Investigating the potential utility of the goat's ruminal adaptation strategies to tannins present in *Acacia* and other tree fruits[1]

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Abstract

This study investigated the potential of the goat's ruminal adaptation mechanisms to reduce the negative effect of tannins on *in vitro* fermentation. Rumen fluid from goats fed for 85 days on tannin-containing tree fruits (adapted rumen fluid) or a tannin-free commercial diet (unadapted rumen fluid) was used to ferment tannin-rich substrates. Dry and mature fruits of *Acacia nilotica*, *A. erubescens*, *A. erioloba*, *Dichrostachys cinerea* and *Piliostigma thoningii* were used as substrates for the *in vitro* fermentation. The effectiveness of adapted rumen fluid was measured against a known tannin-binding agent, polyethylene glycol (PEG). Although adapted rumen fluid significantly (P<0.05) increased the fermentation of tannin-rich substrates, it was no match for PEG. When PEG was added to adapted rumen fluid a further improvement in extent of fermentation was observed, suggesting that detannifying tannin-rich feedstuffs may still be necessary even when these are offered to adapted animals.

Introduction

Dry and mature tree fruits from *Acacia* and other tree species are potential protein sources for goats in semi-arid areas of Zimbabwe. However, the fruits contain high levels of phenolics, which may limit their utilisation. Many types of tannin are known as anti-nutritional, protein-binding secondary plant compounds, which may reduce availability of dietary protein. To improve the utilisation of tree fruits as protein supplements, tannin-inactivation treatments may be required. However, several researchers have presented findings that suggest that upon prolonged exposure to tanniniferous diets, some rumen microbes acquire mechanisms to deal with tannins. Such mechanisms include secretions that reduce the effect of tannins on microbes (defence) (Chiquette *et al*., 1988) and ability to produce tannin-degrading enzymes (Brooker *et al*., 1999; Wiryawan *et al*., 1999; McSweeney *et al*., 2001). Such microbes become adapted to the presence of tannins in the diet and hence their fermentation activity is less inhibited, allowing better utilisation of tannin-rich feedstuffs. Jones and co-workers (1994) identified *Prevotella ruminicola* and *Ruminobacter amylophylis* as tannin-resistant rumen bacteria. While the degradation of

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hydrolysable tannins in the rumen has been demonstrated (McSweeney *et al.*, 2001), breakdown of condensed tannins is not a confirmed feature of the fore stomach. Ruminal adaptation to condensed tannins is likely to be in the form of defence mechanisms, such as secretions, rather than tannin breakdown. As goats in semi-arid areas of Zimbabwe are continuously exposed to tannin-rich forage they may be considered to be adapted to tannins. It is, therefore, possible that there is little, or no, need to control the amount of tannins they consume through tannin inactivation treatments. However, in addition to the fact that tannin adaptation mechanisms are poorly understood, the same mechanisms may not be sufficient under a semi-intensive feeding regime of tree fruits (200 g/day for a mature goat). The objective of this experiment was to evaluate the effectiveness of adapted rumen fluid in reducing the biological activity of tannins using an *in vitro* tannin bioassay.

Materials and methods

The study tested the hypothesis that adapted rumen fluid is more potent in degrading tanniniferous fruits than unadapted rumen fluid. The second hypothesis was that treatment with polyethylene glycol (PEG) to inactivate tannin does not improve fermentation of tree fruits incubated with adapted rumen fluid.

Six rumen fistulated, castrated male Matebele goats were randomly allocated to supplements (200 g/animal/day) of either a tannin-free commercial protein supplement (CPS) or a fruit mixture (*Dichrostachys cinerea*, *Piliostigma thonningii*, *Acacia nilotica*, *A. erubescens* and *A. erioloba*, in equal proportions). Standing grass hay was offered at the rate of 600 g/animal/day as a basal diet while drinking water was available throughout the experiment. Animals were fed for a period of 85 days after which rumen fluid was collected and used to ferment tree fruits, in the presence and absence of PEG (200 mg/g DM), for 96 h using the Reading Pressure Technique (RPT) (Mauricio *et al.*, 1999). The animals offered a mixed fruit supplement provided adapted rumen fluid while those offered CPS provided unadapted rumen fluid.

The change in gas production in response to source of rumen fluid and PEG inclusion was measured. Dry and mature fruits used in the RPT fermentation procedure were collected from *D. cinerea*, *P. thoningii*, *A. nilotica*, *A. erubescens* and *A. erioloba* tree species. Gas production data were subjected to the analysis of variance using the GLM procedure for a completely randomised design (CRD) with a 5 (species) * 2 (rumen fluid) * 2 (PEG) factorial treatment arrangement. The model for cumulative gas production at 24 and 36 h post-inoculation was:

Y = *Overall mean* + *Species* + *PEG* + *Rumen fluid* + *Species*PEG* + *PEG*Rumen fluid* + *Rumen fluid*Species* + *Species*PEG*Rumen fluid* + *Residual error*,

where Y represented cumulative gas production. Treatment means were compared by applying the probability of difference (PDIFF) option of the least squares means statement in the GLM procedures of SAS (SAS/STAT, 1996).

Results

Table 1 shows the chemical composition of the basal diet and supplements offered to rumen fistulated goats. The grass hay offered to fistulated goats as the basal diet was, typically, low in nitrogen (4.62 g kg^{-1} DM) and high in both NDF and ADF (786 and 562 g kg^{-1} DM, respectively). The mixed fruits and CPS did not differ significantly in N content (Table 1). Mixed fruits supplement, however, had significantly (P<0.05) higher levels of both NDF and ADF.

Table 1 *Dry matter (g/kg) and chemical composition (g/kg DM) of basal diet and supplements (see text for details) fed to rumen fistulated goats*

Constituent	Grass hay	CPS	Mixed fruits	s.e. mean
Dry matter	934^{a}	929^{b}	923^{c}	1.0
Ash	79.7^{a}	93.6^{b}	49.3^{c}	0.97
Nitrogen	4.62^{a}	23.5^{b}	20.8^{b}	1.76
Neutral detergent fibre	786^{a}	449^{b}	479^{c}	9.3
Acid detergent fibre	562^{a}	246^{b}	337^{c}	1.0

In a row, means with different superscripts differ significantly (P<0.05)

Table 2 *Effect of PEG on cumulative gas production (ml/g OM) of tree fruits incubated with adapted and unadapted rumen fluid*

		24 h incubation		36 h incubation	
Species	Rumen fluid	Without PEG	With PEG	Without PEG	With PEG
Acacia erioloba	Unadapted	82.9^{a}	87.8^{b}	120.7^{a}	129.9^{b}
	Adapted	108.7^{c}	115.3^{d}	146.1^{c}	154.2^{d}
A. erubescens	Unadapted	75.9^{a}	74.8^{a}	106.6^{a}	105.1^{a}
	Adapted	87.9^{b}	86.0^{b}	115.0^{b}	113.7^{b}
A. nilotica	Unadapted	42.9^{a}	70.2^{b}	64.3^{a}	106.3^{b}
	Adapted	74.5^{b}	106.6^{c}	132.3^{c}	158.8^{d}
Dichrostachys cinerea	Unadapted	53.2^{a}	96.1^{b}	73.2^{a}	134.4^{b}
	Adapted	77.6^{c}	110.9^{d}	103.9^{c}	139.8^{d}
Piliostigma thoningii	Unadapted	80.9^{a}	122.6^{b}	112.9^{a}	154.8^{b}
	Adapted	100.3^{c}	136.4^{d}	132.7^{c}	165.0^{d}
s.e. mean		2.11		2.33	

Within species and within incubation time, means with different superscripts differ significantly (P<0.05)

Table 2 shows the effect of source of rumen fluid and PEG on cumulative gas production from tree fruits. All comparisons were made using 24 and 36 h incubation data. Adapted rumen fluid was consistently more potent than unadapted rumen fluid across all tree species. The largest increase in gas production between unadapted and adapted rumen fluid was observed with *A. nilotica* fruits (73.7 per cent). The PEG treatment significantly (P<0.05) improved the fermentation of tree fruits in adapted rumen fluid in all tree species except *A. erubescens*. Adapted rumen fluid plus PEG generated more gas than unadapted rumen fluid plus PEG for all tree species except *D. cinerea*. In *D. cinerea*, there was no significant difference between the gas production values for these two treatments.

Discussion

The hypothesis that rumen fluid from adapted animals is more potent in fermenting tanniniferous tree fruits than fluid from unadapted animals was confirmed by the results obtained. However, the hypothesis that PEG inclusion does not improve gas production from tree fruits incubated with adapted rumen fluid was not supported. The ability of rumen microbes to adapt to dietary phenolics has been reported by several workers (Lowry *et al*., 1996; Odenyo *et al*., 1997; Saarisalo *et al*., 1999; McSweeney *et al*., 2001). However, the mechanisms through which microbes adapt to the presence of phenolics in the diet of the animal are poorly understood. Polyethylene glycol has been used to investigate the biological effect of tannins on *in vitro* fermentation thus largely ignoring the effect of low molecular weight phenolics, which may not bind to PEG. These phenolics may affect microbial growth and some even act as nutrients (Lowry *et al*., 1996), and thus adaptation of the microflora may protect the animal from the harmful effects of these phenolics in the rumen.

The higher potency of the adapted rumen fluid is supported by the observation made by Smith (1992), that microbial populations change with prolonged exposure to toxic substances, thus allowing ruminants to increase their tolerance to some potentially poisonous plants. As expected, the PEG effect was higher when applied to unadapted rumen fluid compared to adapted rumen fluid. This suggests that the rumen microbes, either through tannin degradation or through other adaptive mechanisms, are able to inactivate some of the tannins, which in unadapted animals would have been inactivated by PEG (McSweeney *et al*., 2001; Acamovic and Stewart, 1999; Wiryawan *et al*., 1999).

The fact that the increase in cumulative gas production due to the application of PEG is higher than the increase due to the use of adapted rumen fluid for *D. cinerea* and *P. thonningii* fruits may indicate a strong *in vitro* effect of condensed tannins in these tree species. For *A. nilotica*, it appears that hydrolysable tannins, epigallocatechin gallates (Ayoub, 1985), play a much bigger role as anti-nutritive factors. Interestingly, adapted rumen fluid increased gas production by 74 per cent over unadapted rumen fluid when *A. nilotica* fruits were fermented for 24 h post-inoculation. This was higher than the increase in gas production when PEG was used to ameliorate tannin effects in unadapted rumen fluid. However, adapted rumen fluid plus PEG always gave higher cumulative gas production values suggesting an additive effect of the two methods with respect to alleviation of toxicity of phenolics. There is, therefore, evidence to support the observation that PEG may not bind efficiently to all types of tannins (our unpublished data) in the *in vitro* evaluation of forages, such as *A. nilotica* fruits, except in situations where such forages are to be offered to adapted animals. Adapted animals are expected to metabolise low molecular weight phenolics and hydrolysable tannins. Indeed, Distel and Provenza (1991) reported that 'experienced' goats excreted more uronic acids (an indicator of phenol detoxification in animals) per unit body weight than 'inexperienced' goats fed blackbrush (*Coleogyne ramosissima* Torr.). As expected the fermentation response of *A. erubescens* fruits to both PEG and adapted rumen fluid was limited due to its high fibre and low phenolic content. The fermentation pattern of *A. erioloba* fruits followed that of *A. nilotica*, with higher gas production values being obtained with adapted rumen fluid than with PEG treatment.

Although this study focused on ruminal adaptations to tanniniferous forages it is important to note that there are other defence mechanisms that enable goats to utilise tannin-containing forages. Tannin-binding salivary proteins were first discovered by Mehansho *et al.* (1983) and comprise proline rich proteins (PRPs), which have a strong affinity for tannins and may be the first line of defence against dietary tannins (Wróblewski *et al.*, 2001).

Conclusions

Adapted rumen fluid and the addition of PEG alleviated the inhibitory effects of phenolics on *in vitro* fermentation of tree fruits in an additive manner. Because PEG further increased fermentation with adapted rumen fluid, it is suggested that detannifying of fruits may still be necessary even when being fed to 'adapted' goats. It should be noted that feeding equal mixtures of tree fruits might have helped in alleviating anti-nutritional effects of phenolics through dilution caused by co-feeding fruits with high levels and different types of phenolics and those with very low levels. In fact, recent research by a few authors showed that a mixture of tanniniferous browses produces less deleterious effects than sole feeds (Dube & Ndlovu 1995; Patra *et al.*, 2003; Ben Salem *et al.*, 2002; Melaku *et al.*, 2004). Fruits of *A. nilotica* and *A. erioloba* may require treatments that inactivate low molecular weight phenolics when being fed in large quantities or as a sole supplement. Alkaline treatments are potential candidates for this role. Fruits from *D. cinerea* and *P. thonningii* contain appreciable quantities of condensed tannins, which may not be metabolised in the rumen and may thus require treatments. It is important to check, *in vivo,* to what extent the condensed tannins compromise the protein value of fruits from these two species. The role of alkaline treatment in inactivating phenolics and enhancing the protein value of fruits should be evaluated *in vivo*.

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