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# Increasing dietary sodium chloride promotes urine dilution and decreases struvite and calcium oxalate relative supersaturation in healthy dogs and cats

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#### Abstract

Urolithiasis is highly prevalent in dogs and cats, with struvite and calcium oxalate being most commonly diagnosed. Some commercial diets aimed at reducing the risk of urolithiasis are based on inclusion of sodium chloride (NaCl) in an attempt to dilute the urine and the risk of crystallization, but more information on the effect of differing levels of sodium inclusion is needed. The objective of this study was to compare the short-term effect of four diets differing only in NaCl content (base diet with 0.3% sodium and diets with added NaCl to achieve 0.7, 1.0 and 1.3% sodium as fed) on urinary ion concentrations and relative supersaturation (RSS) of struvite and calcium oxalate in dogs and cats. In both species, there was a significant increase in water intake and urine volume as dietary NaCl increased. Urine sodium concentration increased with increasing dietary NaCI. The highest sodium diet increased urinary calcium excretion in dogs only, while decreasing urinary calcium concentration. Calcium oxalate RSS and struvite RSS both significantly decreased, with the lowest RSS values reported on the highest sodium diet in both dogs and cats (p < .001). These results suggest that an increase in dietary NaCl decreases RSS values in both dogs and cats. Despite an increase in urinary calcium excretion in dogs, urinary calcium concentration and calcium oxalate RSS were lower on high sodium diets due to urine dilution. Long-term studies are needed to confirm the relationship between RSS and stone occurrence and recurrence.

#### KEYWORDS

calcium oxalate, crystallization risk, relative supersaturation, sodium chloride (salt), urolithiasis, veterinary nutrition

# 1 | INTRODUCTION

Urolithiasis can develop in various parts of the urinary tract of dogs and cats and lead to significant morbidity and mortality. It accounts for 18% and 7%-22% of cases of lower urinary tract disease in dogs (Lulich, Osborne, Bartges, & Lekcharoensuk, 2000)

and cats (Dorsch, Remer, Sauter-Louis, & Hartmann, 2014; Gerber et al., 2005; Lekcharoensuk, Osborne, & Lulich, 2001; Saevik, Trangerud, Ottesen, Sorum, & Eggertsdottir, 2011) respectively. It can also cause acute or chronic kidney disease when located in the upper urinary tract. In both species, the two most common types of urinary stones are struvite (magnesium ammonium phosphate)

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and calcium oxalate (Osborne, Lulich, Kruger, Ulrich, & Koehler, 2009).

Causes of urolithiasis are multifactorial, including genetic predisposition, acquired or congenital alterations in metabolism, environmental factors and diet (Houston, Moore, Elliott, & Biourge, 2011). The degree of supersaturation of urine with lithogenic substances determines the potential of a crystal to dissolve, form or grow in size. It is best estimated by the relative supersaturation (RSS) value, which takes into account the urine concentration of various ions and urine pH (Robertson, Jones, Heaton, Stevenson, & Markwell, 2002). Limiting the amount of stone precursors excreted in the urine, and promoting urine dilution to reduce their concentration is therefore recommended to reduce the risk of crystallization. In addition, larger urine volumes increase the frequency of micturition and reduce the residency time of crystals in the bladder.

In order to promote urine dilution, dietary strategies have focused on increased moisture or salt to drive water intake. Although a high sodium intake is currently not recommended in humans with CaOx kidney stones because of the potential increase in renal calcium excretion (Frassetto & Kohlstadt, 2011), effects of sodium chloride supplementation have been studied in companion animals with variable outcomes. In studies performed in dogs, lower CaOx RSS has been reported with increases in dietary NaCl (Lulich, Osborne, & Sanderson, 2005; Stevenson, Hynds, & Markwell, 2003), but these studies were conflicting with regards to the urine volume and urine specific gravity (USG), with one reporting an increase in volume and a decrease in USG (Lulich et al., 2005), and another one reporting no change in either (Stevenson et al., 2003).

In a retrospective study analysing 13 different diets fed to healthy adult cats, a higher dietary sodium led to significantly greater water intake and urine volume, and lower USG and CaOx RSS (Hawthorne & Markwell, 2004). Short-term prospective studies have also reported this increase in water intake and/or urinary volume when cats were fed diets supplemented with NaCl (Passlack, Burmeier, Brenten, Neumann, & Zentek, 2014; Tournier et al., 2006; Xu, Laflamme, & Bartges, 2006), but these studies report conflicting results on CaOx RSS.

The studies described based on their conclusions on a great variety of dietary sodium contents, and in some studies, the diets differ in other nutrients in addition to NaCl. This makes extrapolation of these results to a dose-response relationship between dietary NaCl, urine dilution and MAP and CaOx RSS challenging. Based on these inconsistent results in dogs and cats and the various levels of sodium tested, the objectives of this study were to determine the dose-response relationship between dietary NaCl and water intake, urine volume, urinary mineral concentrations and MAP and CaOx RSS in healthy dogs and cats in a short-term, prospective trial. The hypothesis was that increasing dietary sodium would promote higher water intake and urine volume, and lower RSS for both MAP and CaOx.

# 2 | MATERIALS AND METHODS

# 2.1 | Animals

Thirteen healthy adult cats and eight healthy adult dogs were included in the study. The health status of the animals was ascertained Animal Physiology and Animal Nutrition

by daily observations by animal keepers and yearly veterinary checkups including physical examination, blood work (complete blood count and serum biochemistry panels), urinalysis and diagnostic imaging (abdominal radiographs and ultrasound). The breeds represented in the cat population were Ragdoll (n = 6), European Shorthair (n = 6) and Birman (n = 1). All dogs (n = 8) were Miniature Schnauzers. At the beginning of the study, the cats were (mean  $\pm$  SD) 4.1  $\pm$  0.5 years old and weighed 4.79  $\pm$  1.20 kg and the dogs were 1.5  $\pm$  0.2 years old and weighed 5.58 ± 0.93 kg. All animals were in ideal body condition (body condition score, BCS, of 5/9) (Laflamme, 1997a,b), except for one cat that was overweight (BCS 7/9) and maintained its weight throughout the study. There were seven female and six male cats, and only female dogs, all neutered. Cats and dogs were maintained in a temperature-controlled facility, with natural daylight. They were housed collectively during the adaptation phase (one panel of seven cats, one panel of six cats and one panel of eight dogs), and individually during the 72 hr of urine collection, in lodges to which they had been acclimated prior via a habituation programme. The protocol has obtained ethical approval from the internal Royal Canin ethics committee, and housing and urine collection method were in agreement with the Mars Welfare Standards for Cat and Dog Facilities and received approval of the French Government (reference 1595.01).

## 3 | STUDY DESIGN AND PROCEDURES

## 3.1 | Diets

Four experimental dry extruded diets were used for this study. All diets carried code names, and the animal and laboratory technicians were blinded as to which diet was which. Diets were formulated to be nutritionally complete and balanced for adult cats and dogs, according to the NRC recommended allowances (National Research Council. Nutrient requirements of dogs & cats, 2006) and the 2014 FEDIAF (European Pet Food Industry Federation) and AAFCO (Association of American Feed Control Officials) nutrient profiles. The base diet (diet A, target sodium 0.3% as fed) was supplemented with NaCl to achieve 0.7%, 1% and 1.4% sodium as fed in diets B, C and D respectively, all with similar base excess (Kienzle, Schuknecht, & Meyer, 1991). Taking into account the sodium content of ingredients, the predicted sodium levels of diets A, B, C and D were 0.7, 1.7, 2.4 and 3.4 g/1,000 kcal respectively. Ingredient and nutrient profiles of the four 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 and combustion at 550°C, respectively. Crude protein (International Organization for Standardization, ISO, 2008), crude fat (ISO, 1999), total dietary fibre (AOAC, 1995), Ca, Na, Mg and K (ISO, 2000), CI (ISO, 1999) and P (adapted from ISO 6869) were determined. Starch was measured by enzymatic digestion (ISO, 2004). Nutrient analyses of the diets were within analytical variability when compared to expected levels. Slight variation existed between the protein contents of the diets when analysed on a kcal basis due to a difference in energy density. One single batch of each diet was used for the entire trial in dogs and cats.

**TABLE 1**Ingredient composition and analysed nutrient profilesof the four experimental diets

	Diet A	Diet B	Diet C	Diet D
Moisture	5.7	5.2	4.7	5.4
Crude protein	86.5	90.1	94.4	89.1
Crude fat	35.0	35.5	35.6	34.5
Total dietary fibre	15.1	19.4	26.5	16.2
Ash	13.9	15.7	17.1	20.1
Starch	66.6	61.1	59.2	66.8
Calcium	1.85	1.91	1.98	1.89
Phosphorus	1.64	1.63	1.68	1.62
Magnesium	0.16	0.17	0.16	0.17
Potassium	2.45	2.41	2.18	2.55
Sodium	0.67	1.68	2.41	3.27
Chloride	2.22	3.58	4.39	6.05
Metabolizable energy <sup>a</sup>	4,187	4,111	3,984	4,072

*Note*: All nutrient contents are expressed in grams per 1,000 kcal, except for moisture, which is expressed in %, and metabolizable energy, expressed in kcal/kg as fed.

Ingredient composition by order of weight: brewers rice, wheat gluten, poultry meal, corn flour, animal fat, corn gluten, hydrolysed animal proteins, minerals and vitamins, vegetable fibres, beet pulp, fish oil, soya oil, fructo-oligosaccharides.

<sup>a</sup>Calculated with the cat predictive equations from NRC 2006 using total dietary fibre.

## 3.2 | RSS testing

Dogs and cats were fed each diet for 7 days of adaptation, followed by 3 days of urine collection. Quantities fed were based on the individual's energy requirements in order to maintain body weight. Drinking water was offered ad libitum. Quantities offered and refused were weighed and recorded daily during the urine collection phase. All urine produced by natural voiding during the collection period was collected into a clean Erlenmeyer flask. These flasks were checked several times per day to ensure urine was pooled as quickly as possible after confirmation of absence of visual or bacterial contamination. Bacterial contamination was assumed if duplicate pH measurements at two hour time intervals showed over 0.2 units variation. For each animal, urine samples collected over 3 days were pooled into a bottle containing 1 ml of 20% chlorhexidine (Hibitane; Mölnlycke Health Care), stored at 4°C.

#### 3.3 | Urine analysis

The urine weight, density (refractometer Anton Paar DMA 35) and pH (calibrated pH meter Mettler Toledo SevenEasy) of each urine sample and the final urine pool were recorded for each animal with each diet. Urine volume was calculated by dividing its weight by its density. An aliquot was taken from the urine pool, titrated to pH 2.0 with 37% hydrochloric acid in order to dissolve all salts before ion concentrations were determined. The samples were then either analysed or kept at  $-20^{\circ}$ C until analysis. The concentrations of calcium, phosphate, magnesium, sodium, potassium, ammonium, oxalate, citrate, sulphate and uric acid in the urine pool were measured by ionic chromatography (Dionex) as described by others (Markwell, Smith, & McCarthy, 1999). This method was determined to have a variability of less than 5% for the mineral ions and of 10% for the organic ions (oxalate and citrate). The computer software Supersat was then used to calculate MAP and RSS CaOx from the pH and urine concentrations of the ions mentioned above (Robertson et al., 2002). Urine excretion of calcium was calculated as follows: (urine calcium concentration)  $\times$  (3-day urine volume/3)/ (body weight).

### 3.4 | Statistical analyses

Power calculations were performed in G\*Power freeware and based on historic RSS results assuming 80% power and two-sided significance using p < .05 divided by the eventual number of comparisons (six comparisons between four diets creates a p < .008). Historic average (measured between 2004 and 2014) of CaOx RSS mean  $\pm$  *SD* on low sodium diets (~0.3% sodium) was approximately three times as high as CaOx RSS on high sodium diets (~1.3% sodium). This determined a group size of a minimum of eight animals. Because of animal availability, it was decided to use a total of 13 cats and eight dogs.

SAS software was used for the remainder of the statistical analyses. A linear mixed model was used to assess the effect of diet (fixed effect in four levels) including cat or dog as a random term as each animal was its own control. According to residual distribution of each model, data were ranked or not. The difference between two levels of fixed effects was assessed by the Scheffe test (adjustment for multiple comparisons).

Data are expressed as Least Square Means  $\pm$  SE as residuals of the model were normally distributed, except for MAP RSS in both species for which medians are reported. Significance level was set at p < .05.

## 4 | RESULTS

All animals remained healthy over the duration of the study. Caloric intakes were similar among diets (p = .83 in cats, p = .39 in dogs). In both species, there was a significant increase in water intake and urine volumes, and significant lower USG as dietary NaCl increased. The highest water intakes and urine volumes, and lowest USG were obtained with diet D, the diet with the highest NaCl content, while values were intermediate for diets B and C compared with the lowest NaCl diet. Urine pH was significantly lower with diet D in both species.

Urine concentrations of all ions except sodium decreased significantly with increasing levels of dietary NaCl, in both cats and dogs. Urinary sodium concentrations increased with dietary sodium. There was a difference between species with regards to urinary calcium excretion: there was no effect of dietary NaCl in cats, but excretion increased significantly as dietary NaCl increased in dogs. Of note is that in both species, urinary calcium concentration did decrease with an increase in dietary NaCl, like all other minerals.

Overall, RSS decreased with increasing dietary NaCl. In cats, CaOx RSS was the lowest with diet D, and intermediate with diets B and C. In dogs, CaOx RSS was lower with both diets C and D compared with the other two diets. MAP RSS was the lowest with diet D for both species. All data are presented in Tables 2 and 3. Interindividual variation was present for both CaOx and MAP RSS in both dogs and cats. An overview of this can be found in Figures S1–S4.

# 5 | DISCUSSION

This study shows that increasing dietary sodium gradually from 0.67 to 3.27 g/1,000 kcal via the inclusion of salt in a dry diet significantly increased water intake and urine volume, and thereby decreased USG in both cats and dogs. Those findings are in agreement with previous studies in cats (Hawthorne & Markwell, 2004; Passlack et al., 2014) with sodium >2.8 g/1,000 kcal; and in dogs (Lulich et al., 2005; Stevenson et al., 2003) with sodium >3.0 g/1,000 kcal. With the highest sodium diet, urine volume

nearly doubled in both species compared with the lowest sodium diet. As a consequence, the urinary concentrations of all ions but sodium were significantly lower with the highest dietary sodium, in both species. This included the precursors of struvite (magnesium, ammonium and phosphate) as well as calcium and oxalate. Inter-individual variations were apparent in both dogs and cats, but the highest sodium diet consistently led to a lower CaOx and MAP RSS compared with the lowest sodium diet. It is widely accepted that this strategy is central to reduce the risk of crystallization, although organic promoters or inhibitors also play a role (Lulich, Osborne, & Albasan, 2011). Urinary sodium concentrations were expectedly higher with the high sodium diets as this nutrient is readily absorbed in the gastrointestinal tract and predominantly excreted by the kidneys, which adjust the glomerular filtration and tubular reabsorption to maintain homeostasis.

Relative supersaturation, which is used as a risk index for crystallization in the urinary tract, was also affected by dietary NaCl. In both species, MAP RSS decreased as NaCl increased, and was the lowest with the highest sodium diet. This likely resulted from the lower urinary concentrations of precursors, but could also be explained by the lower urine pH induced by NaCl since in acidic urine, protonation makes phosphate unavailable for MAP crystals. The lower urine pH with the high NaCl diet was not expected, as the four diets were formulated to have similar base excess value in order to induce similar urine pH (Kienzle et al., 1991). Physiological

	Diet A	Diet B	Diet C	Diet D	SE	p value
Sodium (g/1,000 kcal)	0.67	1.68	2.41	3.27		
Caloric intake (kcal/BW <sup>0.71</sup> )	88	86	86	86	2.4	.83
Water intake (ml/kg/day)	$24.5^{\dagger}$	28.8 <sup>‡</sup>	30.3 <sup>‡</sup>	36.7 <sup>§</sup>	1.38	<.001
Urine volume (ml/kg/day)	10.9 <sup>†</sup>	13.7 <sup>‡</sup>	15.5 <sup>‡</sup>	19.6 <sup>§</sup>	0.69	<.001
Urine specific gravity	$1.067^{\dagger}$	1.063 <sup>†‡</sup>	1.061 <sup>‡</sup>	1.051 <sup>§</sup>	0.002	<.001
Urine pH	6.36 <sup>†</sup>	6.51 <sup>†</sup>	6.39 <sup>†</sup>	6.27 <sup>‡</sup>	0.05	<.001
CaOx RSS	3.39 <sup>†</sup>	2.80 <sup>‡</sup>	2.41 <sup>‡</sup>	1.64 <sup>§</sup>	0.25	<.001
MAP RSS	0.81 <sup>†</sup>	0.83 <sup>†</sup>	0.41 <sup>‡</sup>	0.16 <sup>§</sup>	-	<.001
Urinary concentrations						
Calcium (mmol/L)	0.66 <sup>†</sup>	$0.58^{\dagger}$	0.48 <sup>‡</sup>	0.41 <sup>‡</sup>	0.03	<.001
Magnesium (mmol/L)	3.53 <sup>†</sup>	3.30 <sup>†‡</sup>	2.97 <sup>‡</sup>	2.38 <sup>§</sup>	0.14	<.001
Sodium (mmol/L)	139 <sup>†</sup>	279 <sup>‡</sup>	359 <sup>§</sup>	375 <sup>§</sup>	8.3	<.001
Potassium (mmol/L)	270 <sup>†</sup>	225 <sup>‡</sup>	177 <sup>§</sup>	159 <sup>¶</sup>	6.0	<.001
Ammonium (mmol/L)	229 <sup>†</sup>	196 <sup>‡§</sup>	184 <sup>§</sup>	136 <sup>¶</sup>	5.1	<.001
Phosphate (mmol/L)	63 <sup>†</sup>	54 <sup>‡</sup>	48 <sup>‡</sup>	36 <sup>§</sup>	1.4	<.001
Sulphate (mmol/L)	113 <sup>†</sup>	95 <sup>‡</sup>	87 <sup>‡</sup>	69 <sup>§</sup>	2.7	<.001
Oxalate (mmol/L)	1.99 <sup>†</sup>	1.67 <sup>†‡</sup>	1.55 <sup>‡</sup>	1.02 <sup>§</sup>	0.07	<.001
Citrate (mmol/L)	1.33 <sup>†</sup>	0.96 <sup>†‡</sup>	0.64 <sup>‡</sup>	0.59 <sup>‡</sup>	0.14	<.001
Urate (mmol/L)	0.96 <sup>†</sup>	0.88 <sup>†‡</sup>	0.79 <sup>‡</sup>	0.56 <sup>‡</sup>	0.03	<.001
Urinary excretions						
Calcium (µmol/kg/24 hr)	7.08	7.85	7.15	7.92	0.86	.25

*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 < .05).

**TABLE 2** Diet and water intakes, and urine composition in cats (n = 13) fed the four diets differing in sodium chloride content

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diurnal variations in urine pH are not likely to be responsible for this finding, as urine collection took place over the course of 3 days. A slight acidifying effect of NaCl, not reflected by its cation and anion contribution to the base excess, could be responsible for this finding, and to the authors' knowledge has not been reported in biological fluids like urine before. Increasing concentration of salt in other solutions, however, has been described to decrease pH (Critchfield, 1959; Robinson, 1929), and this effect is more pronounced in acidic solutions (Critchfield, 1959).

High intake of dietary sodium has been contraindicated in human stone formers because it increases calcium excretion (Frassetto & Kohlstadt, 2011). In the present study, an increased calcium excretion was apparent in dogs, but not in cats. However, due to the concurrent effect of NaCl on urine dilution, calcium concentration in the urine was lower despite a higher excretion, thus decreasing CaOx RSS. In dogs, these findings are consistent with two previous studies, where diets with sodium  $\geq 3.0 \text{ g/1},000 \text{ kcal}$  induced significantly lower CaOx RSS than diets with sodium <1.0 g/1,000 kcal (Lulich et al., 2005; Stevenson et al., 2003). Interestingly, when an additional dietary sodium concentration of 2.0 g/1,000 kcal was tested, CaOx RSS was intermediate (Stevenson et al., 2003). In our study, the RSS values obtained for 0.7 and 1.7 g sodium/1,000 kcal were higher than with 2.4 and 3.3 g sodium/1,000 kcal. This suggests that the threshold of dietary sodium needed to lower CaOx RSS in dogs may be close to 2.0 g/1,000 kcal. In cats, a retrospective study has also found lower CaOx RSS with diets containing >2.8 g sodium/1,000 kcal (Hawthorne & Markwell, 2004). However, a more recent prospective study failed to show an effect of dietary NaCl on CaOx RSS (Passlack et al., 2014). The four diets were formulated to contain sodium levels of 0.9, 1.6, 2.8 and 3.5 g/1,000 kcal, close to the levels in the present study. The methodology was similar except for longer adaptation (21 days) and collection (7 days) periods. Urinary ions were assayed by ion chromatography, and Supersat software was used to calculate RSS. This is an important factor to consider as other software solutions can over- or underestimate RSS (Robertson et al., 2002). Differences between the diet profiles might however explain the discrepancy between the results of this and our current study. The diet used in the current study had a mineral concentration of calcium, phosphorus and magnesium 30 to 60% lower than in the study of Paßlack et al. (Passlack et al., 2014). The diets in the current study were formulated similarly apart from their NaCl content, although one diet (diet C) was slightly higher in total dietary fibre. Diets A, B and D, however, had similar fibre contents. The diets used in the current study had less varied fibre content than the diets used in the Paßlack study. Intestinal calcium absorption and urinary calcium excretion are affected by fibre content of the diet, although variable depending

	Diet A	Diet B	Diet C	Diet D	SE	p value
Sodium (g/1,000 kcal)	0.67	1.68	2.41	3.27		
Caloric intake (kcal/BW <sup>0.75</sup> )	116	120	119	118	-	.39
Water intake (ml/kg/day)	42.0 <sup>†</sup>	45.9 <sup>†</sup>	61.5 <sup>‡</sup>	63.7 <sup>‡</sup>	2.9	<.001
Urine volume (ml/kg/day)	19.9 <sup>†</sup>	27.6 <sup>‡</sup>	30.8 <sup>‡</sup>	39 <sup>§</sup>	2.1	<.001
Urine specific gravity	$1.056^{\dagger}$	1.048 <sup>‡</sup>	1.046 <sup>‡</sup>	1.038 <sup>§</sup>	0.002	<.001
Urine pH	$6.26^{\dagger}$	6.19 <sup>†‡</sup>	6.10 <sup>†‡</sup>	5.98 <sup>‡</sup>	0.13	.022
CaOx RSS	$11.6^{\dagger}$	9.19 <sup>†</sup>	5.83 <sup>‡</sup>	6.40 <sup>‡</sup>	0.80	<.001
MAP RSS	$0.81^{\dagger}$	0.32 <sup>†‡</sup>	0.16 <sup>‡</sup>	0.06 <sup>§</sup>	-	<.001
Urinary concentrations						
Calcium (mmol/L)	$2.4^{\dagger}$	2.16 <sup>†‡</sup>	$1.76^{\dagger\ddagger}$	1.73 <sup>‡</sup>	0.24	.015
Magnesium (mmol/L)	$5.44^{\dagger}$	4.08 <sup>‡</sup>	3.85 <sup>‡</sup>	3.16 <sup>§</sup>	0.22	<.001
Sodium (mmol/L)	$116^{\dagger}$	199 <sup>‡</sup>	256 <sup>§</sup>	281 <sup>§</sup>	11.5	<.001
Potassium (mmol/L)	220 <sup>†</sup>	160 <sup>‡</sup>	124 <sup>§</sup>	115 <sup>§</sup>	6.7	<.001
Ammonium (mmol/L)	$185^{\dagger}$	143 <sup>‡</sup>	124 <sup>‡</sup>	96 <sup>§</sup>	8.9	<.001
Phosphate (mmol/L)	49.8 <sup>†</sup>	37.3 <sup>‡</sup>	35.3 <sup>‡</sup>	25.0 <sup>§</sup>	2.11	<.001
Sulphate (mmol/L)	$118^{\dagger}$	85 <sup>‡</sup>	79 <sup>‡</sup>	61 <sup>§</sup>	4.1	<.001
Oxalate (mmol/L)	$1.57^{\dagger}$	1.11 <sup>‡</sup>	0.8 <sup>§</sup>	0.73 <sup>§</sup>	0.08	<.001
Citrate (mmol/L)	$0.04^{\dagger}$	0.02 <sup>‡</sup>	0.03 <sup>‡</sup>	0.01 <sup>§</sup>	0.001	<.001
Urate (mmol/L)	$1.60^{\dagger}$	1.26 <sup>‡</sup>	1.07 <sup>‡</sup>	0.84 <sup>§</sup>	0.09	<.001
Urinary excretions						
Calcium (µmol/kg/24 hr)	47.3 <sup>†</sup>	57.6 <sup>†‡</sup>	53.0 <sup>†‡</sup>	66.9 <sup>‡</sup>	5.6	.011

**TABLE 3** Diet and water intakes, and urine composition in dogs (*n* = 8) fed the four diets differing in sodium chloride content

*Note:* Data are presented as Least Square Means except for caloric intake and MAP RSS for which medians are indicated. Two different symbols within a row indicate significant difference (Scheffe test, p < .05).

on source of the fibre and other nutrients such as fat (Harrington, Flynn, & Cashman, 2001; Kies, 1985; Shah et al., 2009). The fibre content and source may have affected urinary concentrations of crystal precursors and the CaOx RSS values, which were on average three to five times lower with the diets used in the current study. The higher mineral load and the differing nutrient profiles of the diets used by Paßlack et al. could have led to higher urinary mineral concentrations despite the urinary dilution in response to the sodium, thereby limiting its effect on RSS.

This study has some limitations. The effect of different levels of dietary NaCl was assessed over 10 days for each diet, which is a relatively short time period. Although renal response to dietary sodium intake is rapid, as shown by the significant changes in urinary variables, longer term metabolic adaptations, which would affect urinary composition, cannot be excluded. Another limitation is the absence of Latin Square design to test the four diets, due to logistical constraints in the animal colony. However, sodium balance is rapidly achieved to match excretion to intake via the adaptation of natriuretic systems and sodium and water retaining systems (Guyton & Hall, 2006), and carry-over effects from one treatment period to another are therefore unlikely. A last limitation is the manner in which urine volume was determined. Urine was collected non-invasively, and whilst bladder catheterization at beginning and end of the collection period could be performed for an accurate measurement, this was not done because of animal welfare considerations. Therefore, the volume collected over 3 days may have been under- or overestimated, as well as the calculation of urinary calcium excretion. However, this does not impact the conclusions on urine concentrations and therefore RSS.

In conclusion, this short-term study showed that increasing dietary concentration of NaCl in a dry diet was effective to gradually and significantly increase water intake and urine volume, as well as decrease RSS of struvite and calcium oxalate in healthy cats and dogs. To increase the clinical relevance of these findings, a longer prospective trial in stone forming animals should be carried out to determine whether dietary NaCl supplementation can affect RSS and prevent or delay recurrence of stone formation in such populations.

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#### CONFLICT OF INTEREST

All authors are employees of Royal Canin, Mars Petcare.

#### 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.

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# SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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