

The effect of urine acidification on calcium oxalate relative supersaturation in cats

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Abstract

There is an apparent reciprocal relationship between magnesium ammonium phosphate (MAP, struvite) and calcium oxalate (CaOx) urolithiasis incidence rate in cats. The number of struvite uroliths submitted for analysis over the past 35 years has been decreasing, with an increase in CaOx urolith submissions. Commercial diets aimed to dissolve struvite uroliths are typically acidified, and it has been suggested that dietary acidification increases urinary calcium excretion and the risk of CaOx crystallization. The objective of this study was to assess the effect of urine acidification on the relative supersaturation (RSS) of CaOx in cats, as a representation of crystallization risk. Four diets were extruded to contain identical nutrient contents, but with gradual acidification (0, 0.6, 1.3 and 1.9% sodium bisulphate substituted sodium chloride in diets A, B, C and D respectively). Thirteen adult cats were fed each diet sequentially for a minimum of 10 days. Average urine pH was 6.4, 6.2, 6.0 and 5.9 on diets A, B, C and D respectively ($p < 0.0001$). Struvite RSS decreased on diets inducing more acidic urine pH ($p < 0.0001$). Urinary calcium excretion and concentration increased with diets inducing lower urine pH ($p < 0.0001$), but oxalate excretion and concentration decreased ($p < 0.001$). CaOx RSS was not different between diets ($p = 0.63$). These results suggest that a lower diet base excess and resulting urine pH to support struvite dissolution do not increase the risk for CaOx crystallization in the range of urine pH representative of most commercial feline diets. Long-term studies are needed to confirm this.

KEYWORDS

calcium oxalate, crystallization risk, relative supersaturation, urine acidification, urolithiasis, veterinary nutrition

1 | INTRODUCTION

Over the past 35 years, there has been a shift in the mineral composition of uroliths diagnosed in cats. Reports from the 1980s describe the majority of uroliths being composed of magnesium ammonium phosphate (MAP, or struvite) (Cannon et al., 2007; Houston & Moore, 2009; Osborne et al., 2009). However, in the mid-1990s, MAP only represented a third of submitted uroliths, and the vast majority was

analyses as calcium oxalate (CaOx). The decrease in MAP uroliths seemed mirrored by the increase in CaOx, with uroliths of other mineral types remaining fairly consistent (Cannon et al., 2007; Houston & Moore, 2009; Osborne et al., 2009). The apparent reciprocal relationship between MAP and CaOx has been hypothesized to result from the increasing use of struvite calculolytic and preventative commercial diets. The acidifying properties of these diets have been hypothesized to increase urine calcium excretion, a risk factor for

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CaOx stones. Moreover, epidemiological studies identified acidifying diets as a risk factor for CaOx urolithiasis in cats (Kirk et al., 1995; Lekcharoensuk et al., 2001).

To assess the risk of urolith formation, the crystallization potential of a given salt in urine can be determined with relative supersaturation (RSS) (Robertson et al., 2002). RSS calculations are based on the measurement of the urinary pH and the concentrations of calcium, magnesium, sodium, potassium, ammonium, phosphate, oxalate, citrate, sulphate, uric acid and chloride. Commonly used software programs to determine RSS in dogs and cats, SUPERSAT and EQUIL2, calculate the activity products of various stone forming salts (Robertson et al., 2002). The ratio of the activity product to the thermodynamic solubility product of the salt yields an RSS value, which defines three levels of saturation for the salt in the urine: undersaturation, metastable supersaturation or labile supersaturation (J. Bartges, 2011; Robertson et al., 2002). Small prospective studies in cats report conflicting results on the relationship between urine pH and the risk of CaOx crystallization as assessed by RSS (J. W. Bartges et al., 2013; van Hoek et al., 2010). In one study in five cats, there was no effect of urine pH on CaOx RSS, but nutrient composition differed slightly between diets due to the use of different acidifying mineral source (Hoek et al., 2010). A longer-term study evaluated diets with similar nutrient composition but promoting acid, neutral or alkaline urine pH in three groups of four cats. CaOx RSS was significantly lower with the more alkalinizing diet, but struvite RSS was not different between diets (J. W. Bartges et al., 2013).

It has been hypothesized that urine acidification can increase the risk of CaOx by promoting calciuria, as a result of bone calcium and phosphate resorption in chronic metabolic acidosis, and by decreasing citruria (Buffington et al., 1990; S V Ching et al., 1989). In humans, urinary calcium excretion increases after an oral acid load. In cats, however, findings are inconsistent (Houillier et al., 1996). Calcium excretion is either increased (S V Ching et al., 1989) or unchanged (J. W. Bartges et al., 2013; Dow et al., 1990; Fettman et al., 1992) with varying degrees of acidification. In one study reporting urine calcium concentration rather than excretion, acidification of a controlled diet with magnesium chloride increased calciuria, but a different diet promoting the same acidified urine pH did not (Buffington et al., 1990).

The aim of this study was to prospectively assess the effect of urine pH on urine ion excretions and concentrations, as well as on MAP and CaOx RSS in a larger group of animals, using diets similar in nutrient composition. The hypothesis was that gradual dietary urinary acidification (pH < 6.5) would improve MAP RSS without affecting CaOx RSS in healthy cats.

2 | MATERIALS AND METHODS

2.1 | Animals

Thirteen adult cats (7 males, 6 females) aged 1.7 ± 0.2 years, from different breeds (6 Ragdolls, 3 Birmans, 2 British Shorthaired, 1 Maine Coon and 1 Domestic Shorthair), were included. The health status of

the animals was ascertained by daily observations by animal keepers and yearly veterinary check-ups including physical examination, blood work (complete blood count and serum biochemistry panels), urinalysis (including measurement of urine specific gravity (USG), urinary pH, and urine microscopy and bacterial culture) and diagnostic imaging (abdominal radiographs and ultrasound). Cats were housed in a group in a temperature-controlled facility with natural daylight during the adaptation phase to each diet, but individually during the urine collection phase. The urine collection phase had a duration of 72 hours and took place in lodges specifically designed for urine collection, which they had been acclimatized to prior to the study. During the urine collection phase, the animals were allowed to interact with each other under supervision for 1 hour per day.

2.2 | Diets

Four experimental dry extruded diets were used for this study. All 4 formulations were complete and balanced for adult cats, according to the NRC (National Research Council, 2006) recommended allowances and the 2014 FEDIAF (European Pet Food Industry Federation) and AAFCO (Association of American Feed Control Officials) nutrient profiles. They contained identical ingredients and nutrient content except for sulphur and chloride, as sodium bisulphate substituted sodium chloride for gradual acidification with levels of 0, 0.6, 1.3 and 1.9% as fed (diet A, B, C and D respectively). This altered the base excess of the diets with a predicted range of pH of 0.6 point between the least and the most acidifying diets (Kienzle et al., 1991). Analysed nutrient profiles of the 4 diets are reported in Table 1. The diets were analysed for dry matter (DM) and ash by drying to a constant weight at 103°C followed by combustion at 550°C. Crude protein (ISO, 2008), crude fat (ISO, 1999a), total dietary fiber (AOAC International, 1995), calcium, sodium, magnesium, potassium (ISO, 2000), chloride (ISO, 1999b) and phosphorus (adapted from ISO 6869, 2000) were determined. Starch was measured by enzymatic digestion (ISO, 2004). Nutrient analyses of the diets (including sodium and sulphate) were consistent with expected concentrations. One single batch of each diet was used for the entire trial.

2.3 | Urine collections

The 4 diets were fed sequentially, and each diet was fed to weight maintenance, for 7–9 days of adaptation, followed by a 3–5 days of individual urine collection. Quantities of food offered and refused were weighed and recorded daily during the urine collection phase. Drinking water was available ad libitum. All urine was collected by natural voiding into a clean Erlenmeyer flask. These flasks were checked several times per day. Urine was discarded—but its weight recorded—if visibly contaminated with food or faeces.

The protocol has obtained ethical approval from the internal Royal Canin ethics committee. Housing and urine collection method were in agreement with the Mars Welfare Standards for Cat and Dog

TABLE 1 Ingredient composition and analysed nutrient profiles of the four experimental diets

	Diet A 0% NaHSO ₄	Diet B 0.58% NaHSO ₄	Diet C 1.25% NaHSO ₄	Diet D 1.92% NaHSO ₄
Moisture	5.6	5.2	5.5	5.7
Crude protein	91.1	89.4	85.6	82.9
Crude fat	36.2	36.1	35.8	36.8
Total dietary fibre	29.0	26.5	27.1	24.4
Ash	17.5	16.9	17.4	17.4
Calcium	2.08	2.58	2.30	2.65
Phosphorus	2.49	2.50	2.50	2.47
Magnesium	0.23	0.23	0.23	0.20
Potassium	1.80	1.77	1.81	1.64
Sodium	1.72	1.69	1.76	1.71
Chloride	2.39	1.99	1.66	1.21
Oxalate	391	387	426	372
Metabolizable energy*	3895	3960	3915	3968

Note: All nutrient contents are expressed in grams per 1000 kcal, except for moisture, which is expressed in %, oxalate which is expressed in ppm, and metabolizable energy, expressed in kcal/kg as fed. Crude protein was slightly higher in diets A and B (least acidic) compared to diets C and D (most acidic). Chloride decreased with increasing acidity, in line with the replacement of sodium chloride with sodium sulphate. Ingredient composition by order of weight: cereals, meat products, oil and fat, vegetable protein extract, vegetable by-products, minerals and vitamins, yeast.

*Calculated with cat predictive equations from NRC 2006 using total dietary fibre.

Facilities and received approval of the French Government (reference 1595.01).

2.4 | Urinalysis

The urine weight, specific gravity (refractometer Anton Paar DMA 35, Graz, Austria) and pH (calibrated pH meter Mettler Toledo SevenEasy, Port Melbourne, Australia) of each urine sample and the final urine pool were recorded for each animal with each diet. Bacterial contamination was assumed if duplicate pH measurements at two-hour time intervals showed over 0.2 units variation and that urine was not kept in the final pool. Urine volume was calculated by dividing its weight by its specific gravity. For each animal, urine samples collected were pooled into a bottle containing 1 mL of 20% chlorhexidine (Hibitane; Mölnlycke Health Care, Gothenburg, Sweden), and urine was stored at 4°C. An aliquot from the urine pool was titrated to pH 2.0 with 37% hydrochloric acid, to dissolve all salts before ion concentrations were determined. Samples were kept at -20°C until batch analysis.

2.5 | RSS determination

Urinary calcium, magnesium, sodium, ammonium, phosphate, citrate, sulphate, oxalate and urate concentrations were measured in each pool by ionic chromatography (Dionex, Port Melbourne, Australia) as described by others. The variability of this method

was determined to be less than 5% for the mineral ions and uric acid and 10% for oxalate and citrate. Daily urinary excretion of anions and cations ($\mu\text{mol}/\text{kg BW}$ per 24 h) was calculated as (anion or cation concentration in the urine ($\mu\text{mol}/\text{ml}$) \times total urinary volume ($\text{ml}/24 \text{ h}$))/BW (kg). Using the pH and the urine concentrations mentioned above, CaOx and MAP RSS were calculated using the computer software SUPERSAT.

2.6 | Statistical analyses

Group size was determined in G*power freeware. An estimated difference in urine pH of 0.6 with a standard deviation of 0.5, power of 0.80 and P of 0.05 divided by 6 comparisons between the 4 diets ($p < 0.008$) were used as inputs. This gave a group size of 13 cats.

All data were analysed using commercially available statistical software (SAS). The data were assessed for normality using visual plotting of data. The RSS values for MAP were not normally distributed, and values were log-transformed for inclusion in the models. Mixed effects models were used to assess the effect of diet (fixed effect in 4 levels) on food intake, weight, urine volume, urinary mineral excretion and concentration, and CaOx and MAP RSS using cat as a random term. The absence of heteroscedasticity and the normal distribution of residuals were checked in order to verify that assumptions of one-way repeated measures model were met. Data are expressed as LSmeans \pm SE (standard error) except for MAP RSS which is presented as median [25th, 75th percentile]. Significance level was set at $p < 0.05$.

3 | RESULTS

3.1 | Food intake and body weight maintenance

There was no difference in food consumption by the cats between the 4 diets. Body weight was maintained throughout the trial. One cat had to be excluded from the analysis of Diet A due to soft faeces.

3.2 | Diet profiles

Diet nutrient analyses (Table 1) were consistent with predicted levels and within analytical tolerance for each nutrient, except for crude protein which was 6.5% lower in diets C and D when compared to A and B, and calcium which was 22% lower in diet A compared to diets B and D.

3.3 | Urinary minerals, pH and RSS

All results are presented in Table 2. Diets A, B, C and D induced urine pH of (LSmeans±SE) 6.37 ± 0.03 , 6.23 ± 0.03 , 6.02 ± 0.03 and 5.93 ± 0.03 , respectively, with a significant difference between diets ($p < 0.0001$). Urinary volumes (mL/kg/day) produced during the collection period did not differ between diets ($p = 0.45$).

Urinary calcium, ammonium, oxalate, citrate, sulphate, and uric acid excretions were affected by diet, whereas urinary magnesium, sodium, potassium, phosphate and creatinine excretions did not differ. Excretions of calcium, ammonium and sulphate were higher, and oxalate and citrate excretions were lower in the diets inducing a lower pH ($p < 0.0001$).

Urinary concentrations of all ions but uric acid differed significantly between diets. Urinary calcium concentration was significantly higher, and urinary oxalate concentration significantly lower,

Sodium bisulphate (%)	Diet A	Diet B	Diet C	Diet D	SE	P value
	0	0.58	1.25	1.92		
Urine pH	6.37 [†]	6.23 [‡]	6.02 [§]	5.93 [§]	0.034	<0.0001
CaOx RSS	3.17	3.35	3.37	3.38	0.25	0.63
MAP RSS	0.91 [†]	0.72 [†]	0.29 [‡]	0.25 [§]	n/a	<0.0001
Urine volume (mL/kg/d)	12.5	12.4	11.9	11.4	0.8	0.45
Urine specific gravity	1.066 [†]	1.068 [†]	1.072 [‡]	1.076 [§]	0.002	<0.0001
Urinary concentrations:						
Calcium (mmol/L)	0.48 [†]	0.60 [†]	0.72 [‡]	0.89 [§]	0.05	<0.0001
Magnesium (mmol/L)	3.96 [†]	4.65 ^{†‡}	4.55 ^{†‡}	5.03 [‡]	0.43	0.017
Sodium (mmol/L)	304 [†]	320 ^{†‡}	333 ^{‡§}	353 [§]	11.5	<0.0001
Potassium (mmol/L)	153 [†]	164 ^{†‡}	166 [‡]	167 [‡]	4.7	0.0034
Ammonium (mmol/L)	172 [†]	193 [‡]	219 [§]	261 [¶]	6.8	<0.0001
Phosphate (mmol/L)	91 [†]	100 [‡]	102 [‡]	106 [‡]	2.7	<0.0001
Sulphate (mmol/L)	76 [†]	120 [‡]	168 [§]	230 [¶]	5.2	<0.0001
Oxalate (mmol/L)	2.50 [†]	2.49 [†]	2.21 [‡]	2.07 [‡]	0.08	<0.0001
Citrate (mmol/L)	1.00 [†]	0.98 [†]	0.81 ^{†‡}	0.62 [‡]	0.18	0.0002
Urate (mmol/L)	1.04	1.11	1.07	1.01	0.14	0.26
Urinary excretions						
Calcium (µmol/kg/24 h)	6.12 [†]	7.46 ^{†‡}	8.70 ^{‡§}	10.09 [§]	0.83	<0.0001
Oxalate (µmol/kg/24 h)	31.0 [†]	31.2 [†]	26.3 ^{†‡}	23.3 [‡]	2.0	0.0003
Citrate (µmol/kg/24 h)	11.9 [†]	11.6 [†]	9.2 ^{†‡}	6.7 [‡]	2.0	0.0008

TABLE 2 Urine composition in cats (n = 13) fed the four diets differing in base excess in a sequential order

Note: Data are presented as least square means except for MAP RSS for which medians are indicated. Two different symbols within a row indicate significant difference (Scheffe test, $p < 0.05$).

Abbreviation: SE, Standard Error.

in the most acidic diets (diets C and D) compared to diets A and B. Urinary citrate concentration was significantly lower with the most acidic diet (D) compared to diets A and B.

Struvite RSS decreased with acidification (Diet A: 0.91[0.60, 1.20], Diet B: 0.72[0.54, 0.83], Diet C: 0.29[0.24, 0.47], Diet D: 0.25[0.16, 0.39]) ($p < 0.001$). Calcium oxalate RSS values were not significantly different from one another (Diet A: 3.17 ± 0.25 ; Diet B: 3.35 ± 0.25 ; Diet C: 3.37 ± 0.25 ; Diet D: 3.38 ± 0.25) ($p = 0.63$). Figure 1 depicts the CaOx RSS in relation to urinary pH on each diet in individual cats.

4 | DISCUSSION

The findings in this study provide evidence that diet base excess and urinary pH affect MAP RSS, but not CaOx RSS. Acidification of the diet caused a change in the excretion of several minerals that could be involved in stone formation. Urinary calcium excretion and concentration increased with the most acidic diets, but urinary oxalate concentration decreased, which could be a potential explanation for the lack of an effect of acidification on CaOx RSS. The diets tested in the current study were considered to be representative of commercially available dry diets and induced a range of urine pH that was therefore deemed to be clinically relevant. Previous studies that have been performed on this topic included either a small number of animals, or reported on diets yielding urinary pH irrelevant for most current commercial dry feline diets (i.e. >7.0). The current study

had a relatively small group size, but a strength is the study design in which each animal was fed all diets, which limits interindividual variation.

The findings in this study differ from a previous report in cats fed 3 diets inducing different urine pH for 12 months (J. W. Bartges et al., 2013). Relative supersaturation for CaOx was reported to increase with urine acidification. Although longer in duration, that study was done in small groups of 4 cats, fed in a parallel design, thereby making the results more sensitive to interindividual variation. Baseline values would also have been valuable to be able to interpret the results and wide standard deviations (Bartges et al., 2013). Previously published data on individual urines obtained with 341 dry feline diets showed no correlation of urine pH with CaOx RSS (Tournier et al., 2006), similar to the findings reported here. This was, however, not a controlled or prospective study and included a large number of differently formulated diets. Therefore, the current study is the first of its kind to compare the effect of dietary—and therefore urinary—acidification on CaOx RSS in a more robust fashion.

This study assesses the short-term effect of acidification of diet on CaOx RSS. The urinary excretion of minerals involved in the RSS calculation responds rapidly to dietary changes, but some—especially calcium—have been hypothesized to be altered over time secondary to bone turnover. To date, studies assessing bone turnover have, however, not confirmed a change in calcium excretion after 12 months due to the consumption of an acidified diet (Bartges et al., 2013; Fettman et al., 1992).

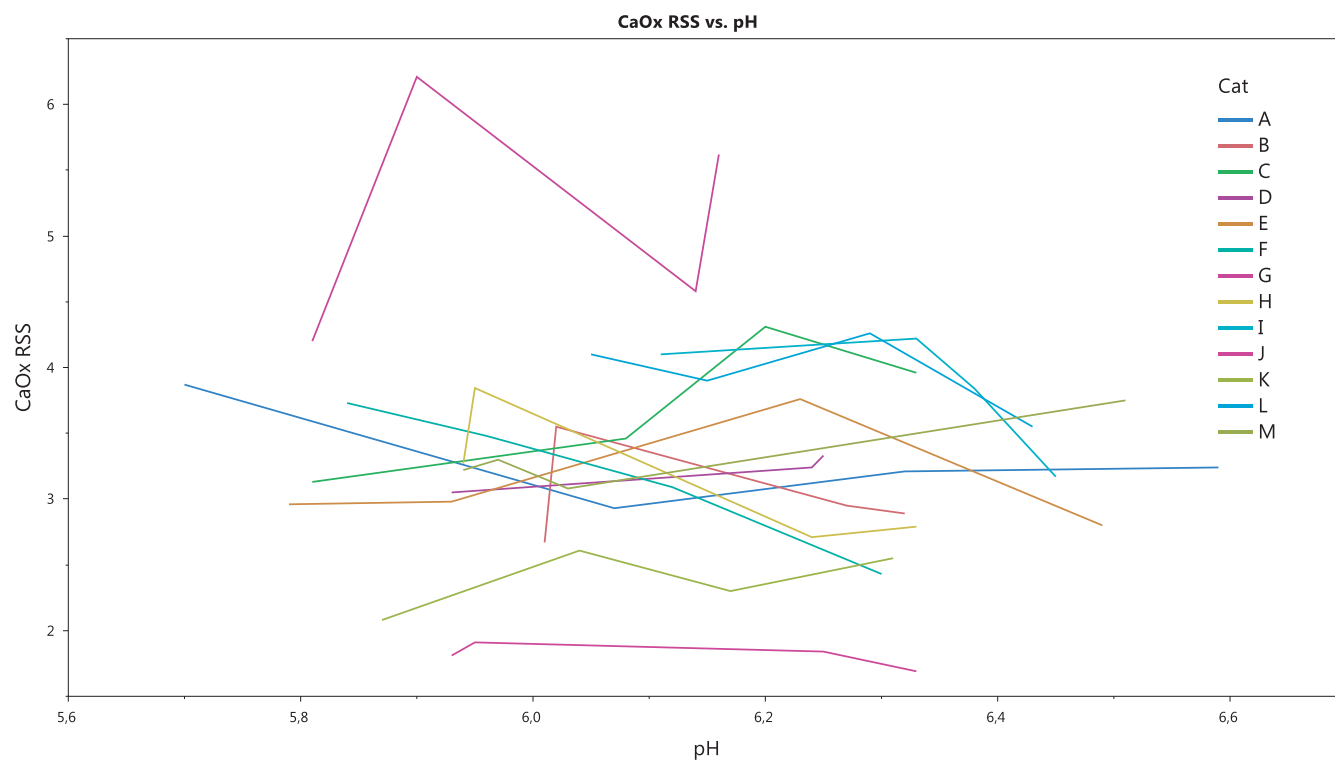


FIGURE 1 Urine pH versus calcium oxalate relative supersaturation (CaOx RSS). Each cat is represented by a different colour. A marked interindividual variation is evident, whereas intra-individual variation is less pronounced [Colour figure can be viewed at wileyonlinelibrary.com]

As urinary pH induced by the diet decreased, urinary calcium excretion and concentration increased, as previously described in humans (Dow et al., 1990). In humans, an increase in urinary calcium concentration is considered a risk factor for the development of calcium oxalate uroliths. The same has been stated for dogs (Stevenson et al., 2004) and hypercalcaemic cats (Osborne et al., 1996). A feline study comparing a purified diet with added magnesium and a commercial diet found a significant difference between urinary calcium despite the diets inducing a similar urine pH (Buffington et al., 1990). This illustrates to the limit of comparing studies, as macro- and micronutrient dietary profiles can influence calcium absorption and excretion. It also indicates that the risk of CaOx urolithiasis is not linear, nor monofactorial, and is influenced by a complex interplay between other urine components aside from pH. Other anions that influence CaOx RSS value are oxalate and citrate (Robertson et al., 2002). By its ability to chelate calcium, urinary citrate can affect calcium oxalate crystallization. Low urinary citrate has been associated with an increased risk in calcium oxalate urolithiasis (Zuckerman & Assimos, 2009). In the current study, a lower urinary pH was associated with a lower urinary citrate concentration. The association between a higher urinary calcium concentration and excretion, lower urinary citrate concentration and CaOx risk was, however, not found in this study, as CaOx RSS did not significantly differ between diets. Alkalinization may have been insufficient to see an effect on urinary citrate. This could also be explained by the decrease in urinary oxalate concentration that was seen with lower urine pH, thus compensating an increase urinary calcium in its effect on RSS. The decrease in urinary oxalate concentration with diet inducing lower urinary pH cannot completely be explained. In a study comparing diets with different macronutrient profiles and inducing different urine pH, urinary oxalate excretion was unaffected (Dijcker et al., 2012). A follow-up study identified multiple factors contribute to variation of feline urinary oxalate excretion, but a considerable part of urinary oxalate excretion remained unexplained (Dijcker, Hagen-Plantinga, Everts, et al., 2014). The observations in the current study might therefore be due to endogenous oxalate metabolism rather than a direct effect of diet acidifying properties.

Calcium concentrations of the four diets varied up to 26% from diet A to diet D, and resulting calcium to phosphorus ratios also varied from 0.84 to 1.08, which may have confounded the results on urinary calcium excretion. However, as the four diets were manufactured based on the same formula to minimize variation apart from sodium bisulphate and sodium chloride, the observed dietary calcium differences (and Ca:P ratios) were likely a result of laboratory analytical variability rather than a true difference. Measured ash was identical across diets, and the lowest calcium concentration was obtained for diet A, which also had the highest protein concentration. Since all calcium was provided from meat products, the highest protein diet should also have been the highest in calcium. This highlights the need for safety margins when formulating diets for long-term feeding, in order to ensure a Ca:P ratio >1.0 as recommended by the NRC or FEDIAF. Even if those

small differences in dietary calcium were indeed real, they would have been unlikely to affect urinary calcium concentrations and excretions as observed in this study. Indeed, prior studies in cats suggest that a variation in dietary Ca does not result in urinary Ca excretion changes, but rather impacts fecal Ca excretion, whether the Ca:P ratio is maintained (Paßlack et al., 2016), increased (Pastoor et al., 1994) or decreased (Pastoor et al., 1995). Moreover, the increase in urinary calcium excretion was linear from diet A to diet D, following the pattern of dietary acidification and not that of dietary Ca content or Ca:P ratios. A lower urine pH is associated with an increased calcium concentration in humans (Lemann, 1999) due to a direct effect of pH on renal tubular calcium reabsorption (Blaine et al., 2015).

Crude protein was slightly higher in diets A and B (least acidifying) vs diets C and D (most acidifying). In humans, an increase in dietary animal protein is associated with an increase in risk of CaOx stone formation, which is partly explained by acidosis followed by increased urinary calcium excretion (Zemel et al., 1981). It should be noted that although sulphur amino acids from animal protein sources can have an acidifying effect, those are not the sole determinants of the acidifying properties of commercial feline diets. They are also supplemented with other acidifying or alkalinizing mineral sources. In the current study, the variation of sulphur amino acids methionine and cysteine resulting from the variation in crude protein content was simulated (results not shown) and would have been responsible of a negligible change in diet base excess when compared to the addition of sodium bisulphate (which had an effect 10 times greater). Conflicting reports have been published on the effect of dietary protein on CaOx risk in cats. One study found that a higher content decreased the risk of CaOx urolith formation in cats (Lekcharoensuk et al., 2001). A study assessing urinary oxalate excretion did not find a difference between a high protein, a high fat or a high carbohydrate diet. However, plasma oxalate concentration was the lowest when fed the high protein diet (Dijcker et al., 2012). Additionally, protein quantity and quality is known to affect urinary oxalate excretion (Zentek & Schultz, 2004). Hydroxyproline, an amino acid found in high concentration in the collagen, is a precursor of oxalate (Dijcker, Hagen-Plantinga, Thomas, et al., 2014), but was not measured in the diets used for this study. The protein level differences between the diets of this study might thus explain in part the lowest urinary oxalate concentration.

RSS calculation is based on urinary ion concentrations; hence, it is affected by urine dilution. Concentrating the urine increases RSS and is a risk factor for the development of CaOx stones (Robertson et al., 2002). Previous studies have shown that USG is an important determinant for CaOx urolithiasis risk, with increased water intake decreasing USG and CaOx RSS (Buckley et al., 2011). Despite a statistically significant difference in USG, the greatest difference in USG was 13% (between diets A and D) which was less than the difference observed in urine calcium concentration for those diets (46% lower for diet A). The difference in USG is therefore unlikely to be a confounding factor in the assessment of calciuria and RSS in this study.

This study has a number of limitations. All cats were healthy, with no prior history of CaOx formation. They were also young, and it is known that an older age is associated with an increased risk to develop CaOx uroliths (Kirk et al., 1995). Studies need to be performed in healthy subjects before investigating CaOx forming cats, allowing a better understanding of the influences of different factors on the urine composition of normal subjects, such as diet. The duration of the study (10 to 14 days per diet) may also be considered short to see potential compensatory mechanisms of acidification, especially as it relates to calcium and phosphorus metabolism. Changes in urinary calcium and other ions were seen after 7 days of feeding, similar to studies performed in other species. Humans show increases in urinary calcium concentrations within 5 days of acidification of diet (Martin & Jones, 1961), and a study in sheep showed an increase in renal calcium excretion within 60 minutes after an intravenous acid-load (Stacy & Wilson, 1970). To date, longer-term studies have shown no deleterious effects of chronic acidification on bone calcium turnover in adult cats (J. W. Bartges et al., 2013; Shelley V. Ching et al., 1990; Fettman et al., 1992).

Another limitation is the range of urine pH evaluated (5.93–6.37), which may have been too narrow to see an effect on CaOx RSS. Most dry commercial feline diets induce a mean urine pH <6.5 to limit the risk of struvite, which makes the range tested here relevant. Finally, RSS was chosen in this study as a risk index for CaOx crystallization. Other risk indices have been used in veterinary research but lack extensive validation to date (Queau, 2019). One limitation of RSS for predicting the risk of CaOx is that it does not take into account organic promoters and inhibitors of stone formation, some of which may be influenced by urine pH, such as Tamm Horsfall protein, an inhibitor of CaOx crystallization (Hess, 1994). However, the relative importance of those organic molecules versus the mineral precursors of CaOx is unknown.

The results of this study indicate that CaOx RSS, and therefore the risk of CaOx crystallization, does not increase with a decrease in urine pH when feeding a diet similar to commercially available feline diets. This supports the prescription of slightly acidifying diets that induce low MAP and CaOx RSS, to ensure both types of uroliths are prevented when eating the diet. However, further studies are needed to confirm this finding in cats already prone to forming CaOx uroliths and during long-term feeding.

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ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

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