Rates of oxalic acid degradation in the rumen of sheep and goats in response to different levels of oxalic acid administration

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Abstract

Oxalic acid is found in high concentrations in some plants consumed by ruminants and may cause renal toxicity. To determine whether exposure to oxalic acid affects the capacity of the rumen of sheep and goats to degrade the compound, 20 animals (10 sheep and 10 goats) were dosed with free oxalic acid by gelatin capsule twice daily for 3 weeks at one of five levels (0.0, 0.3, 0.6, 0.9 and 1.2 mmol/kg live weight (M) per day). Rumen samples were collected by stomach tube in the week prior to the start of dosing and in each week of the 3-week experiment. Oxalic acid degradation capacity was measured by adding ^{14}C -labelled oxalic acid to rumen fluid in vitro and capturing evolved $^{14}\text{CO}_2$. Rates of degradation increased with increasing level of administration (2.30, 4.71, 6.74, 9.83 and 13.90 mmol of oxalic acid degraded per l rumen fluid per day for doses 0.0, 0.3, 0.6, 0.9 and 1.2 mmol/kg M per day, respectively; $P < 0.001$). Rates of degradation increased during the dosing period ($P < 0.001$) with the largest increases occurring in the 1st week of dosing. Goats showed a greater response than sheep, with a higher mean oxalic acid degradation capacity (9.04 v. 5.95 mmol of oxalic acid degraded per l rumen fluid, $P < 0.05$). Oxalic acid administration did not influence plasma calcium concentration or cause renal function impairment as measured by plasma creatinine concentrations. The experiment demonstrated adaptation in the rumen to potential toxins in the host diet and suggests that the rumen micro-organisms of goats may have been more adapted to degrading oxalic acid than sheep.

Keywords: goats, oxalic acid, rumen degradation, sheep.

Introduction

The rumen environment represents an efficient site of biotransformation for a range of secondary plant compounds (Carlson and Breeze, 1984). Toxicity of plants consumed by ruminant animals can be significantly modified following chemical changes to potentially toxic compounds in the rumen (Lanigan and Smith, 1970; Majak and Cheng, 1987; Duncan and Milne, 1992). The composition of the rumen microbial population is labile and may differ between species, between populations and over time. The abundance of particular microbial species in the rumen environment may therefore have a strong influence on secondary compound biotransformation and hence on the ultimate toxicity of particular food items (Allison *et al.*, 1981). Species differences in rumen biotransformation reactions have been reported previously and cited as one potential factor underlying species differences in dietary preference (Kronberg and Walker, 1993).

Although nutritional differences between sheep and

goats are the subject of debate, it is generally accepted that goats consume shrubby vegetation and browse plants more readily than sheep (Howe *et al.*, 1988; McCabe and Barry, 1988). Since these food sources also tend to be richer in secondary plant compounds than graminaceous species, the question arises as to whether goats possess more efficient physiological means for digesting and metabolizing plant secondary compounds and hence minimizing their detrimental effects. Various experiments lend support to this possibility (Kronberg and Walker, 1993; Narjisse *et al.*, 1995).

Oxalic acid is a simple organic acid occurring as its salt in a range of plant species commonly consumed by ruminants. For example, the leaves of fodder beet (*Beta vulgaris*) which may contain up to 10% soluble oxalates (Libert and Franceschi, 1987) are commonly given to ruminants in the United Kingdom (Roberts and Martindale, 1990). *Halogeton glomeratus* may contain even higher levels (up to 35%; Libert and Franceschi, 1987) and this plant is an important food

source for free-ranging ruminants in the United States (Pemberton, 1986). Many of the tropical panicoid grasses such as those of the genera *Pennisetum*, *Setaria*, and *Cenchrus* which represent a major food source for wild and domestic livestock in the tropics also accumulate oxalates to potentially toxic concentrations. Oxalic acid, consumed in sufficient quantity can precipitate as its calcium salt causing capillary obstruction and hence tissue damage particularly in the rumen wall and the kidney tubules (James, 1972). Furthermore, formation of calcium salts of oxalic acid may lead to hypocalcaemia in the short term (Watts, 1959). Oxalic acid is readily degraded in the rumen, following a period of adaptation, by the ruminal bacterium *Oxalobacter formigenes* to yield formic acid and CO₂ (Allison *et al.*, 1985). The extent to which the rate of rumen microbial breakdown of oxalic acid changes with the level of exposure to oxalic acid and hence protects the host animal from toxic consequences has not been quantified. Furthermore, species differences in the efficiency of oxalic acid degradation have not, thus far, been assessed in comparative experiments. The current experiment was therefore conducted to examine the response of the rumen environment of sheep and goats to different levels of oxalic acid administration and to determine the development of post-absorptive physiological changes.

Material and methods

Experimental design

Ten sheep (Scottish Blackface or Scottish Blackface × Border Leicester ewes, mean live weight (M) 55.3 (s.e. 2.03) kg) and ten goats (Scottish Cashmere nannies, mean M 38.6 (s.e. 0.82) kg) were used for the experiment. The animals were individually given dried grass pellets (crude protein 156.9 g/kg dry matter (DM); neutral-detergent fibre 497.0 g/kg DM; acid-detergent fibre 274.9 g/kg DM) at 1.2 times their estimated maintenance requirements for energy and were given 100 g hay per head per day to maintain rumen function, for 2 weeks prior to commencing the experiment and throughout the experimental period.

The experiment took the form of a 2 × 5 factorial design with factors consisting of two species (sheep *v.* goats) and five levels of oxalic acid administration. The dose rates of oxalic acid were as follows: 0.0 mmol/kg M per day; 0.3 mmol/kg M per day; 0.6 mmol/kg M per day; 0.9 mmol/kg M per day; 1.2 mmol/kg M per day.

Oxalic acid (Aldrich, Dorset, UK) contained in gelatin capsules (Davcaps, Monmouth, UK), was administered by twice daily oral dosing at 08.00 and 16.00 h for 22 days, with the dose being increased from zero to proportionately 1.0 of the final dose in

equal stages over the first 5 days. Rumen samples were collected, under anaerobic conditions, by stomach tube 1 h after the morning dose of the oxalic acid 3 days prior to the start of dosing and on days 5, 12 and 19 of the dosing period to measure oxalic acid degradation capacity.

Blood samples were collected from the jugular vein into heparinized evacuated tubes (Vacutainer, Becton Dickinson, Oxford, UK) on days 0, 7, 14 and 21 of the dosing period to measure plasma calcium concentration as an indicator of oxalic acid toxicity.

Plasma creatinine concentrations were also measured in the same samples as an indicator of kidney function impairment.

Measurements of rates of oxalate metabolism

Samples of ruminal contents obtained by stomach tube were kept at 37°C and processed within 60 min of collection. Rates of oxalic acid degradation were measured by a modification of the method of Allison *et al.* (1985). Fluid obtained by filtration through one layer of muslin was dispensed (1 ml) into a McCartney bottle with 1 ml of CO₂ saturated artificial saliva (McDougall, 1948). An isotopically labelled substrate was provided by adding 14.8 kBq of 0.2 mol/l ¹⁴C-labelled oxalic acid (100 µl) to the McCartney bottle while flushing with CO₂. Tubes were stoppered with butyl rubber-lined caps and incubated at 37°C in a water bath for 1 h. Reactions were stopped, after 1 h, by injecting 1 ml of 1 mol/l NaOH through the rubber stopper and the bottles left overnight. Bottles were then uncapped, a 5 ml scintillation vial containing 1 ml of 3 mol/l NaOH inserted, and the bottles recapped. After injecting 1 ml of 5 mol/l HCl into the outer well and agitating gently, the tubes were left overnight again. The 3 mol/l NaOH containing CO₂ trapped as bicarbonate was then flushed into a scintillation vial with 15 ml scintillation fluid (NE 260, Nuclear Electric Industries, Edinburgh) and counted on a Packard (TRICARB. 1900 CA) liquid scintillation analyser.

Plasma creatinine and calcium measurements

Plasma creatinine concentration was determined using a commercial diagnostic method (Sigma Procedure number 555). Plasma calcium concentration was determined by the method of Voth (1981).

Statistical analysis

The experiment was analysed using a hierarchical analysis of variance with a factorial treatment structure. Week was nested within individual animals. The treatment structure employed a linear contrast for dose level and a contrast for week such

Table 1 Effect of daily oral dosing with different amounts of oxalic acid on rates of oxalic acid degradation in the rumen, plasma calcium concentrations and plasma creatinine concentrations in sheep and goats. Data represent means across the whole experiment (s.e.d. denotes standard error of difference for means in the same row)

		Dose rate (mmol/kg M per day)					s.e.d.
		0	0.3	0.6	0.9	1.2	
Rate of oxalic acid degradation (log mmol/l per day) (untransformed values in parenthesis)	sheep	0.38 (1.55)	1.18 (4.26)	1.35 (5.30)	1.31 (4.26)	2.37 (14.39)	0.433
	goats	0.97 (3.04)	1.45 (5.17)	1.83 (8.17)	2.46 (15.41)	2.16 (13.42)	0.433
	mean	0.67 (2.30)	1.31 (4.71)	1.59 (6.74)	1.88 (9.83)	2.26 (13.90)	0.306
Plasma calcium concentration (mg/l)	sheep	99.5	87.4	95.6	95.0	86.0	10.32
	goats	91.5	92.3	94.3	89.4	83.8	10.32
	mean	95.5	89.8	94.9	92.2	84.9	7.29
Plasma creatinine concentration (mg/l)	sheep	8.10	7.89	8.13	7.12	7.25	1.229
	goats	5.45	8.73	6.64	6.74	7.18	1.229
	mean	6.77	8.31	7.39	6.93	7.21	0.869

that week 0 values were compared with combined values for weeks 1 to 3. Variance associated with dose and species factors was estimated at the animal level. Variance associated with week and interactions with week was estimated within animals. Rates of degradation of oxalic acid were log transformed to improve the homogeneity of the variance. All animals consumed their full food allowance throughout the experiment except one sheep (treatment 0.9 mmol/kgM) which stopped eating 6 days into the dosing period and two goats (both 1.2 mmol/kgM treatment) which stopped eating 6 and 9 days into the dosing period respectively. These animals were removed from the experiment and all data from these animals were excluded from the analysis. Data were analysed using GENSTAT 5 (Lawes Agricultural Trust, 1989).

Results

The level of administration of oxalic acid had a marked effect on the rate of degradation of oxalic acid by rumen microbes (Figure 1; $P < 0.001$) with the logarithm of rate of degradation increasing in a linear fashion with level of administration (Table 1; linear contrast $P < 0.001$). The contrast for week 0 *v.* weeks 1 to 3 was highly significant at the week by dose interaction level ($P < 0.001$) demonstrating that degradation rates prior to administration of oxalic acid were significantly different from those during the oxalic acid administration phase. The deviation from the week contrast was not significant indicating that the response of the rumen microbes to differing levels of oxalic acid administration did not change with time following changes elicited during week 1. Mean rates of oxalic acid degradation were higher in the rumen of goats than in sheep (mean values: 9.04 mmol/l per day *v.* 5.95 mmol/l per day respectively;

$P < 0.05$) but the response to increasing levels of oxalic acid administration did not differ between species (dose $\times$ species interaction; $P > 0.05$).

Plasma calcium concentrations were not significantly affected by level of oxalic acid administration or by species (Table 1). There was an overall significant effect of week ($P < 0.001$); the week contrast (week 0 *v.* weeks 1, 2 and 3) was not significant but the deviation from the contrast was significant ($P < 0.001$) indicating that plasma calcium

Figure 1 Effect of daily oral dosing with different amounts of oxalic acid on rates of oxalic acid degradation in the rumen of sheep and goats. Week 0 values (□) represent degradation rates prior to the start of oxalic acid dosing. Mean values are presented for weeks 0 (□), week 1 (▨), week 2 (▩) and week 3 (▧). Error bar denotes standard error of difference (s.e.d.) for the interaction between week and dose.

concentrations changed with time but that this change was not attributable to the administration of oxalic acid. Average plasma calcium concentrations for weeks 0 to 3 were 93.9, 78.2, 111.5 and 84.1 mg/l respectively (s.e.d. for week = 4.37 mg/l).

Similarly, plasma creatinine concentrations were not significantly affected by oxalic acid dosing level (Table 1). Creatinine concentrations showed a tendency to differ between weeks ($P < 0.1$) but this was not attributable to oxalic acid dosing; the week contrast (week 0 *v.* weeks 1 to 3) was not significant but the deviation from this contrast was significant ($P < 0.05$) indicating that there were non-treatment related changes in plasma creatinine concentrations over time. Average plasma creatinine concentrations for weeks 0 to 3 were 7.38, 7.63, 6.56 and 7.72 mg/l respectively (s.e.d. for week = 0.441).

Discussion

The rumen environment of both sheep and goats responded rapidly to the administration of oxalic acid with changes occurring within a week of the onset of oxalic acid dosing. Similarly rapid changes in rates of oxalic acid degradation have been found when oxalic acid-containing plants have been offered to sheep (Allison *et al.*, 1977).

Rumen degradation of oxalic acid appeared to protect animals from detrimental post-ingestive consequences. There was no evidence of hypocalcaemia even at the highest oxalic acid dose level. This contrasts with the results of another experiment in which sheep which received 1.3 mmol/kg M per day oxalic acid for 2 days abruptly without an adaptation phase, showed mild hypocalcaemia (Kyriazakis *et al.*, 1997). Furthermore the lack of treatment-related changes in plasma creatinine concentrations gave no indication of chronic kidney function impairment as a result of the formation of calcium oxalate crystals in renal capillaries.

Sheep and goats differed in their ability to degrade oxalic acid with the rumen microbial population of goats showing a greater capacity to catabolize the compound. It is difficult to envisage mechanisms whereby different ruminant species could 'manipulate' the composition of their rumen microflora except by altering rumen conditions. Various studies indicate that goats digest low quality roughage more efficiently than do sheep, possibly due to greater rates of nitrogen recycling to the rumen (Domingue *et al.*, 1991b). Such subtle differences in rumen conditions might favour the particular species of bacteria responsible for oxalic acid degradation. Little is known of the effects of

rumen conditions on the success of *Oxalobacter formigenes* beyond the fact that oxalic acid is required as a substrate. The current results indicate that the tendency of goats to select diets containing a higher proportion of non-grass plants than sheep may have an underlying physiological basis with goats having a rumen microflora which is more responsive to the presence of secondary compounds in the diet. This idea is attractive but it would be unwise to extrapolate the observed differences between sheep and goats in ability to degrade oxalic acid to other groups of secondary compounds without specific experiments.

The levels of oxalic acid administered during the experiment were chosen based on concentrations found in plants together with their likely ingestion rates. Consideration of the potential toxicity of plant oxalates must take into account the form in which the compound is present in plants since different salts have markedly different rates of availability to ruminant animals (James, 1972). Calcium oxalate for example is relatively unavailable due to its low solubility at the range of pH found in the digestive tract. Sodium and potassium oxalate, on the other hand are freely soluble and therefore present a greater toxic challenge to the animal. Free oxalic acid was given in the current experiment although presumably some of this was transformed into its various salts in the digestive tract prior to absorption. Libert and Franceschi (1987) reviewed the concentrations of oxalates in a range of crop plants. One or two of the species reviewed, often associated with toxicity problems, had extremely high concentrations of soluble oxalates in their leaf material. For example, *Halogeton glomeratus*, common on North American rangelands contains up to 350 g/kg of soluble oxalate in the leaves and *Oxalis cernua* (sour sob) contains up to 150 g/kg of soluble oxalates. The majority of plant species commonly consumed by domestic livestock which accumulate oxalates contain much lower concentrations, typically around 10 to 20 g/kg which are similar to the levels tested in the current experiment. For example, *Cenchrus ciliaris* (Buffalo hay) was reported to contain 11 g/kg of soluble oxalates while in *Oryza sativa* (paddy straw) the reported concentrations were 10 to 25 g/kg (Libert and Franceschi, 1987).

The results of the current experiment suggest that at the levels of oxalate found in commonly consumed crop plants, the rumen microbial population of both sheep and goats adapts effectively to the amount of ingested oxalic acid thus protecting the host animal from toxic symptoms. Using measured rates of oxalic acid degradation, together with information on rates of administration it is possible to assess the likely rates of oxalic acid absorption. For example at the

highest rate of administration (1.2 mmol/kg per day) a 50-kg animal would receive 60 mmol of oxalic acid. The mean rate of oxalic acid degradation among animals receiving this dose rate was 13.9 mmol/l rumen fluid per day. Assuming a rumen volume of 5 l (Domingue *et al.*, 1991a) the amount of oxalic acid degraded would be 69.5 mmol. The pulsatile nature of the dosing regime may have resulted in transient ruminal concentrations of oxalic acid which were beyond the capacity of the microbes to degrade but in broad terms the results indicate that the amounts of oxalic acid escaping degradation and being absorbed were minimal. This conclusion is supported by the failure to detect effects on indicators of toxicity such as changes to plasma calcium concentrations and creatinine concentrations.

In conclusion, the results of this experiment demonstrate the adaptability of the rumen microbial population of sheep and goats to the presence of oxalic acid in the host diet. At the levels tested here, which are representative of likely ingestion rates by ruminants in conventional agricultural practice, rates of degradation of oxalic acid were sufficient to protect the host animal from obvious detrimental effects. Furthermore the results indicated that the rumen of goats may harbour a microbial population which degrades oxalic acid more rapidly than that of sheep.

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