Effects of a high-protein diet versus dietary supplementation with ammonium chloride on struvite crystal formation in urine of clinically normal cats

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Objective—To evaluate the effects of a high-protein diet versus dietary supplementation with ammonium chloride (NH_4Cl) on struvite crystal formation in the urine of clinically normal cats by measuring the urine concentration of hydrochloric acid (HCl)-insoluble sediment, urine pH, struvite activity product (SAP), number of struvite crystals in urine, and urine volume.

Animals—23 healthy adult cats.

Procedure—Urine was fractionated by centrifugation with subsequent extraction of the sediment with 1 N HCl (study 1). Diets containing either 29% crude protein or 55% crude protein were fed to cats in a crossover trial of 3 weeks/period (study 2). Diets supplemented with either sodium chloride (NaCl) or NH_4Cl were fed, by use of a 3 × 3 Latin-square design with 3 wk/period (study 3). In studies 2 and 3, urine samples were collected for the last 7 days of each period.

Results—The HCl-insoluble sediment contained Tamm-Horsfall glycoprotein (THP; study 1). The high-protein diet (study 2) and dietary supplementation with NH_4Cl (study 3) resulted in a decrease in urine pH, SAP, and the number of struvite crystals in urine. However, the high-protein diet decreased urine concentrations of HCl-insoluble sediment containing THP (study 2), in contrast to the NH_4Cl supplementation that increased urine volume without a significant effect on the urine concentration of the HCl-insoluble sediment (study 3).

Conclusions and Clinical Relevance—Our results indicate that compared with dietary supplementation with NH_4Cl, the high-protein diet is preferable as a urine acidifier for the prevention of struvite crystal formation in clinically normal cats. (*Am J Vet Res* 2003;64:1059–1064)

Uroliths and urethral plugs in cats usually contain minerals. One of the major mineral components is struvite, a magnesium-ammonium-phosphate complex ($MgNH_4PO_4 \cdot 6H_2O$). In a study by Osborne et al,[1] 64.5% of feline uroliths were found to be composed of struvite. Results of epidemiologic studies[2-5] indicate that there is a decrease in the incidence of struvite-associated lower urinary tract disease in recent years. Nevertheless, there is still substantial occurrence of struvite urolithiasis.[4,5] Solubility of struvite crystals is negatively related to the **struvite activity product** (**SAP**; $[Mg^{2+}] \times [NH_4^+] \times [PO_4^{3-}]$).[6] Urine pH affects concentrations of the constituents; lowering the urine pH results in a decrease in the total phosphorus concentration (present as PO_4^{3-}) that is proportionately greater than the increase in NH_4^+ concentration.[6,7] Thus, nutritional management to decrease the SAP may be helpful in the prevention of struvite uroliths.

Formation and growth of struvite crystals, however, are not determined solely by SAP. Several inorganic and organic electrolytes and organic compounds, including proteins and glycosaminoglycans, can modulate growth of struvite crystals in vitro.[8-10] Among these molecules, the **Tamm-Horsfall glycoprotein** (**THP**), which was isolated from feline urine,[8,11] increases the number of struvite crystals and promotes growth of struvite crystals in feline urine in vitro.[8] These findings suggest that increasing THP in urine is a risk factor for struvite uroliths and, therefore, it would be useful to clarify factors affecting THP concentrations in urine.

In a previous study,[12] we found that high-protein diets increased urine volume and decreased the SAP in healthy adult cats. These results have led us to hypothesize that high-protein diets are effective for the prevention of struvite crystals in clinically normal cats. However, high-protein diets may increase urine concentrations of organic compounds, including glycoprotein, a possibility yet to be determined. The purpose of the study reported here was to evaluate the effects of a high-protein diet versus dietary supplementation with **ammonium chloride** (**NH_4Cl**) on struvite crystal formation in the urine of clinically normal cats by measuring the urine concentration of **hydrochloric acid** (**HCl**)-insoluble sediment, urine pH, SAP, number of struvite crystals in urine, and urine volume.

Materials and Methods

Cats—A total of 23 mixed-breed cats considered to be clinically normal on the basis of findings on physical examination, hemograms, serum biochemical analysis, and urinalysis were used for 3 studies. Different cats were used in each study. All cats were individually housed in metabolic cages

Received June 14, 2002.
Accepted January 23, 2003.
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continuously throughout each study and cared for according to the principles outlined in the Institute of Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals.[13] They were kept in a temperature-controlled room (24 ± 2°C) with artificial light provided from 6 AM to 6 PM daily. In studies 2 and 3, health of cats was monitored every day on the basis of appearance, appetite, and the condition of feces.

Study 1—Voided urine samples were collected from 6 sexually intact male adult cats (3 to 8 years old; mean body weight, 5.64 kg [range, 4.57 to 6.35 kg]). These cats were fed a commercial dry food[a] containing 29% crude protein on a dry matter basis. The diet and water were available ad libitum. Urine that was freshly voided between 5 and 8 AM was collected. All urine samples from all cats were pooled and kept at 4°C for a few hours, until total urine from all cats exceeded 50 mL.

To characterize the different types of protein in the urine sediment of healthy cats, collected urine samples (50 mL) were immediately centrifuged at 4,200 X g for 30 minutes at 4°C and separated into supernatant and sediment. The sediment was air-dried at 45°C for 24 hours and weighed. Next, 1 N HCl (10 mL) was added to the dried sediment and mixed by a gentle shaking for 24 hours at 25°C. The mixture was again centrifuged at 4,200 X g for 30 minutes at 4°C, and the sediment was air-dried at 45°C for 24 hours and weighed. The weight of HCl-soluble sediment in urine was obtained by deducting the weight of HCl-insoluble sediment from that of total sediment.

The HCl-insoluble sediment was assumed to be organic compounds that were insoluble in urine, because the organic compounds, including protein, are generally HCl-insoluble. In contrast, because the inorganic matter including mineral is HCl-soluble, the HCl-soluble sediment was assumed to be inorganic matter that was insoluble in urine. To verify these assumptions, protein concentrations of whole urine, supernatant, and HCl-soluble sediment after centrifugation were measured by a protein assay with bicinchoninic acid.[14] Bovine serum albumin was used for quantification as a protein standard. Protein concentrations of total and HCl-insoluble sediment after centrifugation were estimated as differences between protein concentrations of whole urine and the supernatant and between protein concentrations of total sediment and HCl-soluble sediment, respectively. In addition, protein in each fraction of urine was separated by **sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)**. Ten micrograms of the protein from whole urine, supernatant, and sediment after centrifugation and from the HCl-soluble and HCl-insoluble sediment were subjected to 12.5% SDS-PAGE,[b] by use of bovine serum albumin (66.2 kd) and phosphorylase B (97.4 kd) as a marker of molecular weight; gels were stained with Coomassie brilliant blue R-250.

Study 2—Eight adult cats (4 sexually intact males and 4 sexually intact females; 1 to 6 years old; mean body weight, 3.81 kg [range, 2.58 to 4.90 kg]) were used in a crossover trial of 3 wk/period. To check the effect of high-protein diet on urine pH, urine concentrations of struvite crystals, total sedimrnt,HCI-soluble sediment, and HCI-insoluble sediments, cats were fed diets with low (**28.6% crude protein [CP29 diet]** on a dry matter basis) or high (**54.9% crude protein [CP55 diet]** on a dry matter basis) crude protein content (**Appendix**). In the CP55 diet, fish meal and corn gluten meal substituted for a part of the corn and tallow in the CP29 diet to increase the crude protein content. The determined contents of nutrients met or exceeded the requirements for maintenance.[15,16] The Ca and P contents between diets were made similar by addition of appropriate amounts of $CaCO_3$ and $Ca(PO_4)_2$. Food was provided daily at 4 PM. Intakes of food and water were recorded each day of the study.

For the last 7 days of each 3-week period, urine samples were collected every 24 hours at 4 PM into a bottle containing 10 mL of 10% (vol:vol) sulfuric acid to prevent possible loss of ammonia. Urine samples taken from each cat were pooled and stored at –20°C until analysis of Mg, P, and total ammonia. On 1 day of the sample collection period, first-voided urine after 5 AM was also obtained from each cat as fresh urine. At 5 AM on that day, the usual urine-collection bottle was replaced with a bottle without sulfuric acid. Within 10 minutes after urination, the fresh urine was used for determination of pH, the number of struvite crystals, and fractionation into total, HCl-soluble, and HCl-insoluble sediment. Struvite crystals in 1 to 5 µL of fresh urine were counted under an optical microscope at a magnification of 100X.[10,17] Five milliliters of fresh urine was used in duplicate for fractionation. Because of the small volume of urine samples, the fractionation method was modified as follows: 1 mL of 1 N HCl was added to the total sediment obtained by centrifugation of 5 mL of urine at 4,200 X g for 30 minutes at 4°C. Remaining fresh urine was poured into a bottle containing sulfuric acid. Our experience indicates that 10 mL of 10% (vol:vol) sulfuric acid is enough to prevent possible loss of ammonia from daily urine samples of adult cats.

Study 3—Nine adult cats (6 sexually intact males and 3 sexually intact females; 2 to 7 years old; mean body weight, 4.11 kg [range, 2.92 to 5.09 kg]) were used in a 3 X 3 Latin-square design of 3 wk/period. To check the effect of dietary supplementation of NH_4Cl on urine pH, struvite crystals, and sediment quantity, 3 diets were prepared as follows: control diet that was the same as the CP29 diet (Appendix), 1.5% NH_4Cl supplemented diet, and 0.75% **sodium chloride (NaCl)** supplemented diet to equalize additional inorganic electrolytes with the NH_4Cl diet. The NH_4Cl and NaCl diets were made by adjustment of the corn content of the control diet. The determined contents of nutrients met or exceeded the requirements for maintenance.[15,16] Dietary base excess calculated according to Kienzle et al[18] was 350 mmol/kg on a dry matter basis for the control and NaCl diets, and that of the NH_4Cl diet was 69 mmol/kg on a dry matter basis. Diets and water were available ad libitum throughout the study. Food was provided daily at 4 PM. Intakes of food and water were recorded every day. For the last 7 days of each sample collection period, pooled urine and fresh urine were collected as described for study 2. The modified fractionation method was also used in study 3.

Analyses—Chemical composition of the diets was determined according to the Association of Official Analytical Chemists.[19] Protein content in the fractions of urine was measured by a protein assay with bicinchoninic acid.[14] Mineral (Na, K, Ca, P, Mg, and Cl) contents of the diets, and concentrations of struvite constituents (Mg, P, and total ammonia) in urine collected in the presence of sulfuric acid were measured, as previously described.[12,17] For estimating SAP, Mg was assumed to exist as ionic forms in urine. In addition, **ammonium ion (NH_4^+)** and **phosphoric acid ion (PO_4^{3-})** concentrations in the urine were estimated from the urine pH and determined concentrations of total ammonia and P in urine as described previously.[6,12,17] For convenience, SAP is expressed as **the negative logarithm of SAP (pSAP)**.[6,12,17]

Analysis of data—Data were analyzed by use of an ANOVA.[20,c] The model included diet, animal, and period. When the effect of diet was significant ($P < 0.05$), differences among cats fed the control, NH_4Cl supplemented, or NaCl supplemented diets in study 3 (control, NH_4Cl, and NaCl) were evaluated by the Dunnett multiple-range test. Mean (±

SEM) values are presented in tabular format, and results were considered significant at $P < 0.05$.

Results

Study 1—On the basis of bicinchoninic acid protein assay results, 97.2% of urine total protein was fractionated in the supernatant, and protein content of the HCl-insoluble sediment fraction was only 2.0% of total protein **(Table 1)**. The SDS-PAGE profiles **(Fig 1)** of whole urine and of supernatant revealed a single band at 66 kd. In contrast, the SDS-PAGE profile of total sediment revealed 2 bands, at 66 kd and 95 to 100 kd. The band at 95 to 100 kd was not detected in whole urine, probably because of a low concentration of this molecule in urine. The SDS-PAGE profile of the HCl-insoluble sediment was similar to that of total sediment, whereas specific bands were not detected on the SDS-PAGE profile of HCl-soluble sediment.

Study 2—Daily food and water intakes and urine volume in cats given the CP29 or the CP55 diets were monitored **(Table 2)**. Dietary protein content had no effect on daily food and water intakes. Urine volume was higher in cats fed the CP55 diet, compared with cats fed the CP29 diet, although this difference was not significant ($P = 0.12$).

Urine pH, concentrations of struvite constituents, pSAP, number of struvite crystals, and concentration of sediment (total, HCl-soluble, and HCl-insoluble) in cats given the CP29 or CP55 diet were determined **(Table 3)**. Urine pH was significantly ($P = 0.006$) lower in cats fed the CP55 diet, compared with cats fed the CP29 diet. Urine Mg^{2+} concentration was also significantly ($P = 0.04$) lower in cats fed the CP55 diet, compared with cats fed the CP29 diet, whereas urine NH_4^+ concentration was significantly ($P = 0.005$) higher in cats fed the CP55 diet. Total P concentration in urine was not significantly different between cats fed the CP55 and those fed the CP29 diet. Because of the decrease in urine pH, urine PO_4^{3-} concentration was lower in cats fed the CP55 diet, compared with cats fed the CP29 diet, although this difference was not significant ($P = 0.20$). As a result, a significant ($P = 0.02$) increase in the pSAP and a significant ($P = 0.02$) decrease in the number of struvite crystals in urine were detected in cats fed the CP55 diet, compared with cats fed the CP29 diet. The concentration of total sediment in urine was significantly ($P = 0.02$) lower in cats fed the CP55 diet, compared with cats fed the CP29 diet. Decreases were significant in not only the HCl-soluble sediment ($P = 0.04$) but also in the HCl-insoluble sediment ($P = 0.002$).

Study 3—Daily food and water intakes and urine volume in cats given the diet supplemented either with 0.75% NaCl or 1.5% NH_4Cl were monitored **(Table 4)**.

Table 1—Fractionation of protein in urine* (study 1)

	Protein	
Fraction	**Urine (mg/mL)**	**%**
Whole urine	8.14	100.0
Supernatant	7.91	97.2
Total sediment	0.23	2.8
HCl-soluble sediment	0.07	0.8
HCl-insoluble sediment	0.16	2.0

*Mean values of duplicates.

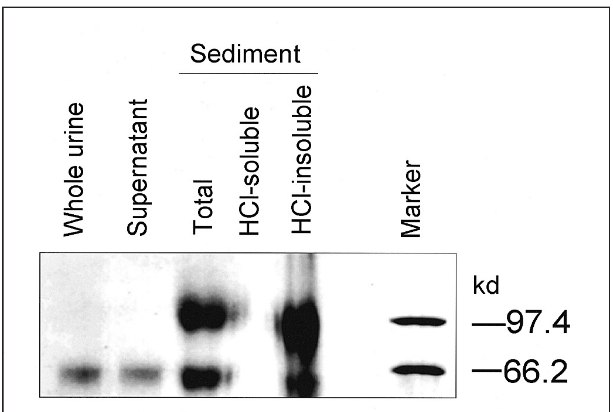

Figure 1—Sodium dodecyl sulfate-polyacrylamide gel electrophoretic profiles of fractionated urine (study 1). Urine was fractionated, and 10 µg of protein from each fraction was loaded on a 12.5% sodium dodecyl sulfate-polyacrylamide gel. Bands were observed after being stained with Coomassie brilliant blue R-250.

Table 2—Mean (± SEM) values of daily food and water intakes and urine volume in 8 cats fed the CP29 or the CP55 diets (study 2)

	Diets		
Variables	**CP29**	**CP55**	***P* value**
Food intake (g/kg of body weight/d)	24.1 ± 2.2	23.1 ± 1.5	NS
Water intake (mL/kg of body weight/d)	51.4 ± 4.3	43.1 ± 3.8	NS
Urine volume (mL/kg of body weight/d)	22.0 ± 1.6	29.1 ± 3.9	NS

CP29 diet = Diet with 28.6% crude protein on a dry matter basis.
CP55 diet = Diet with 54.9% crude protein on a dry matter basis.
NS = No significant ($P < 0.05$) difference between diets.

Table 3—Mean (± SEM) values of urinary variables in 8 cats fed the CP29 or CP55 diet (study 2)

	Diets		
Variables	**CP29**	**CP55**	***P* value**
pH	7.25 ± 0.18	6.63 ± 0.11	0.006
Magnesium (mM)	7.31 ± 0.60	5.75 ± 0.58	0.04
Ammonium ion (mM)	92.6 ± 14.3	168.6 ± 21.4	0.005
Total phosphorus (mM)	40.3 ± 4.4	42.6 ± 4.3	NS
Phosphoric acid ion ($\times 10^{-3}$mM)	5.12 ± 3.47	0.94 ± 0.69	NS
pSAP	9.08 ± 0.24	9.71 ± 0.22	0.02
Struvite crystals (No./µL of urine)	116.2 ± 23.2	32.4 ± 9.2	0.02
Total sediment (mg/mL of urine)	5.79 ± 0.77	3.98 ± 0.40*	0.02
HCl-soluble sediment (mg/mL of urine)	4.78 ± 0.66	3.61 ± 0.37	0.04
HCl-insoluble sediment (mg/mL of urine)	1.01 ± 0.13	0.36 ± 0.04	0.002

*Value affected by rounding.
pSAP = The negative logarithm of the struvite activity product.
See Table 2 for remainder of key.

Daily food intake was comparable among cats fed the control, NaCl supplemented, or NH_4Cl supplemented diets. Daily water intake was significantly higher for cats fed the NaCl supplemented or NH_4Cl supplemented diets, compared with cats fed the control diet, whereas no significant difference in water intake was found between cats fed the NaCl supplemented or NH_4Cl supplemented diets. Urine volume was also significantly higher in cats fed the NaCl supplemented or NH_4Cl supplemented diet, compared with cats fed the control diet. Urine volume was also significantly higher in cats fed the NH_4Cl supplemented diet, compared with cats fed the NaCl supplemented diet.

Urine pH, concentrations of struvite constituents, pSAP, number of struvite crystals, and concentration of sediment (total, HCl-soluble, and HCl-insoluble) in cats given the diet supplemented either with NaCl or NH_4Cl were determined (**Table 5**). Urine pH was significantly lower in cats given the diet supplemented with NH_4Cl, compared with cats fed the control or NaCl supplemented diets. Urine concentrations of Mg^{2+} and total P were significantly lower in cats fed the NaCl supplemented or NH_4Cl supplemented diet, compared with cats fed the control diet. No significant difference in urine concentrations of Mg^{2+} was detected between cats fed the NaCl supplemented or NH_4Cl supplemented diets. Conversely, urine PO_4^{3-} concentration, which was calculated from determined urine pH and total P concentration, was significantly lower in cats fed the NH_4Cl supplemented diet, compared with cats fed the control or NaCl supplemented diet. Although urine NH_4^+ concentration increased in cats fed the NH_4Cl supplemented diet, the magnitude of the increase was much smaller than that of the decrease in the PO_4^{3-} concentration. This resulted in a significant increase in pSAP (ie, a decrease in SAP) in cats fed the NH_4Cl supplemented diet, compared with cats fed the control or NaCl supplemented diets. The number of struvite crystals in the urine significantly decreased in cats fed the NH_4Cl supplemented diet, compared with cats fed the control or NaCl supplemented diet. Compared with cats fed the NaCl supplemented diet, the HCl-soluble sediment in the urine of cats fed the NH_4Cl supplemented diet was lower, although this difference was not significant. Neither NaCl nor NH_4Cl dietary supplementation affected the urine concentration of HCl-insoluble sediment.

Table 4—Mean (± SEM) values of daily food and water intakes and urine volume in 9 cats fed a diet supplemented with either NaCl or NH_4Cl (study 3)

Variables	Diets: Control	NaCl	NH_4Cl
Food intake (g/kg of body weight/d)	13.1 ± 0.9	14.5 ± 0.7	12.6 ± 0.8
Water intake (mL/kg of body weight/d)	28.2 ± 3.0[b]	34.0 ± 4.3[a]	34.9 ± 3.5[a]
Urine volume (mL/kg of body weight/d)	16.6 ± 2.8[c]	20.0 ± 3.5[b]	23.2 ± 3.8[a]

[a,b,c]Superscript letters that differ within a row indicated mean values that are significantly ($P < 0.05$) different among diets.

Table 5—Mean (± SEM) values of urinary variables in 9 cats fed a diet supplemented either with NaCl or with NH_4Cl (study 3)

Variables	Diets: Control	NaCl	NH_4Cl
PH	7.28 ± 0.12[a]	7.35 ± 0.14[a]	6.13 ± 0.08[b]
Magnesium (mM)	5.85 ± 0.60[a]	4.98 ± 0.62[b]	4.83 ± 0.60[b]
Ammonium ion (mM)	153.6 ± 23.2[b]	129.4 ± 16.0[b]	255.4 ± 30.0[a]
Total phosphorus (mM)	48.1 ± 4.7[a]	36.3 ± 4.3[b]	32.3 ± 4.3[b]
Phosphoric acid ion ($\times 10^{-3}$mM)	2.90 ± 0.92[a]	3.74 ± 1.55[a]	0.04 ± 0.02[b]
pSAP	8.81 ± 0.15[b]	9.00 ± 0.25[b]	10.56 ± 0.22[a]
Struvite crystals (No./µL of urine)	82.2 ± 21.6[a]	88.8 ± 33.7[a]	1.6 ± 1.2[b]
Total sediment (mg/mL of urine)	2.93 ± 0.41*	3.30 ± 0.57	2.48 ± 0.49*
HCl-soluble sediment (mg/mL of urine)	1.35 ± 0.35	1.97 ± 0.38	0.98 ± 0.24
HCl-insoluble sediment (mg/mL of urine)	1.59 ± 0.26	1.33 ± 0.28	1.49 ± 0.29

See Tables 3 and 4 for key.

Discussion

Consumption of a urine acidifying diet effectively solubilizes struvite crystals, and recent prevalence of diets controlling urine pH has decreased the occurrence of struvite urolithiasis.[2-5] However, cats still frequently suffer from struvite urolithiasis,[4,5] suggesting that factors other than urine pH affect the formation of struvite uroliths. The THP, a glycoprotein with bands at 95 to 100 kd,[8] has been reported to increase the number of struvite crystals and stimulate crystal growth in vitro.[8] In our first study, a protein band at 95 to 100 kd was only detected from the sediment fraction of urine, although soluble protein at 66 kd was the most abundant in urine (97.2%). Rhodes et al[11] obtained a feline THP-rich fraction from urine sediment after centrifugation, although human THP was originally isolated by salt precipitation (0.58M NaCl).[21] In view of the molecular size of the proteins, bands at 66 kd and 95 to 100 kd were considered to be albumin and THP, respectively.[8] Albumin is soluble and is the major protein in urine at 66 kd.[22]

In our study, the band at 95 to 100 kd was detected in the HCl-insoluble sediment, and no specific bands were detected in the HCl-soluble sediment. These results indicate that THP is fractionated to HCl-insoluble sediment. Considering that the major band of HCl-insoluble sediment migrated at 95 to 100 kd, the substance being measured in the HCl-insoluble sediment is likely to be THP. In view of the extraction procedure, the HCl-soluble sediment would be mainly composed of inorganic compounds insoluble in urine. In fact, the SDS-PAGE profile revealed that no specific bands were detected in the HCl-soluble sediment fraction. In our second study, cats fed the CP55 diet had decreases in urine pH and the concentration of HCl-insoluble sediment. In our third study, however, dietary supplementation with NH_4Cl acidified the urine but did not decrease the urine concentration of HCl-insoluble sediment. These results indicate that high protein intake is desirable for the prevention of struvite crystal formation in clinically normal cats.

Consistent with findings in our previous study,[12] the high-protein diet resulted in an increase in urine volume (Table 1). However, the decrease in the urine concentration of HCl-insoluble sediment in cats given the high-protein diet could not only be a result of an increase in urine volume. In cats fed the CP55 diet, daily quantity of the HCl-insoluble sediment per kilogram of body weight was less than a half of that in cats fed the CP29 diet (10.5 vs 22.2 mg/kg/d). Considering that carnivorous cats originally preyed on small rodents containing 50 to 55% crude protein on a dry matter basis,[23] feeding a high-protein diet to cats may be rather natural, even when a part of the dietary protein is of plant origin. However, protein source of dry cat foods could also affect the urine concentration of HCl-insoluble sediment. In a previous study,[24] urine concentration of this fraction was lower in cats fed a diet containing fish meal as a main protein source, compared with cats fed a diet containing corn gluten meal with the same crude protein content. In our study, the CP55 diet was prepared by increasing the content of fish meal and corn gluten meal. Compared with the CP29 diet, the fish meal and corn gluten meal contents of the CP55 diet were 4.4 and 2.7 times higher, respectively.

Osmotic diuresis generally occurs when reabsorption of water from renal tubules is inhibited by the intratubular presence of substances, such as mannitol and sucrose, that are not reabsorbed.[25] Although glucose and electrolytes can be reabsorbed from renal tubules, even NaCl and glucose could result in osmotic diuresis when a large amount is ingested.[25] In our third study, cats fed the NH_4Cl supplemented diet had an increase in water intake and urine volume, which was reproducibly observed (data not shown). Although the increase in water intake in response to dietary supplementation with 1.5% NH_4Cl was comparable to that caused by dietary supplementation with 0.75% NaCl, urine volume was significantly higher in cats fed the NH_4Cl supplemented diet. This increase in urine volume may be the result of osmotic diuresis caused by electrolytes and urea. The effect of producing less concentrated urine by dietary supplementation with NH_4Cl indicates that this urine acidifier is superior to D,L-methionine, another urine acidifier, because the addition of the latter to diets does not result in an increase in water intake or urine volume.[26]

When SAP is lower than the solubility product of struvite (ie, 1.15×10^{-13}),[27,28] struvite does not crystallize, and preformed struvite crystals dissolve. In turn, when SAP is higher than the formation product of struvite (ie, 2.5×10^{-13}),[29] growth and aggregation of preformed struvite crystals occur spontaneously. The negative logarithms of the solubility and formation products of struvite are 12.9 and 12.6, respectively. Dietary NH_4Cl supplementation and the high-protein diet increased the pSAP in our study, but the value was higher than the negative logarithm of formation product of struvite. Therefore, our results indicate that dietary NH_4Cl supplementation and high-protein intake do not solubilize preformed struvite crystals completely but reduce the extent of oversaturation of struvite, which could contribute to reduction of spontaneous struvite crystallization. However, the number of struvite crystals in the urine was remarkably decreased, not only by dietary NH_4Cl supplementation but also by high protein intake. It is likely that there are inhibiting molecules on struvite crystallization in urine, and these putative molecules would contribute to struvite solubilization in response to decreasing SAP, even within the range of oversaturation.

In our study, we show that high protein intake has preventive effects on formation of struvite crystals in healthy adult cats. Unlike dietary NH_4Cl supplementation, high-protein diet inhibits the formation of struvite crystals through a decrease not only in SAP but also in organic molecules believed to contribute to struvite crystallization. Although feline THP has not been well characterized yet, several reports reveal that human THP affects crystallization of calcium oxalate monohydrate. Monomeric human THP is adsorbed to calcium oxalate monohydrate and inhibits the aggregation of calcium oxalate monohydrate crystals,[30,31] whereas aggregated human THP has a decreased ability to inhibit calcium oxalate monohydrate self-aggregation.[32] These results indicate that human THP may act as inhibitor of calcium oxalate uroliths.

[a]Canet Chip (formulated in April 2000), Petline, Tokyo, Japan.
[b]Mini-Protean II, Bio-Rad Laboratories, Hercules, Calif.
[c]PROC GLM, SAS Institute Inc, Cary, NC.

Appendix

Dietary ingredients and composition of diets

Components	Diets	
	CP29 (control)*	CP55
Ingredients (g/kg of diet)	-	-
Fish meal	50	220
Corn gluten meal	150	400
Soybean meal	140	140
Taurine	1	1
Corn	420	75
Wheat flour	100	100
Beef tallow	65	24
Vitamins and minerals†	20	20
NaCl	8	5
$Ca(PO_4)_2$	26	-
$CaCO_3$	5	-
Flavor‡	15	15
Metabolizable energy (kcal/100 g)	368	384
Moisture (% as fed)	6.4	7.0
Crude protein (% of dry matter)	28.6	54.9
Acid ether extract (% of dry matter)	10.9	7.2
Sodium (% of dry matter)	0.43	0.46
Potassium (% of dry matter)	0.84	0.79
Calcium (% of dry matter)	1.50	1.48
Phosphorus (% of dry matter)	0.99	1.01
Magnesium (% of dry matter)	0.13	0.12
Chloride (% of dry matter)	0.66	0.71
Amino acids (% of dry matter)	-	-
Methionine	0.61	1.36
Cystine	0.41	0.69
Base excess (mmol/kg of dry matter)§	350	205

Dietary base excess of NaCl supplemented diet in study 3 is 350 mmol/kg on a dry matter basis, and that of NH_4Cl supplemented diet in study 3 is 69 mmol/kg on a dry matter basis. *The CP29 diet in study 2 is the same as the control diet in study 3. †One kilogram of the vitamin and mineral mixture contains 22,500 IU of vitamin A, 35 g of vitamin E, 2.2 g of vitamin B_1, 2.3 g of vitamin B_2, 1.6 g of vitamin B_6, 8.5 mg of vitamin B_{12}, 20 g of nicotinic acid, 5 g of panthothenic acid, 22 mg of biotin, 185 g of chlorine, 10 g of inositol, 450 mg of folic acid, 600 mg of Mn, 6.5 g of Fe, 33 mg of Co, 420 mg of Cu, 500 mg of I, and 500 mg of taurine. ‡Spray dried fish extract. §Base excess was calculated as 2[Ca] + 2[Mg] + [Na] + [K] – 2[P] – 2[methionine] – [Cl].

See Table 2 for remainder of key.

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