Oxalate degradation by intestinal lactic acid bacteria in dogs and cats

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Abstract

This study evaluated the ability of the lactic acid bacteria (LAB) component of canine and feline feces to degrade oxalate in vitro. Oxalate degradation by individual canine-origin LAB was also evaluated. The effects of various prebiotics on in vitro oxalate degradation by selected oxalate-degrading canine LAB was also evaluated. Canine fecal samples reduced oxalate levels by 78 ± 12.2% (mean ± S.D.; range: 44–97%; median: 81%). Feline results were similar, with oxalate reduction of 69.7 ± 16.7% (mean ± S.D.; range: 40–96%; median: 73%). Thirty-seven lactic acid bacteria were isolated from canine fecal samples. Mean oxalate degradation was 17.7 ± 16.6% (mean ± S.D.; range: 0–65%; median: 13%). No oxalate degradation was detected for four (11%) isolates, and 10/37 (27%) degraded less than 10% of oxalate. The effects of lactitol, arabinogalactan, guar gum, gum Arabic, inulin, maltodextrin or a commercial fructooligosaccharide (FOS) product on in vitro oxalate degradation by five canine LAB isolates were highly variable, even within the same bacterial species. Overall, in vitro degradation was significantly greater with guar gum compared to arabinogalactan (P < 0.05), gum Arabic (P < 0.05), and lactitol (P < 0.01). This study suggests that manipulation of the LAB component of the canine and feline gastrointestinal microflora may decrease intestinal oxalate, and correspondingly intestinal oxalate absorption and renal excretion, thus potentially reducing oxalate urolithiasis.

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1. Introduction

Calcium oxalate urolithiasis in an increasingly common and frustrating problem in dogs and cats. Lulich et al. (1999) reported that calcium oxalate accounted for 35.4% of canine uroliths in 1997, up from 5.3% in 1981 (Lulich et al., 1999). Similarly, two Canadian studies reported that 49.8% of feline and 41.5% of canine bladder uroliths were comprised of calcium oxalate (Houston et al., 2003, 2004). The pathogenesis of calcium oxalate urolith formation is complex and only partially understood, although familial, gender, age and dietary factors have been identified (Lulich et al., 1999; Leckhoorn et al., 2000a,b). Urine relative supersaturation (RSS) is the driving force for crystal formation in the urinary tract (Balaji and Menon, 1997; Ogawa et al., 2000) and hyperoxaluria is believed to play the most important role in influencing urinary calcium oxalate saturation (Ogawa et al., 2000). Urinary excretion of oxalate is dependent on dietary intake, in—
intestinal absorption, renal tubular secretion and the rate of endogenous synthesis (Ogawa et al., 2000). Intestinal absorption of oxalate is dependent on the amount of unbound oxalate in the intestinal tract (Ogawa et al., 2000). Very little is known about the role of the intestinal microflora in oxalate degradation in dogs and cats, but there is increasing evidence in other species that the intestinal microflora can have a significant impact on intestinal oxalate levels. *Oxalobacter formigenes*, an oxalate degrading anaerobic bacterium, has been identified in the intestinal tract of rats, sheep, pigs and humans (Allison et al., 1985, 1986; Daniel et al., 1987). This bacterium uses oxalate as its sole energy source and a correlation between the absence of *O. formigenes* and hyperoxaluria and calcium oxalate renal disease has been reported in rats and humans (Sidhu et al., 1998, 1999; Troxel et al., 2003). Administration of *O. formigenes* resulted in reversal of hyperoxaluria in rats (Sidhu et al., 2001).

The ability of other intestinal microorganisms to degrade oxalate has been studied to a lesser degree. Differences in the presence of oxalate degrading bacteria in feces have been reported between calcium oxalate stone formers and normal humans (Ito et al., 1996; Mikami et al., 2003); however, the organisms responsible for oxalate degradation were not evaluated. A limited number of studies have evaluated lactic acid bacteria (LAB), a diverse group of largely non-pathogenic bacteria. A small study in humans reported that administration of high doses of a combination of LAB resulted in a reduction of oxaluria (Campieri et al., 2001). These studies indicate that changes in the intestinal microflora can impact oxalate absorption and excretion.

One method of manipulation of the intestinal microflora, particularly the LAB, is via administration of prebiotics. Prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating growth or activity of certain bacterial components of the intestinal microflora (Gibson and Robertfoid, 1995). Fructooligosaccharides (FOSs) are likely the most widely used prebiotic in dogs and cats, and are incorporated into some commercial pet foods. The effect of prebiotics on oxalate degrading organisms, however, has not been reported.

The objectives of this study were to evaluate the role of the lactic acid bacteria component of the intestinal microflora of dogs and cats in in vitro oxalate degradation, and to evaluate the effects of various prebiotics on oxalate degradation by selected lactic acid bacteria.

2. Material and methods

2.1. Oxalate degradation by canine and feline feces

Fecal samples were collected from 20 dogs and 19 cats. All animals were part of a research colony and were fed standard commercial diets (Iams Chunks, The Iams Company, Dayton, OH; Meow Mix Original Choice, The Meow Mix Company, Secaucus, NJ). Animals did not have a history of dietary change, antimicrobial administration, probiotic supplementation or diarrhea within 30 days of fecal sample collection. Samples were collected and stored at 4°C until submission to the laboratory, which occurred within 48 h of collection.

One hundred milligrams of feces was inoculated into 8 ml MRS broth, a selective LAB medium, that was supplemented with 10 mmol/L sodium oxalate (Sigma Chemical Co., St. Louis, MO) (MRSox) and incubated aerobically at 37°C for 48 h. Tubes were then centrifuged at 4400 × g for 5 min and the oxalate concentration of the supernatant was determined using a commercial colorimetric assay (Oxalate assay, Sigma Diagnostics Inc.). All testing was performed in triplicate and oxalate degradation was expressed as the percent decrease in oxalate compared to the control.

To confirm that *O. formigenes* could not grow in MRSox and was not responsible for oxalate degradation, pure growth of *O. formigenes* (ATCC 35274) was inoculated into MRSox and incubated aerobically for 48 h, at which point the oxalate concentration of the supernatant was determined.

2.2. Oxalate degradation by selected lactic acid bacteria of canine origin

Fecal samples from clinically normal client owned dogs with no recent (<30 days) history of dietary change, antimicrobial administration, probiotic supplementation or diarrhea were collected. Fecal
samples were inoculated onto MRS agar and incubated anaerobically at 37 °C for 48 h. Gram-positive, catalase-negative bacteria growing on MRS agar were presumptively identified as LAB. Isolates were grown in pure culture on MRS agar for 48 h and 100 μl of a MacFarland 2.0 suspension in phosphate-buffered saline (PBS; pH 7.2) was added to 8 ml of MRSox and incubated at 37 °C for 48 h. Oxalate level of the supernatant was determined as described above. Testing was performed in triplicate.

2.3. Effects of various prebiotics on oxalate degradation in vitro

Five LAB, demonstrated in the above experiment to be able to degrade oxalate in vitro, were chosen for further study. They were identified via a commercial biochemical assay (API 50 CHL, BioMerieux, St. Laurent, Quebec, Canada) and grown in pure culture on MRS agar. A MacFarland 2.0 suspension of 48 h growth was made in sterile PBS. One hundred microliters of bacterial suspension was added to 8 ml of MRSox, and MRSox with 1 g/L of lactitol (Lactitol monohydrate, Aldrich Chemical Co., Milwaukee, WI), arabinogalactan (Arabinogalactan, Aldrich Chemical Co.), guar gum (Guar gum, Sigma Chemical Co.), gum Arabic (Gum Arabic, Sigma Chemical Co.), inulin (Inulin, Sigma Chemical Co.), maltodextrin (Maltodextrin, Aldrich Chemical Co.) or a commercial fructooligosaccharide product (Fruitafit Inulin Tex, Sensus, Monmouth Junction, NJ) was added to 8 ml of MRSox. Tubes were incubated aerobically at 37 °C for 48 h, and then the concentration of oxalate in the supernatant was determined. Testing was performed in triplicate. The effect of the prebiotics was expressed as percent increase in oxalate degradation compared to the control (MRSox). A pilot study was performed to demonstrate that the individual prebiotics did not affect the oxalate assay (Weese, Unpublished data).

2.4. Statistical analysis

Comparison of mean oxalate degradation by canine and feline feces was performed using a paired t-test. Overall comparison of the different prebiotics, and within-groups comparison of prebiotics were performed using repeated measures ANOVA with Tukey-Kramer multiple comparisons test. Within-species comparison of oxalate degradation in the presence of different prebiotics was performed using an unpaired t-test. A statistical software package (InStat, GraphPad Software, San Diego, CA) was used and a P-value of <0.05 was considered significant for all comparisons.

3. Results

3.1. Oxalate degradation by canine and feline feces

All canine and feline fecal samples degraded oxalate to some degree. Canine fecal samples reduced oxalate levels by 78 ± 12.2% (mean ± S.D.; range: 44–97%, median: 81%). Feline results were similar, with oxalate reduction of 69.7 ± 16.7% (mean ± S.D.; range: 40–96%, median: 73%). There was no significant difference between canine and feline results (P = 0.082).

No oxalate degradation was evident in MRSox inoculated with O. formigenes, confirming that this organism did not play a role in the degradation of oxalate that was noted here.

3.2. Oxalate degradation by selected lactic acid bacteria of canine origin

Thirty-seven lactic acid bacteria were isolated from canine fecal samples. There was marked variation in the ability to degrade oxalate in vitro. Mean oxalate degradation was 17.7 ± 16.6% (mean ± S.D.; range: 0–65%, median: 13%). No oxalate degradation was detected for four (11%) isolates, and 10/37 (27%) degraded less than 10% of oxalate.

3.3. Effects of various prebiotics on oxalate degradation in vitro

Five of the 37 isolates evaluated above were chosen for further study based on subjectively superior oxalate degradation ability. These isolates were identified as two strains of Leuconostoc lactis, two strains of Lactobacillus acidophilus and one strain of Lactobacillus plantarum. Effects of prebiotics are displayed in Fig. 1. Overall, in vitro degradation was significantly greater with guar gum compared to arabinogalactan (P < 0.05), gum Arabic (P < 0.05), and...
Fig. 1. Increase in in vitro oxalate degradation by canine-origin lactic acid bacteria via addition of prebiotics (Within isolates, different superscripts indicate a significant difference ($P < 0.05$). Similar or no superscripts indicate no significant differences.)

lactitol ($P < 0.01$). Within-group differences are presented in Fig. 1. Within-species differences were also apparent. There were significant differences in oxalate degradation by the two *L. lactis* isolates in the presence of FOS ($P = 0.016$) and guar gum ($P = 0.037$). There were also differences between the two *L. acidophilus* strains in the presence of FOS ($P = 0.039$) and lactitol ($P = 0.013$).

4. Discussion

This study has demonstrated that the LAB component of the intestinal microflora of dogs and cats can degrade oxalate in vitro. Oxalate degradation occurred in MRS broth, an optimal nutritional medium for LAB, indicating that oxalate degradation does not occur only when an appropriate nutritional source is not available. This point is important because a previous study reporting oxalate degradation by *Enterococcus faecalis* was performed in a nutrient-restricted medium and reported a loss of oxalate degrading activity in routine culture media (Hokama et al., 2000). The variability in oxalate degradation by the different canine-origin LAB was interesting. Stevenson et al. (2003) reported a high degree of variability in oxalate relative supersaturation in dogs in response to oral oxalate loading. Differences in oxalate degrading organisms was one of the proposed reasons for this variation, which is consistent with the variability in oxalate degradation by fecal samples observed in this study. The mechanism of oxalate degradation and the reason it was apparent in only some LAB strains is unclear. The extent of oxalate degradation by fecal samples was somewhat surprising, especially considering that samples were incubated for only 48 h versus...
There was significant variation in the response of the LAB tested against different prebiotics. This was not surprising as interspecies variation in the response to prebiotics is well documented (Kaplan and Hutkins, 2000; Rycroft et al., 2001; Olano-Martin et al., 2002). That different strains of \( \text{L. acidophilus} \) and \( \text{L. lactis} \) responded differently to the different prebiotics re-enforces the notion that strain-specific, not just species-specific, testing should be performed when evaluating probiotic or prebiotic effects. The increase in oxalate degradation noted with different prebiotics was assumed to be the result of increased bacterial growth. The differences in prebiotic response suggest that a combination of prebiotics such as guar gum and FOS might result in the optimal increase in oxalate degradation. Further in vitro and in vivo study is required.

Reduction in the recurrence of calcium oxalate uroliths via dietary means would be highly desirable yet has proven difficult to date. Results of this study suggest that manipulation of the LAB component of the gastrointestinal microflora could help reduce oxalate RSS and calcium oxalate urolithiasis. Within isolates, different superscripts indicate a significant difference (\( P < 0.05 \)). Similar or no superscripts indicate no significant differences.

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