gradient of 1 ml/min of 10 mM to 80 mM sodium hydrochloride and identified using conductivity detection. The gradient was run over ten minutes

for the elution of chloride, sulphate and phosphate and over 22 minutes for the elution of oxalate, citrate and pyrophosphate. On both of the described systems helium was used for degassing the eluents and pressurising the reservoirs at an operating pressure not exceeding 0.56kg/cm^2. The concentrations of analytes determined by these procedures were entered into a microcomputer based program, Equil 2,[12] which calculates urinary relative supersaturations (activity product/solubility product) for struvite and calcium oxalate.

Urine pH, urinary concentrations of calcium and citrate, and urinary calcium oxalate and struvite relative supersaturations were summarised as mean values ± standard deviations (SD) for each group of cats. Results from the three diets were compared by analysis of variance statistical techniques. $P<0.05$ was considered significant.

RESULTS

The addition of NH_4Cl tended to reduce urine pH, and resulted in production of a urine with a significantly higher CaOx RSS and urinary calcium concentration compared to Diet C (**Table 1**). Addition of $NaHCO_3$ significantly increased urine pH and urinary citrate concentration, and resulted in a significant increase in struvite RSS. There was no effect on calcium concentration or CaOx RSS.

DISCUSSION

Acidification of urine has been recommended for the management and prevention of struvite urolithiasis in cats.[4] Manipulation of urine pH has a major effect on the activity product of struvite, primarily because pH influences the concentrations of the different ionic forms of phosphate (although it also influences the proportion of ammonia present as ammonium). The moderately acidic urine produced by cats fed Diet C resulted in a low struvite saturation, close to the solubility product. Increasing the dietary acid load did not significantly change struvite saturation. However, an increase in urine pH towards 7.0 (due to the addition of $NaHCO_3$) resulted in a significant increase in struvite saturation, and thus an increased risk of struvite formation.

Urinary acidification has been implicated as a risk factor for CaOx urolithiasis in epidemiological studies of cats.[13] One mechanism may be increased urinary calcium excretion as a consequence of increased dietary acid load. Increased urinary calcium excretion has been reported as a consequence of dietary supplementation with a urinary acidifier resulted in a urine pH less than 6.[14] When the cats in the study reported here were fed the supplemented diet resulting in a urine pH below 6, the urinary calcium concentration significantly increased, compared with the other two diets, resulting in a significantly higher CaOx saturation. No differences in urinary calcium concentration or CaOx saturation, were seen between the moderately acidic urine and when the urine pH was close to neutral. It is likely that effects of dietary acid load on urinary calcium excretion in cats may not become marked until the acid load is sufficient to result in a risk of metabolic acidosis.[14]

Urinary acidification may also alter the concentration of citrate. Citrate is thought to act as an inhibitor of CaOx crystallisation, primarily through complex formation, and the potency of this inhibitor may be increased by a more alkaline urine pH.[15] Urinary concentrations of citrate significantly increased when the cats received the $NaHCO_3$ diet. However, this did not result in a CaOx saturation that differed from Diet C.

Supersaturation of urine with calcium oxalate is a prerequisite for crystal formation, either by heterogeneous or homogeneous nucleation; crystals would not form in undersaturated urine. Diet C resulted in production of moderately acidic urine undersaturated with calcium oxalate. This diet could therefore, be fed with the expectation of preventing calcium oxalate formation. This level of acidification would also be appropriate for the prevention of struvite formation especially when compared with a urine of near neutral pH, as resulted from feeding the NaHCO3 supplemented diet. This study, therefore, indicates that it is possible to design one diet to aid in the control of both calcium oxalate and struvite urolithiasis.

Table 1: Mean urine pH, calcium oxalate and struvite relative supersaturations (RSS) and urinary concentrations of citrate and calcium produced by six cats fed a control diet (C) or the control diet plus NH_4Cl or $NaHCO_3$

Diet	Mean trial urine pH (±s.d.)	Calcium oxalate RSS (±s.d.)	Struvite RSS (±s.d.)	Urinary concentration (mmol/l±s.d.)	
				Citrate	Ca
C	6.18±0.26[a]	0.71±0.28[a]	1.61±1.11[a]	0.71±0.72[a]	0.35±0.19[a]
NH4Cl	5.81±0.14[a]	1.66±0.58[b]	1.16±0.25[a]	0.05±0.04[a]	0.62±0.29[b]
NaHCO3	6.81±0.33[b]	0.78±0.53[a]	7.98±4.62[b]	1.83±1.36[b]	0.35±0.15[a]

Different superscript letters within a column indicate significant differences

CONCLUSIONS

This study showed that, when compared to a diet designed to produce a moderately acidic urine pH within the range of 6.0-6.5:

1) the addition of a urinary acidifier, (resultant urine pH<6.0), increased urinary calcium concentration, indicating that overacidification of the urine may be a risk factor for CaOx formation,
2) the addition of a urinary alkaliniser (resultant urine pH around 6.8), did not reduce CaOx RSS, and resulted in production of a urine oversaturated with struvite.

[a] Ultrawave Limited, Cardiff, UK
[b] Dionex (UK) Ltd, Camberley, Surrey, UK

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