



## Short term effects of increasing dietary salt concentrations on urine composition in healthy cats



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### ABSTRACT

High dietary salt (NaCl) concentrations are assumed to be beneficial in preventing the formation of calcium oxalate (CaOx) uroliths in cats, since increased water intake and urine volume have been observed subsequent to intake. In human beings, dietary NaCl restriction is recommended for the prevention of CaOx urolith formation, since high NaCl intake is associated with increased urinary Ca excretion. The aim of the present study was to clarify the role of dietary NaCl in the formation of CaOx uroliths in cats. Eight cats received four diets that differed in Na and Cl concentrations (0.38–1.43% Na and 0.56–2.52% Cl dry matter, DM). Each feeding period consisted of a 21 day adaptation period, followed by a 7 day sampling period for urine collection. Higher dietary NaCl concentrations were associated with increased urine volume and renal Na excretion. Urinary Ca concentration was constant, but renal Ca excretion increased from 0.62 to 1.05 mg/kg bodyweight (BW)/day with higher dietary NaCl concentrations ( $P \leq 0.05$ ). Urinary oxalate (Ox), citrate, P and K concentrations decreased when NaCl intake was high ( $P \leq 0.05$ ), and urinary pH was low in all groups (6.33–6.45;  $P > 0.05$ ). Relative supersaturation of CaOx in the urine was unaffected by dietary NaCl concentrations. In conclusion, the present study demonstrated several beneficial effects of high dietary NaCl intake over a relatively short time period. In particular, urinary Ca concentration remained unchanged because of increased urine volume. Decreased urinary Ox concentrations might help to prevent the formation of CaOx uroliths, but this should be verified in future studies in diseased or predisposed cats.

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### Introduction

A high concentration of dietary salt (NaCl) is assumed to be beneficial for the prevention of calcium oxalate (CaOx) urolith formation in cats (Lekcharoensuk et al., 2001), since increased water intake and urine volume have been associated with high NaCl intake, resulting in more dilute urine (Luckschander et al., 2004; Chandler, 2008). High urine volume lowers the total urinary concentrations of Ca and Ox, and therefore the risk of urolith formation (Dijcker et al., 2011), assuming that renal Ca and Ox excretions remain constant.

However, in human beings a high intake of NaCl has been associated with increased renal Ca excretion, which can increase the risk of formation of CaOx uroliths (Castenmiller et al., 1985; Kok et al., 1990; Sakhaee et al., 1993). It is hypothesised that high Na intake can lead to an upregulation of specific Ca transport molecules in the

distal tubule of the nephron (Lee et al., 2012). Thus, dietary NaCl restriction is recommended for the prevention of formation of CaOx calculi in humans (Frassetto and Kohlstadt, 2011). In cats, there is little published information on the effects of dietary Na on renal Ca excretion. In one study, dietary Na had no observable effect on urinary Ca concentration; however, data on renal Ca excretion were not reported (Hawthorne and Markwell, 2004).

In human beings, NaCl is thought to lower urinary pH (Frassetto and Kohlstadt, 2011), but, to the author's knowledge, comprehensive data for cats remain unpublished. One study reported that cats receiving high Na diets (13.37 g/MJ metabolisable energy) had a mean urinary pH of  $6.26 \pm 0.03$ , while cats receiving lower Na diets (4.40 g/MJ metabolisable energy) had a mean urinary pH of  $6.38 \pm 0.06$  ( $P > 0.05$ ; Hawthorne and Markwell, 2004). Urine pH is a critical factor in the formation of uroliths and a pH  $< 6.2$  is considered to be a risk factor for the development of CaOx uroliths (Kirk et al., 1995).

The role of dietary NaCl as a potential risk factor for the formation of CaOx uroliths in cats remains unclear. The aim of the present study was to evaluate the effects of four diets of varying NaCl concentrations on urinary pH, urine volume and urine composition in cats, with special emphasis on renal Ca and Ox excretion.

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## Materials and methods

### Study design

The study was approved by the Animal Welfare Committee (Landesamt für Gesundheit und Soziales, Berlin, Germany, G 0004/08). Eight adult cats (European Short-hair; four male, four female; aged 12–25 months) were housed in a room with a constant light (12 h light/12 h darkness) and temperature (20 °C) regimen. Cats were fed four experimental diets (Table 1) over four feeding periods and all cats received the same diet during the same feeding period. With each consecutive feeding period, the NaCl content of the diets increased, reaching dietary Na:Cl concentrations of 0.38:0.56, 0.65:0.95, 1.14:1.90 and 1.43:2.52% dry matter (DM). The experimental diets were formulated to fulfil the requirements of adult cats as specified by the National Research Council (NRC, 2006).

Each feeding period was divided into a 21 day adaptation period, where the cats were housed in groups, and a 7 day collection period, where cats were housed individually. Cats were allowed to socialise throughout the study period. Purpose built cat litter boxes were used with plastic pellets as litter and urine collection containers were connected to the litter boxes to separate urine from faeces. Each urine collection container was treated with one drop chlorhexidine-digluconate to prevent bacterial growth in the urine. The cats were fed once daily in the morning. Tap water was provided ad libitum and individual amounts of food offered were calculated according to NRC (2006) recommendations. Daily water and food intake were recorded during each collection period.

Urine and faeces were collected three times per day during the collection period and stored in a refrigerator (4 °C) until the evening. After pH measurements were recorded in the evening using a pH meter (Seven Multi, Mettler-Toledo GmbH), urine and faecal samples were stored at –80 (urine) or –20 °C (faeces) prior to further analysis.

### Analysis of diets, urine and faeces

A detailed description of the nutrient analysis of the experimental diets and the urine and faecal analysis can be found elsewhere (Paßlack and Zentek, 2013; Paßlack et al., 2014). Dietary crude nutrient concentrations were measured using the Weende analysis of feed method (Naumann and Bassler, 2004), while crude fat was measured using a modified method (Paßlack et al., 2014). The analysis of the dietary mineral concentrations was performed as will be described later for faecal minerals.

The 7 day urine samples were pooled separately for each cat, mixed thoroughly, and an aliquot of 12 mL was subsequently removed for the further analysis. Hydrochloric acid (37%) was added to adjust to a pH of 2 (pH-meter Seven Multi, Mettler-Toledo GmbH). Three hundred microlitres of the acidified urine was used to measure urinary N with the Vario MAX CN Makro-Elementaranalysator (Elementar Analysensysteme GmbH). The remaining urine was filtered (Syringe Filter, Bulk, SFCA, surfactant-free cellulose acetate, 0.2 µm, 25 mm non-sterile, Thermo Scientific) before further analyses of urea and creatinine (high performance liquid chromatography, HPLC, method, Agilent 1100 with ultraviolet, UV, detector, Agilent Technologies), SO<sub>4</sub>, PO<sub>4</sub>, Ox and citrate (ion exchange HPLC system, Dionex DX-500, Dionex), and Na, K, NH<sub>4</sub>, Mg and Ca (ion exchange HPLC system, Dionex DX-120, Dionex). The relative supersaturation of urinary CaOx (RSS CaOx) was determined using the Supersat-Program (Robertson et al., 2002).

Faecal samples were weighed and lyophilised in a vacuum freeze dryer (Lyovac GT2, LC Didactic). The lyophilised samples were ground to a particle size of 0.25 mm (Mill: ZM 100, Kurt Retsch). Faecal Cl concentrations were measured using an ion exchange HPLC system (Dionex DX-500, Dionex), and P concentrations were measured spectrophotometrically (Gericke and Kurmies, 1952; Ultrospec 2000, Pharmacia Biotech). Faecal Ca, Na, K and Mg concentrations were detected by atomic absorption spectrometry (flame atomic absorption spectrometer type Vario 6 with an Autosampler AS 52, Analytik Jena AG).

**Table 1**  
Nutrient analysis of experimental diets.<sup>a</sup>

Composition		Concentration of Na in diet (%)			
		0.38	0.65	1.14	1.43
DM <sup>b</sup>	g/kg	922	919	937	927
Crude protein	g/kg DM	401	438	435	433
Crude fat	g/kg DM	120	124	115	119
Crude fibre	g/kg DM	13.0	22.8	17.1	5.39
Crude ash	g/kg DM	63.8	67.7	81.2	86.4
Ca	g/kg DM	11.5	11.3	11.2	9.88
P	g/kg DM	10.9	11.0	11.0	10.9
Na	g/kg DM	3.82	6.51	11.4	14.3
K	g/kg DM	4.69	5.24	6.54	7.43
Cl	g/kg DM	5.58	9.50	19.0	25.2
Mg	g/kg DM	1.16	1.12	1.07	1.06
ME <sup>c</sup>	MJ/kg DM	17.3	17.2	17.0	17.2

<sup>a</sup> List of ingredients: corn, poultry meal, corn gluten, greaves meal, pig fat, rice gluten, dried beet pulp, rice, digest, salt, yeast, marigold meal, minerals, vitamins.

<sup>b</sup> Dry matter.

<sup>c</sup> Metabolisable energy calculated according to NRC (2006).

sured spectrophotometrically (Gericke and Kurmies, 1952; Ultrospec 2000, Pharmacia Biotech). Faecal Ca, Na, K and Mg concentrations were detected by atomic absorption spectrometry (flame atomic absorption spectrometer type Vario 6 with an Autosampler AS 52, Analytik Jena AG).

### Statistical analysis

Statistical Package for the Social Sciences (SPSS 15, IBM) was used for statistical analysis. Repeated measures analysis of variance (ANOVA) was performed (fixed factor dietary Na concentration) and within-subject comparisons (simple contrasts; dietary Na concentrations, four levels; six tests per parameter) were undertaken to detect group differences. Correlation coefficients (Pearson for normally distributed data and Spearman for nonparametric data) were calculated to detect dependencies between increasing dietary Na concentrations and measured urinary and faecal parameters.

## Results

There were small variations in BW and feed intake during the study (Tables 2 and 3). Urine volume increased with increasing dietary NaCl (correlation coefficient 0.586;  $P = 0.001$ ) and differed significantly ( $P \leq 0.05$ ) between the groups with the lowest and the highest dietary NaCl concentration. Water intake increased linearly from 29.8 to 34.8 mL/kg BW/day when groups were fed increasing concentrations from 0.65 to 1.43% Na ( $P \leq 0.05$ ). However, there was no significant difference in water intake after feeding diets with the lowest and the highest NaCl concentrations ( $P > 0.05$ ). Urinary pH was 6.33–6.45 and was not linearly affected by dietary NaCl concentration ( $P > 0.05$ ).

Urinary concentrations of anions and cations were markedly affected by dietary NaCl concentrations. Decreased urinary P, K, creatinine, Ox, citrate, NH<sub>4</sub> and N concentrations were observed with increasing dietary NaCl concentrations ( $P \leq 0.05$ ). In contrast, urinary Na concentrations increased from 1768 to 3886 mg/L with increasing dietary NaCl concentrations ( $P \leq 0.05$ ). Urinary Ca, Mg and SO<sub>4</sub> concentrations were not affected by dietary NaCl concentration ( $P > 0.05$ ). Urinary urea concentrations were highest in the 0.38% Na group and lowest in the 0.65% Na group ( $P \leq 0.05$ ). Because of decreasing urinary Ox concentrations in the face of unchanged urinary Ca concentrations, urinary Ca:Ox increased with increasing dietary NaCl concentrations (correlation coefficient of 0.477;  $P = 0.006$ ). There were no significant changes in urinary Ca: citrate with increasing NaCl concentration ( $P > 0.05$ ). RSS CaOx was unaffected by dietary NaCl concentration ( $P > 0.05$ ).

Renal Ca excretion increased from 0.62 to 1.05 mg/kg BW/day with increasing dietary NaCl concentration (correlation coefficient of 0.454;  $P = 0.012$ ). Renal Na, P, Mg and SO<sub>4</sub> excretion increased with increasing concentrations of dietary NaCl ( $P \leq 0.05$ ). Renal urea excretion increased from 0.56 to 1.13 mg/kg BW/day with increasing Na concentration from 0.65% to 1.43% Na ( $P \leq 0.05$ ), but no difference was observed between cats fed 0.38% and 1.43% Na ( $P > 0.05$ ). Renal excretion of K, Ox, creatinine, citrate, NH<sub>4</sub> and N was not affected by the dietary NaCl concentration.

Faecal DM output was highest after feeding the diet with the lowest NaCl concentration (correlation coefficient of –0.434;  $P = 0.013$ ). Faecal DM concentration decreased with increasing dietary NaCl concentrations (correlation coefficient of –0.484;  $P = 0.005$ ). Faecal Ca and K concentrations were the lowest in the group fed 0.38% Na compared to all other groups ( $P \leq 0.05$ ; Table 4). Faecal Cl concentrations were lowest in the group fed 1.14% Na (1.11 mg Cl/g DM) and highest in the group fed 1.43% Na (2.06 mg Cl/g DM;  $P \leq 0.05$ ). Faecal P, Mg and Na concentrations were not affected by dietary NaCl concentrations ( $P > 0.05$ ).

The highest faecal excretion of Ca, P and Mg was observed in the group that received the diet with the lowest NaCl concentration (0.38% Na;  $P \leq 0.05$ ; Table 5). The group fed 0.38% Na also had higher faecal Na excretion than the groups fed 0.65% and 1.14% Na ( $P \leq 0.05$ ), higher faecal Cl excretion than the group fed 1.14% Na ( $P \leq 0.05$ ) and

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