26

The use of wood ash to overcome detrimental effects of tannins on *in vitro* fermentation of tree fruits

V. Mlambo, F. L. Mould, T. Smith, E. Owen and I. Mueller-Harvey

School of Agriculture, Policy and Development, The University of Reading, Earley Gate, P O Box 237, Reading, RG6 6AR, UK.

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 presence of high levels of phenolics limits their utilization. Tannins are known anti-nutritional protein binding secondary plant compounds, which reduce availability of dietary protein. To improve the utilization of tanniniferous tree fruits, tannin-inactivating treatments are required. The use of polyethylene glycol is well documented but not affordable for subsistence farmers. In addition the effect of PEG-tannin complexes on the soil is largely unknown, hence caution is required where PEG is used on a large scale. Work on high-tannin sorghum has provided evidence that alkali treatments can be used to inactivate tannins. Sources of alkali include expensive, and relatively dangerous, sodium hydroxide and ammonium hydroxide as well as inexpensive wood ash and sodic soils. Wood ash is non-corrosive and subsistence farmers have experience in handling it as most of their fuel is provided by firewood. The use of wood ash solution to treat tree fruits can provide a cheap and safe way of improving their utilization as protein supplements during the dry season. The effectiveness of alkaline treatments on forage tannins is difficult to estimate because of confounding with the effect on fibre. Colorimetric, protein precipitation and gravimetric tannin assays on alkali treated substrates are complicated by residual alkalinity in the extraction medium, which may cause an overestimation of the effectiveness of the alkali (Makkar and Becker, 1996). Another problem with these tannin assays is that they do not always reflect the biological activity of the tannins. The objective of this experiment was to evaluate the effectiveness of wood ash solutions in reducing the biological activity of tannins in fruits from *Acacia nilotica* and *Dichrostachys cinerea* tree species using an *in vitro* tannin bioassay.

Material and Methods Wood from an *A. nilotica* tree was made into a fire and 2 kg of ash was collected. A 20 % wood ash solution (20WA) was prepared by dissolving 800g of wood ash in 4 L of distilled water. The mixture was allowed to settle for 1 h and then filtered. A 0.6 % NaOH solution was prepared by dissolving 3 g NaOH powder in 500 ml of distilled water (NaOH). This solution was found to be the most effective in inactivating tannins in tree fruits in a previous study and thus was used in this experiment for comparison purposes. The concentration of OH^- ions was estimated in the wood ash solution through titration with 0.2 M HCl. The 20 % wood ash solution was diluted with distilled water to give a 5 % wood ash solution (5WA) and a solution with the same OH^- ion concentration as the 0.6 % NaOH solution (13.9WA). Five millilitres of each solution was used to treat 1 g fruit samples in serum flasks using the Reading Pressure Technique (RPT) (Mauricio *et al.*, 1999). Distilled water was used to wet the samples, which were not assigned to any treatment. The treatment lasted for 24 h after which 85 ml of the RPT buffer was added to stop the reaction. Half of the flasks received buffer carrying PEG while the other half received no PEG. The PEG was used to detect residual detrimental effect of tannins after treatment with alkalis. This was compared to the detrimental activity in untreated fruits. *In vitro* fermentation was carried out using the RPT by inoculating the flasks with 10 ml of rumen fluid from a cow feeding on grass and maize silage and concentrate. Gas production and *in vitro* organic matter degradability (iOMD) were estimated.

Results Table 1 shows the cumulative gas production in response to alkali and PEG treatments at 24 and 48 h post-inoculation. Generally the responses to alkali treatments were less pronounced in *A. nilotica* compared to *D. cinerea* fruits. In *A. nilotica*, the NaOH solution was most effective in reducing the detrimental effect of tannins on *in vitro* fermentation. Gas production was increased by 29 % as a result of tannin inactivation (PEG) while treatment with 20 % wood ash solution caused a 26 % increase. For *D. cinerea*, the 20 % wood ash solution was the most effective tannin inactivating alkali causing a 23 % increase in gas production. Wood ash solutions with the same OH^- concentration as NaOH solution were less effective when compared to the NaOH solution for both species. Alkali treatments alone did not cause any improvement in degradability of *A. nilotica* fruits at 24 h post-inoculation, however, PEG treatment improved iOMD. Both wood ash and NaOH caused an improvement in the iOMD of *D. cinerea* fruits.

Table 1 Cumulative gas production (ml g^{-1} OM) for tree fruits treated with wood ash and sodium hydroxide solutions incubated with and without PEG (+/-) at 24 and 48h post-inoculation (see text for description of treatments)

		24h		48h	
Species	Treatment	-	+	-	+
Acacia nilotica	NaOH	59.5^{Ab}	63.3^{Aa}	93.6^{Ab}	105^{Ba}
	13.9WA	54.8^{Aa}	69.2^{Bb}	84.1^{Aa}	117^{Bb}
	20WA	55.9^{Aab}	61.1^{Ba}	88.5^{Aab}	102^{Ba}
	5WA	56.8^{Aab}	77.0^{Bc}	84.8^{Aa}	123^{Bbc}
	Untreated	59.0^{Ab}	80.6^{Bc}	89.1^{Aab}	126^{Bc}
Dichrostachys cinerea	NaOH	61.2^{Ab}	59.5^{Aa}	92.2^{Aa}	96.5^{Aa}
	13.9WA	62.2^{Ab}	67.8^{Bb}	93.3^{Aa}	104^{Bb}
	20WA	60.8^{Ab}	57.7^{Aa}	92.7^{Aa}	94.4^{Aa}
	5WA	61.4^{Ab}	69.4^{Bb}	92.8^{Aa}	108^{Bbc}
	Untreated	55.8^{Aa}	74.2^{Bc}	90.6^{Aa}	113^{Bc}
s.e. mean		2.04		2.93	

All comparisons using superscripts were performed within species and incubation time
Means with different superscripts differ significantly ($P < 0.05$)
Uppercase superscripts were used to compare PEG effect within a treatment (rows) while lower case superscripts were used to compare alkali treatments within a PEG level (columns)

Conclusions Wood ash effectively reduced the detrimental effect of tannins on *in vitro* fermentation. However, wood ash is less effective when compared with NaOH presumably because of the lower caustic properties of wood ash. Higher concentrations of wood ash may be required for improved effectiveness. There is potential for wood ash to be used by farmers to detannify tree fruits before feeding goats in order to improve utilization and protect animals from long-term effects of phenolic compounds. There is a strong possibility that alkali treatment enhances oxidation of low molecular weight phenolics which may not be bound by PEG but are still toxic to rumen microbes and impair post-rumen digestion and absorption as well as nutrient metabolism.

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