Effect of Supplementation of Dry Cat Food with D,L-Methionine and Ammonium Chloride on Struvite Activity Product and Sediment in Urine

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ABSTRACT. Feeding dry foods supplemented with urine acidifier (D,L-methionine (Met) or ammonium chloride) decreased urinary pH and struvite activity product in clinically normal cats. As a result, the number of struvite crystals in urine was greatly reduced. Supplementation with 3% Met but not 1% Met caused decrease in the urinary concentration of sediment, which resulted from a reduction in the HCl-soluble fraction. The concentration of HCl-insoluble sediment was not affected by supplementation with the urine acidifier.

KEY WORDS: feline, urinary sediment, urine acidifier.

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Struvite uroliths are frequently seen in cats fed dry foods, when urinary pH exceeds 7.0. Therefore, supplementation of dry food with a urine acidifier such as D,L-methionine (Met) or ammonium chloride (NH_4Cl) has been suggested to prevent struvite uroliths, although an excess of the acidifiers could cause harmful effects such as depressed food intake and metabolic acidosis [4], but urinary pH is not the sole determinant of the occurrence of struvite uroliths. It is known that organic compounds including proteins and glycosaminoglycans could also regulate struvite crystallization as a matrix [3]. Tamm-Horsfall protein, which is glycoprotein at 95–100 kDa, is assumed to be one of the candidates for the matrix [3], and a protein at 95–100 kDa was detected mainly in urinary sediment fraction [1], so that it would be necessary to pay attention to this fraction as a risk factor for uroliths. The present study was conducted to examine the effect of urine acidifier (D, L-Met or NH_4Cl) on the amount of sediment in the urine of clinically normal cats.

A total of 12 adult cats, which were clinically normal on the basis of physical examination, were used. All the cats were cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals [8]. The diet and water were available on an *ad libitum* basis throughout the study. Nutrients found in all diets used in this study exceeded the requirements for the maintenance of mature cats [9]. Food was provided daily at 4 PM. The cats were kept in a temperature-controlled room (24 ± 2°C) with artificial light provided from 6 AM to 6 PM daily.

In experiment 1, the effect of supplemental Met was examined. Six adult cats (mean body weight with standard error, 4.1 ± 0.4 kg) were divided into 3 groups of 2 cats (one male and one female), and fed experimental diets for 2 weeks. They were fed diets supplemented with 0%, 1% or 3% Met to commercial dry food (Table 1). For the last 3 days of the 2nd week of feeding, urine was collected every 24 hr at 4 PM. During this period, first-voided urine after 5 AM was also obtained to measure urinary pH, the number of crystals and the amount of sediment. After immediately measuring the pH of freshly voided urine, sulfuric acid was added to the urine collection bottle to prevent electrolytes from crystallization and to prevent possible loss of ammonia.

Because of the limited number of cats in experiment 1, experiment 2 was conducted to examine the effect of another urinary acidifier, NH_4Cl, in 6 adult cats (two male and four female; mean body weight with standard error, 3.8 ± 0.4 kg). They were used in a 3 week cross-over trial.

Table 1. Dietary ingredients and composition of basal diets

	Experiment 1[a)]	Experiment 2
Ingredients (%)		
Corn gluten meal		15.0
Soybean meal		14.0
Fish meal		5.0
Taurine		0.1
Corn		42.0
Wheat flour		10.0
Beef tallow		6.5
Vitamins and minerals[b)]		2.0
NaCl		0.8
$Ca(PO_4)_2$		2.6
$CaCO_3$		0.5
Flavor[c)]		1.5
Composition (% of dry matter)		
Crude protein	31.2	26.9
Ca	1.86	1.61
P	1.02	0.95
Mg	0.13	0.11

a) Commercial dry cat food containing corn, fish meal, meat meal, wheat flour, rice flour, beef tallow, flavor, vitamins, minerals, and taurine. b) 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 B1, 2.3 g of vitamin B2, 1.6 g of vitamin B6, 8.5 mg of vitamin B12, 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.
c) Spray dried fish extract.

They were fed diets with or without NH_4Cl (1.5% of the diet) (Table 1). The NH_4Cl was supplemented by decreasing corn content. For the last 7 days of each sampling period, urine was collected as described in experiment 1.

The intake of food and water was recorded every day. Urine samples during the sampling period from each cat in experiments 1 and 2 were pooled and stored at –20°C until analysis. The amount of mineral in the diets and urine and urinary total ammonia were measured as described previously [7]. According to Buffington *et al.* [5], the solubility of struvite crystals was determined on the basis of struvite activity product (SAP, $[Mg^{2+}] \times [NH_4^+] \times [PO_4^{3-}]$). In estimating the SAP, Mg and P were assumed to exist as ionic forms and inorganic forms in urine, respectively. In addition, NH_4^+ and PO_4^{3-} concentrations in urine were estimated from urinary pH and total concentrations of ammonia and inorganic P in urine as described previously [7]. For convenience, the SAP is expressed as pSAP, the negative logarithm of SAP, because the SAP value is usually less than 10^{-8}. Preformed crystals are more easily solubilized with decreasing SAP, i.e., increasing pSAP. Crystals in freshly voided urine were counted by light microscopy. The amount of sediment in freshly voided urine was determined; the sediment after centrifugation (12,000 × g for 20 min at 4°C) was air-dried for 24 hr at 45°C, and the resultant sediment was weighed. The sediment was further fractionated by using 1 *N* HCl, and after extraction with 1 *N* HCl for 24 hr at room temperature, the HCl-insoluble sediment was measured as described above. The amount of the HCl-soluble fraction of the urine was determined by subtracting HCl-insoluble sediment from total sediment. Consistent with the results of previous studies [3, 10], protein at 95–100 kDa was detected by SDS-PAGE only from urinary sediment (data not shown). In addition, this protein was further detected in the HCl-insoluble fraction of the sediment but not in the HCl-soluble fraction (data not shown), although it was expected in view of the extraction procedure.

Data in experiment 2 were analyzed by ANOVA, with the General Linear Model procedures [11]. The model included diet, animal and period.

All cats used in every experiment appeared to be healthy and did not manifest any clinical abnormalities throughout the study. Table 2 shows daily intakes and urinary excretion in cats fed diet supplemented Met. Daily food and water intake was similar in all groups at the end of the 2nd week of feeding, although cats in the 3% Met group exhibited a transient decrease in food intake within the first week (data not shown). Fau *et al.* [6] also reported that the addition of 2 to 4% L-Met to cat food resulted in reduced food intake, although adaptation occurred after 10 d except when 4% L-Met was added. In our previous study [2], food intake and body weight decreased but adaptation did not occur when 6% Met was added to the diet. The transient decrease in food intake resulting from an addition of 2 to 3% Met may be due to a Met imbalance rather than Met toxicity, which could occur when more than 4% Met was added to the diet. Urinary pH in the 3% Met group but not in the 1% Met group tended to be lower than that in the control group. The urinary concentrations of Mg^{2+} and total P were not changed by the additional Met, in contrast to an 80–90% increase in the NH_4^+ concentration due to the Met supplementation. The 3% Met supplementation tended to decrease the urinary concentration of PO_4^{3-}, suggesting increased P excretion as titratable acids. As a result, pSAP tended to increase in the 3% Met group. The increase in pSAP, i.e., decrease in SAP, suggests solubilization of struvite crystals [5], and, in fact, the number of struvite crystals remarkably decreased in the 3% Met group.

The urinary sediment concentration tended to be lower in the 3% Met group than in the control group. There was also a tendency for the HCl-soluble fraction in the 3% Met group to be lower than that in the control group, which suggested dissolution of preformed struvite crystals caused by the supplementation with 3% Met, but the urinary concentration of the HCl-insoluble fraction was not affected by dietary Met content. These results suggest that the reduction in the amount of urinary sediment was mainly due to the reduction in the HCl-soluble fraction.

Similar results were obtained in experiment 2, where NH_4Cl was added to the diet as a urine acidifier. Urinary pH was significantly decreased by supplementation with 1.5%

Table 2. Daily intake and urinary excretion of cats fed a diet supplemented with D,L-methionine

Met:	0%	1%	3%	SEM
Food intake (g/d)	62.7	55.6	62.6	15.0
Water intake (m*l*/d)	129.7	105.4	117.4	31.5
Urinary pH	6.82	6.86	6.12	0.35
Urinary concentration				
Mg^{2+} (mM)	7.0	6.8	7.4	2.3
NH_4^+ (mM)	61.3	110.8	116.2	20.5
Total P (mM)	50.0	50.4	36.4	11.4
PO_4^{3-} (μM)	0.60	2.49	0.04	1.39
pSAP	9.71	9.46	10.61	0.46
Struvite crystals (no./μl)	172.5	113.7	2.3	103.0
Total sediment (mg/m*l*)	2.22	2.28	1.43	0.58
HCl-soluble	1.83	2.02	1.00	0.64
HCl-insoluble	0.38	0.23	0.43	0.09

Table 3. Urinary excretion of cats fed a diet supplemented with NH_4Cl

NH_4Cl:	0%	1.5%	SEM	P <
Urinary pH	7.34	6.29	0.06	0.001
Urinary concentration				
Mg^{2+} (mM)	6.9	6.1	4.0	NS
NH_4^+ (mM)	241.6	468.9	23.9	0.001
Total P (mM)	50.2	45.9	4.1	NS
PO_4^{3-} (μM)	5.57	0.22	0.82	0.001
pSAP	8.43	9.65	1.24	0.001
Struvite crystals (no./μl)	447.9	80.0	24.1	0.001
Urinary sediment (mg/m*l*)				
HCl-insoluble	1.38	1.13	0.18	NS

NS: P>0.10.

NH_4Cl, leading to an increase in pSAP mainly resulting from a decrease in the PO_4^{3-} concentration (Table 3). Struvite crystals in urine were greatly reduced by the NH_4Cl supplement, but supplemental NH_4Cl did not affect the urinary concentration of the HCl-insoluble fraction.

The current study showed that supplementation of dry cat food with urine acidifiers such as Met and NH_4Cl decreased urinary pH and SAP, but the supplementation failed to reduce the urinary concentration of organic fraction that may be a candidate for the matrix of struvite uroliths. Therefore, prevention of struvite uroliths by supplementation with urinary acidifiers may not be completely effective.

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