DICHROSTACHYS CINEREA PODS AS A PROTEIN SUPPLEMENT FOR GOATS FED ON HAY BASED DIETS

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Abstract

Studies were conducted to investigate the potential of *Dichrostachys cinerea* pods as protein supplements. Bulk *D. cinerea, Acacia erioloba, A. erubiscens, Piliostigma thonningii* and *A. nilotica* pods harvested in August 2000 were used for chemical analyses and an *in vitro* tannin bioassay. Chemical analyses were carried out to estimate the level of N, tannins and fibre in the pods. The pods were then evaluated using the *in vitro* gas production technique as a tannin bioassay, by incubating pods, with or without a tannin-binding agent, polyethylene glycol (PEG). Increase in gas production upon adding PEG was used as a measure of tannin anti-nutrient activity. *Dichrostachys cinerea* pods gave the highest response to tannin inactivation (over 200% increase in cumulative gas production), followed by *A. nilotica*. *Dichrostachys cinerea* and *A. nilotica* pods were selected for further evaluation of tannin inactivation methods *in vivo,* however, due to limited resources only one species could be evaluated at a time. This paper reports the *in vivo* evaluation of *D. cinerea.* Alkaline and PEG treatments were evaluated *In sacco* and in a nitrogen balance trial with goats offered *D. cinerea* pods. Milled (2 mm screen size) treated and untreated pods were incubated in nylon bags in the rumens of goats fed on a grass hay diet supplemented with mixed pods. Treated pods had significantly higher soluble fractions, rates of degradation and effective degradabilities of nitrogen (outflow rate 0.05) compared to the untreated pods. Treated pods were used as supplements for goats fed on a basal diet of grass hay. The supplements were compared to a commercial goat feed (goat meal) and a control treatment where goats were unsupplemented. Supplementation significantly increased both the intake of hay and N retention. The control group were in negative N balance. Supplementing with PEG treated pods significantly increased hay intake compared to the untreated pods, while there was no difference between the alkaline treated pods and the untreated ones. However, untreated pods gave a higher N retention value compared to the treated pods and this value was the same as that with goat meal. This suggests that there is no need to ameliorate the tannins in *D. cinerea* pods before feeding, as these are beneficial to the protein nutrition of the animal. PEG treatment may have resulted in excessive protein degradation in the rumen and increased N loss through the urine.

Introduction

The utilisation of protein rich leguminous trees and shrubs as protein supplements can counter the seasonal shortage of good quality forage for livestock in Zimbabwe. The long dry seasons (up to 7 months) are characterised by reduced quantity and quality of feed resources. In semi-arid areas, trees and shrubs are the only cheap source of protein for

livestock, thus maximising their use can help improve the productivity of livestock in the smallholder farming communities. Due to perennial feed shortages, the semi-arid areas have a large goat population whose productivity is considered to be low (Kusina and Kusina, 1999). The goat has been ranked the most important livestock species in these areas. Browse leaves are mostly available to the animals during the wet season and early in the dry season. However, as the dry season progresses, browse leaves become scarce and pods /fruits become available. Dry and mature pods are, therefore, important as a dry season protein supplement. However, the potential of tree pods to be used on a large scale is limited by the presence of anti-nutritional factors such as tannins (Tanner *et al.,* 1990; Mlambo *et al.,* 2000) and cyanogenic glycosides.

It is important that the nutritional effect of tannins is well understood before the intensive use of tree pods as protein supplements is recommended. The role of tannins in livestock nutrition is poorly understood. This is compounded by the use of quantitative assays that fail to relate to the biological activity of the tannins. It is important to understand the structure/activity relationship of tannins but this may not be possible in the often poorly equipped laboratories found in agricultural research institutions in developing countries. It is, therefore, imperative that simple, cheap and reliable tannin assaying techniques are developed that can be routinely used to screen the diverse tree species, which are potential protein sources for the resource-poor livestock keepers. In addition, it is also important to develop and evaluate cheap ways of ameliorating the anti-nutritional effects of tannins in those tree species where the utilization of pods is limited by the presence of tannins.

In this study the effect of tannin inactivation on the nitrogen balance of goats fed *D. cinerea* pods as a supplement is evaluated. They are compared with a commercial goat feed (goat meal). Polyethylene glycol (PEG) and sodium hydroxide (NaOH) are used as tannin inactivation agents. It is important to note that these two tannin-inactivating agents are expensive and, therefore, it is not practical for poor farmers to use them. Cheaper substitutes, such as wood ash, are available for alkaline treatments and these are being evaluated.

Materials and Methods

Pods

Dichrostachys cinerea pods used for chemical analyses and *in vitro* tannin bioassay were harvested at Matopos Research Station, Bulawayo, Zimbabwe, in August of 2000. Pods used for the nylon bag and nitrogen balance trials were harvested from the same site in August of 2001. Pods were knocked off trees with the aid of sticks and collected into sacks. Samples intended for N and tannin analyses were ground through a 1 mm screen while samples for the *in vitro* tannin bioassay, nylon bag trial and other chemical analyses were ground through a 2 mm screen. Pods and grass hay used in the nitrogen balance trial were ground through a 15 mm sieve.

Chemical analyses

Dry matter (DM) content was determined by drying 1 g of sample in an oven set at 100 °C overnight. Nitrogen content of nylon bag residues was determined by the macro-Kjeldahl method while the N content of other samples was determined using the total combustion method with the Dumas Elemental Analyser. Neutral detergent fibre (NDF) and acid detergent fibre content (ADF) were determined by the method of Goering and Van Soest, (1970), while acid detergent insoluble nitrogen (ADIN) content was obtained by determining the N remaining in the ADF fraction using the total combustion method. Tannin analyses were carried out on a 70% aqueous acetone extract. Total phenolics were determined colorimetrically by reacting the extract with the Folin-Ciocalteau reagent and measuring absorbance at 650 nm. Gallic acid was used as a standard. Ytterbium precipitable phenolics

were determined by precipitating phenolics in the extract with trivalent ytterbium in the presence of triethanolamine at 4 °C for 2 hours according to the method of Reed *et al.* (1985).

In vitro tannin bioassay

Pods were incubated, with or without PEG, using the Reading Pressure Technique (Mauricio *et al*., 1999). Approximately 1 g of sample was weighed into each serum bottle and incubated with a buffer and rumen fluid for 96 hours. PEG-4000 was dissolved in the buffer to give an inclusion rate of 200 mg PEG per gram sample. Two sets of buffer were prepared, one with PEG and the other without. Gas pressure was measured at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, 96 hours post-incubation. Gas pressure readings were then converted into gas volume using a predictive relationship between gas pressure and gas volume derived by Mauricio *et al.* (1999):

$\mathbf{G_p} = 0.18 + 3.697\ \mathbf{P_t} + 0.0824\ \mathbf{P_t^2}$,

where $\mathbf{G_p}$ = predicted gas volume (ml)

$\mathbf{P_t}$ = gas pressure reading at time t (psi)

Gas volume was corrected for substrate dry matter (DM) and gas produced from blanks.

In sacco N degradation

Based on the results of the *in vitro* tannin bioassay, it was concluded that the tannins, which were likely to cause problems if tree pods were to be fed on a large scale, were those from *D. cinerea* and *A. nilotica* tree species. However, these are the two most common tree species whose yields are consistent annually and, therefore, they were selected for further evaluation. Due to limited resources, each will be evaluated separately, making a between species comparison impossible.

Alkaline treatment was carried out by soaking 1 kg of *D. cinerea* pods overnight in 2 l of distilled water (this was sufficient to wet the entire sample, producing a thick paste, ensuring that no leaching of the sample occurred) in which 60 g of NaOH pellets had been dissolved to give 6% NaOH on a weight-to-weight basis. Treatment with PEG consisted of dissolving 200 g of PEG-4 600 (Aldrich Chemical Co. Inc, USA) in 2 l of distilled water and mixing the solution with 1 kg of *D. cinerea* pods to give a PEG application rate of 200 mg/g. The mixture was left to react overnight. The treated material was then spread onto polythene sheets and sun-dried for 24 hours.

To determine *In sacco* degradation of DM and N, about 5 g of each treated pod sample was weighed into a pre-weighed nylon bag (6 * 12 cm, pore size 40 µm). Duplicate bags were then incubated in the rumens of goats. The goats were fed on a grass hay diet supplemented with 200 g mixed pods per day, for 85 days before the trial started. All the bags were placed in the rumen at the same time (except zero-hour bags) and withdrawn sequentially at 4, 6, 12, 24, 36, 48, and 72 hours. They were then frozen until all bags had been withdrawn at the end of the period. Together with the 0 h bags, incubated bags were washed, in a semi-automatic washing machine with cold water, three times in cycles of ten minutes. Washed nylon bags were then dried in a forced-draught oven at 60 °C for 48 hours. The bags were then cooled in a desiccator and weighed. About 1 g of the residue was used to determine N disappearance.

Calculations and statistical analysis

The disappearance of DM and N was calculated using the following formulae:

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

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	=	N in original feed sample (%)
OSW	=	Original sample weight (g)
DM1	=	Dry matter of feed sample (%)
N2	=	N in residue sample (%)
RSW	=	Residual sample weight (g)
DM2	=	Dry matter of residue sample (%)

A non-linear model (Orskov and McDonald, 1979) was fitted to the degradation data. This gave estimates for the soluble and insoluble fractions as well as the rate of degradation. Effective degradability of N was calculated after assuming a 0.05 outflow rate. The effect of pod treatments was obtained by subjecting the data to a one-way analysis of variance using the general linear models (GLM) procedure. Separation of treatment means was done using the Bonferroni t-test.

N balance trial

Material and methods

Thirty castrated Matebele goats weighing on average 27.4 kg (s.d. = 2.5) were divided into five weight-balanced groups. Five diets were randomly allocated to the five groups of goats (Table 1). The goats were then penned individually in metabolism crates measuring 120 cm long, 54 cm wide, and 90 cm high and raised 90 cm above the floor. The crates were fitted with feeders and drinkers. 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% m/v (Cooper Zimbabwe, Pvt Ltd) using a 10 ml syringe.

Groups 1, 2 and 3 received supplements of either treated or untreated, ground (15 mm screen) D*. cinerea* pods. Group 4 received a goat meal supplement (National Foods Pvt Ltd,

Bulawayo, Zimbabwe) as a positive control, while group five received no supplement, as the negative control. Pod treatments were done as outlined above for the nylon bag trial. The adaptation period was 21 days, while the collection period lasted 7 days.

Feed, faeces and refusals were both analysed for OM, N, NDF, ADF, and ADIN to get an estimate of the intake and digestibility of these constituents while urine was analysed for N only. The difference between N intake and N output in faeces and urine was used as the measure of N retention.

Statistical analysis

Analysis of covariance was carried out to check if initial weight had a significant effect on any of the measured parameters. The effects of supplements on all variables were obtained by a one-way analysis of variance using PROC GLM of SAS. The following linear contrasts were analysed:

1. No supplement versus all supplements
2. Untreated pods versus treated pods
3. Alkaline treated pods versus PEG treated pods
4. Commercial goat meal versus untreated pods

Results and Discussion

Chemical analysis and *in vitro* bioassay

The chemical composition of tree pods are shown in Table 1, while Table 2 shows the fermentation responses of pods from different tree species to inclusion of a tannin binding agent, PEG. The results in Table 1 show that the six tree species differ significantly in chemical composition. *Dichrostachys cinerea* and *A. nilotica* have the least quantities of both NDF and ADF while in terms of N content these two species ranked third and fourth. *Acacia erubescens* had the highest N content followed by *A. erioloba*. More than half (53%) of *A. nilotica*'s N content is insoluble in acid detergent solution, suggesting low availability to microbial fermentation in the rumen. About 29% of total N in *D. cinerea* is bound to ADF. The level of ytterbium precipitable phenolics in *A. nilotica* (61%) should be a cause for concern as the tannins could reduce the productivity of goats if fed in large quantities over a long period. It is evident that there is a positive correlation between ytterbium precipitable phenolics and total phenolics assayed by the Folin-Ciocalteau reagent.

Table 1 Nitrogen, Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Acid detergent insoluble nitrogen (ADIN), Ytterbium precipitable phenolics (YbPh) (g/kg DM) and total phenolics (TPFOL) (µg gallic acid equivalent /mg DM) content of *Acacia* and other tree pods

Species	NDF	ADF	Nitrogen	ADIN	YbPh	TPFOL
Acacia erubescens	542.6	326.1	27.1	6.7	149.2	1.4
A. erioloba	415.1	248.2	21.3	3.8	359.3	5.6
A. nilotica	236.4	150.6	14.6	7.8	607.0	11.6
Piliostigma thonningii	493.4	284.3	13.5	4.2	370.2	6.4
Dichrostachys cinerea	441.3	230.6	19.9	5.7	370.2	6.6
S.E	7.63	3.11	1.13	0.62	10.41	0.32

Table 2 Cumulative gas production of PEG treated and untreated tree pods after 12, 24 and 36 hours of incubation

	Cumulative gas production (ml/g OM)								
Species	**12h**			**24h**			**36h**		
	No PEG	PEG	% [1]	No PEG	PEG	%	No PEG	PEG	%
Acacia sieberiana	44	81	*86*	73	142	*95*	101	178	*76*
A. erubescens	40	50	*25*	73	86	*19*	93	106	*14*
A. erioloba	52	76	*45*	86	121	*40*	114	147	*29*
A. nilotica	28	64	*130*	49	102	*110*	65	129	*98*
Piliostigma. thonningii	51	115	*126*	98	164	*68*	132	184	*40*
Dichrostachys cinerea	17.0	64	*276*	31	107	*242*	43	138	*224*

[1]% percentage increase in gas production due to PEG treatment

Results in Table 2 show an increase in gas production when PEG was included in the fermentation bottles for all the species. As expected, *A. erubescens*, whose tannin levels were the least, had the lowest response to PEG inclusion. However, *A. nilotica,* with the highest level of tannins had a lower response to PEG inclusion than *D. cinerea* (224% increase in gas production at 36 h compared to 98% for *A. nilotica)*. This seems to suggest that tannins from *D. cinerea* are more reactive as inhibitors of *in vitro* fermentation than tannins from *A. nilotica*. It might also mean that most tannins in *A. nilotica* are bound to protein (high NDIN content), thus inactivating them exposes protein to fermentation. Fermentation of protein causes less gas production than that of carbohydrates (Cone and van Gelder, 1999) hence the response to PEG inclusion was not as high as expected.

The use of PEG revealed that tannins reduced fermentation of tree pods either through direct toxicity on microbes or by making the fermentation substrate unavailable to the microbes. However, it is important to note that the inhibitory effect of tannins *in vitro* might be more severe than *in vivo*. Although PEG was effective in reversing the suppressive effects of tannin on *in vitro* fermentation, its use could lead to excessive protein degradation in the rumen resulting in low dietary rumen-escape protein. It is still not clear whether the ability of tannins to improve the supply of protein post-ruminally is a function of quantity or type of the tannin.

In sacco degradability

Table 3 shows degradabilities of DM and N while Figure 1 shows the N degradability curves of treated and untreated *D. cinerea* pods.

Table 3 *In sacco* disappearance of dry matter and N of treated and untreated *Dichrostachys cinerea* (Dci) pods incubated in the rumen of Matebele goats

Component	Parameter‡	Untreated	NaOH treated	PEG treated	SEM
Dry matter	a	26.4[a]	29.3[b]	42.8[c]	0.69
	b	48.3 [a]	36.4 [b]	32.8 [b]	2.21
	c	0.032 [a]	0.039 [a]	0.058 [b]	0.0049
	PD	74.7	65.7	75.6	-
	ED	44.5 [a]	43.5 [a]	59.7 [b]	1.22
Nitrogen	a	47.5 [a]	52.8 [b]	61.8 [c]	1.71
	b	43.1 [a]	29.0 [b]	27.9 [b]	1.71
	c	0.036 [a]	0.068 [b]	0.116 [c]	0.0151
	PD	90.6	81.8	89.7	-
	ED	64.7 [a]	68.8 [b]	81.0 [c]	0.53

In a row, means with the same superscript are not significantly different (*P*<0.05*)*

‡Units: For Dry matter a, b, PD and ED are measured as% of DM, for Nitrogen a, b, PD and ED are measured as% of N incubated. PD : Potential degradability (PD = a + b)

ED: Effective degradability ($ED = a + \frac{b * c}{k + c}$) (outflow rate of solids assumed to be 0.05)

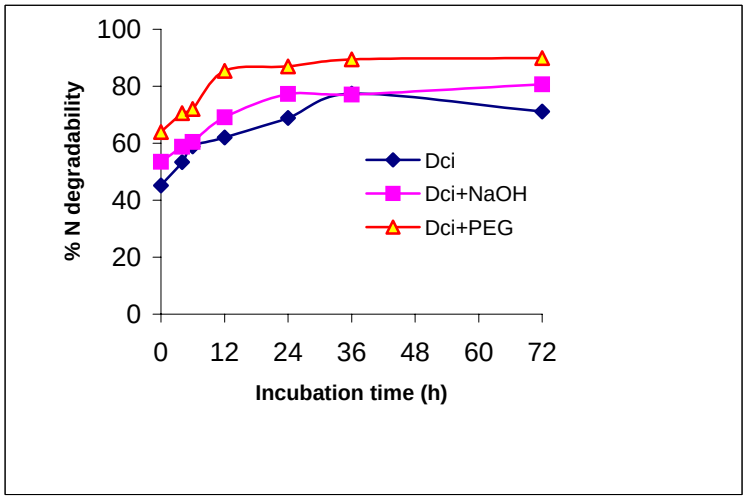

Figure 1. *In sacco* nitrogen disappearance for treated (NaOH and PEG) and untreated *Dichrostachys cinerea* (Dci) pods

Dry matter degradability

Treating *D. cinerea* pods increased the soluble (a) and decreased the slowly degradable (b) fractions, the effects of PEG being greater than NaOH. Alkaline treatment did not significantly increase the rate of degradation of the slowly degradable fraction and effective degradability. Pods treated with PEG had significantly higher rates of degradation and effective degradability.

Nitrogen degradability

PEG and alkaline treatments significantly increased the soluble fraction, rate of degradation and effective degradability of N. Between the treatments PEG treated pods had significantly higher values than alkaline treatments. All the treatments decreased the slowly degradable fraction to a similar extent.

These findings suggest that tannin inactivation using PEG improves the fermentation of protein in *D. cinerea* pods. Although alkaline treatment improves some degradation parameters, the small level of improvement makes practical application questionable.. However, it is possible that at 6%, the sodium hydroxide level used could have been too high to have resulted in the formation of artefacts, which could have resulted in lower degradability parameters. We are currently carrying out experiments in which the optimum level of sodium hydroxide for tannin inactivation is being sought.

N balance trial

Organic matter and N intake

Goats consumed all their supplements after the 21-day adaptation period. Supplementation significantly ($P < 0.05$) increased grass hay intake with goats on PEG treated pods and goat meal supplements consuming most hay. Total N intake varied between groups due to differences in N content of the supplements. Alkaline and PEG treated pods had less N compared to untreated pods, due to the dilution effect. Goats on untreated pods consumed the highest N intake (9.6 g/day) followed by goats on goat meal, while goats on alkaline and PEG treated pods consumed 7.8 and 7.7 g/day N respectively. Unsupplemented goats consumed 1.6 g N/d.

Table 4 Daily organic matter (OM) and N intakes and flows in goats fed grass hay with treated (NaOH, PEG) and untreated *Dichrostachys cinerea* pods

	Untreated	NaOH	PEG	Goat Meal	None
No. of animals	5	6	6	6	6
Average weight (kg)	26.9	27.6	27.6	27.7	27.0
	± 1.10	± 1.21	± 1.21	± 1.21	± 0.89
OM intake					
Supplement (g)	172.6	126.8	178.4	163.3	-
Grass Hay (g)	445.3[a]	466.4 [ab]	474.8[b]	474.3[b]	333.9[c]
Total OM intake (g)	617.9	593.2	653.2	637.7	333.9
N intake					
Supplement (g)	6.5	4.6	4.4	5.9	-
Grass Hay (g)	3.1	3.2	3.3	3.2	1.6
Total N intake (g)	9.6 [a]	7.8[b]	7.7[b]	9.1[c]	1.6[d]
Faecal output					
OM (g)	278.2 [a]	293.3 [a]	297.3 [a]	275.6 [a]	132.6 [b]
Nitrogen (g)	3.9 [a]	3.9 [a]	4.0 [a]	3.6 [a]	1.5 [b]
Urine					
Nitrogen (g)	0.7 [a]	0.7 [a]	1.5 [b]	0.4 [c]	1.9 [d]
OM digestibility	0.55[ac]	0.50 [b]	0.54 [a]	0.57 [c]	0.60 [d]
N retention (g)	5.0 [a]	3.2 [b]	2.3 [c]	5.1 [a]	-1.8 [d]

In a row, means with the same superscript are not significantly different (P<0.05)

N output

Faecal N output was similar for the supplemented groups but was lower (P<0*)* in the group that did not receive any supplements. Goats on the goat meal supplement had the least urinary N while the unsupplemented group had the highest amount of N in urine. Among the pod supplemented groups, goats on PEG treated pods had twice the level of urinary N (1.5 g/day).

Organic matter digestibility and N retention

Organic matter digestibility (OMD) was highest in the unsupplemented group while OMD for the groups on goat meal and untreated pods did not differ significantly. While N retention was negative for the unsupplemented group, goats on the commercial goat meal and untreated pods had the highest N retention values. Goats on treated pods retained less N compared to those on untreated pods. PEG treated, therefore, did not improve N retention. Among goats fed supplements, the group fed on PEG treated pods retained least N. This could have been due to excessive protein degradation in the rumen and subsequent N losses through urine. This could also be the reason why goats on PEG treated pods had higher urinary N values. Tannins inhibit protein fermentation in the rumen and N excretion is shifted from urine to faeces (Reed and Soller, 1987; Mishra and Rai, 1996). Indeed, Carulla (1994) reported that, at similar N intakes, PEG addition was linearly associated with reduced N (microbial, non-microbial and total) flow to the duodenum, reduced faecal excretion and reduced N retention. PEG treatment result in an increase in the rumen degradable protein (RDP) fraction as seen in the *In sacco* experiment. Waghorn *et al*. (1987) found that PEG treatment resulted in higher rumen ammonia, less nitrogen reaching the abomasum and ileum. Nunez-Hernandez *et al*. (1991) reported that sheep and goats fed PEG-treated forage at a rate of 2.3 g/g tannins had

higher rumen ammonia concentrations than those fed control forage. The animals also had lower faecal but higher urinary N excretion than those fed control forage. They however, could not demonstrate any differences in N retention. If a high concentration of rumen ammonia is not synchronised with a good supply of energy, blood urea recycling/turnover to the rumen is low. N losses are more likely to occur through urinary excretion. In this experiment, no effort was made to match N supply with energy because the objective was to simulate likely feeding conditions affordable to smallholder farmers. However, it is likely that the poor N retention in the group fed PEG treated pods was a result of increased N supply, which was not synchronised with energy supply in the rumen.

Conclusions

The use of PEG for *in vivo* tannin inactivation needs to be further evaluated especially with regards the rate of application. High rates of PEG are likely to reduce the quality of protein by allowing excessive protein fermentation. However, it is important to note that the beneficial effects of tannins may not be a function of quantity per se. In light of the findings in this experiment, results from *in vitro* tannin bioassays need careful interpretation. However, the fact that untreated D. cinerea pods matched a commercial feed, goat meal, in terms of N retention, OM digestibility and grass hay OM intake suggest that this tree species can be used by smallholder farmers in place of expensive commercial products. The only inputs required are milling of the pods and storage.

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