The relative effects of supplemental dietary calcium and oxalate on urine composition and calcium oxalate relative supersaturation in healthy adult dogs

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Abstract

The aim of this study was to establish the relative effects of dietary calcium and oxalate (in the form of oxalic acid) on the composition of urine produced by healthy adult Cairn Terriers and Miniature Schnauzers. A nutritionally complete dry dog food was fed to 7 dogs (4 Cairn terriers and 3 Miniature schnauzers) for 24 weeks. The dogs were fed the diet alone, or supplemented with six different combinations of dietary calcium (as carbonate and sulphate) and oxalate (as oxalic acid) commonly found in dry commercially prepared dog foods. Urine pH, volume, specific gravity, and concentrations of 12 analytes were measured for each dog; urinary relative supersaturation (RSS) with calcium oxalate (CaOx) was calculated from these values. The effects of supplemental calcium and oxalate were established using two-way analysis of variance and multiple range tests (least significant difference); \( P < 0.05 \) was considered significant. The lowest level of dietary calcium and oxalate resulted in the lowest CaOx RSS. The high calcium, low oxalate diet resulted in the highest CaOx RSS. The low calcium diet with increased dietary oxalate also tended to increase CaOx RSS although results were highly variable. Urinary calcium concentration increased significantly with dietary calcium; urinary oxalate increased, although inconsistently, with dietary oxalic acid only when dietary calcium was low. Measures to reduce both calcium and oxalate should be considered when implementing dietary changes to reduce the risk of calcium oxalate formation in dogs. A reduction in dietary calcium without a concomitant decrease in dietary oxalate may increase the risk of CaOx crystallisation in susceptible dogs.

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1. Introduction

Calcium oxalate (CaOx) urolithiasis appears to be a growing problem in dogs (Lulich et al., 1999b). The proportion of this urolith type submitted for analysis at one centre in the USA increased from 5.3% in 1981 to 35.1% in 1997 (Lulich et al., 1999a). Recent research has also shown that small dogs have a higher risk of CaOx crystallisation than do large dogs fed the same diet (Stevenson and Markwell, 2001). Many articles have been written evaluating aspects of diet and specific nutrients, including calcium, oxalate, protein, sodium, phosphorus and potassium, in the formation and subsequent prevention of CaOx uroliths in dogs (Lulich et al., 1992, 1999a; Osborne et al., 1995). However, information has often been extrapolated from studies in humans.

Much research has been conducted in humans to evaluate the relative importance of dietary calcium and oxalate on the risk of CaOx urolith formation. In humans, urinary calcium is largely dependent upon dietary calcium at least at low to moderate dietary intakes, (Marshall et al., 1976; Lemann et al., 1979). Urinary calcium, in humans, rises sharply up to intakes of around 10 mg/kg body weight/d, after which it levels off unless individuals are hyperabsorbers of calcium (Lemann et al., 1979; Robertson, 1993). However, hypercalciuria as a consequence of dietary intake alone is unusual in humans (Robertson, 1993). Calcium is absorbed in the ionic state, and therefore substances that complex with calcium either in the diet, or gut, such as phosphate, citrate, sulphate,
oxalate and fatty acids decrease the availability of calcium for absorption (Menon et al., 1998). If dietary calcium is restricted in humans without a concomitant decrease in dietary oxalate, passive intestinal absorption of oxalate in the colon followed by an increase in urinary oxalate may occur (Zarembski and Hodgkinson, 1969; Hodgkinson, 1978). Thus, in human CaOx stone formers severe calcium restriction is not recommended (Bataille et al., 1983; Jaeger et al., 1985). In some human studies a high dietary calcium intake actually decreased the risk of CaOx formation (Marshall et al., 1972; Curhan et al., 1993, 1997). The mechanism for this is thought to be the binding of calcium with oxalate in the intestinal lumen leading to excretion in the stools rather than the urine (Zarembski and Hodgkinson, 1969; Marshall et al., 1972).

Oxalate is a simple organic dicarboxylic acid present in many foods, particularly cereal grains and leafy plants, as oxalic acid (Holmes and Kennedy, 2000). Humans cannot metabolise oxalate directly, and thus with the possible exception of bacterial action in the gut, renal excretion becomes the sole source of oxalate elimination (Asplin et al., 2000). Although it was originally thought that a large amount of the oxalate found in human urine (up to 80%) was derived from endogenous production (Hagler and Herman, 1973; Menon et al., 1998), recent studies have shown that the contribution of dietary oxalate to urinary oxalate may be underestimated (Holmes et al., 1995; Holmes and Kennedy, 2000). No such studies have been conducted in dogs. In humans, primary hyperoxaluria can occur as a result of metabolic errors, inherited through an autosomal recessive pattern (Williams and Wilson, 1990). However, a number of workers within the human field have proposed that mild hyperoxaluria, often as a result of a high dietary oxalate to calcium ratio is secondary in importance only to urine volume as a risk factor for CaOx stone formation in individuals without primary hyperoxaluria (Robertson et al., 1978; Robertson and Peacock, 1980; Bataille et al., 1983; Larsson and Tiselius, 1987). To date, no such research has been conducted in dogs.

To our knowledge the relative effects of dietary calcium and oxalate on composition of urine in healthy small breed dogs have never been examined. In addition, there appears to be very little information available concerning the amount of oxalate commonly encountered in dry commercially prepared dog foods. Thus, the purpose of the study reported here was to compare the relative effects of dietary calcium (in the form of carbonate and sulphate) and oxalate [in the form of oxalic acid, H₂C₂O₄] on urine composition when fed to healthy small breed dogs at levels commonly encountered within commercially prepared dog foods.

### 2. Materials and methods

#### 2.1. Dogs

Healthy adult dogs consisting of 4 Cairn Terriers and 3 Miniature Schnauzers (5 spayed females, 2 castrated male; mean age (SD) 5.2 (1.0) years).

#### 2.2. Study design

Dogs were fed a nutritionally complete dry dog food (Table 1) twice daily at 08.30 and 15.30. Before starting the study all dogs received a veterinary examination and dogs had no previous history of long-term illness. Food allowances were calculated according to adult maintenance energy requirements (110 W 0.75 kcal/d where W is body weight expressed in kg) (Burger, 1995), and adjusted during the studies to ensure body weight maintenance within ±5% of original weight. All dietary nutrients were analysed by previously described methods (Stevenson et al., 2000b). Faeces quality was monitored daily using the WALTHAM faeces scoring system (Moxham, 2001). Tap water was provided ad libitum, and voluntary water intake was measured daily when dogs were individually housed.

#### 2.3. Diet details

Seven different combinations of dietary calcium and oxalate were examined (Table 2). The maximum levels were selected from the analysis of 30 different dry dog foods from 13 different manufacturers in eight different countries including USA (n = 8), Australia (n = 3), Japan (n = 3), Italy (n = 4), Germany (n = 2), Netherlands (n = 3), UK (n = 4) and France (n = 3), designed for adult small breed dogs. Dietary calcium and oxalate were highly variable between diets (calcium: range

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Unit</th>
<th>Amount (per 100 kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>g</td>
<td>2.15</td>
</tr>
<tr>
<td>Protein</td>
<td>g</td>
<td>3.94</td>
</tr>
<tr>
<td>Fat</td>
<td>g</td>
<td>3.42</td>
</tr>
<tr>
<td>Ash</td>
<td>g</td>
<td>1.74</td>
</tr>
<tr>
<td>Calcium</td>
<td>g</td>
<td>0.18</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>g</td>
<td>0.15</td>
</tr>
<tr>
<td>Ca:P ratio</td>
<td>g/g</td>
<td>1.20</td>
</tr>
<tr>
<td>Sodium</td>
<td>g</td>
<td>0.28</td>
</tr>
<tr>
<td>Potassium</td>
<td>g</td>
<td>0.21</td>
</tr>
<tr>
<td>Magnesium</td>
<td>g</td>
<td>0.02</td>
</tr>
<tr>
<td>Iron</td>
<td>mg</td>
<td>3.78</td>
</tr>
<tr>
<td>Copper</td>
<td>mg</td>
<td>0.17</td>
</tr>
<tr>
<td>Manganese</td>
<td>mg</td>
<td>0.28</td>
</tr>
<tr>
<td>Zinc</td>
<td>mg</td>
<td>5.57</td>
</tr>
<tr>
<td>Oxalate</td>
<td>mg</td>
<td>9.76</td>
</tr>
</tbody>
</table>
0.18–0.75 g/100 kcal, mean 0.35 (0.10) g/100 kcal; oxalate range 4–25 mg/100 kcal, mean 11 (5) mg/100 kcal), although calcium content was always higher than oxalate content. Thus the maximum levels selected for this study were 0.75 g calcium/100 kcal and 25 mg oxalate/100 kcal. Each dog received each combination for a 12-day period in a Latin square design. Each study period was separated by an 8-day interval during which time the dogs received only the dry nutritionally complete diet. During each study period the dogs either received a nutritionally complete dry dogfood containing a low level of both dietary calcium (0.18 g/100 kcal) and oxalate (10 mg/100 kcal), (LCa–LOx); or the complete diet plus one of six combinations of supplementary calcium and oxalate (Table 2). Supplements were made into stock solutions with deionised water once weekly and added to the food directly before feeding. HCa–HOx delivered the maximum amounts of both calcium and oxalate. Dogs receiving all other diets were given deionised water up to the total volume supplied by HCa–HOx. The calcium supplement consisted of a combination of calcium sulphate (BDH Laboratory Supplies, Poole, UK) and calcium carbonate (BDH Laboratory Supplies, Poole, UK), dosed at a level to deliver equal amounts of sulphate and carbonate ions to minimise the influence of supplementation on urine pH. Oxalate was given in the form of oxalic acid (BDH Laboratory Supplies, Poole, UK), a form commonly used in human studies examining the influence of dietary oxalate on urine parameters (Marshall et al., 1972; Liebman and Chai, 1997; Liebman and Costa, 2000). All supplements were at least 98% pure and of general purpose grade. To ensure that all liquid supplements soaked completely into the diet 10% of each ration was ground into a crumb format, supplements were added to the crumbs and then mixed with the remaining 90% of the daily food ration, ensuring complete ingestion of all supplements.

2.4. Housing details

Dogs were housed separately, as previously described (Stevenson et al., 1998), for three 48 h periods (days 3–4, 7–8, 11–12) during each treatment phase. During the remaining days the dogs were housed in pairs, although the pen design still allowed individual feeding. Whilst in pairs all dogs were walked once daily for approximately 15 min and group exercised in grass paddock areas for 1–2 h.

2.5. Blood measurements

At the start of the study all dogs were fasted for 24 h and a blood sample was collected from the jugular vein of all dogs and analysed for haematology (Baker system 9000 automated cell counter, Serno-Baker Diagnostics, Pennsylvania, USA), biochemistry (Cobas MIRA Plus Biochemistry Analyser, Roche Diagnostic Systems, Branchburg, NJ USA) and blood gas parameters (AVL Omni 288 Blood Gas System, Graz, Austria).

2.6. Urinary measurements

Urine pH was continuously measured using the non-invasive automated urine pH monitoring system (Stevenson et al., 1996; Stevenson et al., 1998), and specific gravity and urine volume were measured daily during days 3–4 and 7–8 of every period. Over days 11–12, a 48 h urine sample was collected from each dog and immediately frozen, as previously described (Stevenson et al., 1998).

2.7. Urinalysis

Samples were prepared and analysed for 12 parameters: urine pH, and concentrations of calcium, sodium, potassium, oxalate, magnesium, ammonium, phosphate, sulphate, urate, citrate, and pyrophosphate, by previously described methods (Markwell et al., 1999). Urinalysis data were then entered into the SUPERSAT computer program (Robertson et al., 2002), and the concentrations of the numerous soluble ion complexes that formed between these various ions were calculated by means of an iterative computer program. The activity products of CaOx were calculated, and the ratios of these activity products to the CaOx solubility product gave the CaOx RSS on a scale where an RSS of 1 was

<table>
<thead>
<tr>
<th>Diet</th>
<th>Calcium (g/100 kcal)</th>
<th>Oxalate (mg/100 kcal)</th>
<th>Calcium: oxalate mmol/mmol</th>
<th>Oxalate: calcium mmol/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCa–LOx*</td>
<td>0.18</td>
<td>10</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>LCa–MOx</td>
<td>0.18</td>
<td>17.5</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>LCa–HOx</td>
<td>0.18</td>
<td>25</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>MCa–LOx</td>
<td>0.45</td>
<td>10</td>
<td>45</td>
<td>101</td>
</tr>
<tr>
<td>MCa–MOx</td>
<td>0.45</td>
<td>17.5</td>
<td>26</td>
<td>58</td>
</tr>
<tr>
<td>HCa–LOx</td>
<td>0.75</td>
<td>10</td>
<td>75</td>
<td>169</td>
</tr>
<tr>
<td>HCa–HOx</td>
<td>0.75</td>
<td>25</td>
<td>30</td>
<td>68</td>
</tr>
</tbody>
</table>

* LCa–LOx was unsupplemented.
the solubility product, an RSS of < 1 defined undersaturated urine and RSS > 1 was supersaturated with respect to CaOx.

2.8. Statistical analysis

Data were compiled into means ± standard deviations (SD). A Goodness of Fit test was conducted to check normality of the data. Since data were found to be normally distributed two-way analysis of variance, (with dog and diet as factors) and multiple range tests (least significant difference) were used to test the significance of calcium and oxalate supplementation on CaOx RSS, urine pH, and urinary concentrations of calcium, oxalate, sulphate, phosphate and ammonia. The level of significance was taken as \( P < 0.05 \).

3. Results

3.1. Food intake and body weight maintenance

All food offered to the dogs each day was consumed, ensuring the dogs always received the correct treatment. Body weight remained stable with an overall weight change of 1%. Faeces quality remained consistently good throughout the study, as measured using the WALTHAM Faeces Scoring system (Moxham, 2001).

3.2. Water intake

Water intake was unaffected by dietary calcium or oxalate supplementation (Table 3).

3.3. Urine measurements

Urine volume and specific gravity were not affected by dietary calcium or oxalate supplementation (Table 3). Urine pH was not affected by supplementary oxalate alone, but was significantly reduced during calcium supplementation (Table 3).

3.4. Urinary RSS

Urine produced when LCa–LOx was fed had the lowest CaOx RSS (Table 3, Fig. 1). HCa–LOx produced the highest CaOx RSS. Over the range used in this study dietary calcium tended to have a greater influence over the CaOx RSS than dietary oxalate.

3.5. Urinary concentrations

Urinary calcium concentration was significantly increased by dietary calcium supplementation only; dietary oxalate had no effect on this parameter (Table 3, Fig. 1). The standard deviation for urinary calcium concentration between dogs became high as dietary calcium increased indicating a greater degree of variability between individuals (Fig. 1). Supplementing dietary oxalate while maintaining low dietary calcium produced variable and inconsistent results. Urinary oxalate concentration increased significantly with LCa–MOx compared with LCa–LOx, with one dog showing an increase in urinary oxalate concentration by 400% of the original urinary oxalate concentration, although individual variability was high and one dog showed no response. In contrast, the LCa–HOx diet produced no

Table 3
Mean (SD) calcium oxalate relative supersaturation (RSS) A and mean urinary concentrations (mmol/l) of calcium, oxalate, ammonium, phosphate and sulphate produced by 7 dogs fed a commercially prepared dog food containing different combinations of dietary calcium and oxalate (see Table 2 for diet details)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diet</th>
<th>LCa–LOx</th>
<th>LCa–MOx</th>
<th>LCa–HOx</th>
<th>MCa–LOx</th>
<th>Mca–MOx</th>
<th>HCa–LOx</th>
<th>Hca–Hox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume, ml/d</td>
<td></td>
<td>230</td>
<td>223</td>
<td>295</td>
<td>240</td>
<td>280</td>
<td>265</td>
<td>237</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(123)</td>
<td>(122)</td>
<td>(170)</td>
<td>(125)</td>
<td>(142)</td>
<td>(129)</td>
<td>(126)</td>
</tr>
<tr>
<td>Urine specific gravity</td>
<td></td>
<td>1.013</td>
<td>1.016</td>
<td>1.013</td>
<td>1.014</td>
<td>1.016</td>
<td>1.016</td>
<td>1.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.005)</td>
<td>(0.007)</td>
<td>(0.004)</td>
<td>(0.006)</td>
<td>(0.006)</td>
<td>(0.004)</td>
<td>(0.005)</td>
</tr>
<tr>
<td>Water intake, ml/d</td>
<td></td>
<td>468</td>
<td>460</td>
<td>506</td>
<td>464</td>
<td>488</td>
<td>458</td>
<td>505</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(221)</td>
<td>(246)</td>
<td>(276)</td>
<td>(245)</td>
<td>(279)</td>
<td>(199)</td>
<td>(203)</td>
</tr>
<tr>
<td>Urine pH</td>
<td></td>
<td>6.34</td>
<td>6.17</td>
<td>6.15</td>
<td>5.86</td>
<td>5.99</td>
<td>5.55</td>
<td>5.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.42)d</td>
<td>(0.65)d</td>
<td>(0.54)d</td>
<td>(0.43)ab</td>
<td>(0.71)bcd</td>
<td>(0.34)a</td>
<td>(0.44)ab</td>
</tr>
<tr>
<td>Urinary ammonia</td>
<td></td>
<td>31.76</td>
<td>36.24</td>
<td>37.75</td>
<td>40.70</td>
<td>43.10</td>
<td>56.91</td>
<td>53.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.62)a</td>
<td>(16.84)ab</td>
<td>(18.83)bc</td>
<td>(28.38)abc</td>
<td>(15.85)abc</td>
<td>(27.32)c</td>
<td>(21.64)bcs</td>
</tr>
<tr>
<td>Urinary sulphate</td>
<td></td>
<td>11.80</td>
<td>11.63</td>
<td>11.77</td>
<td>33.20</td>
<td>29.56</td>
<td>44.59</td>
<td>43.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.48)a</td>
<td>(5.03)b</td>
<td>(4.19)a</td>
<td>(16.71)bc</td>
<td>(10.09)b</td>
<td>(19.66)d</td>
<td>(17.32)bcd</td>
</tr>
<tr>
<td>Urinary phosphate</td>
<td></td>
<td>11.16</td>
<td>15.65</td>
<td>18.82</td>
<td>2.52</td>
<td>4.04</td>
<td>1.36</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.45)b</td>
<td>(8.92)b</td>
<td>(10.29)</td>
<td>(3.98)b</td>
<td>(3.22)b</td>
<td>(2.22)b</td>
<td>(2.44)a</td>
</tr>
</tbody>
</table>

CaOx, calcium oxalate.

a,b:Within a row values with different superscripts are significantly different (\( P < 0.05 \)).

A. Urinary relative supersaturation is calculated as activity product/solubility product.
significant increase in urinary oxalate compared with LCa–LOx. No change in urinary oxalate concentration occurred with oxalate supplementation when calcium was also increased. When the diet was supplemented with both calcium and oxalate, urinary oxalate, and the variability between dogs, remained low (MCa–MOx and HCa–HOx). Urinary ammonium concentration increased with calcium supplementation and was significantly greater on the HCa–HOx and HCa–LOx than LCa–LOx diets (Table 2). Urinary phosphate concentration significantly increased when dietary oxalate only was supplemented, but decreased when dietary calcium was added (Table 3). Supplementary oxalate had no effect on urinary sulphate, while calcium supplementation significantly increased urinary sulphate. Urinary citrate, potassium, magnesium, sodium and creatinine concentrations were unaffected by dietary calcium or oxalate supplementation.

3.6. Blood measurements

All blood parameters were within the reference ranges for normal healthy dogs. Mean total serum and ionised calcium concentrations (SD) were consistent between dogs with little variability between individuals, being 2.55 (0.09) and 1.37 (0.04), respectively.

4. Discussion

This study was conducted with the hypothesis that dietary calcium restriction without concurrent oxalate restriction would increase the risk of calcium oxalate formation in dogs. The levels of dietary calcium and oxalate used within this study were based upon the analysis of 30 different dry dog foods designed for feeding to adult small breed dogs. Thus, in this study, dietary calcium always exceeded dietary oxalate although dietary calcium to oxalate ratio ranged between 7 and 75 on a weight basis, or from 16 to 169 on a molar basis. Calcium usually considerably exceeds oxalate in the diet of humans in most developed countries (Marshall et al., 1972). Average daily intakes are 2–3mg oxalate/kg/d and 15–20mg calcium/kg/d with a molar calcium to oxalate ratio of around 18 (Marshall et al., 1972; Holmes et al., 1995). At the lowest levels within this study the dogs consumed 130mg/kg/d calcium and 7mg/kg/d oxalate (molar calcium:oxalate ratio = 40).

Most prepared petfoods contain levels well above these values and thus dogs are relatively loaded with both these minerals, and particularly calcium, compared to humans.

The LCa–LOx diet used in this study is designed to produce urine with a low risk of CaOx formation. Manipulation of dietary calcium and oxalate did not influence water turnover since water consumption, urine volume and specific gravity remained constant throughout the study. One study conducted in rats demonstrated an increased urine volume, and a reduction in CaOx supersaturation with increased dietary oxalate (supplied as sodium oxalate) supplementation (Bushinsky et al., 1999). Miniature Schnauzers supplemented with sodium chloride were also observed to increase urine output (Stevenson et al., in press). Thus, oxalic acid was selected as an alternative source of oxalate in the hope that this supplement would not influence urine output, as changes in this parameter would also influence CaOx RSS. Since urine volume and specific gravity remained unchanged throughout this study, oxalic acid does not appear to affect urine output in dogs.

At the levels investigated in this study, dietary calcium had a far greater effect on the CaOx supersaturation, and therefore the risk of CaOx crystallisation, than did changes in dietary oxalate. However, when dietary calcium was kept low, an increase in dietary oxalate, as
shown during the feeding of LCa–MOx, resulted in an increase in urinary oxalate concentration and CaOx RSS, implying that dietary oxalate may become a more important risk factor for CaOx formation when dietary calcium is low. However, dietary oxalate supplementation (when dietary calcium was low) also produced highly variable and inconsistent results in these dogs since the LCa–H0X diet resulted in no significant change in urinary oxalate compared to LCa–LOx. The relative importance of dietary calcium and dietary oxalate has been studied extensively in humans (Zarembski and Hodgkinson, 1969; Marshall et al., 1972; Bataille et al., 1983, 1985; Nakada et al., 1988; Holmes et al., 1995; Masai et al., 1995; Curhan et al., 1997; Liebman and Chai, 1997; Messa et al., 1997; Hess et al., 1998; Liebman and Costa, 2000; Holmes et al., 2001). It was common practice between 1970 and 1990 for clinicians to recommend restriction of dietary calcium in human CaOx stone formers based upon the fact that a large proportion have elevated urinary calcium concentrations (Pak et al., 1974), and dietary restriction reduces urinary calcium excretion (Messa et al., 1992, 1997; Curhan, 1997). However, it has been demonstrated in both humans (Marshall et al., 1972; Messa et al., 1997; Bataille et al., 1983) and rats (Morozumi and Ogawa, 2000) that restricting dietary calcium actually causes an increase in urinary oxalate because the amount of oxalate available for absorption within the colon increases. Hyperabsorption of calcium, a possible mechanism leading to the hypercalciuria, has been observed in a small number of Miniature Schnauzers with calcium oxalate stones (Lulich et al., 1991). Calcium hyperabsorption may leave little available in the gut to bind with oxalate, inducing a greater intestinal absorption of oxalate leading to subsequent hyperoxaluria (Morozumi and Ogawa, 2000). The same effect was also observed in rats when dietary calcium was low (Morozumi and Ogawa, 2000). In a study comparing the relative influence of mild hyperoxaluria and hypercalciuria on a number of parameters involved in stone formation in humans, mild hyperoxaluria had a far more marked effect on urinary supersaturation with respect to CaOx, and on the maximum amount of crystalluria produced, than hypercalciuria (Robertson and Peacock, 1980). No such relationships were seen with hypercalciuria, except at very low urinary oxalate concentrations (Hällson, 1988). The results from dogs presented here are contrary to the observations in humans, with urinary calcium being a greater influence on CaOx RSS, and therefore the crystallisation potential of the urine, than oxalate, at least across the dietary levels tested.

A high degree of variability in the response to oxalate loading was seen in this study. Variability was observed both between individuals on the same diet and between the same individual on different diets, particularly when calcium was low. However, no differences were noted between the two dog breeds with both breeds previously being shown to produce urine with a higher risk of CaOx formation than other breeds such as the Labrador Retriever (Stevenson and Markwell, 2001; Stevenson et al., 2001). Variability between individuals has also been observed in humans during oxalate loading, but not on a low oxalate diet (Holmes et al., 2001). The reasons for the variability in oxalate absorption during oxalate loading on otherwise controlled diets remain unclear although it could be related to differences in gastrointestinal absorption of calcium and oxalate, different activities of oxalate-degrading organisms, differences in transport processes, or renal handling of oxalate. In our study, significant differences in urinary oxalate concentration were observed between LCa–LOx and LCa–MOx, but not LCa–LOx and LCa–HOx. In addition to reasons suggested above it is possible that using a greater number of dogs may have resulted in more consistent results with lower variability.

Urinary oxalate concentration did not increase when oxalate was supplemented in the presence of moderate or high calcium. It is likely that the supplementary oxalate given under these circumstances was bound by calcium within the intestine leaving the dietary oxalate unavailable for absorption. Thus, at any level of calcium supplementation, urinary oxalate concentrations were low and consistently between 0.21 to 0.23 mmol/l, equating to a daily excretion of between 0.6 and 0.7 mg/kg/d (0.01 mmol/kg/d). The authors suggest that this oxalate was derived predominantly from endogenous production, as the level remained low and stable irrespective of dietary oxalate content. One study in humans estimated endogenous production was between 0.15 and 0.2 mg/kg/d (Holmes et al., 1995). However, endogenous oxalate production itself is not fixed and varies with dietary protein consumption (Hess et al., 1998), since glycollate, a product of protein metabolism, is a precursor for endogenous oxalate production (Assimos and Holmes, 2000). Other possible influences include vitamins B6 and C (Assimos and Holmes, 2000). However, consumption of dietary protein and vitamins B6 and C remained constant throughout this study, and thus endogenous production probably remained unchanged, although it is possible that other unknown factors were involved.

Urinary calcium and oxalate were not the only parameters to be affected by the dietary supplementation. Urinary phosphate concentration increased as a result of oxalate supplementation. In humans, intestinal calcium binds with phosphate preventing its absorption (Slatopolsky et al., 1989). However, in rats the preferential binding of calcium with the increasing amounts of dietary oxalate was shown to decrease the calcium available for phosphate binding (Bushinsky et al., 1999). Our study, in dogs, demonstrated that as dietary oxalate increased it is likely that more phosphate became
available for intestinal absorption, resulting in a significant increase in urinary phosphate. When increased dietary oxalate was given with additional calcium these effects appear to have been abolished, as calcium was present in amounts large enough to meet requirements for both oxalate and phosphate binding.

Although the study reported here was designed so that the sulphate and carbonate ions contributed equal amounts to the diet, calcium supplementation resulted in a reduced urine pH, and consequently an increased amount of urinary calcium. However, calcium carbonate is known to be poorly absorbed in both rats and humans (Harvey et al., 1988; Classen et al., 1995). When administered to rats and cats this salt did not significantly affect urine pH (Pastoor et al., 1994b; Classen et al., 1995). The acidifying effect observed in this study is, therefore, likely to have been caused by calcium sulphate supplementation. The increased urinary excretion of sulphate will lower the excretion of bicarbonate, lower the urine pH and increase ammonium excretion. These measures are a normal response, ensuring the maintenance of a constant acid–base balance within the body. The lower urine pH will stimulate ammonium production within the kidney to buffer H⁺ ions as ammonium (Pitts, 1948; Good, 1989). A similar effect was found in a study replacing dietary calcium carbonate with calcium chloride in cats (Pastoor et al., 1994a). It is also likely that reduced urinary phosphate, as a result of intestinal binding of dietary phosphate and calcium, contributed to a reduction in urine pH since this would reduce the buffering capacity of the urine.

During metabolic acidosis, acidifying metabolites are neutralised by phosphates and carbonates mobilised from bone. Bone calcium is released with the phosphorus resulting in hypercalciuria (Lulich et al., 1999a), thus it could be suggested that the reduction in urine pH observed in this study contributed to the increased urinary calcium concentration. Recent data from studies in cats suggested, however, that urinary calcium concentrations may only be increased as urine pH approaches the level at which there is a risk of metabolic acidosis (Stevenson et al., 2000a). Whilst similar studies appear not to have been reported in dogs, urine pH in this study remained within the normal range for healthy dogs, (Bush, 1991), and the dogs would not be considered at risk of metabolic acidosis. In addition, both in this study and those of Pastoor et al. (1994a) in cats there was no correlation between urinary calcium concentration and urine pH, suggesting that within the normal urine pH range this was not the major factor driving the increase in urinary calcium concentration.

In summary, a diet containing low levels of both calcium and oxalate produced the lowest CaOx RSS. Within the range found in commercially available dog foods increases in dietary calcium produced a larger and more consistent increase in CaOx RSS and, therefore, the risk of CaOx crystallisation, than increases in dietary oxalate. Dietary oxalate resulted in variable and inconsistent increases in urinary oxalate when dietary calcium was low. Measures to reduce dietary levels of both calcium and oxalate should be considered when implementing strategies to reduce the risk of CaOx formation in dogs since reducing dietary calcium without controlling dietary oxalate intake may increase the risk of CaOx crystallisation in susceptible individuals.

References


