

# Urinary relative supersaturation and urolithiasis risk



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## ■ Introduction

Urinary stones represent 18% and 15% of all lower urinary tract diseases in dogs and cats respectively (1). In various veterinary stone analysis laboratories, dogs account for 72-81% of total stone submissions, much

more than cats (2-4). Struvite and calcium oxalate (CaOx) uroliths represent 80-90% of all stones in both species, but data from North America and Europe show that relative proportions of each type have varied over the years. Struvite predominated in the 1980s, but was progressively outnumbered by calcium oxalate in the mid-1990s. An inverse shift has now been reported in the last few years (2,5-7).

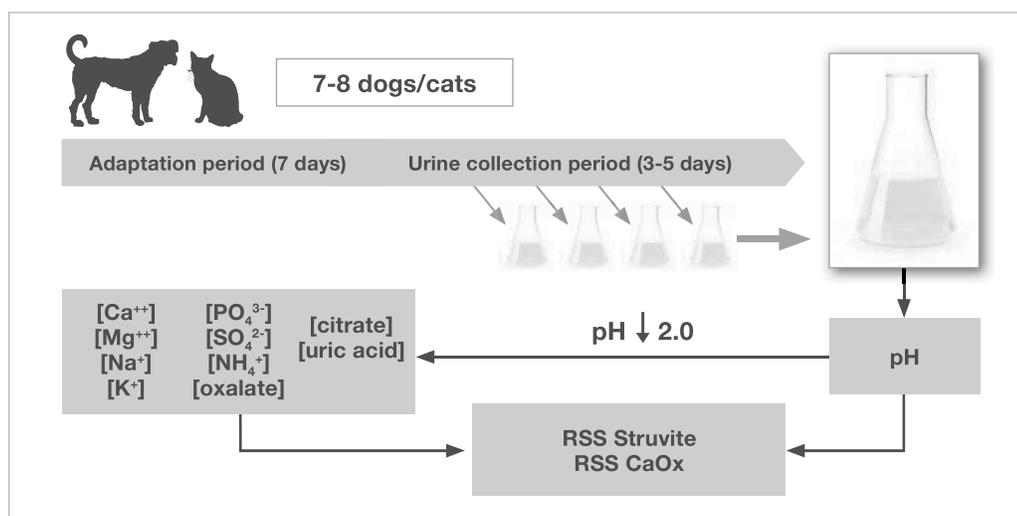
## KEY POINTS

- Relative supersaturation (RSS) remains to date the best tool to assess the risk of crystal formation in urine. It is used to optimize diet formulations, but is unfortunately too cumbersome to be measured in a clinical setting.
- Data obtained in healthy dogs and cats show that urinary pH is an important determinant of struvite RSS, but not calcium oxalate RSS.
- Urinary pH should be interpreted with caution by practitioners when assessing patients at risk for urolithiasis, due to within-day variability and the poor correlation with calcium oxalate RSS.
- Increasing dietary sodium or moisture is effective in decreasing RSS of both struvite and calcium oxalate. Urine specific gravity can be used by practitioners to monitor patients at risk of urolithiasis.

Veterinarians in practice have limited access to urine samples and tools when it comes to assessing the risk of an animal forming uroliths. Urine specific gravity (USG) and pH, as well as microscopic examination of the sediment to visualize crystals, are frequently performed on "spot" urine samples, and while they can be informative, such tests are unfortunately imperfect when considering the risk of various types of urolithiasis. For instance, stones can be present when visualized crystals are absent (and vice-versa), and urine pH varies greatly over the course of the day (8). This paper reviews the relationship between urinary relative supersaturation (RSS) and the risk of urolithiasis in dogs and cats, and identifies recent advances in this area.

## ■ Why use relative supersaturation?

Supersaturation of the urine is the physico-chemical prerequisite for stones to develop. In this state, crystal-



**Figure 1.** Schematic representation of the protocol currently used at the Royal Canin Research Center to obtain representative urine samples for RSS.

lization can occur because the concentration of the crystallizing ions is higher than their solubility product (*i.e.*, the concentration at which the components of a crystal will precipitate in a solvent (like water), at a defined temperature, and - depending on the nature of the crystals - a defined pH). The degree of supersaturation affects crystal nucleation, growth and aggregation, the three steps that precede the formation of macroscopic stones. Therefore the degree of urine supersaturation for a given crystal (*e.g.*, calcium oxalate) is a good indicator of the risk that formation of this salt will occur in the urine, although it does not take into account some other influences such as the presence of organic promoters or inhibitors of crystallization. Estimation of urinary RSS was the most widely used and recognized method in human medicine until less expensive prediction models became available, and RSS has now been used for more than a decade in dogs and cats after validation in these species (9).

## ■ Methodology: how to evaluate and interpret RSS

### Animal phase

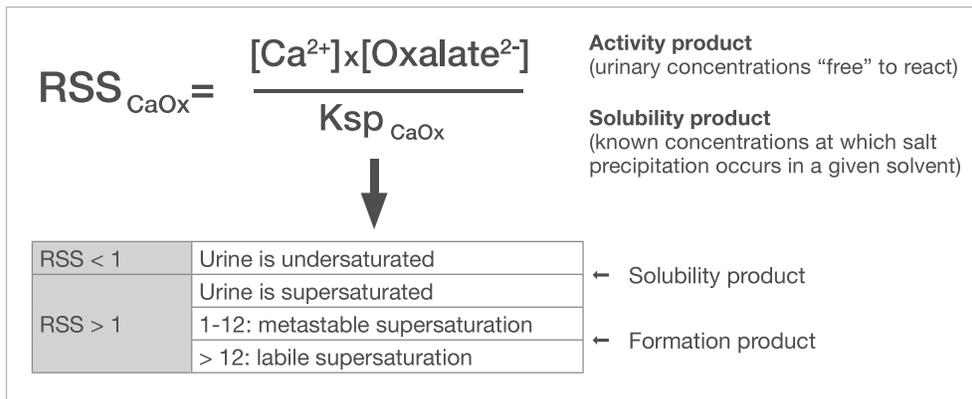
To be relevant, RSS must be estimated on a representative urine sample. In humans, one or two 24-hour urine collections are required for comprehensive metabolic evaluation of patients and for RSS calculation (10). In dogs and cats fed canned diets, 48-hour urine collection periods have been described when performing pH monitoring and RSS estimation (11,12), as urine can be more concentrated than in humans. When dry diets are fed, even longer collection periods (*e.g.*, 72 hours) may be necessary if the diets are not specifically formulated to increase diuresis.

The protocol currently used at the Royal Canin Research Center (as approved by the Ethics Committee) to obtain representative urine samples is shown schematically in **Figure 1**. Briefly, urine is collected from individual animals by a non-invasive method for at least 72 hours (after the animals have had 7 days of adaptation to the new diet). The individual urine samples are then pooled and stored at 4°C; pH is measured and then adjusted to 2.0 with hydrochloric acid in order to dissolve all salts before ion concentrations are determined.

### Analytical phase and RSS calculation

The concentrations of calcium, phosphate, magnesium, sodium, potassium, ammonium, oxalate, citrate, sulfate and uric acid in the urine pool are measured by ionic chromatography; all of these are required to calculate the RSS of a given urine sample. Various computer software packages have been developed and validated for both dogs and cats, allowing RSS to be calculated from the urinary concentrations and urine pH (9). Urine pH is required for the calculation as it influences the form in which certain ions will be present; the overall effect of pH on RSS will be discussed later.

Briefly, the computer program determines the concentrations of the solutes that remain free to interact (the activity products) to form various crystals - including calcium oxalate and struvite - taking into account all possible interactions between the ions and the complexes that could be formed. These activity products are then compared to the solubility products (see above) of the given salt to predict whether the urine is undersaturated or supersaturated for this salt. The ratio is known as the RSS (**Figure 2**).



**Figure 2.** Calculation and interpretation of RSS values. The example is given for calcium oxalate, but the principle is identical for other salts such as struvite (magnesium ammonium phosphate) or calcium phosphate. Only solubility and formation products can vary according to the salt.

**Interpretation**

Based on the average RSS value of the animals in a test panel, it can be concluded whether a specific diet has the potential to induce undersaturated or supersaturated urine. If the urine is undersaturated for a given salt (e.g., struvite), crystals will dissolve. On the other hand, supersaturated urine does not always lead to stone formation. Indeed, the supersaturation state is not uniform: it can be metastable or labile (unstable). In the metastable zone, spontaneous crystallization does not occur as it requires a nucleus to grow, while in the labile (unstable) zone, crystallization does occur. The RSS limit value between those two states is defined as the formation product of the given salt.

■ **RSS and diet testing**

After 15 years of experience with RSS and diet testing, the definitive conclusion is that RSS is more accurate than urinary pH when assessing the risk factors for urolithiasis.

**Struvite**

Struvite stones have long been known to be affected by urinary pH. Indeed, in an acid urine environment, phosphate ions (PO<sub>4</sub><sup>3-</sup>) within the struvite stones are protonated (HPO<sub>4</sub><sup>2-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, H<sub>3</sub>PO<sub>4</sub>), making them unavailable to form complexes with ammonium and magnesium. Various studies and epidemiological observations have led to the classical recommendation that diets which induce a urine pH of < 6.5 should be fed. RSS studies at the Royal Canin Research Center have allowed refinement of this relationship between pH and the risk of struvite formation. Derived from multiple feeding trials in adult cats over several years, the relationship between struvite RSS and urinary pH is shown in **Figure 3**.

As can be seen, very acid urine (pH < 6.2) always promotes RSS values in the undersaturated zone, which is

favorable for struvite prevention and dissolution. For the classically recommended pH range (6.2-6.5), RSS values are either in the undersaturated or metastable supersaturated zone for dry diets, allowing prevention of spontaneous struvite crystallization under controlled conditions (i.e., absence of urinary tract infection or preformed crystal nuclei). Results for pH > 6.5 are much more variable. Some cats with very alkaline urine still maintain undersaturated urine, which can be explained by the other determinants of RSS calculation, the ion concentrations. Indeed, with conditions that promote urine dilution, as in moist diets (wet food), urine pH becomes less critical.

The fact that RSS is a better predictor of struvite dissolution has been demonstrated in *ex vivo* dissolution studies. Such studies showed that with identical urine pH and struvite stone shapes and weights, a lower RSS promoted a more rapid dissolution, whereas dissolution times were identical in urines with similar RSS but different pH (13).

It should be emphasized that unlike cats, dogs almost always develop struvite stones secondary to urinary tract infections with urea-splitting bacteria (*Klebsiella*, *Proteus*, *Pseudomonas*, *Staphylococcus* and *Mycoplasma*) (14). In this situation the diet alone, even if shown to promote a low RSS in healthy dogs, will not be sufficient to dissolve and prevent recurrence of struvite stones if the infection is not appropriately and concurrently controlled (**Figure 4**). The rare occurrence of canine sterile struvite stones (only described so far in one family of cocker spaniels (15)) might be explained by a lesser ability of dogs compared to cats to concentrate urine.

**Calcium oxalate**

The role of pH in calcium oxalate urolithiasis is more controversial. It has been suggested that acidifying diets

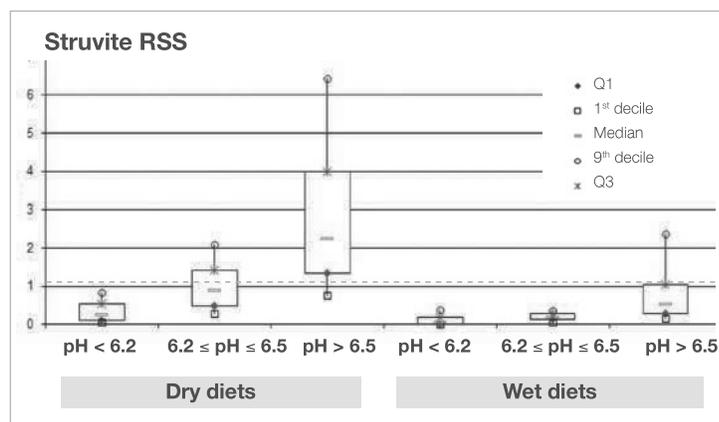
increase the risk of CaOx stone formation, based on epidemiological data (16) and the reciprocal shifts between struvite and CaOx stone prevalence over the years. One of the predominant hypotheses is that promotion of acid urine also promotes calciuria and reduces citraturia, both factors potentially favoring CaOx crystallization.

However, CaOx RSS measurements obtained over the years do not support a strong association with urinary pH (Figure 5). A recent prospective study showed that in cats, gradual acidification of a basal diet which altered the pH from 6.4 to 5.9 increased calcium excretion in the urine, but the RSS remained unchanged (17). It is possible that a much higher pH may be effective, as suggested by one study (18) where the effect on CaOx supersaturation was seen over a broader range of pH values (up to 7.9), but not for moderately acid urine as commonly induced by most maintenance and urinary feline diets. Based on those data, it appears that diets can successfully be formulated to induce a urine pH allowing undersaturation for struvite, with no detrimental effect on CaOx RSS.

### RSS and dilution

Increased levels of dietary salt have been used to stimulate water intake and promote urine dilution. The lower urinary ion concentration resulting from this strategy decreases RSS, as evidenced by several studies in dogs and cats (19-21). In dogs, raising the sodium level of a dry expanded diet from 0.5 to 3.0 g/1000 kcal significantly increased water intake and decreased CaOx RSS (19). The same effect was observed in dogs when the sodium was increased from 0.6 to 3.0 g/1000 kcal in a canned diet (20). It is important to distinguish dry from wet diets, as dietary moisture also affects urine concentration and therefore CaOx RSS. In a study with miniature schnauzers and Labrador retrievers comparing two diets that differed only in their moisture content, the 73% moisture diet resulted in lower USG, oxalate concentration and RSS than the 7% moisture diet in the schnauzers (19). Those changes were not significant in Labradors, which had higher urinary volumes and lower urine concentration than the schnauzers regardless of diet.

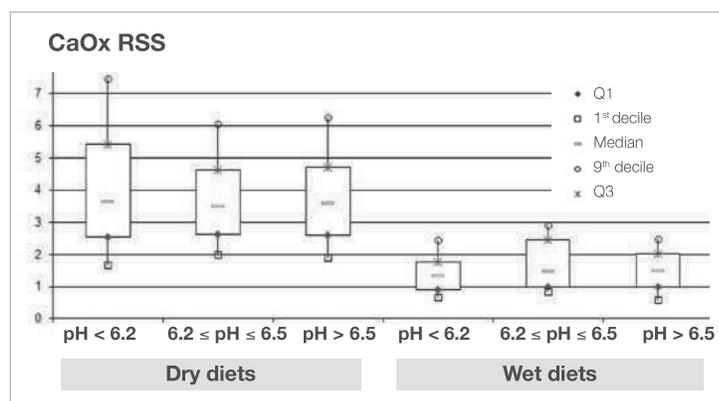
Similarly in cats fed four diets that differed only in moisture content (6%, 25%, 53% and 73%), the 73% moisture diet resulted in a higher water intake, lower USG and lower CaOx RSS than the other diets (22). This latter study also underlines that the effect of moisture on urine dilution is only obtained with a high level of moisture. Dietary strategies to promote urine dilution in order to



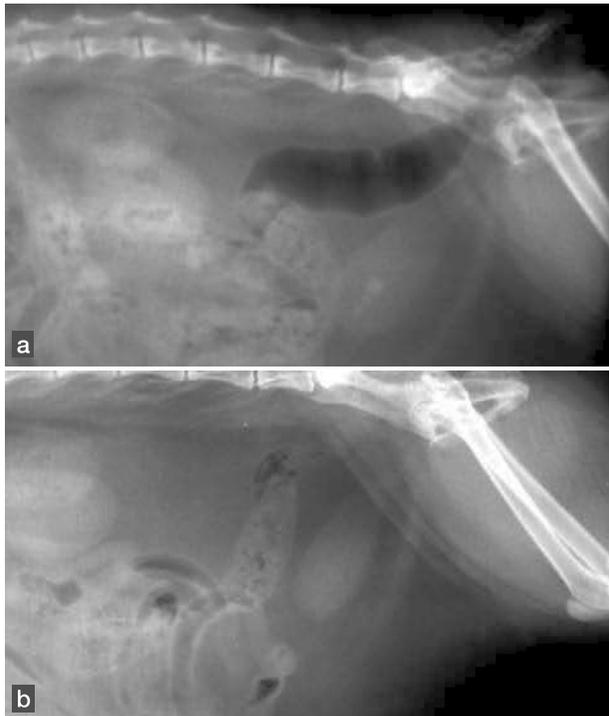
**Figure 3.** The relationship between struvite RSS and urinary pH in 142 cats fed dry (n = 481) and wet (n = 27) diets. The green dashed line represents the solubility product for struvite (the limit between the undersaturated and the supersaturated zones).



**Figure 4.** Struvite uroliths are radiopaque and thus can be seen on technically correct survey radiographs. Note that even if a diet promoting a low RSS is fed, this will not be sufficient to dissolve and prevent recurrence of the stones if the concurrent bacterial infection is not appropriately controlled.



**Figure 5.** The relationship between CaOx RSS and urinary pH is less well-defined (Data obtained from the same cat population as Figure 3).



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**Figure 6.** Radiographs of a cat with suspected struvite uroliths in the bladder before (a) and after (b) 15 days on a diet (wet or dry) formulated to induce a RSS < 1. At day 15, the uroliths are no longer visible (25).

decrease CaOx RSS have been supported by studies in both dog (23) and cat (24) stone formers, although more data is required in those populations.

### ■ How can the practitioner use these results?

From the data, it can be concluded that urine pH and USG provide only limited information to the practitioner in estimating the risk of stone formation or recurrence in a patient. Those parameters can however provide some insight in specific situations:

- A fasted urine pH < 6.2 in a cat indicates a low risk of struvite stone formation, since most RSS values will be under the formation product below this pH.
- A fasted urine pH > 6.5 is not always indicative of a high risk of struvite crystalluria, especially in animals fed canned diets that promote urine dilution.
- A urine pH obtained in the hours following a meal can be elevated and unrepresentative of the average urine pH, due to the postprandial alkaline tide. A pH measurement obtained after a night of fasting is more relevant, but ideally urine should be collected for at least 48 to 72 hours.
- Urinary pH is not a good predictor of CaOx RSS.
- USG, a marker of urine dilution, remains a good,

although imperfect, tool to assess the relative risk of stone formation, especially during follow-up of stone formers to prevent recurrence. The thresholds classically recommended (< 1.020 in dogs and < 1.025 in cats) can be difficult to achieve in some instances (e.g., in cats fed a dry diet).

- It is possible to formulate diets that will both promote the dissolution of struvite stones and low urinary saturation of struvite and CaOx.

### ■ RSS: how it translates to *in vivo* observations

While RSS is obtained from analyses run on urine samples obtained *in vivo*, and describes physicochemical reactions occurring in the urine, it can be argued that this risk index does not take into account other factors known to influence the different crystallization steps, such as organic promoters or inhibitors. Therefore the question of its relevance and accuracy to predict stone formation *in vivo* can be raised. This is partially answered by studies in humans and animals; both stone-forming humans and dogs (especially those with recurrent CaOx or calcium phosphate uroliths) have higher corresponding RSS values than healthy individuals, despite overlap between the two groups (9,23). A recent study has demonstrated the validity of using a diet known to induce a struvite RSS < 1 to predict and achieve struvite stone dissolution in cats (25) (Figure 6).

Another potential limit of RSS measured in research centers is the health status of the animals studied. Metabolic differences between animal populations may explain the appearance of the disease and could affect the RSS value. However, it is difficult to obtain and process 48-hour urine samples in privately-owned animals for RSS. Studies in both dogs (23) and cats (24) not only showed that stone formers have higher CaOx RSS on their usual diet compared to healthy animals, but that when fed diets promoting urine dilution the CaOx RSS decreased, and also that (in dogs) feeding such diets for a year was not associated with clinical recurrence of uroliths.

### ■ Conclusion and perspectives

Today, RSS remains the best tool to predict the potential for a diet to induce urinary stone formation or dissolution. Several years of research in this field in cats and dogs have allowed a better comprehension of the limitations in using urinary pH as the sole risk factor for urolithiasis (especially CaOx), and assessment of the effect of urinary dilution (via dietary sodium or moisture)

on the risk of stone formation, amongst other findings. However, more research is still needed, especially when it comes to understanding the effect of specific nutrient

modifications on urine composition and RSS, and to investigate the metabolic differences between normal animals and stone formers.

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