Urinary pH in Cats: Evaluating a Minimally Invasive Method for Testing the Efficacy of Feeds and Supplements Formulated to Modify Urinary Acidity

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Master's Thesis

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Summary

Urolithiasis, or the presence of calculi in the urinary tract, is one of the main causes of feline lower urinary tract disease. These calculi may form when urine is oversaturated with specific calculogenic crystalloids. Urinary pH seems to influence solubility of these crystalloids. A favourable urinary acidity may prevent, or in some cases even reverse, crystal formation. In response, many prescription diets and dietary supplements have been formulated to affect urinary pH, but studies proving the efficacy of these products often are not available.
The aim of this pilot study is to test a minimally invasive method for evaluating the effect of oral supplements and prescription diets on urinary pH in the cat. Eight cats were given six subsequent interventions: dietary supplements potassium citrate, Urical, Vétoquinol urinary paste and ascorbic acid, and prescription diets Royal Canin S/O and Hill's W/D. Spontaneously voided urinary samples were collected using modified litter boxes, and urinary pH was measured using both a benchtop pH meter and commercial test strips.
Unfortunately, the current study design proved to be inadequate to test the efficacy of interventions. Urinary pH showed large variations between and within individual cats, likely due a relatively small amount of collected samples and a lack of consistency in the time of voiding. For future research, we strongly recommend creating day curves for urinary pH using a more reliable sample collection method, to allow for a more detailed comparison of changes in pH value over the day with or without interventions.

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Introduction

Urolithiasis and crystalluria in the domestic cat: a definition

Uroliths, or calculi present in the urinary tract, are mineral concretions typically accompanied by a small amount of organic matrix. Such mineral concretions may be formed when the urine is oversaturated with the specific components a given urolith consists of. The constituents initially form microcrystals, a sign also called crystalluria. The crystals may grow or aggregate, eventually developing into mature urinary stones (Forrester et al., 2010).

Urolithiasis is a common cause of feline lower urinary tract disease (FLUTD). In discussing epidemiologic data, Forrester et al. reveal that in 1995 the prevalence of FLUDT in 15.226 cats presented to veterinary hospitals in the USA was found to be 3.0%. Lekcharoensuk et al. found a FLUDT prevalence of 8%. In 10-44% of these cats, urolithiasis was identified as a cause for the FLUDT. It must be noted that in this study, cats with urethral plugs were included in the total number of cats classed to have uroliths. Forrester et al. report the occurrence of uroliths among cats with FLUDT to be 15-23%. A 2014 retrospective study of 302 cats diagnosed with FLUDT presented to a veterinary hospital between 2000 and 2007 reports that urolithiasis was diagnosed in 7.0% of cases (Dorsch, et al., 2014)(Forrester et al., 2010)(Lekcharoensuk et al., 2001).

Clinical signs of urolithiasis are related to the size, quantity and location of the calculi. Signs can be non-specific, typically including hematuria, dysuria or stranguria. If nephroliths are present, symptoms such as vomiting, anorexia and apathy can also be observed. The presence of uroliths in the bladder may lead to partial or complete urethral obstruction, particularly in the male cat, whose urethra is both longer and more narrow. Some animals remain asymptomatic. (da Rosa Gomez et al., 2018)(Lekcharoensuk et al., 2001).

Calculi can be present in all parts of the urinary tract and are named accordingly: nephroliths in the kidney, ureteroliths in the ureters, urocystoliths in the bladder and finally urethroliths in the urethra. It is assumed that calculus formation occurs in the kidneys or bladder, and from there are able to flow down to the ureters or urethra (da Rosa Gomez et al., 2018).

Urolith composition

In feline urolithiasis, typically one type of mineral is predominantly present. Stones may also consist of different minerals, either mixed throughout or compounded in separate layers. Studies conducted on data available from veterinary urolith analysis centres find that the relative incidence of different stone types has shifted over recent decades. However, two types of uroliths remain consistently at the top: struvite and calcium oxalate, together comprising more than 90% of uroliths committed to a Canadian urolith centre in a recent study by Houston et al. Struvite (magnesium ammonium phosphate hexahydrate ($Mg^{2+}NH^{4+}PO_4^{3-}\cdot 6H_2O$)) and calcium oxalate uroliths will be the focus of further discussion in this paper. The third most common urolith contains urates. Other much less common uroliths are comprised of cysteine, xanthine, calcium phosphate, silica and sodium pyrophosphate (Houston et al., 2016)(Houston and Moore, 2009).

Analyses of data from urolith centres that type many thousands of urinary stones indicate a decrease in the relative amount of struvite stones, and an increase in calcium oxalate uroliths. Forrester et al. report that in the Minnesota Urolith Centre in 1981, 78% of feline uroliths were classed as struvite and 2% were classed as calcium oxalate. Between 1994 to 2001 however, calcium oxalate made up 55% of cases and struvite only 33%. From 2001 onwards the Minnesota Urolith Centre has been seeing more struvite and less calcium oxalate stones again. A similar shift was observed in uroliths of Benelux cats: in 1994 77% of uroliths were found to be struvite, and 12% calcium oxalate; by 2003 struvite had decreased to 32% and calcium oxalate had increased to 61%. Studies conducted at the Canadian Veterinary Urolith Centre found a similar trend to that in Benelux cats (Picavet et al., 2007)(Forrester et al., 2010)(Houston et al., 2016).

It is important to note that data from urolith centres are not necessarily an accurate estimate of the incidence of uroliths in feline patients, as not all cats are diagnosed or treated, not all stones need to be surgically removed and if they do, some types of stones may be more likely to be submitted than others. For example, struvite uroliths can be diagnosed relatively easily using radiography, and the availability of diets that facilitate the dissolution of struvite uroliths may make them more medically manageable and thus less likely to be submitted for typing (Forrester et al., 2010)(Houston et al., 2016).

Urinary relative supersaturation (RSS) and activity product ratio (APR)

Several factors contribute to urolith formation, most notably (1) urinary oversaturation with the specific calculogenic crystalloids, potentially resulting from increased renal excretion of these components or from increased urinary concentration, and (2) decreased solubility of these calculogenic crystalloids, as may be caused by changes in urinary pH. Additionally, the presence of inhibitors or promoters of crystallization influences the process. These inhibitors or promoters may be ions other than the ones that constitute the urolith, or large-molecular-weight proteins. The precise role of these influencers in cats is, as of now, poorly understood (Forrester et al., 2010)(Bartges and Callens, 2015).

A risk index of crystallization based on mineral saturation often used in research is relative supersaturation (RSS). In order to calculate RSS, urine pH and the concentration of specific solutes are measured in the urine sample and entered into a computer algorithm. Urinary RSS may be classed as (1) undersaturation, a situation in which crystal dissolution may take place, (2) metastable supersaturation, a state in which no spontaneous crystal formation or dissolution occurs, and (3) labile supersaturation, a state in which spontaneous crystal formation and growth may occur (Queau, 2019). Calculating RSS does not take into account the presence of crystallization promoters or inhibitors. Another, more comprehensive, predictive method is called activity product ratio (APR). APR involves incubating urine with a seeding crystal. Urinary analytes are measured before and after incubation. Comparing analyte concentrations before and after incubation potentially approximates the in vivo effect more closely, as the effect of crystal growth inhibitors or promoters, even if not specifically studied, is part of the measured effect (Forrester et al., 2010).

Decreasing urinary RSS

Many studies have been conducted on interventions aimed at lowering RSS of particular stone types. Particular focal points have been the effects of water intake, diet composition and urinary pH.

Water intake

Increased water uptake may result in a decrease in urinary mineral concentration, thus decreasing the risk of precipitation of minerals uroliths consist of. Furthermore, subsequent enhanced diuresis may lead to more frequent micturition, decreasing time for crystal aggregation to take place. Water intake can be increased effectively by feeding a wet, canned food that should have a moisture content of at least 70% (Forrester et al., 2010)(Bartges, 2016)(Buckley et al., 2011). Higher dietary sodium concentration may also increase water consumption, but based on human studies concerns have been raised regarding blood pressure and kidney and cardiac function (Bartges and Callens, 2015)(Queau, 2019).

Diet composition

Diet composition has been a particular focus of studies on feline urolithiasis. Dietary components, both urolith constituents and other molecules thought to affect crystal formation have been evaluated for their effect on urinary stone formation. Information on the effect of diet composition has given rise to many prescription diets aimed at dissolving or preventing particular uroliths.

A 2019 prospective study by Kaul et al. followed 21 cats diagnosed with urolithiasis for recurrence of FLUTD symptoms. They report symptom recurrence in 52.4% of cats, an increase relative to three other studies they cite, which report a 5.5%, 27.6% and 38.4% recurrence rate respectively. Kaul et al. find that cats that are subjected to prophylactic measures, including the introduction of a prescription diet, significantly lower chance of symptom recurrence (Kaul et al., 2019).

Struvite - Struvite stones have been shown to be amenable for dissolution via dietary intervention. It has been shown that high levels of dietary phosphorus and magnesium increase urinary excretion of these elements, and thereby struvite RSS. High amounts of calcium, sodium, chloride or potassium have also been identified as risk factors for struvite formation. Higher fat content was associated with a decreased risk for struvite urolith formation. Both high and low amounts of protein seemed to increase the risk of struvite urolithiasis, and thus a moderate amount is recommended (Queau, 2019)(Lulich et al., 2016)(Funaba et al., 2001). Additionally, feeding cats larger meals less frequently increases the risk of struvite formation by increasing the magnitude of the postprandial alkaline tide (Bartges and Callens, 2015)(Queau, 2019). The alkaline tide will be discussed in more detail in the discussion section of this thesis.

Calcium oxalate - In contrast to struvite uroliths, calcium oxalate uroliths are not thought to be amenable to dissolution following dietary changes. Dietary intervention in patients with calcium oxalate uroliths is centred mainly around preventing recurrence (Forrester et al., 2010)(Hall et al., 2017).

It is recommended that the diet contains moderate levels of calcium, as high calcium levels increase absorption and subsequently urinary excretion, leading to higher calcium RSS. Hypercalcemia suppresses the release of PTH and the production of 1,25-vitamin D, decreasing bone mobilization and intestinal calcium absorption, and increased urinary excretion of calcium. Hypercalcemia is reported in 35% of cats with this type of urolith, and may be secondary to diseases such as primary hyperparathyroidism, idiopathic hypercalcemia and hyperparathyroidism (da Rosa Gomez et al., 2018)(Bartges and Callens, 2015)(Bartges, 2016).

Oxalic acid is a metabolic end product of ascorbic acid and several amino acids (Bartges, 2016). The amount of calcium and oxalic acid in the diet should be balanced, as calcium oxalate combinations may be formed in the GI tract preventing absorption (Bartges and Callens, 2015)(Queau, 2019).

Higher protein, sodium and potassium levels seem to be associated with a lower risk of calcium oxalate occurrence (Funaba et al., 2001). Large amounts of animal protein may contribute to calcium oxalate urolithiasis by increasing calcium excretion in the urine, and also by decreasing citrate excretion. Citrate is thought to be an inhibitor of calcium oxalate stone formation (Lulich et al., 2016).

The effect of dietary magnesium concentration remains controversial. A sufficient amount of magnesium may be desirable, as urinary magnesium forms complexes with oxalic acid, reducing available oxalic acid for calcium oxalate formation (Bartges and Callens, 2015)(Queau, 2019). However, Gomez et al. identify high magnesium content as a possible risk factor in calcium oxalate formation. As magnesium is also a compound of struvite uroliths and the risks and benefits in magnesium supplementation are not entirely clear, care should be taken increasing magnesium intake (Bartges, 2016)(da Rosa Gomez et al., 2018).

Lastly, low dietary phosphorus may be a risk factor in calcium oxalate urolithiasis, as it may be associated with vitamin D-activation, which promotes hypercalciuria (Bartges, 2016). However, Gomes et al. find both high and low dietary phosphorus to be a risk factor for calcium oxalate formation. Lekcharoensuk et al. also report moderate phosphorus content to be associated with decreased risk of calcium oxalate formation (Lekcharoensuk et al., 2001)(da Rosa Gomez et al., 2018).

Urinary pH

Many studies have demonstrated urinary pH to be a strong predictor of crystal formation for different stone types, although interestingly in the aforementioned Benelux urolith centre study urinary pH at the time of urolith removal was measured in 92% of cats, but no significant correlation was found between urinary pH and urolith composition (Picavet et al., 2007).

Studies have shown that dietary changes may influence urinary pH, but as there is no standard protocol for measuring urinary pH, care should be taken interpreting and comparing the results of different studies (Forrester et al., 2010). The acidifying potential of a diet is thought to be based on the balance between acidifying compounds, such as methionine, calcium, sodium sulfate and ammonium chloride, and alkalizing compounds, such as calcium carbonate and potassium citrate (Queau, 2019). Calculating food base excess (BE) is a practical tool for predicting the effect of a certain diet on urinary pH. Several formulas to calculate urinary pH from food BE have been proposed. Wagner et al in 2006 consecutively fed nine diets to eight cats, and published the following formula for calculating urinary pH: pH=6.25+0.0023*BE; correlation coefficient r = 0.74. Seven years later, Jeremias et al. consecutively fed nine diets to nine cats and published the following formula: $pH = 6.269 + [0.0036 \times BEs] + [0.000003 \times BEs^2]$; correlation of determination R^2 = 0.91. (Wagner et al., 2006)(Jeremias et al., 2013). These formulas could help pre-estimate the acidifying or alkalizing effect on urine pH. However, as base excess is measured in the food and the formulas do not take into account the degree of absorption and subsequent metabolization of certain compounds, these formulas do not completely remove the need for in vivo pH measurements.

Struvite - Struvite crystals form more readily in alkaline urine, as higher pH levels cause phosphate to be in its trivalent state (PO_4^{3-}), prone to crystallisation. Even though acidification of the urine may increase ammonium concentration, another constituent of the struvite urolith, the presence of trivalent phosphate ions seems to be of greater influence. Thus, a more acidic urine in effect still reduces the risk of struvite urolith formation (Forrester et al., 2010).

The presence of urease-positive bacteria in urine, as can be the case in urinary tract infection with for example Staphylococcus spp., Enterococcus spp. and Proteus spp., may increase the risk of struvite urolithiasis. The urease enzyme of the bacteria hydrolyses urea to ammonia and bicarbonate. The presence of bicarbonate raises the urinary pH, which decreases the solubility of the involved minerals. The other product, ammonia, is one of the constituents of struvite. In cats, struvite urolithiasis seems to be related to urinary tract infection in only 5% of cases (da Rosa Gomez et al., 2018)(Bartges and Callens, 2015).

Recommendations on target urinary pH differ, but in general a more acidic pH is preferred. Lekcharoensuk et al. report that diets formulated to produce a pH value between 6.5 and 6.9 were more likely to be related to struvite uroliths than diets formulated to produce a pH between 5.99 and 6.15 (Funaba et al., 2001). Bartges identifies a target urinary pH under 6.8, and Queau recommends a urinary pH between 6.0 and 6.3 (Queau, 2019)(Bartges and Kirk, 2006). Forrester et al. state that for dissolving struvite crystals, pH should be 5.8 to 6.2, and to decrease the risk of recurrence pH should be maintained between 6.0 and 6.4 (Forrester et al., 2010).

Calcium oxalate - Significant aciduria, with a pH lower than 6.2, may increase urinary calcium concentration. A possible explanation for this is that acidemia may promote mobilization of carbonate and phosphate from the bone to buffer the hydrogen ions. Simultaneous bone calcium mobilization may cause hypercalciuria. Also, metabolic acidosis may decrease urinary citrate concentration, increasing chances of calcium oxalate stone formation as citrate is an inhibitor of calcium oxalate urolith formation. However, whether consumption of acid precursors is associated with hypocitraturia in cats remains unclear (Funaba et al., 2001). Even though some studies also suggest calcium oxalate RSS does not increase with a more acidic pH (Queau, 2019), a more alkaline urine may prevent calcium oxalate crystal formation, albeit only of relatively slim influence (Bartges and Callens, 2015)(Bartges, 2016)(Funaba et al., 2001).

As in the case for struvite urolithiasis, recommendations on target urinary pH differ between authors. Bartges et al. find a urine pH above 7.0 to be beneficial for decreasing the risk of this type of calculus forming, and also propose a target pH of 6.6 to 7.5 (Bartges and Kirk, 2006)(Bartges et al., 2013). Forrester et al. state that urinary pH for the prevention of recurrent calcium oxalate urolithiasis should be at least 6.2. Following the recommendations of Forrester et al. a urinary pH between 6.2 and 6.4 could be considered safe for the prevention of both calcium oxalate and struvite stone formation (Forrester et al., 2010).

Aim of the present study: evaluating a minimally invasive method for the determination of urinary pH as influenced by feeds and supplements formulated to modify urinary pH

Many prescription diets and dietary supplements have been created to medically dissolve or prevent recurrence of uroliths. Many of these feeds and supplements claim to affect urinary pH, but studies validating these claims often are not available for online reference. Dutch legislation around health claims on pet food and pet food supplements often lacks specificity, complicating enforcement around scientific substantiation (Kasper et al., 2007).

The aim of this pilot study is to test a minimally invasive method for evaluating the effect of oral supplements or prescription diets on urinary pH in the cat. Six interventions are studied: the alkalizing supplement potassium citrate, acidifying oral supplements Urical, Vétoquinol urinary paste and ascorbic acid, and acidifying prescription diets Royal Canin S/O and Hill's W/D.

Materials and Methods

Cats - Eight castrated cats (5 male, 3 female, all 3 years of age) participated in the study. Mean body weight at the beginning of the study was 4.94 kg (range 4.0 - 5.6). Cats were selected from the cat kennel at the Faculty of Veterinary Medicine, Utrecht University, where a steady population of eleven cats is kept for research and educational purposes. The eight most stress-resistant cats, as judged by the kennel supervisor, were selected to participate in the study. A working protocol for this study was approved by IvD Utrecht on 3/10/2019 (AVD1080020184847WP1-7).

Housing – Normally, all eleven cats are housed in group accommodation divided by gender (6 male, 5 female). Within groups cats share litter boxes. Cats have been trained to eat from an individual bowl and are fed twice daily, at 8.00 and 15.00. Prior to the start of the study all cats, with the exception of one non-participating female cat are fed Hill's Science Plan Adult Cat Food with Chicken. The other cat is fed Hill's W/D. Portion size is based on individual weight and body condition score. Cats were also given this food during baseline measurements and on wash-out days.

For the duration of the study, participating cats were fed their individual meals in group accommodation. On measuring days, they were placed in individual cages after the first meal. Individual cages are located in the same room as the group accommodation, and non-participating cats (1 male, 2 female) remain in the group accommodation. Individual cages included a cat bed with a fleece blanket, a litter box filled with non-absorbent plastic beads (UriCatKit), a bowl of fresh water and a toy. Every morning, the fleece blanket was sprayed with pheromone spray (Feliway Classic). Cats were kept in individual cages until 14.45, and then were returned to group housing to receive the second meal and stay the night. The two daily meals are equal in quantity. The cat kennel is cleaned every Monday. In the case Monday is a test day, cats are kept in group accommodation until 11.00 so as to prevent added stress from being in the individual cage as the cleaning takes place.

When participating cats were individually housed, non-participating cats had access to regular litter boxes. When participating cats were in the group accommodations, regular group litter boxes were substituted with UriCatKit-containing group litter boxes.

Study design - The present study is set up as a single-arm prospective study. All participating cats undergo the same treatment at the same time. Treatment schedule is constructed so that cats remain in group accommodation on weekends, to accommodate the schedule of kennel workers. On day -4 cats are first placed in the individual cages for a baseline measurement. On day 0, cats are given the first intervention treatment. All interventions (either feeds or oral supplements) are separated by a wash-out period in which the cats receive their normal food and no supplement.

Treatment protocol is as follows:

Day no.	Treatment
-4, -3	Baseline measurement
0	Potassium citrate[1]
1	Wash-out 1
2	Ascorbic acid[2]
3 to 5	Wash-out 2
6 to 8	Royal Canin S/O[3]
9 to 13	Wash-out 3
14, 15	Vetoquinol urinary paste[4]
16 to 20	Wash-out 4
21, 22	Urical[5]
23 to 26	Wash-out 5
27 to 29	Hill's W/D[6]

[1]Potassium citrate was administered orally via a syringe, once in the morning of day 0, dosed 150 mg per kilogram of body weight

[2]Ascorbic acid tablets were pulverized and mixed with 2 ml water, and the mixture was administered orally via a syringe, once in the morning of day 2, dosed 500mg per cat (approximately 100mg/kg body weight)

[3]Royal Canin S/O diet was provided twice a day, in the same volumetric quantity as the pre-study diet because of the similar energetic value

[4]Vétoquinol urinary paste was dosed in accordance with guidelines provided by the manufacturer (6 gram per cat, twice a day) from the morning of day 14 to the morning of day 15. For most cats, Vétoquinol urinary paste was mixed with a tablespoon of Royal Canin Recovery to make the paste more palatable. In one cat, Vétoquinol paste needed to be administered via a syringe

[5]Urical was dosed in accordance with guidelines provided by the manufacturer (0.5 ml/kg body weight, administered once daily) on days 21 and 22. For most cats, Urical was mixed with a tablespoon of Royal Canin Recovery to make the supplement more palatable

[6]Hill's W/D was provided twice a day and, having a lower energetic value per volume, the amount of food was calculated so that the energetic value was equal to the pre-study diet

Information on the composition of feeds and supplements, as provided by the manufacturer, can be found in the appendix.

Sample collection - During individual housing, UriCatKit litter boxes were checked every hour. Urine was collected with a pipette and stored in a plastic test tube. Soiled litter boxes were thoroughly cleaned with water, refilled with new UriCatKit litter beads, and returned to the cats. Samples from group UriCatKit litter boxes, as used by all cats in the group accommodation between 15.45 and 8.00, were taken when removing the UriCatKit litter boxes after moving cats to their individual cages. If a cat was seen voiding urine on the ground or on bedding, urine was also collected and analysed. For each sample, time and place of collection were listed, to see if it would be possible to visualize diurnal pH fluctuations.

Sample analysis - Collected samples were analysed twice a day, at approximately 10.00 and 15.00. In the time between sample collection and analysis samples were stored in closed plastic test tubes at room temperature.
Urinary pH was measured using a urinary test strip (Arkray AUTION Sticks 10 EA) and a benchtop electronic pH measurer. When using the reagent strip, the value was estimated to the nearest 0.1 pH unit. The benchtop pH measurer provided values to two decimal points.

For individual urine samples but not for group samples, urinary sediment was evaluated for crystalluria by transferring urine to an Eppendorf cup and centrifuging for 3 minutes. Sediment was pipetted onto an object glass and studied under a microscope. After analysis, urine samples were discarded.

Additional experiments - In addition to the main experiment, pH values obtained using test strips and the electronic pH measurer were compared. Urinary samples were also used to evaluate the effect of storage time on urinary pH and the effect of washing and reusing UriCatKit beads.

To test the effect of storage time, we measured pH using only the benchtop pH measurer in three urinary samples within 90 minutes after voiding, every hour for six hours, then twice a day for the next day and finally once after 48 hours. Urine was collected from individual cages and stored in the same way as the samples included in the main experiment.
The effect of washing UriCatKit beads was evaluated by filling small containers with beads. Half of these beads were wetted with one of six urinary samples of known pH (5.94, 6.14, 6.17, 6.23, 6.30 and 7.02). Beads were then rinsed with cold water over a strainer and dried using clean paper towels. The beads then were wetted again with one of three urinary samples (pH 5.94, 6.17, 6.30) and pH values were measured again using only the benchtop pH measurer, and compared to control values obtained by measuring the same samples over unused UriCatKit beads.

Data analysis - To evaluate the effect of interventions on urinary pH, mean urinary pH was calculated per cat and per study phase (each separate intervention and wash-out period). For most interventions (potassium citrate, Royal Canin S/O, Vétoquinol and Urical) the wash-out period following intervention is used as reference. In the case of ascorbic acid and Hill's W/D the wash-out period preceding intervention is used as a comparison. In many cases, no or only one data point was obtained per cat per study phase. If multiple samples were obtained, the mean value was calculated by taking the antilog of pH values, calculating the mean and taking the logarithm of the resulting value. Samples were designated equal weight. Mean pH values of intervention periods were compared with pH values obtained in the adjacent wash-out period for individual cats. Results were visualized per intervention in bar graphs.

In comparing test strip and benchtop pH measurer values, values from the benchtop meter were considered the standard to which values obtained for the test strip were compared. The mean pH difference between test strip and benchtop measurer over all obtained samples was calculated by subtracting the value from the test strip from the corresponding value from the benchtop measurer, taking the sum of all absolute value differences and dividing by the total number of samples. Additionally, separate mean pH differences were calculated for samples classed as more alkaline ($pH \geq 6.5$) and more acidic ($pH \leq 5.99$). Finally, the percentage of values that differed more than 0.25 pH units was calculated.

Data points obtained in the experiment on storage time were evaluated for any values differing more than 0.05 point on the pH scale, compared to the first measured value.

Data points obtained in the experiment on washing UriCatKit beads were evaluated for any values differing more than 0.05 points on the pH scale, compared to control values.

Results

A total of 109 samples of sufficient volume for pH measurement were collected. Of these, 76 were individual samples and 33 were samples from group litter boxes. Of individual samples, 53 were voided on litter boxes in individual housing and 23 were collected from other places when cats were seen urinating. On five occasions, two samples were obtained from one cat on the same day.

Some cats provided urinary samples more often than others. Table 1 shows the number of collected samples per cat. Samples were collected more frequently as time progressed, likely due to cats gradually getting used to individual housing and modified litter boxes, but also to the researchers' increasing familiarity with the voiding habits of particular cats and being more alert to the times and places cats may urinate. Table 2 shows the gradual increase of individually obtained samples over the weeks.

Cat	1	2	3	4	5	6	7	8	total
N	6	5	9	20	11	5	10	10	76

Table 1: amount of individual samples collected from each cat

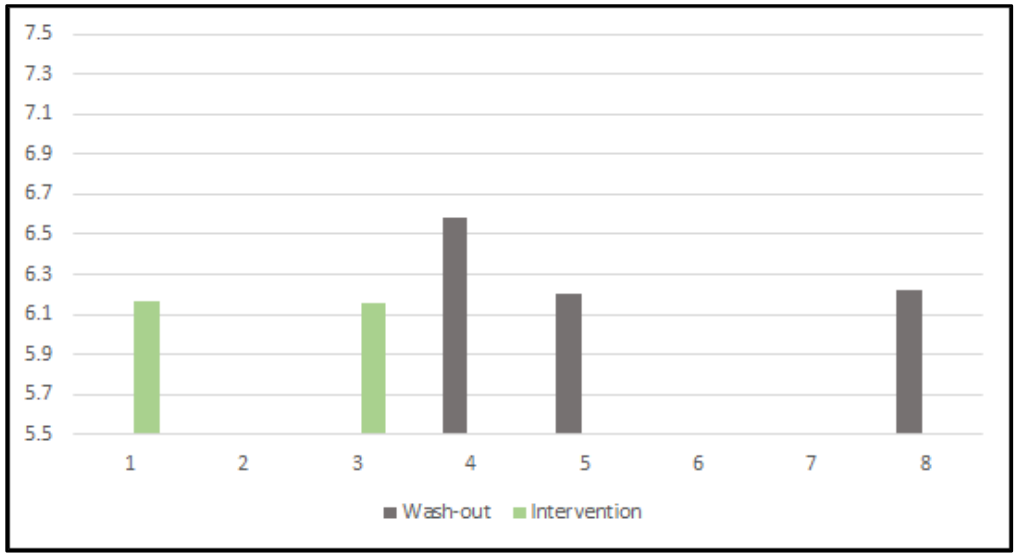
Week	Number of individual samples	Number of measuring days	Mean samples/day
1	11	4	3
2	14	5	3
3	18	4	5
4	19	4	5
5	14	3	5

Table 2: Mean amount of individually collected samples per day

The effect of interventions on urinary pH - For each intervention, mean pH values per cat are compared to pH values obtained in the adjacent wash-out period. Calculated means, as well as the number of samples and the range, are shown per cat per study phase. In the bar graphs mean pH is shown on the y-axis and individual cats are shown on the x-axis.

Potassium citrate

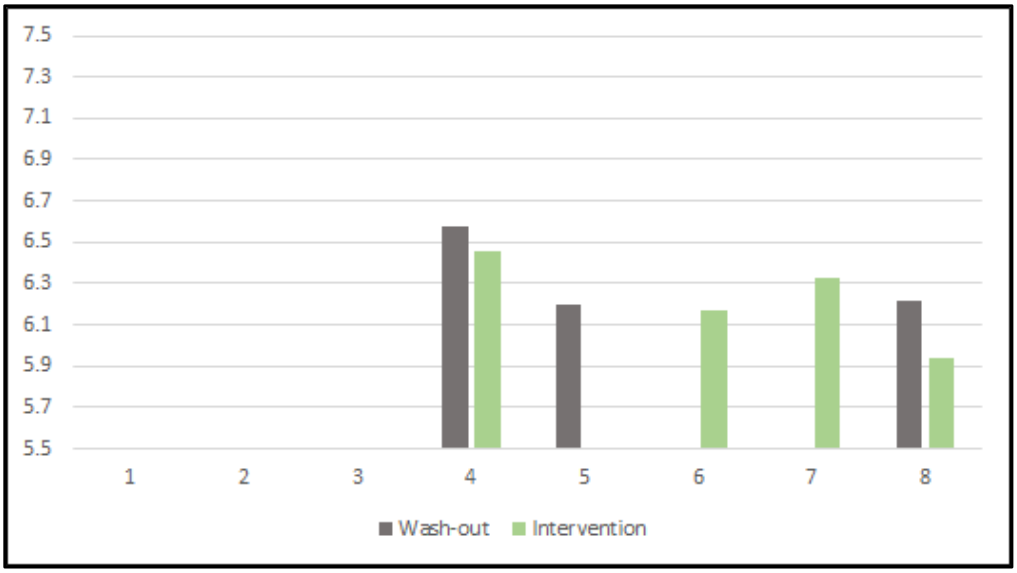
Cat	Intervention mean pH	No. of samples	Range	Wash-out mean pH	No. of samples	Range
1	6.17	1	-		-	-
2		-	-		-	-
3	6.16	1	-		-	-
4		-	-	6.58	1	-
5		-	-	6.20	1	-
6		-	-		-	-
7		-	-		-	-
8		-	-	6.22	1	-



Cut-off time between intervention and wash-out was set at 24 hours after potassium citrate supplementation. With the data points obtained for potassium citrate intervention, unfortunately no direct comparisons can be made between means for individual cats.

Ascorbic acid

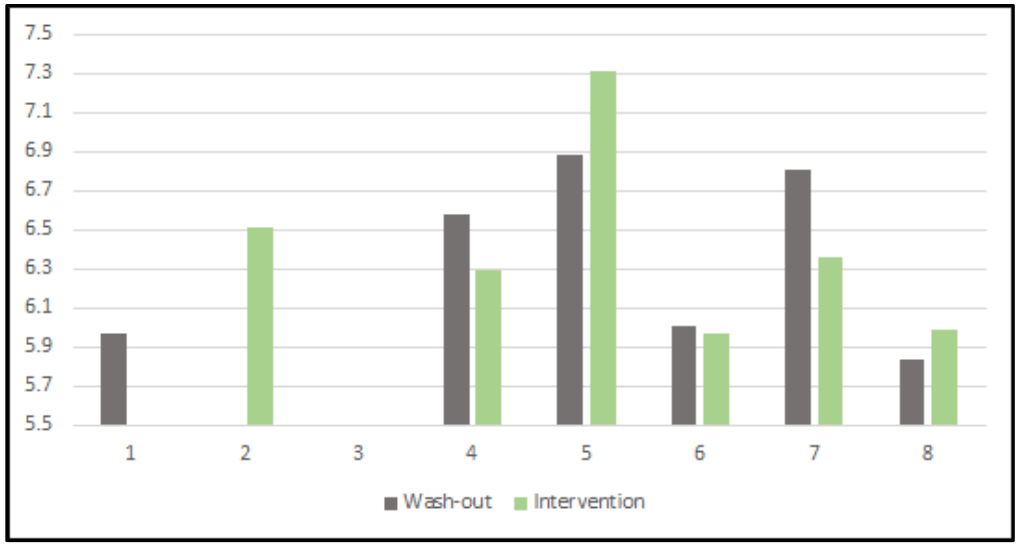
Cat	Intervention mean pH	No. of samples	Range	Wash-out mean pH	No. of samples	Range
1		-	-		-	-
2		-	-		-	-
3		-	-		-	-
4	6.46	2	6.45-6.48	6.58	1	-
5		-	-	6.20	1	-
6	6.17	1	-		-	-
7	6.33	2	6.25-6.40		-	-
8	5.94	1	-	6.22	1	-



All samples obtained within 48 hours of vitamin C supplementation were classed as intervention samples. With the data points obtained for ascorbic acid intervention, a comparison of mean pH values can be made for two cats. Both cats showed a slight decrease in urinary pH value.

Royal Canin S/O

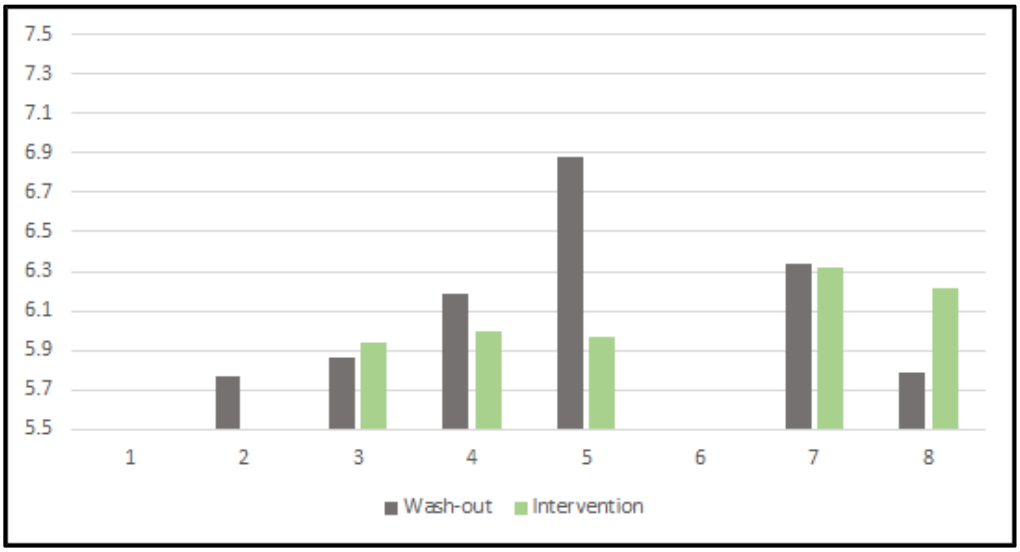
Cat	Intervention mean pH	No. of samples	Range	Wash-out mean pH	No. of samples	Range
1			-	5.97	1	-
2	6.51	1	-		-	-
3			-		-	-
4	6.29	2	6.23-6.35	6.58	2	6.27-6.76
5	7.31	1	-	6.88	1	-
6	5.97	1	-	6.01	1	-
7	6.36	1	-	6.81	1	-
8	5.99	1	-	5.84	1	-



The cut-off time between intervention and cut-off data points was chosen at 24 hours after the last Royal Canin S/O meal. With the data points obtained for Royal Canin S/O intervention, a comparison of mean pH values can be made for five cats. Two cats showed a slight decrease in urinary pH value, two cats showed a slight increase, and in one cat mean pH values for intervention and wash-out differed less than 0.1 point on the pH scale.

Vétoquinol urinary paste

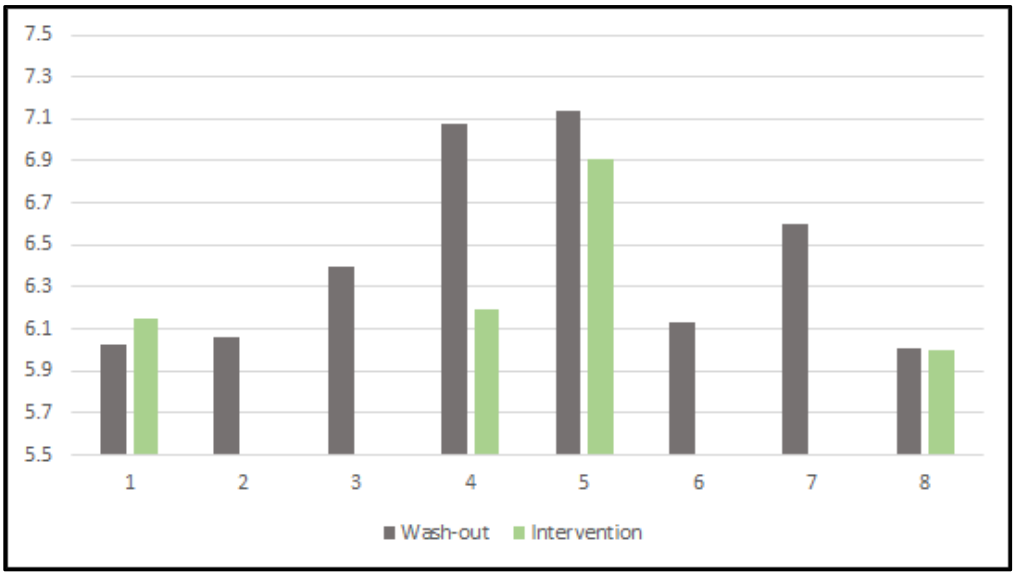
Cat	Intervention mean pH	No. of samples	Range	Wash-out mean pH	No. of samples	Range
1		-	-		-	-
2		-	-	5.77	1	-
3	5.94	1	-	5.86	2	5.85-5.86
4	6.00	2	5.83-6.12	6.19	3	6.14-6.25
5	5.97	2	5.89-6.04	6.88	2	6.48-7.08
6		-	-		-	-
7	6.32	1	-	6.34	2	6.1-6.5
8	6.21	1	-	5.79	1	-



The cut-off point between intervention and wash-out samples was set at 24 hours after the last supplementation. With the data points obtained for Vétoquinol urinary paste intervention, a comparison of mean pH values can be made for five cats. Again, two cats showed a slight decrease in urinary pH value, two cats showed a slight increase, and in one cat mean pH values for intervention and wash-out differed less than 0.1 point on the pH scale.

Urical

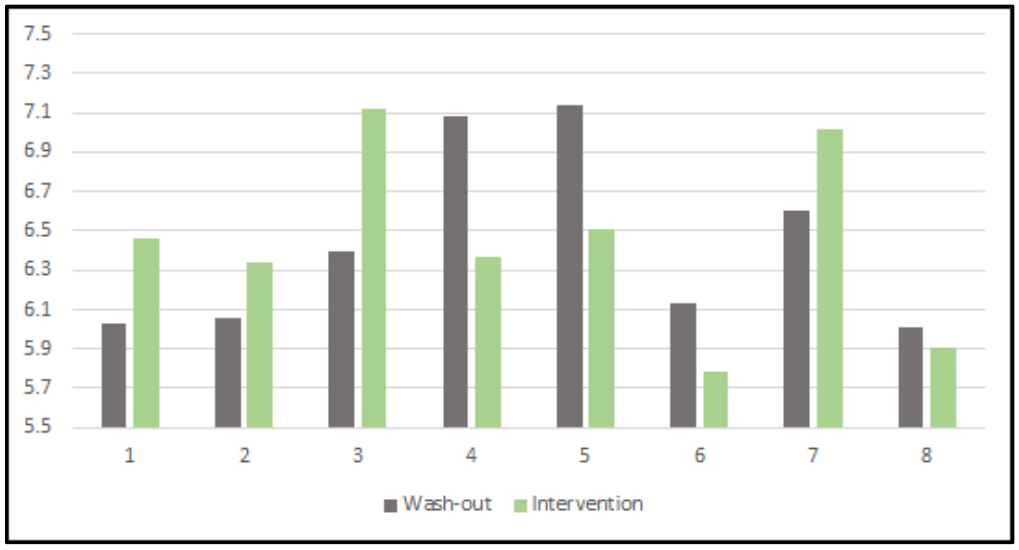
Cat	Intervention mean pH	No. of samples	Range	Wash-out mean pH	No. of samples	Range
1	6.15	1	-	6.03	1	-
2		-	-	6.06	1	-
3			-	6.4	2	6.2-6.53
4	6.19	4	5.89-6.44	7.08	1	-
5	6.91	1	-	7.14	2	7.05-7.21
6		-	-	6.13	1	-
7		-	-	6.6	2	6.38-6.74
8	6.00	2	5.94-6.05	6.01		-



The cut-off point between intervention and wash-out samples was set at 24 hours after the last supplementation. With the data points obtained for Urical intervention, a comparison of mean pH values can be made for four cats. Two cats showed a decrease in urinary pH value, one cat showed a slight increase, and in one cat mean pH values for intervention and wash-out differed less than 0.1 point on the pH scale.

Hill's W/D

Cat	Intervention mean pH	No. of samples	Range	Wash-out mean pH	No. of samples	Range
1	6.46	2	6.23-6.61	6.03	1	-
2	6.34	2	6.01-6.53	6.06	1	-
3	7.12	3	6.52-7.34	6.4	2	6.2-6.53
4	6.37	3	6.26-6.48	7.08	1	-
5	6.51	1	-	7.14	2	7.05-7.21
6	5.78	1	-	6.13	1	-
7	7.02	1	-	6.6	2	6.38-6.74
8	5.91	1	-	6.01		-



All samples collected after giving the first portion were considered intervention samples. Intervention samples were compared to wash-out samples collected after the previous intervention. With the data points obtained for Hill's W/D intervention, a comparison of mean pH values can be made for all eight cats. Four cats showed a decrease in urinary pH value, while the other four cats showed an increase.

Comparison between values obtained from test strips and benchtop pH measurer - The mean difference between benchtop meter and reagent strip values was 0.13, with a very slight bias towards a more acidic pH value. Of 109 data points, 21 were classed as more alkaline (pH≥6.5) and 20 were classed as more acidic (pH≤5.99). Disagreement between the benchtop meter and the reagent strip was more pronounced in alkaline samples, with a mean difference of 0.19 points on the pH scale. In more acidic samples the mean difference was 0.13, and in samples with a moderate pH value (n=68) mean difference was 0.12 points on the pH scale. In 12 of 109 samples (11.0%), pH values differed more than 0.25 pH units.

The effect of storage time on urinary pH - pH values obtained from the same sample tested on multiple time points remained virtually identical. None of the values differed more than 0.05 points on the pH scale compared to the first obtained value.

The effect of washing UriCatKit beads - pH values obtained from the same sample tested on new or washed UriCatKit beads were virtually identical. None of the values differed more than 0.05 points on the pH scale compared to the corresponding control value.

Discussion

The effect of interventions on urinary pH
The effect of interventions in the present study and in cited literature are summarized in table 3 at the end of this section.

Potassium citrate - With the data points obtained for potassium citrate intervention, unfortunately no direct comparisons can be made between means for individual cats. This is attributable to the small amount of samples collected during potassium citrate intervention and the associated wash-out period, and to the large variation in pH values between samples between and within individual cats. Possible explanations for the small amount of data points, the large variation and suggestions for further research are discussed under 'further comments on study design'.

Several articles discuss potassium citrate as an alkalising agent to be used in the prevention of calcium oxalate crystallization. Potassium citrate has several proposed working mechanisms. Citrate ions from orally ingested potassium citrate bind to calcium ions in the intestinal lumen, decreasing calcium absorption. Additionally, metabolization of citric acid in the tricarboxylic acid cycle produces bicarbonate, which in turn causes metabolic alkalinisation. This in turn results in a more alkaline urine, as well as in an increase of calcium reabsorption in the distal tubule, thereby reducing urinary calcium excretion (Stevenson et al., 2000). Furthermore, in urine, citric acid combines with calcium to form soluble complexes, reducing ionic calcium concentration (Forrester et al., 2010)(Bartges and Callens, 2015)(Bartges, 2016) (Bartges and Kirk, 2006).

Literature on the efficacy of potassium citrate in cats and dogs is scarce. In a 2000 study by Stevenson et al., healthy dogs were given 150 mg potassium citrate per kilogram body weight twice a day. Potassium citrate supplementation in these dogs increased urinary pH by 0.2 units, but this change was not statistically significant. Both the intervention and control diets produced a postprandial alkaline tide, but dogs on the intervention diet maintained a slightly higher urinary pH later in the day. Additionally, supplementation did not influence the mean urinary RSS of either calcium oxalate or struvite components (Stevenson et al., 2000). In cats very few data on the effectiveness of potassium citrate supplementation is available. Forrester et al. note that it has been shown that feeding citric acid up to 100mg/kg has little effect on urinary citrate concentration. Still, they recommend considering potassium citrate supplementation of 50 to 75mg/kg twice a day to cats that have recurrent calcium oxalate uroliths despite other dietary measures to prevent this (Forrester et al., 2010).

Ascorbic acid - With the data points obtained for ascorbic acid intervention, a comparison of mean pH values can be made for two cats. Both cats showed a slight decrease in urinary pH. The low number of data points and large variation seen between and within cats throughout this study decrease the value of these findings. Still, these findings are in line with literature finding some evidence for ascorbic acid as a urinary acidifier.

In a 1994 study on ascorbic acid metabolism in cats of which only the abstract is available, it is reported that urinary ascorbic acid increases with higher ascorbic acid intake, but the magnitude of this increase is not described. In this study cats were given single doses of ascorbic acid of 100 and 1000 mg/kg, or a dose of 100 mg/kg every day for 10 days (Maiwald, 1994). In 1998 Kienzle and Maiwald replicated finding a dose-dependent increase in renal excretion of ascorbic acid after a single dose of 100 and 1000mg/kg. Renal excretion was still elevated on the second day after supplementation. Ascorbic acid plasma concentration peaked at 4 hours after ingestion (Kienzle and Maiwald, 1998). It is not unlikely that the peak ascorbic acid plasma level coincides with the alkaline tide from the first meal after supplementation.

Kienzle et al. also tested the effect of oral supplementation with ascorbic acid on urinary pH by adding increasing amounts of ascorbic acid to a basal diet without vitamin C (200, 400, 600, 800 and 1000 mg/kg body weight per day) or to minced beef (200, 400, 1000 mg/kg body weight per day). In comparison the dose in the present study, approximately 100mg/kg body weight added to the amount of vitamin C present in the baseline feed, was relatively low. In the basal diet by Kienzle, urinary pH decreased only starting at 800 mg/kg, whereas in the minced beef diet changes occurred in response to lower doses and were more marked. The researchers note that highly mineralized diets may induce higher buffering capacity for the urine (Kienzle and Maiwald, 1998).

As cats also do not have a dietary vitamin C requirement and increased amounts of oxalic acid, one of the metabolic end products of ascorbic acid, may promote calcium oxalate formation, ascorbic acid supplementation should be avoided in patients at risk for this type of urolith (Bartges and Callens, 2015)(Bartges, 2016).

Royal Canin S/O - With the data points obtained for Royal Canin S/O intervention, a comparison of mean pH values can be made for five cats. Two cats showed a slight decrease in urinary pH value, two cats showed a slight increase, and in one cat mean pH values for intervention and wash-out differed less than 0.1 point on the pH scale. The absence of a resultant effect of feeding Royal Canin S/O may be attributable to the small amount of data points and large variation within and between cats found with the current study design.

On the website of Royal Canin, the S/O diet is advertised for use in struvite stone dissolution, and it is said to contain urine acidifying components. Indeed, Forrester et al. report a target urinary pH for the dry Royal Canin S/O food of 6.0-6.3 (Forrester et al., 2010). Using the equations for calculating base excess (with sulphur) and expected pH as proposed by Jeremias at al. on data on the ingredients provided by the manufacturer we find a base excess of -34.377 and an expected urinary pH of 6.15.

A 2011 study conducted under a grant of Royal Canin tested the in vivo effects of both the wet and dry version of the S/O diet. In the publication, they report to have shown previously that the dry S/O food causes a struvite RSS <1. This previous study, titled *''RSS is a better predictor for struvite dissolution than urine pH''* does not seem to be accessible online. In line with this title, a 2015 study by Pineda et al. saw the Royal Canin S/O diet fed for 60 days, and concluded that urinary pH was not significantly decreased after this period (Pineda et al., 2015). Still, in another study following feline patients presenting with signs of FLUTD, both the wet and dry diets effectively dissolve struvite uroliths. Unfortunately, urinary pH is not reported in this study, enrolled cats receive additional medical care such as antibiotic treatment and are not housed under laboratory conditions (Houston at al., 2011). The chapter on medical management of urolithiasis of the BSAVA manual of canine and feline nephrology and urology reports both the wet and dry S/O diet to be proved effective in the dissolution of struvite uroliths (Elliott et al., 2017).

Vétoquinol paste - With the data points obtained for Vétoquinol urinary paste intervention, a comparison of mean pH values can be made for five cats. Again, two cats showed a slight decrease in urinary pH value, two cats showed a slight increase, and in one cat mean pH values for intervention and wash-out differed less than 0.1 point on the pH scale.

The website of this Vétoquinol product states that the paste has acidifying properties on the urine of the patient, mostly by virtue of it containing DL-methionine (80g grams per kg of product). The paste is aimed at both cats and dogs, and is advertised for use in animals in which a more profound change of diet is not possible, for example when the patient is a picky eater or lives in a multi-pet household. In the present study, cats were supplemented with 6g of product (corresponding with 0.48g DL-methionine) twice a day, in accordance with recommendations by the manufacturer. Compared to other studies testing the effect of DL-methionine on urinary pH, this is a relatively low amount.

Funaba et al. tested the effect of DL-methionine supplementation in six clinically healthy adult cats. Cats were divided into three groups, and given food supplemented with 0%, 1% (approximately 0.6g) and 3% (approximately 1.8g) DL-methionine in approximately 60 g food, respectively. Cats were fed the diets ad libitum for two weeks, and samples of freshly voided urine were collected on the last three days. Study groups contained only a very limited number of cats and urine collection time was not standardized, but urinary pH in cats fed 3% but not 1% DL-methionine seemed to decrease compared to the control diet (Funaba et al., 2001). In 1990 Denhart conducted an experiment in which healthy cats were given three different doses (2.5, 5.0 and 10.0 mEq/kg, corresponding to 0.37, 0.75 and 1.49 g/kg, respectively) of DL-methionine. Cats were fed ad libitum and urine was collected after spontaneous voiding in individual cages. Urinary pH measurements demonstrated methionine supplementation was effective in lowering urinary pH. During the intervention period urine was collected at day 3 and 7. No significant difference in urinary pH was found between day 3 and 7, and a dose related decline in pH was also not demonstrated (Denhart, 1990). Skoch et al supplemented cats with 151, 329 and 492 mg/kg of DL-methionine, and found a dose dependent acidification of the urine in both meal- and ad libitum fed cats (Skoch et al., 1991).

Urical - With the data points obtained for Urical intervention, a comparison of mean pH values can be made for four cats. Two cats showed a decrease in urinary pH value, one cat showed a slight increase, and in one cat mean pH values for intervention and wash-out differed less than 0.1 point on the pH scale. Only few data points were obtained and values showed large variation in and between individual cats. Because of this, reaching a conclusion on the efficacy of Urical as a urinary acidifier based on present study results is not possible.

Urical is a liquid feed supplement claimed to lower urinary pH (below 6.5), by virtue of containing the acidifying ingredients ammonium chloride and methylthionine. The product also contains ferrichloride, citric acid, methionine and dextrose, claimed by the manufacturer to form complexes and thereby inhibit crystal formation. The respective amounts of ingredients is not presented on the website nor disclosed by the manufacturer, making it impossible to compare doses of active ingredients with those found in literature, or to calculate base excess.

Funaba et al. fed a diet supplemented with 1,5% ammonium chloride and a control diet to six cats in a three week cross-over design. Spontaneously voided urine was collected on the last seven days of each trial period. A significant decrease in urinary pH was found between control and intervention diets (Funaba et al., 2001). Another study performed by the same research group in 2003 also found urinary pH to be significantly lowered in a group of 9 cats on a 1,5% ammonium chloride supplemented diet (Funaba et al., 2003). Taton et al. in a 1984 study of which only the abstract is available online also found a significant decrease in urinary pH when supplementing cats with 1.5% ammonium chloride. Urinary pH was tracked for 11 months, during which time urinary pH remained stable at around 5.9 in the intervention group and 7.0 in the control group (Taton et al., 1984). Skoch et al supplemented cats with 84, 166 and 250 mg/kg of ammonium chloride and observed a dose dependent acidification of the urine in both meal and ad lib-fed cats (Skoch et al., 1991).

Literature on the effectiveness of methylthionine in reducing urinary pH could was not found. A review on nephrolithiasis therapy names methylthionine, or methylene blue, saying it has been reported to counteract urolith formation by disordering the matrix or changing the matrix surface, but notes that it is not widely used (Erwin, 1976). Another paper published in 1973 of which only the introduction is available online also notes methylene blue could partially inhibit urolithiasis of both struvite and calcium oxalate (Rollins and Finlayson, 1973).

Hill's w/d - With the data points obtained for Hill's w/d intervention, a comparison of mean pH values can be made for five cats. Again, two cats showed a slight decrease in urinary pH value, two cats showed a slight increase, and in one cat mean pH values for intervention and wash-out differed less than 0.1 point on the pH scale. Similarly to the other interventions, the absence of a resultant effect of this feed may be attributable to the small amount of data points and the large variation between pH values within and between cats.

At the time the study was set up, it was stated on the official Hill's website that their prescription W/D diet had an acidifying effect on the urine. Currently, this statement is not displayed on the website. Upon contacting Hill's to ask why this statement was removed, the employee stated that the W/D diet was formulated to create a urinary pH between 6.2 and 6.4, and the fact this effect was not stated on the website anymore was likely incidental after a recent update of the website.

Hill's W/D is not mentioned in the 2017 BSAVA manual of canine and feline nephrology and urology as one of the diets proven to be effective in the dissolution of struvite uroliths (Elliott et al., 2017). However, Forrester et al. report a target urinary pH for the Hill's W/D diet with chicken of 6.22 (Forrester et al., 2010).

Intervention	Study	Dose	Effect and additional remarks
Potassium citrate	Present study	150 mg/kg, single dose	- No data points to compare intervention and wash-out per cat
	Stevenson et al., 2000	150 mg/kg, 2dd	Urinary pH increased by 0.2 (not statistically significant) Study in dogs, intervention was associated with a slightly higher pH later in the day
Ascorbic acid	Present study	500 mg per cat, single dose	Slight decrease of pH in two cats, no data points to compare intervention and wash-out in others
	Kienzle and Maiwald, 1998	200, 400, 600, 800 and 1000 mg/kg added to a basal diet or minced beef diet	A decrease in urinary pH was seen starting at 800mg/kg when ascorbic acid was added to a basal diet, and at lower doses when it was added to a minced beef diet. In the basal diet, 1000 mg/kg ascorbic acid significantly decreased urinary pH to 6.85±0.24 (control 7.12±0.22). In a minced beef diet a change was seen with lower doses (control: 6.93±0.54, 200 mg/kg 6.77±0.51, 400 mg/kg 6.62±0.4, 1000 mg/kg 6.47±0.39).
Royal Canin S/O	Present study	Fed to fill energy requirement, two portions of equal size per day	Slight pH decrease in two cats, slight increase in two cats, no change in one cat, no data points to compare intervention and wash-out in three cats.
	Pineda et al., 2015	Fed to fill energy requirement, ad libitum	No significant decrease in urinary pH after 60 days
	Houston et al., 2011	Food provided by owner	Effective in dissolving struvite uroliths, no data on urinary pH provided
Vétoquinol paste (DL-methionine)	Present study	0.48 g/cat, 2dd	Slight pH decrease in two cats, slight increase in two cats, no change in one cat, no data points to compare intervention and wash-out in three cats.
	Funaba et al., 2001	Approximately 0.6 g and 1.8 g per cat	Urinary pH was lowered after supplementation with 1.8 g DL-methionine (pH 6.12), but not after 0.6 g (pH 6.86, control 6.82).
	Denhart, 1990	0.37, 0.75 and 1.49 g/kg	Effective in lowering urinary pH, no dose effect found Low dose pH levels: Baseline: 6.55, day 3: 5.98, day 7: 6.17 Medium dose pH levels: Baseline: 6.71, day 3: 5.87, day 7: 5.92 High dose pH levels: Baseline: 6.58. day 3: 5.82, day 7: 5.93
	*Skoch et al., 1991	151, 329 and 492 mg/kg	Dose-dependent acidification of the urine: Meal-fed cats: Control pH~7.8, low dose pH~7.6, medium dose pH~7.4, high dose pH~6.8 Ad libitum fed cats: Control pH~7.3, low dose pH~6.3, medium dose pH~6.25, high dose pH~6.2

Table 3a: Overview of intervention doses and results in the present study and cited literature
* pH values estimated from a line graph in the paper, as values were not written in the text

Urical (ammonium chloride)	Present study	0.5 ml/kg 1dd of Urical (dose of separate ingredients unknown)	pH decrease in two cats, slight increase in one cat, no change in one cat. No data points to compare intervention and wash-out in four cats
	Funaba et al., 2001	1.5% of total food weight	Significant decrease in urinary pH between control and intervention diets (intervention urinary pH: 6.29, control 7.34)
	Funaba et al., 2003	1.5% of total food weight	Significant decrease in urinary pH between control and intervention diets (intervention urinary pH: 6.63±0.11, control 7.25±0.18)
	Taton et al., 1984	1.5% (presumably of total food weight)	Stable urinary pH of 5.9, compared to 7.0 in the control group, over 11 months
	*Skoch et al., 1991	84, 166 and 250 mg/kg	Dose-dependent acidification of the urine Meal-fed cats: Control pH~7.8, low dose pH~7.7, medium dose pH~7.5, high dose pH~7.2 Ad libitum fed cats: Control pH~7.3, low dose pH~6.5, medium dose pH~6.4, high dose pH~5.9
Hills' W/D	Present study	Fed to fill energy requirement, two portions of equal size per day	Slight pH decrease in two cats, slight increase in two cats, no change in one cat. No data points to compare intervention and wash-out in three cats.

Table 3b: Overview of intervention doses and results in the present study and cited literature

Comparison between values obtained from test strips and benchtop pH measurer
Findings from the present study on differences between pH values obtained by test strips and benchtop pH measurer are in accordance with data published by Raskin et al., who also compared findings from reagent strips with a benchtop pH meter, using the benchtop pH meter as the gold standard. The benchtop pH meter used was deemed to be accurate at the 0.01 pH unit level, and the reagent strip was used to estimate acidity to the nearest 0.5 pH unit. They reported a negative bias of 0.12 units and a wide disagreement between methods particularly at more alkaline pH levels. Only 50% of values were in the 0.25 pH unit range of each other (Raskin et al., 2002).

Both the data from the present study and the data found by Raskin support the recommendation that reagent sticks may be useful in obtaining estimates, such as in a routine urinalysis, but should not be used in situations where more careful monitoring is preferred.

	Present study	Raskin et al.
Accuracy benchtop measurer	0.01	0.01
Accuracy estimation reagent strip	0.1	0.5
Mean disagreement	0.13	-
Mean disagreement ≥6.5	0.19	-
Mean disagreement ≤5.99	0.13	-
Mean disagreement 5.99<pH<6.5	0.12	-
Mean bias	-0.02	-0.12
% values within 0.25 pH units	89	50

The effect of storage time on urinary pH
In the present study, collected urine samples were tested twice a day, around 10AM and around 3PM. Samples from individual cages collected after 3PM were stored until 10AM the next day, making 19 hours the maximum storage time. Samples were kept in closed plastic test tubes by room temperature and no preservative was added.

Forrester et al. propose that during storage CO_2 may dissolve into the atmosphere, causing the urine to be more alkaline (Forrester et al., 2010). Also, if the urinary sample is contaminated with urease-producing bacteria, increased storage time may cause bacteria to proliferate and also make urine more alkaline. As described in the Materials and Methods section, we evaluated the effect of storage duration by measuring pH in three urinary samples at different time points. Urinary pH remained virtually identical in all samples for the duration of this test.

These findings are in accordance with data published by Albasan et al., who studied the effect of storage time and temperature on pH, specific gravity and crystalluria in fresh urine samples of 31 dogs and 8 cats. The exact method of sample collection in this study is not described. The obtained samples were aliquoted into 5 units; one of which was evaluated within 60 minutes of collecting and regarded as a baseline measurement, two were stored for 6 hours (refrigerated or at room temperature), and the remaining two were stored for 24 hours (refrigerated or at room temperature). Urinary pH and specific gravity did not seem to be affected by storage time or temperature. Crystalluria did increase with increasing storage time and decreasing storage temperature (Albasan et al., 2003).

We consider the effect of storage time in these individually obtained samples to be negligible. However, in samples obtained from group litter boxes any effect loss of CO_2 and bacterial proliferation may be more pronounced as the urine is left in the litter boxes in open air, making CO_2 dissolution and faecal contamination more likely to occur.

The effect of washing UriCatKit beads

pH values obtained from the same sample tested on new or washed UriCatKit beads remained virtually identical. None of the values differed more than 0.05 points on the pH scale compared to the corresponding control value. No literature on the effect of washing plastic litter beads was found, but our results support the notion that UriCatKit beads can be reused after washing when measuring pH. Reusing beads may mitigate waste generation and reduce total cost in future studies.

Further comments on study design

Acclimatization period - The present study did not include an acclimatization period. Cats were housed in individual cages for baseline measurement for only one day, after which the first intervention treatment was started. Looking at the number and pattern of data points obtained over the study, it seems unlikely that adding an acclimatization period to in the current study setup would have significantly improved data collection. Cats seldom urinated in the individual cages on the first day after having access to a regular litter box, and started urinating more regularly on the following days. Including an acclimatization period would most likely not change this pattern.

An acclimatization period might however reduce any influence acute stress from a change in housing and litter boxes has on urinary pH. One abstract from a 1996 case report suggested that stress from being transported to the veterinarian caused a cat to have alkaline urine as a result of respiratory alkalosis (Buffington et al., 1996). Another team of researchers considers the possible effect of stress before sampling (researchers captured and euthanized feral cats before collecting their urine) causing lower urinary pH by means of an increase in protein catabolic conversion because of an increased metabolism, increasing sulfuric acid production and thereby lowering urinary pH (Cottam et al., 2002). The effect of different types of stress on urinary pH seems to be poorly understood and thus it is difficult to say if stress has caused a shift in urinary pH in the present study.

Including an acclimatization period could be useful in a study setup where cats are either permanently housed in individual cages or presented with a modified litter box only, to bridge the time in which cats refuse to urinate before they take on a more natural urinating pattern. An acclimatization period could also add value when it is used to train cats to urinate on the modified litter boxes. Additionally, any samples collected in the acclimatization period could help to obtain more solid baseline values for urinary pH in these cats.

Baseline measurement and pH reference values - Baseline urinary pH measurements were collected on the first day of placing the cats in individual housing. As described in the previous section, stress from this may or may not affect urinary pH levels. If stress does alter pH value, the effect of this might have been strongest on the first day of the study. On this first day, none of the cats urinated in their individual cages, but urine was found in both the group litter boxes. Because of the limited amount of data points and large variation in urinary pH between samples of different cats on the same day, we chose not to compare data from intervention periods to the baseline measurement, but to compare data from an intervention period to an adjacent wash-out period. In the case of potassium citrate intervention, the two data points collected in the baseline measurement phase were as reference. For ascorbic acid and Hill's W/D interventions the foregoing wash-out period provided reference, and for Royal Canin S/O, Vétoquinol urinary paste and Urical the following wash-out period was used as reference.

Using reference values from literature for urinary pH in cats is complicated, as the amount of acid eliminated by the kidneys is very much influenced by metabolism and diet. In cats fed a diet of small mammal carcasses, the urinary pH averages between approximately 6.2 and 6.4. Due to the postprandial alkaline tide, time of pH measurement relative to the time of feeding is a crucial variable (Timothy, 1996). Another study attempting to provide baseline data on urinary composition and pH investigated urinary samples from feral cats. They found female cats to have significantly lower urinary pH of about 5.97 (range 5.54-6.57) than male cats, whose urinary pH on average was 6.37 (range 5.73-7.39). As the cats were feral and only one sample was taken per cat, the effect of feeding and the timing and intensity of the alkaline tide cannot be taken into account (Cottam et al., 2002).

Duration of intervention and wash-out periods - The duration of both intervention and wash-out periods in the present study are relatively short compared to those reported in literature on the respective interventions. In part, this approach was chosen because we were primarily interested in a pH change in urine, and not in a change in other parameters that were studied in referenced literature, such as urinary RSS for different compounds or the time to dissolution of struvite uroliths. We expected it would take more time for these changes to manifest, and for urinary pH change to be apparent more quickly. Support for short intervention periods from literature is scarce, but some studies on ascorbic acid and DL-methionine support this expectation, and a rapid, short-lived effect seems to match with recommendations to dose supplements daily or even multiple times per day. In a 1996 article Timothy discusses a testing procedure in which cats were fed a diet for 8 days, and urinary samples were collected on days 5 to 8 of the trial, by expressing the bladder manually. He reports that studies using this protocol have shown that the duration of feeding (one up to five weeks) does not affect urinary pH, although these data are unpublished (Timothy, 1996). In 1991 Skoch et al. report that in cats fed alkalizing and acidifying diets, changes in urinary pH are observed within 24-48 hours if feeds are switched abruptly (Skoch et al., 1991).

For future research, we recommend choosing regular time frames for both intervention and wash-out procedures to facilitate differentiating between intervention and wash-out data points. When selecting a method for urine collection that is more reliable in terms of data quantity and quality, but more invasive, a choice could be made to measure urinary pH only the last days of the intervention or wash-out period. As cats could be housed normally on all other days, prolonging intervention or wash-out times could be achieved without much added distress to animals and workload to staff and researchers. Study designs in which urinary samples are only obtained at the end of the testing period have also been used by Spears, Funaba, and Jeremias. Spears et al. in their 2010 research of acidifying dietary supplements, opted for a study design in which cats were fed the wash-out or intervention diet for a full week, and urinary samples were only taken at day 7 of each week via cystocentesis, at three different time points on that day (Spears et al., 2003). Funaba et al studied urinary pH on several diets as well, feeding the diets for 3 weeks and collecting urine on the last 7 days of this period (Funaba et al., 2003). Jeremias et al. used study periods for 10 days, with seven days of diet adaptation and three days for urine collection (Jeremias et al., 2013).

Sample collection - In the present study design, urine samples were obtained by placing cats in individual housing with modified litter boxes. One of the main aims of this study was to evaluate if collecting spontaneously voided urine this way could be feasible for testing our hypotheses. Collecting spontaneously voided urine could be considered a relatively non-invasive method of obtaining samples. We have to conclude that collecting urine is this way is not a feasible method for evaluating the effect of several feeds and supplements in a short time span. Several issues with this method of sample collection presented themselves.

Lack of consistency in number of samples and time of voiding - The main problem with this method of data collection was the lack in consistency of samples. Many cats did not urinate on a time or place a sample could be collected. For many of the study days, no samples were collected from multiple cats. In multiple instances, individual cats did not provide urine at all on one or even both the intervention or wash-out period. Moreover, the timing of urination could not be controlled. This is problematic because urinary pH naturally fluctuates over the day. As urine typically remains in the bladder for several hours, a spontaneously voided sample may represent urine produced at very different pH levels initially. As cats were not individually housed and observed for 24 hours per day, in most cases the amount of time that has passed between sampling and the previous urination is not known. pH differences found between samples of the same cat, if samples represent different time intervals, cannot be ascribed only to the applied intervention or lack thereof. This was illustrated by one cat in the present study that urinated twice on the same day, first at 13.00 (five hours after feeding, urinary pH 7.08) and a second time at 15.00 (seven hours after feeding, urinary pH 6.48). One notable cause of circadian fluctuation is a phenomenon named the postprandial alkaline tide.

The postprandial alkaline tide is a temporary rise in pH caused by increased secretion of alkaline ions by the kidney, in response to the loss of gastric acid that is secreted after meal ingestion. The magnitude of the alkaline tide increases as cats are fed only one or two meals over the day, as is the case in the present study, as opposed to cats consuming smaller portions of food throughout the day (Spears et al., 2003)(Timothy, 1996)(Taton et al., 1984). In a 1992 study of which only the abstract is available online, Finke and Litzenberger present 90 cats with different portion sizes of food and measure urinary pH in a sample obtained 4 hours after feeding. They find a linear function predicting pH value: urine pH = 6.15 + [food intake (g) x 0.015] (Finke and Litzenberger, 1992).

The exact timing of the alkaline tide is not easy to pinpoint, and is most likely influenced by feeding habits and food composition. An abstract available from Taton et al. describes urinary pH increasing to 7.6 two hours after feeding the only daily meal, and pH remaining between 7.6 and 6.6 for 9 hours. Therefore it seems likely that the peak of the postprandial tide occurs within the first two hours after meal ingestion (Taton et al., 1984). Stevenson et al. created a day curve of urinary pH in dogs that are fed twice daily, around 8.30 and around 15.30, very similar to the cats in the present study. Urinary pH steeply increases after the first meal, peaking around 2-3 hours after feeding. After the second meal a similar alkaline tide with a slightly lower peak can be seen. In the study potassium citrate supplemented dogs are compared to control dogs, and the curves mostly overlap except during the second alkaline tide, during which the pH values of supplemented dogs exceed those of control dogs (Stevenson et al., 2000). An alkaline tide was also visualized in a day curve of feline urine samples by Skoch et al. Cats were fed ad libitum, complicating a comparison between data found in the study by Skoch and the present study. Furthermore, the method of obtaining the samples for the day curve are not described (Skoch et al., 1991). Creating day curves for meal-fed and possibly also ad libitum fed cats on different interventions would be a very interesting topic for future research. It seems likely that some feeds or supplements only significantly alter urinary pH during certain times of the day. Day curves of all participating cats can be compared and evaluated for a general shift, or a more delicate change feeds or supplements may cause, such as the specific change in the magnitude of the postprandial tide found by Stevenson et al.

A day curve is an excellent tool for evaluating such subtle and transient changes, and could be of great use for clinicians advising clients on the use of these feeds and supplements.

Added value of group samples - In addition to the samples from individual cats, samples were also taken from group litter boxes. UriCatKit litter boxes were available to cats overnight. The value of group samples is very limited, as we have found large differences between individual cats urinating on similar time points, on the same day and on the same intervention. Moreover, three cats in the group housing were not enrolled in the study and did not receive intervention treatments, but they did have access to the group litter boxes. Even though these three cats had access to normal litter boxes during the time the enrolled cats were in individual housing, and seemed to greatly prefer using these normal litter boxes, it cannot be ruled at that some of the urine in the group boxes was (partly) from these non-enrolled cats. For these reasons, group samples could not be used to test our hypotheses.

However, group litter boxes proved to be helpful in collecting individual samples in two ways. Firstly, providing a normal litter box to cats, as was done over weekends, strongly prompted cats to urinate there and not urinate at all on the first day they were presented with UriCatKit litter boxes again. This interfered with our ability to reach conclusions on the efficacy of studied supplements and feeds. This effect was most prominently seen during potassium citrate intervention. Secondly, often cats urinated on the group litter boxes right before being transferred to individual housing. When we observed cats urinating and the urine did not mix with other urine in the litter box, urine was collected and classed as an individual sample.

Sample contamination and concentration - Urine was voided spontaneously and thus came into contact with the environment before collection. The maximum time between voiding and sample collection was one hour for individual samples. During this time samples were potentially subjected to environmental bacteria, and, in the case of samples collected from places other than the litter box potentially residues from cleaning agents. Furthermore, samples that were not immediately collected were exposed to the air, leading to possible concentration of the sample or diffusion of CO^2 into the atmosphere.

Samples could have been bacterially contaminated by contact with faecal material or other environmental bacteria. It is also possible cats developed a bacterial infection of the urinary tract, although we observed no clinical signs of this. It is known that several bacteria (for example Staphylococcus spp., and Proteus spp.) alkalinise urine. Other bacteria, such as E.coli, may acidify urine (Forrester et al., 2010)(Sink and Weinstein, 2012). Using dyes to visualize bacteria in the sample could be helpful in evaluating the possible influence of bacterial urinary tract infection when urinary samples are obtained through catheterization or cystocentesis.

On the potential effect of dilution or concentration very little information was found. In one study, six cats were subsequently fed four diets that differed in moisture content, but were otherwise identical: in these cats, urinary specific gravity decreased with higher moisture content, but urinary pH did not change significantly. This may be an indication that urinary dilution does not greatly influence urinary pH, but in the present study setup, urine (especially samples collected from group litter boxes) may have undergone stronger concentration. Measuring urine specific gravity could help to more accurately estimate the effect of urinary dilution or concentration on urinary pH (Buckley et al., 2011).

Microscopic evaluation of samples - In the present study, urinary sediments were evaluated for the presence of urinary crystals. We observed a single calcium oxalate crystal in one sample, and struvite crystals in many samples. The presence and amount of struvite crystals seemingly increased with more alkaline pH levels, as was the expectation. However, Albasan et al. report an increase in the amount of urinary crystals with increased time between sample collection and evaluation. In this study, samples were evaluated 6 and 24 hours after collection (Albasan et al., 2003). Perhaps in vitro crystallization could also take place within the first 6 hours. Therefore we advise studying urine sediment directly after obtaining the sample.

Conclusions

The aim of this pilot study was to test a minimally invasive method whereby cats urinate spontaneously for evaluating the effect of oral supplements and prescription diets on urinary pH. Unfortunately, the current study design proved to be inadequate to test the efficacy of our interventions, mostly due to a lack of data points on all interventions from all cats and a lack of control on the timing of voiding. Often, no or only one sample was obtained per study phase per cat. Time of and between urinating varied between and within cats.

It is possible that pH changes caused by interventions are subtle, or present only in a relatively short time frame or during certain times of day. In combination with expected diurnal changes, especially in meal-fed cats, any changes in urinary pH we found cannot be directly attributed to the presence or absence of an intervention.

For future research, we recommend using a sample collection method that is more controllable. Ideally, a day curve of urinary pH is made for all cats for each intervention, and as a baseline measurement. Catheterization would be ideal for creating a day curve, as after placing the catheter urine can be taken in regular intervals, and the bladder can be emptied between samples. When using a catheter, there is very little risk of losing urine due to cats urinating between sample collection times. Another collection method that can be considered is obtaining urinary pH day curves for all cats using a catheter as a baseline measurement, gaining insight into the timing and magnitude of diurnal changes, and taking samples on subsequent measuring days using cystocentesis at set times derived from baseline day curves.

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Appendix

Composition of feeds and supplements used in this study as provided by the manufacturer

Hill's Science Plan Adult Cat Food with Chicken - Chicken and turkey meal, wheat, maize, animal fat, maize gluten meal, brewers' rice, digest, minerals, dried beet pulp, fish oil.

Royal Canin S/O - rice, wheat gluten, dehydrolysed poultry protein, maize meal, animal fat, hydrolysate of animal protein, maize gluten, minerals, plant fiber, fish oil, soy oil, fructo-oligosaccharides, tagetes extract (source of lutein)

Hill's W/D - Chicken: Chicken- (42%) and turkey meal, maize, maize gluten meal, cellulose, brewers' rice, protein hydrolysate, minerals, animal fat, soy oil, L-carnitine, taurine, vitamins, trace elements and beta carotene. With a natural antioxidant (mixed tocopheroles).

Urical - Ammonium chloridum, Acidum citrricum, Ferri chloridum, Glucosum, Kalii chloridum, Methioninum, Methylthioninum, Natrii chloridum,Flavorum agétia, Aqua, Zinci sulfas.

Potassium Citrate - Potassium citrate (144mg/ml), Citric acid-1-hydrate, glycerol, saccharose, methylparahydroxybenzoate, water

Ascorbic acid - 500 mg ascorbic acid per tablet

Vetoquinol Care Urinary paste (Blaasgruis), 120 g - Glucose syrup, malted barley, soy oil, fish oil, sugar cane molasses, hydrolysed pork collagen; additives/kg: DL-methionine (3c301) 80000 mg.

Nutrient	Dry Matter %	Dry Matter %	Dry Matter %
	Hill's Science plan adult	Hill's W/D	Royal Canin S/O
Protein	33.7	39.4	34.5
Fat	21.4	9.5	15
Crude fibre	1.4	8.9	2.9
Carbohydrate (NFE)	37.8	35.7	
Calcium	0.79	1.07	0.9
Phosphorus	0.78	0.86	0.9
Sodium	0.32	0.31	1.3
Potassium	0.85	0.83	1
Magnesium	0.088	0.081	0.05
L-carnitine	21.3 ppm	501.9 ppm	
Vitamin C	121 ppm	110 ppm	
Vitamin E	675 IU/kg	596 IU/kg	
Omega-6-fatty acids	3.32	2.57	
Crude Ash	5.6	6.5	8.9
Vitamin A	6789 IU/kg	5344 IU/kg	21.500 IU/kg
Vitamin D	642 IU/kg	503 IU/kg	800 IU/kg (D3)
Taurine		0.20	0.23
Omega-3-fatty acids		0.19	0.37 (EPA/DHA)
Beta-carotene		3.95 ppm	
Starch (analysed)		25.8 %	
Total sugar (analysed)		0.7 %	
Chloride			2.26
Sulphur			0.7
DL-methionine			0.39
Calcium sulphate			1.25