Alleviating seasonal nutrient fluctuations in semi-arid areas of Zimbabwe: potential for tree fruits as protein supplements for goats

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Abstract

This paper presents the studies undertaken to evaluate the potential of locally available tree fruits as supplements for goats in the dry season in semi-arid areas of Zimbabwe. Initially the evaluation process was carried out in laboratories. This process entailed chemical characterization with emphasis on protein and phenolic content, these being the two main constituents that affect the value of fruits as protein supplements. Chemical composition and *in vitro* fermentation of fruits and separated fruit fractions from *Acacia nilotica*, *A. erubescens*, *A. erioloba*, *Piliostigma thoningii* and *Dichrostachys cinerea* tree species were determined. The presence of phenolics reduced fermentation *in vitro* and interfered with the determination of *in vitro* organic matter degradability, due to their solubility in the fermentation medium. The nutritional effects of tannins were investigated using an *in vitro* tannin bioassay where fruit substrates were incubated with and without tannin-binding polyethylene glycol (PEG). Treatment with PEG increased the fermentation of tree fruits suggesting that tannins limit their fermentation.

Wood ash solution, a locally available alkali, inactivated tannins in *D. cinerea* and *A. nilotica* fruits, resulting in improved fermentation *in vitro*. The effect of alkali and PEG treatments on nitrogen balance of goats was evaluated using *D. cinerea* fruits as protein supplements. Goats were offered a basal diet of standing grass hay. Fruit supplements were compared to a commercial protein supplement (CPS). Treatment with PEG caused excessive protein degradation in the rumen resulting in nitrogen loss through the urine. Goats offered untreated fruits had the same nitrogen retention as those offered CPS. *Dichrostachys cinerea* fruits used in this study did not require tannin inactivation treatment. A feeding trial in which does were offered fruit supplements showed that supplemented does had higher conception rates, weaning weights and fewer kid mortalities.

Introduction

Zimbabwe's smallholder agricultural sector, based in the communal lands, holds about 97 per cent of the estimated 4.7 million goats (CSO, 1997) in the country. Most of these animals are in the dry and less productive agro-ecological zones (Kusina and Kusina, 1999). With the exception of drought tolerant sorghum and pearl millet, crop production in these semi-arid areas is risky due to the low and unpredictable annual rainfall of less than 600mm. The result of recurrent droughts and poor rainfall is that high quality animal feed is always in short supply resulting in the drought tolerant goat and donkey playing a prominent role in the livelihood of the smallholder farmers in semi-arid areas. Despite the potentially high rate of reproduction, the productivity of the goat in Zimbabwe is low (Kusina and Kusina, 1999). This is attributed to dry season malnutrition, high rates of kid mortality and incidence of disease, as well as poor marketing structures (Kindness *et al.*,

1999). Kids that are born during the rainy season (from December to April), when feed quantity and quality is high, show good health and survivability. This suggests a link between nutritional status of the doe and reduced kid mortality, which in turn affects goat productivity. The number of does kidding in October and November is low (Kindness *et al.*, 1999) suggesting poor conception rates due to feed shortages in June and July (dry season).

The *Acacia* thornveld is the main feed resource for goats in the semi-arid areas of Zimbabwe. Goats browse on green leaves for most of the rainy season while in the dry season fallen fruits from the same trees are consumed. In most cases goats and other animals, are given free access to the fruits, hence the fruit supply does not last through the dry season, and under-utilization often occurs, especially in the early dry season when the goats have a wider choice of feed. Fruits from these trees could be used as a protein source for animals feeding on low quality roughage later in the long dry season. Many *acacias* produce potentially nutritious fruits with up to 20 per cent crude protein. While supplementation with fruits has the potential to improve goat productivity little is known about the nutritive value of the fruits. Anti-nutritional factors are known to be a significant component of most browse tree species (Aganga and Mosase, 2001). Caution should be exercised especially on the quantities that are fed to an animal, the frequency of feeding and the form in which the fruits are fed. Feeding large quantities of fruits frequently may result in animals developing haemorrhagic lesions in the gastro-intestinal tract, resulting in the death of the animal (Terblance *et al.*, 1967).

The objectives of the study were to improve the productivity of smallholder owned goats through dry season feeding interventions based on locally available tree fruits. Several studies were undertaken to assess the potential of tree fruits to reduce the fluctuations in nutrient supply experienced during the dry season in semi-arid areas of Zimbabwe.

Material and methods

Chemical characterization

Mature and ripe fruit samples were harvested by hand in June 1999 from *Acacia. nilotica, A. erubescens, A. erioloba, Piliostigma thoningii, Dichrostachys cinerea* and *A. sieberiana* trees growing in the thornveld in Mbembeswana communal areas, about 100 km south west of Matopos Research Station, Bulawayo, Zimbabwe. Annual rainfall in this area averages 400mm. The fruits were bulked by species and stored in brown paper bags until required for use.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by refluxing 1 g samples in neutral and acid detergent solutions, respectively, according to the method of Goering and Van Soest (1970). Acid detergent insoluble nitrogen (ADIN) was determined by nitrogen analysis on ADF, dried at 40°C for 48 hours, using the Dumas total combustion method with a Carlo Erba Elemental Analyser 2100.

Samples used for the analyses of N and phenolics were further ground to pass through a 1mm sieve. Total N was determined on 50mg sample using the Dumas method as for ADIN.

Phenolics were estimated after extraction of 40mg sample three times with 10ml 70 per cent aqueous acetone for a total of 15 minutes. Soluble condensed tannins (SCT) were estimated using the butanol-HCl reagent (95:5 v/v) (Porter *et al.*, 1986). Aqueous acetone extract (0.5ml) was pipetted into a glass screw cap test-tube and 5ml butanol-HCl reagent

added. The test-tube was closed and then placed on a heating block at 100°C for 1 hour. Absorbance was measured after the test tubes had cooled to room temperature. The measurements were reported as absorbance units (au) at 550 nm. Determination of trivalent ytterbium precipitable phenolics was done on a 70 per cent aqueous acetone extract, which was obtained as described above for SCT and insoluble condensed tannins (ICT). Ytterbium precipitable phenolics were gravimetrically determined using the procedure described by Reed *et al*., (1985) Results were expressed in g kg^{-1} DM.

Effect of tannins on *in vitro* fermentation

Microbial fermentation was assessed using the Reading Pressure Technique (RPT) (Mauricio *et al*., 1999). About 1g of sample, ground to pass through a 2mm sieve, was weighed into 125ml serum bottles. Using an automatic dispenser (Jencons, Hemel Hemstead, England), 90ml reduced buffer was added to each serum bottle. The buffer was divided in two and polyethylene glycol (PEG) dissolved in one set to give an application rate of 1g PEG per 1g DM of substrate, based on work done by Makkar *et al*. (1995) and Salawu *et al*. (1997). Serum bottles without samples (blanks) were also included for each of the six withdrawal periods (6, 12, 18, 24, 48 and 96 h post-incubation) to allow correction for gas produced from rumen liquor. After addition of the buffer, the flasks were sealed and stored at room temperature (20°C) before being transferred into the incubators, set at 39°C, 8 h before inoculation with rumen fluid.

Rumen fluid was obtained from a cow fed *ad libitum* on a diet comprised of grass and maize silage and concentrate (wheat, barley and soya bean meal). Rumen fluid was collected at 07.00 h, prior to feeding. Inoculation was complete within 60 minutes of fluid being prepared. Rate of gas production, *in vitro* dry matter degradability (iDMD), *in vitro* organic matter degradability (iOMD) and partition factors were determined.

Detannification: *in sacco* evaluation

Alkaline treatment was carried out by soaking fruits overnight in distilled water, in which NaOH pellets had been dissolved to give a 6 per cent (w/w with the fruit sample) NaOH treatment level. Sufficient water was added to wet the entire sample, producing a thick paste ensuring that no leaching of the sample occurred. Polyethylene glycol-4 600 (Aldrich Chemical Co. Inc, USA) was dissolved in distilled water and the solution mixed with *D. cinerea* fruits. A PEG application rate of 200mg g^{-1} of sample was used. The mixture was left to react overnight. The treated samples were then spread onto polythene sheets and sun-dried for 24 h.

Three adult male Matebele goats fitted with ruminal cannulae and weighing about 25kg live weight were used in this study. These animals had been used in a previous experiment, where diets containing mixtures of fruits were compared. The goats were housed individually in crates under a roofed shed with a concrete floor. They were fed on 200g mixed fruits per animal per day and grass hay *ad libitum*. Water was available at all times. The animals had been on this diet for 85 days. Dry matter loss from the rumen and nitrogen degradability were determined using nylon bags (Lockertex, Cheshire, England), measuring 6cm * 12cm with a pore size of 40 μm. About 5 g of each sample were weighed in duplicate, for every treatment and each incubation time, into nylon bags, which were incubated in the rumen of the fistulated goats (internal diameter of the rumen cannulae was 40mm). The incubation was carried out in a 3*3 Latin square design trial. Each period was of 3 days duration and a 24h changeover period was allowed, to ensure that there was no residual effect of the previous treatment on the following treatment. All bags were inserted into the rumen at the same time (07.00 h) on the first day, before the morning

feeding, and were incubated for 4, 6, 12, 24, 36, 48 and 72 h. The bags were withdrawn at different times (sequential withdrawal). Upon removal from the rumen the bags were cleaned with tap water and immersed in ice water to stop microbial fermentation activity (Shannak *et al.*, 2000). The bags were frozen until all the incubated bags were withdrawn at the end of the period. Together with the 0h bags, incubated bags were washed with cold water three times in cycles of ten minutes, in a twin-tub semi-automatic washing machine (Rotary tub, Goldfish, South Africa). Washed nylon bags were then dried in a forced-draught oven at 60°C for 48 hours and cooled in a desiccator, followed by weighing.

Loss in dry matter (DM) for each incubation period was calculated as follows:

$$\text{DM Disappearance} = \frac{\{\,(OSBW - BW) * DM1\,\} - \{\,(RSBW - BW) * DM2\,\}}{(OSBW - BW) * DM1}$$

where:

OSBW	=	Original sample weight + nylon bag (g)
BW	=	Nylon bag weight (g)
RSBW	=	Residual sample weight + nylon bag (g)
DM1	=	Dry matter of feed sample
DM2	=	Dry matter of residue sample

Loss in N was calculated on the basis of N incubated as follows:

$$\text{N Disappearance} = \frac{(\%N1 * OSW * DM1) - (\%N2 * RSW * DM2)}{\%N1 * OSW * DM1}$$

where:	%N1	=	Percent nitrogen in original feed sample
	OSW	=	Original sample weight (g)
	DM1	=	Dry matter of feed sample
	%N2	=	Percent nitrogen in residue sample
	RSW	=	Residual sample weight (g)
	DM2	=	Dry matter of residue sample

Degradation data were fitted to the Ørskov and McDonald (1979) non-linear model using NEWAY Excel Version 5.0 package (Chen, 1997). The Ørskov and McDonald model reads:

$$p = a + b(1 - e^{-ct})$$

where	p	=	disappearance of DM and N
	a	=	washing loss or soluble fraction
	b	=	degradable part of the insoluble fraction

c = degradation rate of fraction b

This gave estimates for the soluble and insoluble fractions as well as the rate of degradation. Effective degradability (ED) of N was calculated, after assuming a 5 per cent h^{-1} solid outflow rate, according to the following equation:

$$ED = a + \frac{b * c}{k + c}$$

where ***a***, ***b*** and *c* are the constants from the Ørskov and McDonald (1979) equation above and ***k*** is the outflow rate of the solid phase in the rumen.

The effect of treatments on chemical composition (DM, OM, NDF, ADF, N and NDIN) was obtained by subjecting the data to a one-way analysis of variance. The effect of treatments on the *in sacco* degradability was analysed using the general linear models procedures of SAS (SAS/STAT, 1996) for a Latin square.

Detannification: *in vivo* evaluation

Thirty castrated Matebele goats, aged 18 – 22 months and weighing on average 27.4kg, (s.d. = 2.5) were assigned to five diets, using a randomized complete block design after the animals had been blocked according to initial live-weight. Each of the five diets was randomly allocated to the five animals in the six weight-blocks. The goats were then penned individually in metabolism crates measuring 120cm long, 54cm wide, and 90cm high and raised 90cm above the floor. The crates were fitted with feeders and drinking bowls. All the goats were dewormed at the beginning of the adaptation period, by oral administration of 8ml of Systemex liquid, active ingredient, oxfendazole 2.265 per cent m/v (Cooper Zimbabwe, Pvt Ltd), using a 10ml syringe.

The five experimental diets consisted of mixed grass hay in combination with the following supplements:

Diet A - 200 g $day^{-1}$of alkali treated *D. cinerea* fruits

Diet B - 200 g $day^{-1}$of polyethylene glycol treated *D. cinerea* fruits

Diet C - 200 g day^{-1}untreated *D. cinerea* fruits

Diet D - 200 g day^{-1} CPS (National Foods Pvt Ltd, Bulawayo, Zimbabwe) (positive control)

Diet E – 800 g grass hay fed alone (negative control)

All animals received a daily ration of 600 g of mixed grass hay' except for those on Diet E. Fruits were treated with NaOH and PEG in bulk and sun-dried to ensure less variable supplements were offered to goats throughout the trial. The treatment involved soaking fruits overnight at the rate of 0.6kg in a litre of distilled water in which 36g of NaOH pellets had been dissolved to give a 6 per cent NaOH treatment level. Polyethylene glycol treatment was carried out by dissolving 120g polyethylene glycol-4 600 (Aldrich Chemical Co. Inc, USA) in a litre of distilled water and mixing the solution with 0.6kg of *D. cinerea* fruits to give a PEG application rate of 200mg g^{-1} feed. The mixture was left to react overnight and sun-dried to improve intake.

Feed supplements were offered at 0800 h everyday and the animals were allowed 2h to consume them. After 2h, supplement refusals were weighed and removed from the feeding troughs. All the animals were then offered half of the grass hay ration, 300g for animals receiving supplements and 400g for the animals on the negative control. The other half was offered at 1600 h. Refusals were weighed and collected before fresh feed was offered. Clean, fresh, drinking water was offered at 0800, 1400 and 1600 h everyday. Grass hay refusals were weighed in the morning before the feeding of supplements.

Goats were allowed to adapt to the different diets and metabolism crates for 21 days. During this period feed intake was closely monitored to ensure that the goats were eating approximately the same amount everyday.

The collection period lasted seven days. During this period a complete collection of faeces and urine from each experimental animal was made. Sub-samples of faeces were taken for dry matter determination everyday. The DM was determined by drying the faecal samples in an oven at 100°C for 12 h. About 10 per cent of the total faecal collection from each animal was bulked over the entire collection period and stored in a freezer at –4°C to await chemical analyses.

Urine was collected in plastic containers over 25ml of 10 per cent (v/v) sulphuric acid. The volume of the urine was then measured using a measuring cylinder and a 10 per cent aliquot was removed everyday, bulked over the collection period and stored at –4°C pending nitrogen analysis. Refusals from supplements and the basal diet were weighed separately each morning and dried at 60°C for 48 h to determine dry matter refused. Sub-samples of the feed offered were also taken and similarly dried to determine the amount of dry matter offered per day. The difference between dry matter offered and dry matter refused was used as the measure of dry matter intake. Feed, faeces and refusals were both analysed for OM, N, NDF, ADF, neutral detergent insoluble nitrogen (NDIN) and ADIN to estimate the intake and digestibility of these constituents.

The proportion of average daily feed intake not excreted in faeces was used as a measure of apparent dry matter digestibility:

1. DM apparently digested = DM intake (g day^{-1}) – Faecal DM (g day^{-1})

2. Apparent DM digestibility = DM apparently digested / DM $intake^{-1}$ (g g^{-1}DM)

3. Digestibilities of organic matter, NDF, ADF, N, NDIN and ADIN were calculated as in 1 and 2 on a DM basis.

Nitrogen retention was calculated as the difference between total nitrogen intake and the losses through faeces and urine:

N retention (g day^{-1}) = Total N intake – (Faecal N + N in urine)

Intake and digestibility data for OM, N, NDF and NDIN as well as retention of N were statistically analysed using the general linear models procedures of SAS (SAS/STAT, 1996).

Results and Discussion

Table 1 shows the chemical composition of tree fruits from different tree species. Terblance *et al.*, (1967) reported incidences of poisoning leading to deaths when goats consumed excess *A. nilotica* fruits in South Africa. Symptoms observed include abortions, dyspnoea, tachycardia, methyglobinaemia, ruminal atony and hyperglycaemia. Although the toxic principle was not identified at the time, the report indicates that caution should be exercised when feeding *A. nilotica* fruits to goats on a daily basis. Soluble condensed tannin content is much lower in *A. nilotica* fruit while *D. cinerea* and *P. thoningii* fruits have higher levels.

Table 1 Nitrogen (N), acid detergent insoluble nitrogen (ADIN), neutral detergent fibre (NDF), ytterbium precipitable phenolics (YbPh) (g/kg DM) and soluble condensed tannins (SCT) (au) content of tree fruits

Species	N	ADIN	NDF	YbPh[1]	SCT[2]
Dichrostachys cinerea	19.9	5.7	441	485	3.4
Acacia erioloba	21.3	3.9	415	206	0.7
A. erubescens	27.1	6.7	543	175	0.5
A. nilotica	14.7	7.8	236	758	1.2
Piliostigma thoningii	13.5	4.2	493	299	4.1

[1]YbPh – ytterbium precipitable phenolics

[2]SCT – soluble condensed tannins

As shown in Table 2, PEG inclusion increased cumulative gas production in all tree fruits except *A. erubescens*. The highest response was obtained with *D. cinerea* fruits indicating that the tannins in the fruits from this species may reduce the availability of nitrogen to the rumen microbes. Although a 100 per cent increase in cumulative gas production was obtained with *A. nilotica* fruits, it is important to note that the majority of phenolics in this species are not condensed tannins (Table 1). The effect of PEG inclusion on OM degradability was underestimated due to the presence of PEG-tannin complexes in the residue (undegradable material) after filtration. In addition, the procedure of determining *in vitro* degradability means that phenolics that are solubilised in the fermentation medium are erroneously quantified as degradable material since these are lost during filtration.

Table 2 Responses to Polyethylene glycol (PEG) inclusion (+/-) of cumulative gas production (ml/g OM) and organic matter (OM) degradability (g/g OM) at 48 h post-inoculation

	Cumulative gas production		Organic matter degradability	
Species	**-**	**+**	**-**	**+**
Dichrostachys cinerea	48	156	0.37	0.48
Acacia erioloba	130	164	0.54	0.60
A. erubescens	102	115	0.49	0.50
A.. nilotica	78.8	150	0.62	0.76
Piliostigma thoningii	143	193	0.56	0.50

Having established that tannins may reduce the utilisation of some tree fruits (Table 2), an experiment was carried out to evaluate the effect of detannifying *D. cinerea* fruits on nitrogen availability both *in sacco* and *in vivo*. Table 3 shows DM and N disappearance in the rumen of goats. Treatment with PEG caused excessive N loss in the rumen while alkali treated and untreated fruits caused moderate losses of N in the rumen. It is, therefore, likely that PEG treatment of *D.cinerea* fruits will cause a reduction in N retention in animals compared to alkali treatment.

Table 3 *In sacco* disappearance of dry matter and nitrogen from treated and untreated *Dichrostachys cinerea* fruits incubated in the rumen of Matebele goats

	***Dichrostachys cinerea* fruits**			
Parameter‡	**Untreated**	**NaOH treated**	**PEG treated**	**s.e. mean**
a	26.4[a1]	29.3[b]	42.8[c]	0.71
b	48.3[a]	36.4 [b]	32.8[b]	2.22
c (% h^{-1})	3.26[a]	3.92[a]	5.78[b]	0.549
PD[2] (a + b)	74.7	65.7	75.6	-
ED[3]	44.5[a]	43.5[a]	59.7[b]	1.19
a	47.5[a]	52.8[b]	61.8[c]	1.72
b	43.1[a]	29.0[b]	27.9[b]	1.74
c (% h^{-1})	3.59[a]	6.76[b]	11.63[c]	1.463
PD (a + b)	90.6	81.8	89.7	-
ED	64.7[a]	68.8[b]	81.0[c]	0.49

[1]In a row, means with the different superscripts differ significantly ($P < 0.05$)

‡Units: For Dry matter a, b, PD and ED are measured as per cent of DM, for Nitrogen a, b, PD and ED are measured as per cent of N incubated.

[2]PD = Potential degradability

[3]ED = Effective degradability estimated as: $ED = a + \frac{b * c}{k + c}$, k (outflow rate of solids) assumed to be 5 per cent h^{-1}

Table 4 shows the results when detannification treatments were compared in a metabolism trial. Goats offered untreated fruits had significantly ($P < 0.0001$) higher N retention values when compared to those offered treated fruits. There were significant ($P < 0.05$) differences among treated fruits, with alkali treated fruits causing higher N retention values than PEG treated fruits (2.70 vs. 0.96 g N day^{-1} respectively). All supplements increased the goats' intake of grass hay by at least 50 per cent over the unsupplemented animals. This confirms that provision of N to rumen microbes improves the utilization of fibrous poor quality feedstuffs. An increase in grass hay intake was observed when calves were supplemented with *Acacia tortilis* fruits (Coppock, 1993). Tanner *et al.*, (1990) reported similar findings when sheep fed on maize stover were supplemented with *A. tortilis* fruits.

Table 4 Metabolism trial: *in vivo* evaluation of detannification treatment of *D. cinerea* fruits

	Treatments				
	Untreated	**NaOH[2]**	**PEG[3]**	**Goat Meal**	**Unsupplemented**
OM intake (g)	610^{bc1}	598^{b}	640^{c}	622^{bc}	297^{a}
Urine N (g)	0.67^{a}	0.46^{a}	1.68^{b}	0.48^{a}	2.09^{c}
OM dig	0.54^{b}	0.50^{a}	0.56^{b}	0.58^{b}	0.58^{b}
NDF dig	0.46^{b}	0.51^{b}	0.55^{c}	0.51^{b}	0.59^{d}
N balance (g)	3.7^{c}	2.7^{b}	0.96^{a}	4.1^{c}	-3.4^{d}

[1]In a row, means with the different superscripts differ significantly ($P < 0.05$)

[2]NaOH = sodium hydroxide treated *D. cinerea* fruits

[3]PEG = polyethylene glycol treated *D. cinerea* fruits

The unsupplemented animals in this experiment represent the plane of nutrition for smallholder owned goats during the dry season. Negative N balance and low feed intake suggest that the animals gradually lose weight during the dry season and thus are prone to malnutrition and disease. By offering *D. cinerea* fruits as a protein supplement, farmers may be able to maintain their goats through the dry season. In absolute terms, the production improvements, as a result of the N retention, observed in this study are modest but it is important to emphasise that maintenance of animals through the dry season is the most appropriate production objective in the communal farming system. It appears that tannins in *D. cinerea* fruits used in this experiment are beneficial to the animal and hence did not require inactivation. This raises questions about the suitability of *in vitro* tannin bioassays as predictors of *in vivo* tannin effect. There is need to include, as part of *in vitro* tannin bioassays, measures of the effect of tannins on protein degradability. This might be investigated by fermenting tanniniferous forages with and without PEG in a nitrogen deficient medium. This ensures that the nitrogen deficient rumen environment when goats

are consuming low quality fibrous diets in the dry season is adequately simulated. The improvement in fermentation with PEG treatment can then be attributed to the increased availability of nitrogen in the fermentation medium.

Conclusions

This study revealed that tree fruits harvested from Mbembeswana communal lands contain enough protein to improve the utilization of poor quality feeds during the long dry seasons experienced in this area. However, the presence of phenolics reduced protein degradability *in sacco*. Up to 70 and 50 per cent of the dry matter of *A. nilotica* and *D. cinerea* fruits, respectively, were made up of phenolics, which negatively affected *in vitro* fermentation of the fruits. Evaluation of feedstuffs rich in phenolics has concentrated on the colorimetric and gravimetric assays, which unfortunately say little about the potential biological activity of the phenolics.

Alkaline treatments had limited efficiency (up to 30 per cent) on fruit tannins compared to PEG. This could be because tannins in ripe and mature fruits exist in bound form and their reactive sites are not accessible to the alkali. Polyethylene glycol was more efficient because its mechanism of action is mediated through its high affinity for tannins allowing it to bind tannins, which are already bound to other constituents.

Results from the nitrogen balance trial showed that alkaline treatment, unlike PEG, did not cause excessive protein degradation in the rumen.

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