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Farmina Vet Research Journal

***Nutritional management of
struvite urolithiasis in dogs.***

Farmina Vet Research

Farmina Vet Research Group (FVR), with its scientifically demonstrated dietary nutrients (Vet Life Formula), aims to support the Veterinarian with the management of some pathologies commonly occurring in pets.

It also intends to provide valid solutions to dietary problems and scientific consultancy through the cooperation of the Animal Science and Food Control Department of the Faculty of Veterinary Medicine of University of Napoli Federico II.

Farmina Vet Research is capable of maintaining a scientific dialogue with the Veterinary world, delving into clinical and dietary issues.

Farmina Vet Research is part of the scientific division of the Farmina firm supported by the competence and the hard work of several professionals focusing on delivering effective consultancy services.

Farmina Vet Research integrates its production by studying technological innovations and by improving new products through manufacturing processes aimed at keeping up with future challenges. The high quality of its products contributes to the wellbeing and health of our pets.

Urolithiasis

The formation of stones along the urinary tract (urolithiasis) is universally acknowledged as one of the most frequent afflictions of the urinary tract in men and animals. The oldest finding of human stones was discovered in an Egyptian tomb dating back to 5000 B, whereas the first findings of animal urinary stones are even older: the skeleton of a marine reptile,

The first paper documents that testified to the finding of concretions in the kidneys and bladder of animals, which were sacrificed, date back to Herodotus and Aristotle (Anke and Anning, 1973). There are no definite data concerning the nature of these stones. Nevertheless, it was supposed that they could be constituted by calcium carbonate and/or struvite.

Table 1 – Composition of the concretions in different animal species (modified by Anke and Anning, 1973).

Species	Composition
Sturgeon, Carp	Guanine
Frog	Struvite, calcium phosphate
Turtles, Lizards, Snakes	Urate
Flamingos, Cranes, Pigeons	Uric acid, urate
Sheep, Cows	Struvite, silica
Monogastric herbivores	Calcium carbonate
Pigs	Calcium phosphate, calcium carbonate
Cats	Struvite, apatite, calcium oxalate, urate, cystine
Dogs	Struvite, apatite, calcium oxalate, urate, cystine, xanthine, silicate

which lived approximately 80 million years ago, containing a calcium carbonate (calcite) and calcium phosphate (apatite) stone, and that of a bear that lived in the Pleistocene and found in a pit; the stone was made from calcium carbonate in this case too (Hesse et al., 1998).

The various animal species have a greater tendency towards specific types of stones, as stated in Table 1, e.g. monogastric herbivores (horses, donkeys and rabbits) mostly have carbonate calcium urate (Neumann et al. 1994).

The urinary stones in vertebrates, particularly domestic ones, represent an economic problem of a certain magnitude. In domestic carnivores urolithiasis is a recurrent and rather common problem (Bartges, 1998).

The surgical removal of the stones is the most frequent treatment used, whereas in some cases dietetic and/or pharmacological treatment allow the stones to be dissolved and is useful in the prevention of recurrences. The long-term efficiency of an urolithiasis treatment depends on the identification of the type of stone and knowledge of the specific etiopathogenesis of every type of urolithiasis in order to handle the specific factors that arise, which means that the minerals present in the stone must be analysed correctly. In some cases the number and shape of the stone leave no doubts on the composition without requiring specific analysis (Osborne et al. 1989). Nevertheless, it is extremely important to establish the composition of the concretions as the pathogenesis and, consequently, the therapy and prevention of recurrences are extremely different according to the type of urate. The microscopic evaluation of the urinary sediment is only an indicator of suspicion of the composition of any type of stone as its formation, development and dissolution are associated with a complicated set of variables. The definitive identification of the composition of

the stones requires analysis with a polarizing light or diffraction microscope or any other quantitative analysis method (Osborne et al., 1990).

Pathogenesis

Calcium carbonate stones

These stones are the most frequently found in herbivorous mammals. In rabbits it is known that an excessive administration of calcium does not reduce the intestinal absorption of this element, but it may act towards increasing the urinary excretion (Kamphues 1991). Moreover, many edible plants contain significant quantities of oxalate, which may give rise to inclusions of calcium oxalate.

Nevertheless, more so than the level of calcium, the parameter that seems to have a greater effect on the urinary excretion of calcium appears to be the calcium/phosphate ratio. Calcium oxalate stones are not particularly frequent in dogs (Hesse et al., 1998), even though this element is a minor amorphous component in many stones. Even if the pH is reduced to levels less than 8.0, the crystallization of the calcium phosphate in case of excessive concentration of calcium is always possible; only the reduction of the pH to levels less than 6.4 may prevent the

sedimentation of the calcium phosphate, therefore it is indispensable to guarantee a sufficient dilution of the urine, stimulating the thirst to prevent the formation of stones.

Urate stones

As well as lizards, snakes and birds, Dalmatian dogs are particularly predisposed to the formation of urate and ammonium urate concretions especially. Other dog breeds (Pekingese, Fox Terrier, Chow Chow and Pinscher) also produce concretions of urate, albeit in these cases the etiopathogenesis is different.

Different hepatic enzymes, like xanthine-oxidase and uricase, break down the endogenous and nutritional purines into xanthine, uric acid and allantoin. The latter is excreted through the kidneys. Case et al. (1993). The human species does not present uricase, so the purinic derivative that is excreted through the urinary tract is uric acid. In Dalmatians the plasma concentration of uric acid is triple what is found in other dog breeds and the excretion of uric acid is ten times higher. Nevertheless, there's no difference as regards the activity of hepatic uricase, meaning that the high plasma concentration of uric acid, which may only be limited through renal excretion, should mainly be ascribed to the lack of uric acid conveyed to the liver (Giesecke et al. 1985). In Dalmatians, a

significant correlation was found between the purine bases ingestion and the plasma concentration of purine on one hand and the urinary excretion of these elements in 24 hours on the other hand. The high rate of urinary excretion of uric acid may not be enough to explain the formation of stones. This is associated with a greater secretion in terms of the ammonium ion tubes.

Limiting the levels of the purines administered becomes an indispensable condition for prevention. The addition of potassium citrate enables the urinary pH to be reduced to at least 7.0. Nevertheless, it may also be considered that these urinary pH levels encourage the formation of phosphate crystals. Consequently also in this instance the most reasonable thing is to stimulate diuresis. Using allopurinol to suppress the xanthine oxidase, the production of uric acid may be reduced. An appropriate preventive compromise lies in correcting the diet and administering pharmacological treatment.

Cystine uroliths

Cystinuria is a congenital metabolic disorder distinguished by an abnormal reabsorption in the renal tubes of cystine and other amino acids. In humans, other amino acids are also found in this

6 uroliths, such as lysine, arginine and ornithine. In dogs, very different frameworks of amino acidic excretion have been discovered; in addition to the aforesaid amino acids, concretions of citrulline and threonine have been found. Hesse et al. (1998) recorded an incidence of stones exclusively consisting of cystine, amounting to approximately 28% of the cases, whereas they found lysine, arginine and citrulline in the remaining 72%. Even if the only amino acid that is only partially soluble in urine is cysteine, which may crystallise when present in concentrations greater than 200 mg/l (0.65 mmol/l), whereas when the concentration exceeds 340 mg/l (1.3 mmol/l) the formation of crystals is practically guaranteed.

Struvite stones

Struvite ($MgNH_4PO_4 \cdot 6H_2O$) is regarded as the common form of stones in dogs, cats and humans (Hesse et al., 1998; Deng & Ouyang, 2006; Pintilie et al. 2010), even if in recent years some studies have highlighted a decline in the incidence (Osborne et al., 1999; Picavet et al., 2007). These concretions have also been found in sheep and cows.

The over-saturation of urine by the ammonium magnesium phosphate ions is one of the fundamental requisite for the formation of struvite uroliths, although their formation may be influenced by

numerous other factors such as bacterial infections of the low urinary tracts, alkaline urinary pH, unsuitable



diets and genetic predisposition.

The formation of struvite stones was often associated with bacterial infections of the urinary tract (Buffington et al., 1998). In particular the involved bacteria are capable of breaking down the urea into ammonia and carbon dioxide, such as some strains of *Proteus*, *Staphylococcus* and *Klebsiella*. A slight increase in the concentration of ammonium and bicarbonate ions is seen in a marked urine alkalization. A pH urinary higher than 7.0 and the high concentration of ammonium, magnesium, calcium and phosphate. If there is excessive bacterial flora, struvite crystals may rapidly form so as to block the entire renal pelvis. The bacterial inflammation may lead to the formation of a mucoid matrix that gradually incorporates the precipitated crystals so as to form voluminous aggregates. In this case the stones contain the bacteria that during treatment

may suddenly become active again. This explains why it is fundamental that the concretions are removed completely (Sanders et al., 1986; Kienzle et al., 1993). On average, 58% of cases of urolithiasis in dogs involve struvite. Some breeds like Cocker Spaniel, Pekingese, German Shepherd, Shih Tzu, Bobtail and Bernese Mountain seem to be particularly predisposed to the formation of these stones compared with other forms of urolithiasis.

To dissolve struvite concretions caused by bacterial infection it is necessary to treat the infection, dilute the urine, reduce the protein levels of magnesium and phosphates in the diet, increase sodium chloride for thirst and induce polyuria and, lastly, acidify the urine with ammonium chloride or methionine to levels less than 6.2.

Calcium oxalate stones

Calcium oxalate stones are those most frequently found in humans (75% of the total of cases), whereas they are much less frequent in animals (from 3.6% to 17.5% of such Hesse et al., 1998), although a considerable increase of the incidence of such stones has been seen in recent years in cats and dogs. Isolated cases have been documented in hamsters, rabbits, deer and sheep.

Two different types of calcium oxalate concretions

7 have been acknowledged: one characterized by monohydrate calcium oxalate ($CaC_2O_4 \cdot H_2O$) and the other consisting of dihydrate calcium oxalate ($CaC_2O_4 \cdot 2H_2O$), which represent 56.8 and 43.2% of the total of calcium oxalate urolith respectively, stated by Hesse et al. (1998). The morphology of these two types of uroliths is extremely different, what enables the recognition in the urinary sediment. Experimental studies have shown that monohydrate calcium oxalate concretions are somewhat unstable, tending to form in the main with high concentrations of calcium and magnesium, whereas dihydrate calcium oxalate stones come from the stabilization of oxalate and urate (Hesse et al., 1976; Berg et al., 1976). They are the most frequent stones in small-sized breed. 90% of stones were found in male subjects, mostly above the age of 6 years (Hesse et al., 1998).

The use of large quantities of plant foods in the diet carnivores may cause an excessive ingestion of oxalates with consequent hyperoxaluria. In humans, the patients affected by calcium oxalate stones show a greater intestinal absorption of oxalate than healthy subject. This may be due to gut inflammatory phenomena, low calcium and magnesium intakes hyperlipidic diets or enterectomy. In dogs, the urinary excretion of oxalates is 3 to 4 times higher than in humans, whether in healthy subjects or in those affected by urolithiasis, thus why crystals may form

very easily if the predisposed subject.

The subjects that form stones show calcium concentrations in the urine, which are significantly higher than in healthy one, with low concentrations of citrate and high concentrations of magnesium.

At present it is not possible to evaluate whether the growing incidence of calcium oxalate stones in cats and dogs is due to changes in the population, such as a larger number of predisposed species, or lifestyle changes and the type of nutrition. There is, however, an evident need to regularly monitor the urine in the predisposed subjects.

Urolithiasis may be defined as a multifactorial disorder in which predisposing and conditioning factors are recognised that may influence the formation of concretions.

Diverse predisposing factors have been recognised in the formation of stones, such as disturbances of the urodynamics due to pathological and anatomical changes, metabolic disorders, genetic factors, and environmental and nutritional factors.

Over the years various theories have been formulated concerning the origin of the formation of uroliths:

- the so-called *matrix theory*, which is based on the assumption that the stone is formed from the formation of a primary precipitate consisting

of an organic substance, mostly protein and polysaccharides, which could represent the stone's primary nucleus;

- the *crystalization theory*, on the other hand, is based on the assumption that the first step in the urolith formation is represented by the over-saturation of the urine with the formation of an inorganic matrix;
- 3. the *crystalization suppression theory*, according to which there is a partial or total lack of the factors suppressing the crystalization at the basis of the formation of the stones (Osborne et al., 1986).

As is often the case, all of the theories mentioned contain some truth. In fact in different stones an organic matrix consisting of protein, glycoprotein and glycosaminoglycans can be made out with litogenic and inhibitory effects. Likewise, the importance of substances capable of inhibiting or increase the precipitation of crystals cannot be negated. These substances are capable of influencing the intestinal absorption and the renal excretion of the elements that may go towards forming the stone.

Pathogenetic factors that contribute towards the formation of different types of stones can be summarized as follows. The formation of crystals in the urine may only occur in conditions of over-

saturation, therefore the prevention of any type of uroliths requires an adequate level of urinary dilution; the specific weight of the urine should be below 1.010 (Borghi et al., 1996; Hesse et al 1997a,b). The urinary pH is equally fundamental; e.g. calcium carbonate stones only develop in subjects affected by hypercalciuria, if the urinary pH is between 7.5 and 9.5.

Epidemiology of the urolithiasis in dogs

The results of some epidemiological studies published in the last decade are extremely interesting (Hesse et al., 1998; Osborne et al., 1999; Picavet et al., 2007). In particular Picavet et al. (2007) whilst analyzing over 4,000 uroliths taken from dogs (65%) and cats (35%) between 1994 and 2004 saw a significant increase of the cases; whereas in 1994 the authors analyzed 110 stones, of which 85% came from dogs, in 2003 they recorded 1,067 stones with 59% from dogs. The authors also found substantial changes in relation to the types of concretions in the period examined, in particular whereas in 1994 77% of cats were affected by stones consisting of struvite and 12% had stones made of calcium oxalate, in 2003 32% of the uroliths from felines consisted of struvite and 61% of calcium oxalate. The same trend, albeit with less evident differences, was observed in dogs. In 1994 51% of

uroliths consisted of struvite and 33% of calcium oxalate; whereas in 2003 40% consisted of struvite and 46% of calcium oxalate.

The authors also saw that there was a significant effect due to species, size, breed and gender. The average age of the subjects affected by urolithiasis was 7.3 years and 7.2 years in dogs and cats respectively. The authors concluded that the results obtained from samples coming from the Benelux countries were similar to those obtained by Osborne et al. (1999a) with stones taken in North America and that over the last decade in Benelux, as in the USA, the relative incidence of calcium carbonate stones has increased compared with struvite uroliths in both cats and dogs. The number of stones subjected to qualitative analysis also increased significantly in the same period, in confirmation of the greater awareness of veterinarians for a correct investigation about the type of stones removed from pets.

Experimental part

Use of two diets for the management of struvite urolithiasis in dogs

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In dogs, the majority of struvite stones are associated with bacterial infections sustained by the *Staphylococcus* and *Proteus* strains, capable of producing urease by increasing the levels of ammonium, phosphates and carbonates in the urine and, consequently, raising the pH.

Many struvite concretions also contain small quantities of other minerals like calcium phosphate and ammonium urate. Finding sterile struvite stones in dogs is rather rare. In these cases the causes of the uraths formation are due to a genetic predisposition, incorrect nutrition or metabolic disorders (Osborne et al, 1995).

Struvite stones caused by bacterial infection require a treatment based on the association of appropriate antimicrobial agents and diet capable of dissolving the stones. In the case of sterile stones, a dietary therapy is enough, associated (if needed) with the administration of urine acidifying substances (Osborne et al, 1999b; Rinkardt & Houston, 2004). Nevertheless, the majority of commercial diets for the treatment of struvite urolithiasis is integrated with

acidifying substances. The use of therapies for this purpose is therefore useless, if not actually counter-productive.

Once defined, the antibiotic treatment must continue until the stones cannot be seen by x-ray to ensure that vital bacterial capable of causing recurrences cannot linger in the concretions (Seaman & Bartges, 2001).

The diets formulated to dissolve the struvite calculi must contain limited levels of protein (15-20% in a diet of 4000 kcal/kg) so as to limit the quantity of urea available for any urease reducer bacteria. Diets with low concentrations of fibre are also preferred to limit the intestinal reabsorption of water and, at the same time, to maintain a high level of digestibility.

In terms of the mineral component, these diets must contain reduced concentrations of phosphorus and magnesium and are integrated with sodium chloride to stimulate thirst (Lulich et al., 2000).

The nutritional treatment must last for at least a month after stones dissolution in order to guarantee the complete excretion of all fragments, including the not-detectable. It is advisable to regularly monitor

the subject throughout the period using abdominal ultrasound or radiography and urine analysis.

The symptoms usually subside quickly, whereas for the complete dissolution approximately 3 months may be needed. This period may be brief (5-6 weeks) in cases of sterile stones (Osborne et al, 1999b)

The aim of the work was to compare the effects on the urinary pH of two different completely dry compound feeds formulated for the treatment of struvite urolithiasis.

Material and methods

Twelve dogs were recruited for the study (average age 4.3 ± 1.2 ; live weight 20.2 ± 10 kg) of different breeds affected by struvite urolithiasis, confirmed by microscopic analysis of the urinary sediment.

The subjects were divided into two groups, which were fed with diet A and B, respectively for three months. The owners of the dogs were asked not to provide other foods and to stimulate the voluntary water intake and increase the number of outdoor walks to stimulate greater passing of urine.

Throughout the experiment, urine samples were taken from the dogs six times at regular intervals (15

days) in order to determine the physical, chemical and microbiological analysis, whereas blood was only taken at the beginning and end of the experiment.

The two diets were analysed for chemical composition according to AOAC (2006), as well as determining the mineral profile (de Ruig W.G., 1986) by atomic absorption spectrography.

The acid/bases equilibrium was therefore calculated using the equation put forward by Langendorf, H. (1963): $mEq/kg\ ss = 49.9Ca + 82.3Mg + 43.5Na + 25.6K - 64.6P - 62.4S - 28.2Cl$.

The results relating to the parameters of the urine analysis were subjected to variance analysis by PROC. GLM of the statistical program SAS (2000).

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Results

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In Table 2 the ingredients of the two diets are stated. In both products you can make out the addition of sodium chloride to stimulate thirst (Stevenson et al, 2003b).

Table 2 – Ingredients of the diets

Diet	
A	Ground corn, dried chicken and turkey protein, animal fats, hydrolysates, ground soya beans, vegetable oil, whole powdered eggs, linseeds, calcium sulphate, potassium chloride, salt, taurine, trace elements and vitamins, EU-approved antioxidants.
B	Rice, dried chicken meat, animal fats, potatoes, oats, whole powdered eggs, linseeds, hydrolysed animal protein, fish oil, potassium chloride, calcium sulphate, vegetable oil, sodium chloride.

Moreover, it is evident that high-organic protein or in hydrolized form is used in both cases to limit the urinary excretion of nitrogen catabolics.

In Table 3 the average values of the chemical composition of the two feeds used are stated. Both diets have protein and fibre contents in the ranges shown by Lulich et al. (2000). Although diet A had more protein, lipid and raw cellulose contents than diet 2, the energy density values of the both diets were virtually the same.

Table 3 – Chemical composition of the diets

Diet	Raw proteins	Ether extract	Raw cellulose	Ash	Metabolizable energy
		%			MJ/kg
A	21,7	19,6	2,7	4,3	16,6
B	19,5	19,0	1,5	4,4	16,5

The main mineral contents of the two diets are shown in Table 4, as well as the relative acid/bases balances. Diet A registered higher values of calcium, sodium, phosphorus

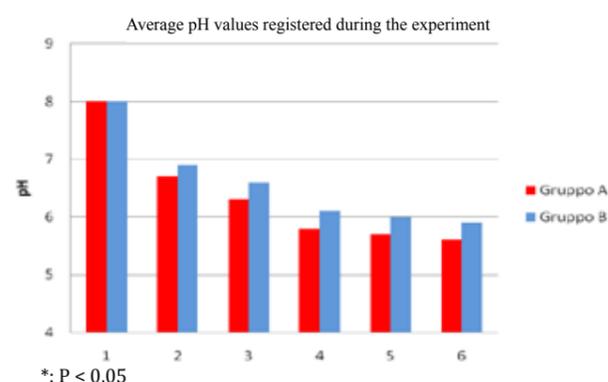
and sulphur than diet B. Both diets registered a negative acid/bases equilibrium, which is plausible when dealing with diets formulated to dissolve struvite stones that requires a reduction of the urinary pH, nevertheless due to higher concentrations of phosphorus and sulphur and a lower magnesium content, diet A showed a ratio of over 10 mEq/kg ss lower than diet B.

Upon the first sampling the urinary pH was rather

Table 4 -Mineral contents of the diets

Age	Ca	Mg	Na	K	P	S	Cl	acid/bases
								mEq/kg ss
A	7,88	1,12	3,67	7,50	9,61	5,76	7,50	- 203
B	6,20	1,40	3,35	7,93	8,63	5,43	7,51	- 192

high in both groups (8.0 ± 0.5 and 8.0 ± 0.8 for groups A and B, respectively); approximately 70% of the samples also had bacterial contamination. It was



consequently deemed advisable to also combine with the nutritional treatment a pharmacological treatment with fluoroquinolone in tablets for one week.

As from the second urine analysis, all the dogs showed a progressive reduction of the pH level in the urine and no sample showed bacterial contamination. As shown in the Figure, after two months of treatment, all the subjects

showed pH values in the range of 5.9 and 6.1 stated by (Stevenson e Rutgers, 2006) as suitable for stones dissolution. The results obtained were in line with those stated by other authors (Osborne et al, 2009; Rinkardt and Houston, 2004).

The average urinary pH values registered at the end of the experiment by both groups (5.6 ± 0.5 and 5.9 ± 0.4 , fro group A and B, respectively) are indicative of the need to block the administration of both diets beyond the three months, so as to avoid creating an environment that supports the formation of other types of stones (urate and/or cystine) (Stevenson e Rutgers, 2006) or repercussions on other systems.

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