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Factors contributing to the variation in feline urinary oxalate excretion rate[1]

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ABSTRACT: This study aimed to identify factors (season, animal, and diet) contributing to the variation in urinary oxalate (Uox) excretion rate, Uox concentration, and urine volume in healthy adult cats. A data set (1,940 observations) containing information on Uox excretion rate of 65 cats fed 252 diets (i.e., each diet was fed to a group of 6 to 8 cats), with known dietary oxalate concentrations, collected over a 6 yr period at a feline nutrition facility, were retrospectively analyzed. Data related to season, animal (i.e., age, gender, body weight, and breed), and diet (i.e., nutrient content) characteristics were subjected to stepwise multivariate regression analysis to identify factors significantly correlated to Uox excretion rate (μmol/(kg BW$^{0.67}$·d)) and concentration (mmol/L) as well as urine volume (mL/(kg BW$^{0.67}$·d)). Independent factors significantly ($P < 0.05$) associated with lower Uox concentration (mmol/L) included greater ash, Ca, and Na intake and lower nitrogen-free extract, total dietary fiber, P, and oxalate intake, and a body weight <5 kg. Factors significantly associated with lower Uox excretion rate (μmol/(kg BW$^{0.67}$·d)) included greater crude fat and Ca intake and lower CP, total dietary fiber, P, and oxalate intake. However, a considerable part of the variation in Uox excretion rate remained unexplained. The majority of the unexplained variation in Uox excretion rate is likely to be related to factors involved in endogenous oxalate synthesis, as the majority of the dietary factors involved in intestinal oxalate absorption were included in the model. Apparent intestinal oxalate absorption was estimated to be 6.2% on average; however, much variation was present. Future research on Uox excretion rate in cats should focus on the influence of dietary protein sources, amino acid composition, vitamin C (that was not included in the present study), and variations in apparent intestinal oxalate absorption.

Key words: cats, diet, feline, urinary oxalate, urolithiasis

INTRODUCTION

Urolithiasis is the condition where stones are present in the urinary tract, which may lead to clinical signs of hematuria, dysuria, pollakisuria, or even obstruction and kidney failure, depending on the location where stones are formed. Over the past 30 yr, a progressive increase in the prevalence of Ca oxalate (CaOx) urolith with a concomitant decrease in struvite has been reported in domestic cats diagnosed with urolithiasis (Cannon et al., 2007; Picavet et al., 2007; Osborne et al., 2009). Considering the relatively short time frame of this observed increase, nutrition (partly by struvite-preventative diet) has been suggested to be a major factor in the aetiopathogenesis (Dijcker et al., 2011).

In humans, much research has been conducted with the aim to reduce urinary oxalate (**Uox**) excretion rate, either by decreasing intestinal absorption of oxalate by diet (Liebman and Chai, 1997; von Unruh et al., 2004) or probiotics (Sidhu et al., 2001; Hatch et al., 2006) or by preventing endogenous synthesis of oxalate (Holmes et al., 1993; Massey et al., 2005; Knight et al., 2006, 2010). To the authors' knowledge, no study investigating the intestinal absorption and only a few studies investigating endogenous synthesis of oxalate have been reported for cats (Bai et al., 1989, 1991; Zentek and Schulz, 2004; Yu and Gross, 2005; Dijcker et al., 2012b). More effort should be directed towards identifying nutritional and other factors contributing

to Uox excretion rate in cats. This would allow the development of feeding strategies to reduce Uox excretion rate and herewith the risk of urolithiasis in cats.

The retrospective cohort study reported here aimed to identify factors affecting variation in Uox excretion rate, Uox concentration, and urine volume in cats. A large data set including information on Uox excretion, season, animal characteristics (i.e., age, gender, BW, and breed), and diet (i.e., nutrient contents) was subjected to multivariate analysis to identify factors significantly contributing to Uox excretion, Uox concentration, and urine volume in adult cats. Furthermore, the relationship between dietary oxalate intake and oxalate excreted in the urine was determined to assess the apparent intestinal oxalate absorption in cats.

MATERIALS AND METHODS

Data

Urine samples from healthy adult colony cats were quantitatively collected from January 2004 until December 2010 with the aim to estimate the crystal forming potential of diets by determining the relative supersaturation of struvite and CaOx in urine as described by Robertson et al. (2002). Diets (i.e., commercial dry diets) were fed to test groups of 6 to 8 cats. In these feeding trials, cats were adapted to the diet for 9 d followed by a quantitative individual urine collection over 5 d. The cats were fed to maintenance energy requirements (50 kcal ME/(kg BW·d)) and water was available at all times.

The cats were grouped together during the adaptation period but individually housed during the collection period. All the voided urine immediately flowed to the lowest point of an empty litter box, emptying directly into an Erlenmeyer flask to avoid urine stagnancy. There were no preservatives added to the urine before pooling of the urine. The urine flasks were checked several times per day to collect the urine as soon as it was produced. To avoid pooling of contaminated urine, the pH was checked a second time after 2 h, and if a difference in pH >0.25 was found, it was not added to the previously collected urine, as the increase in pH might be the result of bacterial growth. Urine pH, density, and weight (to calculate the volume) were measured for all the samples collected throughout the day. The pH was measured with a calibrated pH meter. For each cat, all the samples collected over 5 d were pooled and stored at 4°C in an individual bottle containing 1 mL of 20% chlorhexidine (Hibitane; Mölnlycke Health Care, Gothenburg, Sweden). The use of chlorhexidine as a preservative as well as storage for up to 4 yr at –20°C was previously validated (V. Biourge, unpublished data) to ensure that oxalate concentration as well as the struvite and CaOx relative supersaturation was

unaffected by this method. At the end of the urine collection period, the pooled sample was well mixed and the total volume, density, and pH recorded. An aliquot was taken, titrated to pH 2.0 with 37% hydrochloric acid, and immediately analyzed or kept at –20°C pending analysis.

Data recorded in the feeding trials included date of experiment, animal characteristics (age, gender, breed, and BW), dietary nutrient composition (DM [%] and g/100 kcal ME of ash, CP, crude fat, total dietary fiber [**TDF**], nitrogen-free extract [**NFE**], Ca, P, Na, and oxalate), food and water intake, and urine parameters (pH, density, volume, and oxalate concentration). The data set was examined to ensure that data from cats over 1 yr of age was included, data pertained to cats with a food intake of ≥25 kcal/(kg BW·d), and that data lines (containing all observations per cat within a test) were complete.

Chemical Analysis

The diets were analyzed for DM and ash by drying to a constant weight at 103°C and combustion at 550°C, respectively. The CP (International Organization for Standardization [ISO], 2008), crude fat (ISO, 1999), total dietary fiber (AOAC, 1995), Ca and Na (ISO, 2000), and P (EEC, 1971) were determined. Nitrogen-free extract (g/100 g DM) was calculated as 100 – CP – crude fat – ash – TDF (in g/100 g DM). Metabolizable energy was calculated using the cat equation of the NRC for fiber (NRC, 2006).

Urine samples were analyzed for density (Anton Paar DMA 35, Graz, Austria), pH (Mettler Toledo SevenEasy, Port Melbourne, Australia), and oxalate. Urinary oxalate concentration was determined by ionic chromatography (Dionex, Port Melbourne, Australia) as described by Markwell et al. (1999). This method was estimated to have a method variability of 5.5 and 8.3% (calculated from 2 control samples with mean values of 0.97 and 2.34 mmol/L after 282 and 90 repeated measurements, respectively).

Statistical Analysis

In the current data set, the frequency at which a test group was used differed as well as the cats included in each test group, resulting in individual cats being used from 1 to 95 times in the present data set. In the descriptive statistics and regression analyses, each data line was treated as an independent observation.

Descriptive statistics of the data were performed using SAS (version 9.2 for Windows; SAS Inst. Inc, Cary, NC). Box-and-whisker plots were compiled using R (version 2.13.1; The R Foundation for Statistical Computing, Vienna, Austria) while other plots were compiled using Microsoft Excel (version 2003 for Windows; Microsoft Corp., Redmond, WA). Cats that were fed the

Table 1. Diet composition of the 252 dry diets tested on 65 cats[1] over a 6 yr period

Diet component	Mean	SEM	Minimum	Maximum
Energy, kcal/100 g diet[2]	400.00	0.65	282.30	474.50
DM, g/100 g diet	93.51	0.03	90.50	97.00
CP, g/100 kcal	8.38	0.03	4.20	12.13
Crude fat, g/100 kcal	3.81	0.02	1.66	5.58
Nitrogen-free extract, g/100 kcal	7.21	0.03	1.59	11.81
Total dietary fiber, g/100 kcal	2.40	0.03	0.53	11.41
Ash, g/100 kcal	1.69	0.007	0.86	2.71
Ca, g/100 kcal	0.25	0.001	0.09	0.47
P, g/100 kcal	0.22	0.001	0.07	0.37
Na, g/100 kcal	0.15	0.002	0.05	0.36
Oxalate, g/100 kcal	0.011	0.0002	0.002	0.049

[1]Each diet was fed to a test group of 6 to 8 cats, which consequently resulted in 6 to 8 observations per diet. A number of test groups were used more than once.

[2]Calculated by the following equations: GE (kcal) = $(5.7 \times$ g protein) + $(9.4 \times$ g fat) + $(4 \times$ g nitrogen-free extract + g fiber); percentage energy digestibility = $87.9 - (0.99 \times$ percentage crude fiber in DM); DE (kcal) = (GE × percentage energy digestibility/100); ME (kcal) = DE − (0.77 − g protein).

Table 2. Urine measurements of 65 cats[1] fed in total 252 dry diets (i.e., 1,940 observations) over a 6 yr period

Urine component (or parameter)	Mean	SEM	Minimum	Maximum
Volume, mL/(kg $BW^{0.67}$·d)	23.24	0.23	4.24	83.32
Density, g/mL	1.062	0.0003	1.017	1.108
pH	6.34	0.008	5.52	7.81
Oxalate, mmol/L	1.79	0.02	0.35	7.46
Oxalate, μmol/(kg $BW^{0.67}$·d)	38.54	0.40	5.60	158.97

[1]Each diet was fed to a test group of 6 to 8 cats, which consequently resulted in 6 to 8 observations per diet. A number of test groups were used more than once.

root mean squared error and by plotting the regression-predicted values against the measured values.
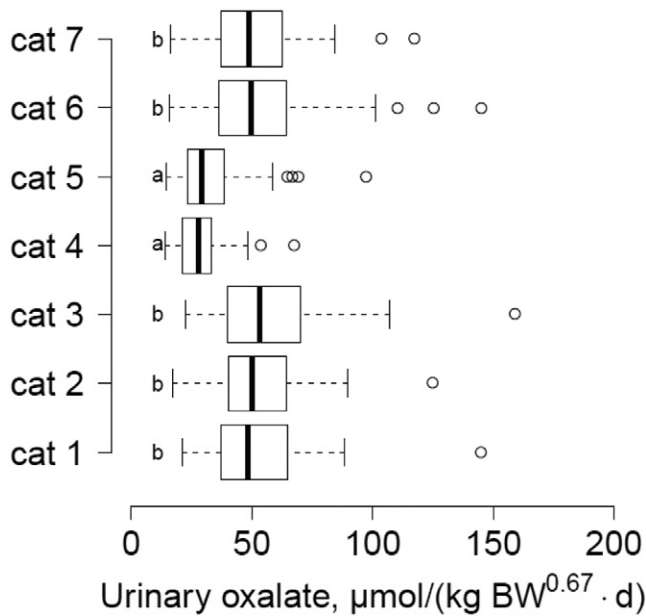
RESULTS

After applying exclusion criteria, the final data set contained 1,940 data lines (hereafter referred to as observations) from a total of 65 cats fed 252 balanced and complete dry extruded diets. Most of the data lines were excluded due to 1 or more missing values with 10 data lines excluded due to a food intake of <25 kcal/(kg BW·d). The mean ± SEM age and BW of the cats in the data set was 5.76 ± 0.05 yr and 4.72 ± 0.03 kg, respectively. Thirty-seven percent of the cats were females of which 37% were spayed while all the male cats were neutered. The cats belonged to the following breeds (number of cats and number of data lines): Japanese Bobtail (6 and 450), Chartreux (12 and 703), domestic shorthair (31 and 713), Bengal (1 and 59), Exotic shorthair (4 and 4), Maine Coon (8 and 8), and Persian (3 and 3). The composition of the tested diets and the associated urine measurements are reported in Tables 1 and 2, respectively.

Urinary oxalate excretion rates from each individual cat of the test group with the greatest number of observations (from 50 diets) are displayed in box-and-whisker plots (Fig. 1). Cats 4 and 5 showed significantly lower Uox excretion rates compared with the other 5 cats in the group ($P < 0.05$). These cats both had a significantly ($P < 0.0004$) lower caloric intake (i.e., 89.7 and 89.4 kcal/(kg $BW^{0.67}$·d), respectively) compared with the other cats in the group (i.e., 110.1 to 128.8 kcal/(kg $BW^{0.67}$·d)) during the tests. Body weight of cats 4 and 5 were not different from the other cats in the group.

To obtain an estimate of the variation for Uox excretion rate within a cat regardless of diet, observations of 22 cats fed the same diet twice were used to calculate the precision error and yielded a mean ± SEM of 14.3 ± 2.36%.

The regression coefficients and SE of the independent variables contributing significantly to the $\log_{10}$ transformed Uox excretion rate, $\log_{10}$ Uox concentration, and $\log_{10}$ urine volume (dependent variables) are reported in Table 3. Decreased Uox excretion rate (μmol/[kg $BW^{0.67}$·d]) was

same diet twice were used to calculate the relative prediction error of the determined Uox excretion rate (%) by (SD/mean) × 100.

To test differences in measured Uox excretion rate (μmol/(kg $BW^{0.67}$·d)) and caloric intake (kcal/(kg $BW^{0.67}$·d)) among 7 cats in 1 test group all fed 50 dry diets, ANOVA was performed using the GLM procedure in SAS. Within the model, differences among cats were compared by the Bonferroni t test.

To describe the relationship between Uox excretion rate and dietary oxalate intake as well as Uox excretion rate and molar dietary Ca to oxalate ratio, ANOVA was performed using the REG procedure of SAS.

Analysis of covariance in the MIXED procedure of SAS was used to identify whether season, animal-related factors, or diet components (independent variables) were associated with changes in Uox excretion rate, Uox concentration, and urine volume (dependent variables). Using the backward stepwise approach and Akaike's information criterion, the best model explaining the variance of each dependent variable was obtained. The dependent variables were transformed by $\log_{10}$ to attain normal distribution of the residuals. Residuals with >2 SD values were studied individually and excluded from data analysis when affecting the outcome. In this analysis, cat was treated as a random effect and the following factors as fixed effect: age (≤7 yr and >7 yr), gender (male castrated, female intact, or female spayed), breed (Bobtail, Chartreux, and cluster of the breeds including European shorthair, Bengal, Maine Coon, and Persians), BW (≤5 or >5 kg), and intake (in g/(kg $BW^{0.67}$·d)) of CP, crude fat, NFE, TDF, ash, Ca, P, Na, and oxalate. Assessment of the accuracy of the model was conducted by calculation of the

Figure 1. Box-and-whisker plots showing the urinary oxalate excretion rate of 7 cats in a test group fed 50 different dry diets. The box represents the lower quartile, median, and upper quartile, the whiskers the 95% confidence interval, and the circles the extreme values. The characteristics of the cats were cat 1) Bengal, male castrated, 4.1 yr, and 4.7 kg; cat 2) Domestic shorthair, male castrated, 4.0 yr, and 4.3 kg; cat 3) Domestic shorthair, female intact, 4.0 yr, and 3.4 kg; cat 4) Domestic shorthair, male castrated, 4.1 yr, and 4.9 kg; cat 5) Domestic shorthair, male castrated, 4.1 yr, and 4.1 kg; cat 6) Domestic shorthair, female intact (40 observations)/female spayed (10 observations), 4.0 yr, and 3.4 kg; and cat 7) Domestic shorthair, female spayed, 3.8 yr, and 3.7 kg. [a,b]Means with different superscripts are significantly different ($P < 0.05$).

associated with having a low intake of CP, TDF, P, and oxalate and relatively high intake of crude fat and Ca. The magnitude of change of the $\log_{10}$ oxalate excretion by dietary CP intake (calculated from data in Table 3 as (18.51–2.28) × 0.018) is 0.292 μmol/(kg BW$^{0.67}$·d). The magnitude of change of the other components in decreasing order of appearance was Ca, –0.342; oxalate, 0.318; P, 0.321; TDF, 0.235; and crude fat, –0.099. Decreased Uox concentration (mmol/L) was associated with cats having a BW <5 kg, a low intake of NFE, TDF, P, and oxalate, and a relatively high intake of ash, Ca, and Na. Decreased urine volume (mL/(kg BW$^{0.67}$·d)) was associated with urine collection during the summer period, having a low CP, ash, and Na intake and a relatively high TDF, P, and oxalate intake. The relationship between the predicted (using the associated factors as provided in Table 3) and the measured $\log_{10}$ transformed Uox excretion rates, concentrations, and urine volumes are illustrated in Fig. 2.

To obtain more insight into the relationship between Uox excretion rate (y) and dietary oxalate intake (x; Fig. 3), univariate linear regression analysis was performed using the entire data set (1,940 observations). This resulted in a significant equation with an intercept of 2.755 mg/(kg BW$^{0.67}$·d) (SE = 0.061; $P < 0.0001$) and a regression coefficient of 0.062 mg/(kg BW$^{0.67}$·d) (SE = 0.004; $P < 0.0001$) and R^2 of 0.093.

The molar Ca to oxalate ratio of the tested diets ranged from 9 to 333 mol/mol (Fig. 4). After conducting univariate linear regression of Uox excretion rate (y) and dietary molar Ca to oxalate ratio (x), a significant equation with a R^2 of 0.011 was obtained. The intercept of this equation was estimated to be 41.157 mmol/mol (SE = 0.694; $P < 0.0001$) and the regression coefficient –0.039 mmol/mol (SE = 0.008; $P < 0.0001$).

DISCUSSION

The aims of this study were to identify factors contributing significantly to the variation in Uox excretion rate, Uox concentration, and urine volume in healthy adult cats and to estimate the contribution of dietary and endogenous oxalates to Uox excretion rate. Such information may be valuable for the development of new strategies to reduce Uox excretion rate by cats.

Urinary oxalate excretion rate showed a wide range for the 65 cats fed 252 dry diets (Table 2) but also for the 7 individual cats all fed 50 different dry diets (Fig. 1). This variation in Uox excretion rate may be explained by animal-related factors but also by external factors such as environment (i.e., season) and nutrition. Animal-related factors tested in this study, such as age, gender, and breed, were not associated with differences in Uox excretion rate (Table 3; Fig. 1). These findings were in agreement with the results of a study with privately owned cats using spot urine sampling (Dijcker et al., 2012a). Season as external factor was also not associated to changes in Uox excretion rate (Table 3). The reproducibility error (i.e., the relative precision error calculated for Uox excretion rate by cats fed the same diet only twice) was 14.3%, which could be caused by methodological errors such as incomplete urine collection and oxalate analysis (relative precision error of 5.5 and 8.3%) as well as a potential effect of time between tests, BCS, activity level of the cats, stress, or individual variation in apparent oxalate absorption, metabolism, or excretion.

Urinary oxalate excretion rate differed significantly among cats receiving the same diet and housed under the same conditions (Fig. 1). There was no influence of season on the Uox excretion rate of these cats as diets were fed to the 7 cats at the same time. The significantly lower Uox excretion rates found in 2 cats (i.e., cats 4 and 5) compared with the remaining 5 cats may be explained by a significantly lower caloric intake (89.7 and 89.4 vs. 110.1 to 128.8 kcal/(kg BW$^{0.67}$·d), respectively). Lower intake would lead not only to lower dietary oxalate intake but also to lower intakes of dietary components potentially affecting endogenous oxalate synthesis. Caloric and/or nutrient intakes may therefore be important factors in Uox excretion rate.

Table 3. Significant ($P < 0.05$) linear regression coefficients and SE for season, animal-related factors, and intake of dietary components on urinary oxalate excretion rate, concentration, and urine volume from urine samples of 65 cats fed 252 diets[1,2]

	Class	n	Log_{10}(oxalate) excretion rate, μmol/(kg BW$^{0.67}$·d)	Log_{10}(oxalate) concentration, mmol/L	Log_{10}(volume), mL/(kg BW$^{0.67}$·d)
Intercept ± SE			1.31 ± 0.02	0.22 ± 0.03	1.06 ± 0.03
Season[3]	Summer	1,173	–	–	–0.01 ± 0.006
	Winter	767	–	–	0
Body weight	>5 kg	638	–[4]	0.04 ± 0.01	–[4]
	≤5 kg	1,302	–[4]	0	–[4]
Component[5]	Minimum	Maximum			
CP	2.28	18.51	0.018 ± 0.003	–	0.017 ± 0.002
Crude fat	0.92	8.93	–0.009 ± 0.003	–	–0.010 ± 0.003
NFE[6]	1.03	18.49	–	0.005 ± 0.002	–
TDF[7]	0.33	11.54	0.020 ± 0.004	0.031 ± 0.003	–0.009 ± 0.003
Ash	0.49	4.45	–	–0.10 ± 0.02	0.09 ± 0.02
Ca	0.06	0.67	–0.56 ± 0.08	–0.74 ± 0.11	0.20 ± 0.10
P	0.06	0.49	0.74 ± 0.10	0.94 ± 0.13	–0.08 ± 0.12
Ca × P			–	0.93 ± 0.34	–1.04 ± 0.30
Na	0.03	0.72	–	–0.90 ± 0.06	0.79 ± 0.06
Oxalate	0.001	0.081	3.94 ± 0.48	5.11 ± 0.42	–1.05 ± 0.38
RMSE[8]			0.15	0.13	0.11

[1]Urinary oxalate excretion rate, oxalate concentration, and urine volume were log_{10} transformed.

[2]The effects of age, gender, and breed were considered but found to not be a significant source of variation.

[3]Summer (April to October); Winter (November to March).

[4]Body weight not included in the model.

[5]The intake of dietary components in g/(kg BW0.67·d).
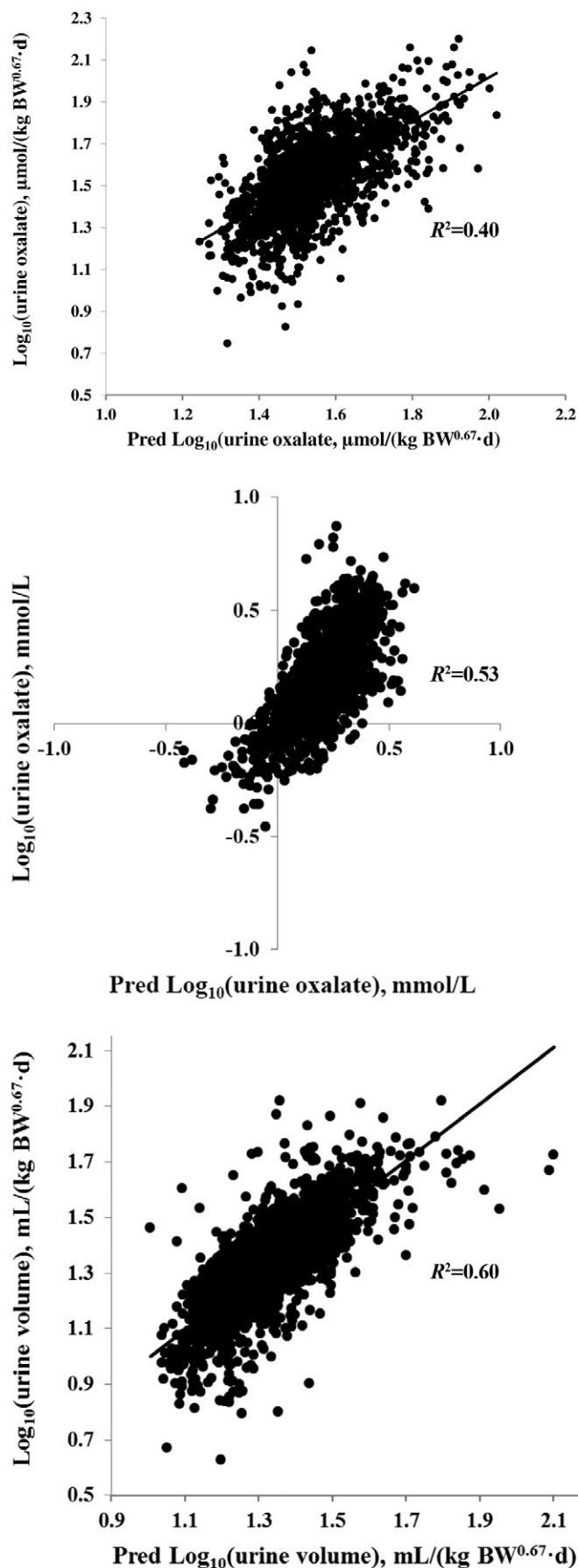
[6]NFE = nitrogen-free extract.

[7]TDF = total dietary fiber.

[8]RMSE = root mean squared error.

By means of multivariate linear regression analyses, intakes of several dietary components (i.e., CP, crude fat, Ca, P, TDF, and oxalate) were found to be significantly related to changes in Uox excretion rate (Table 3). Calcium × P interactions were included in the analysis to compensate for possible correlated effects between these dietary factors. The intake of CP was associated with an increased Uox excretion rate. This association may not be related to the protein content itself because 2 randomized controlled trials reported no effect of diets differing in protein content (and having a similar oxalate concentration) on Uox excretion rate in adult cats (Bai et al., 1991; Dijcker et al., 2012b). However, Uox excretion rate by adult cats has been reported to be affected by diets differing in protein sources, that is, collagen tissue, soy isolate, and horse meat (Zentek and Schulz, 2004). The effect of protein sources on Uox excretion rate may be explained by the presence of amino acids, such as hydroxyproline, serine, and glycine, which can be catabolized in part to endogenous oxalate (Gambardella and Richardson, 1977; Ribaya and Gershoff, 1979, 1981; Takayama et al., 2003). Therefore, the lack of response to CP intake in the above studies (Bai et al., 1991; Dijcker et al., 2012b) may be due to the use of casein, a protein source devoid of hydroxyproline. Dietary crude fat content was weakly and negatively associated with Uox excretion rate. This result is difficult to explain but could be related to the positive association of CP with Uox excretion rate as diets with a greater protein content would be lower in either fat or NFE.

The association of Uox excretion rate with dietary intakes of Ca, P, TDF, and oxalate may be related to the exogenous contribution to Uox. A lower dietary Ca content can be expected to bind less oxalate in the intestinal tract making more oxalate available for absorption (Liebman and Chai, 1997; von Unruh et al., 2004). As P is one of the nutrients able to bind intestinal Ca, a reduction of the potential for Ca to bind oxalate may enhance intestinal oxalate absorption. As Ca × P interaction had a significant positive association with Uox concentration, it may indeed be valid to state that a possible effect of P comes forth from its Ca-binding capacity in the gut. However, much variation was observed in Uox excretion rate depending on the dietary molar Ca to oxalate ratio (Fig. 4) and diets with a moderate instead of low molar Ca to oxalate ratio gave the greatest Uox excretion rates. This lack of relationship ($r^2 = 0.011$, result of univariate analyses) may be due to the relatively great Ca and P content in pet diets compared with human diets. The positive association of TDF with Uox excretion rate may be due to the binding of intestinal Ca and thereby enhance the absorption of oxalate. In addition, fiber may also delay intestinal transit time through the small intestine, which may enhance the absorption processes.
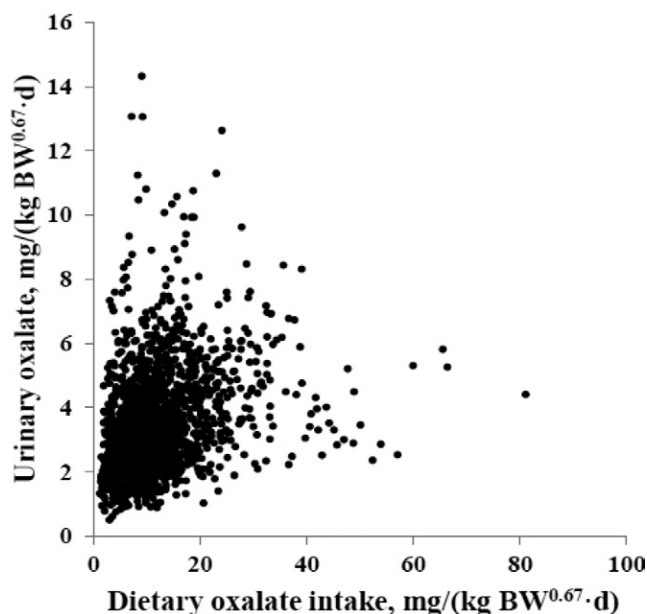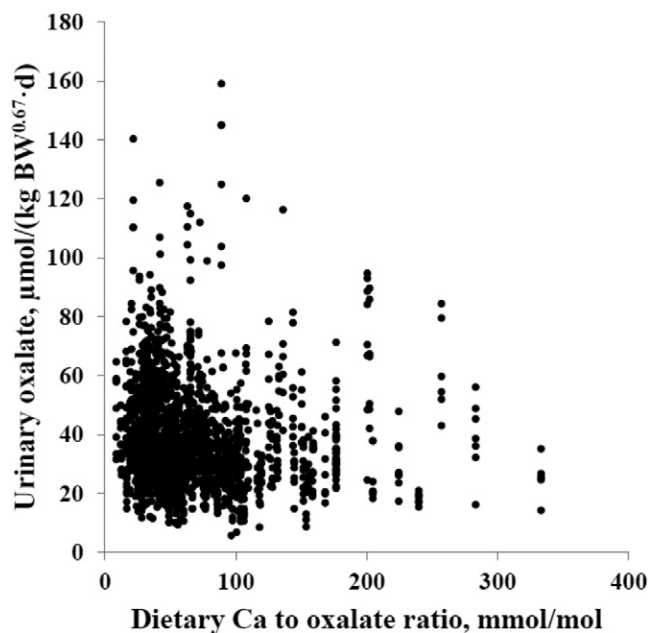
Figure 3. Relationship between dietary oxalate intake and urinary oxalate excretion rate in cats (1,940 observations).

Increased oxalate intake results in greater amounts of oxalate available for absorption and would explain the association of Uox excretion rate and dietary oxalate intake (Table 3). Dietary oxalate can be absorbed via passive diffusion in the stomach and intestines in humans while active transport may occur in the distal parts of the intestines (Hatch and Freel, 2005). To the authors' knowledge, no information is available in the literature on intestinal oxalate absorption in cats. Although not directly determined, in dogs, oxalate absorption appears to be low (Stevenson et al., 2003). In humans, intestinal absorption percentage has been reported to decrease from 55 to 6% with increasing oxalate intake ranging from 2 to 10 mg/(kg $BW^{0.75} \cdot d$; Holmes et al., 2001). The current data set showed that the cats had a mean ($\pm$SEM) oxalate intake of 10 ± 0.2 mg/(kg $BW^{0.75} \cdot d$). This intake corresponds to the greatest level of oxalate intake by humans and resulted in an observed apparent intestinal oxalate absorption of 6%. It can, therefore, be expected that the apparent intestinal oxalate absorption in cats would be in the lower range of that reported in humans (4–16%; Holmes and Assimos, 2004) and similar to dogs (Stevenson et al., 2003). In the present study, the regression coefficient obtained from the univariate regression analysis of Uox excretion rate and dietary oxalate intake was 0.062, indicating a mean apparent intestinal oxalate absorption percentage of 6.2 for cats. However, much variation was observed in Uox excretion rate depending on the dietary oxalate intake (Fig. 3).

In humans it has been estimated that 25 to 68% of the Uox excretion originates from dietary intake (Holmes et al., 2001; von Unruh et al., 2003). In cats, no such data is available. Because the data in the present study shows

Figure 2. Relationship between log_{10} transformed predicted (Pred) values and urinary oxalate excretion rates (A), oxalate concentrations (B), and urine volumes (C) from urine samples of 65 cats fed 252 diets (i.e., 1,940 observations).

Figure 4. Relationship between the molar dietary Ca to oxalate ratio of 252 diets and urinary oxalate excretion rate from 65 cats (1,940 observations).

a great degree of variation in Uox excretion rate, with a considerable part of the variation remaining unexplained after multivariate regression analyses, it is difficult to assess the contribution of exogenous and endogenous oxalates to Uox. Because the majority of known exogenous oxalate-related dietary factors (i.e., Ca, P, fiber, and oxalate) were included in the model (Table 3), it might be suggested that the majority of the unexplained variation in Uox excretion rate are related to factors involved in endogenous oxalate synthesis. As discussed earlier, endogenous oxalate may be formed from amino acids such as hydroxyproline, serine, and glycine. In humans, endogenous oxalate may also be synthesized from glucose and fructose (Nguyen et al., 1989, 1995, 1998). As a randomized controlled trial with cats fed diets containing sucrose and starch was not able to affect Uox excretion rate (Dijcker et al., 2012b), it is unlikely that these components would affect endogenous oxalate synthesis. In humans, oxalates can also be synthesized from dietary ascorbic acid, or vitamin C (Massey et al., 2005; Robitaille et al., 2009). In cats, dietary vitamin C supplementation up to 193 mg/kg did not affect Uox excretion rate (Yu and Gross, 2005); however, supplementations with greater concentrations of vitamin C showed an increase in Uox excretion rate (V. Biourge, unpublished data). Increased endogenous oxalate synthesis has also been reported in cats fed diets deficient in pyridoxine, or vitamin B6 (Bai et al., 1989, 1991). It is unlikely that the tested diets (Table 1) contained insufficient levels of vitamin B6. Therefore, protein sources, or their amino acid profiles, and vitamin C are the most likely cause of the unexplained variation of Uox excretion rate. Future studies are required to determine the absorp-

tion rate of dietary oxalates and thereby indirectly the rate of endogenous oxalate synthesis.

Reduction in Uox excretion rate is important to reduce the risk of CaOx urolithiasis. However, it may be even more important to reduce Uox concentration (mmol/L). As expected, increased Na intake was positively associated with both an increased urine volume and a decreased Uox concentration (Table 3). This effect can be explained by the increased diuresis induced by the increase in tubular excretion of Na and water (Hawthorne and Markwell, 2004). The majority of nutrients associated with Uox excretion rate (i.e., TDF, Ca, P, and oxalate) and urine volume (TDF, ash, P, Na, and oxalate) were also found to be associated with changes in Uox concentration, indicating involvement of both urine volume and Uox excretion rate.

One factor that might have been interesting to be included in the model was the acidifying potential of the diets that were fed. However, due to lacking data on K, Cl, and S content of the diets, it was not possible to estimate this factor for the diets. Interestingly, urine pH showed a significant ($P < 0.0001$) negative ($r = -0.168$) correlation with Uox excretion rate and a nonsignificant ($P < 0.05$) positive ($r = 0.056$) correlation with Uox concentration (mmol/L). However, as various dietary factors both influence Uox excretion rate and urinary pH, it is likely that this correlation comes forth from the effect of dietary factors rather than a direct effect of urine pH on Uox excretion.

In the present study, the identified factors contributing to changes in Uox excretion rate were nutrients both related to apparent intestinal oxalate absorption and endogenous oxalate synthesis. However, a considerable part of the variation in Uox excretion rate remained unexplained. Because the majority of the dietary factors involved in apparent intestinal oxalate absorption were included in our model, it might be suggested that the majority of the unexplained variation in Uox excretion rate is related to factors involved in endogenous oxalate synthesis. Apparent intestinal oxalate absorption was estimated to be 6.2% on average; however, wide variation was present. Future research on Uox excretion rate in cats should focus on the influence of protein sources and their associated amino acid profile and variations in intestinal oxalate absorption.

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