2. Chemical Composition and In Vitro Fermentation Characteristics of Acacia and Other Tree Pods (R7351)

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Abstract

Tree pods (fruits) are being evaluated as dry season protein supplements to complement low quality cereal crop residues and dry veld grass. Antinutritional compounds (e.g. tannins) can occur in large quantities in pods and are being investigated. Polyethylene glycol (PEG) has been used to determine the effects of tannins on *in vitro* fermentation characteristics. Increased gas production and digestible organic matter (OMD) values in response to the addition of PEG showed that tannins inhibited *in vitro* fermentation of fruits (pods) and separated fractions (seeds and hulls). Very low levels of tannins resulted in no response to the addition of PEG. However, response of fruit fractions with higher levels was not directly proportional to tannin quantity. This suggests that the chemical composition of the tannins is important in determining their reactivity *in vitro*. Thus colorimetric assays for phenolic compounds alone do not fully explain the variation in the gas production and degradation characteristics of tree fruits treated with PEG.

Feeding trials are being conducted to assess the nutritive value of pods as supplements for goats. If the presence of tannins causes antinutritional effects in the animal, cheap, locally occurring materials will be sought to inactivate the tannins.

Introduction

In the dry season low quality cereal crop residues and dry grass are usually the only feeds available to livestock in Zimbabwe. Dry, ripe *acacia* and other tree pods (fruits) are potential protein supplements for animals in the dry season. . Secondary plant compounds such as tannins and cyanogenic glycosides may limit the utilisation of tree fruits as protein supplements. Tannins have been associated with a reduced availability of nutrients while cyanogenic glycosides are known to be toxic. Use of *in vitro* gas production techniques has allowed a rapid initial assessment of the fermentation characteristics of feedstuffs and, therefore, their potential nutritive value. As polyethylene glycol (peg) is a highly specific tannin-binding agent, a change in *in vitro* gas production and degradation will indicate the presence of tannins. This study evaluates *in vitro* gas production and degradation characteristics of separated fruit components using peg to establish tannin effects. This information, together with a chemical characterisation of the tree pods, will assist in the interpretation of the responses obtained from feeding the pods as protein supplements..

Materials and Methods

Pods were obtained from *Acacia nilotica*, *A. erubiscens*, *A. erioloba*, *Dichrostachys cinerea*, and *Piliostigma thoningii*. With the exception of *D. cinerea* (pods were not separated), chemical analyses were carried out on the whole fruit and the separated components (seeds, hulls). The seed to pod ratios were determined by weight. The samples were initially ground to pass through a 2mm screen for the analysis of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN) (Goering and Van Soest, 1970), and for *in vitro* gas production and degradation studies. The samples were further ground to pass through a 1mm screen for the determination of nitrogen (N) (Dumas combustion method using the Carlo Erba Elemental Analyser 2100), total and water soluble carbohydrates, total phenolics (Folin-Ciocaltaeu reagent), proanthocyanidins or condensed tannins (CT) (Butanol-HCl), ytterbium precipitable phenolics and neutral detergent fibre-bound proanthocyanidins (NDF-CT) (Butanol-HCl reaction of NDF).

Thin Layer Chromatography (TLC) of the extracts was used to characterise the phenolic compounds further. Phenolic and tannin compounds were extracted from finely ground samples (1mm) using 70% aqueous acetone. The extract was then spotted on 5*5 cm cellulose plates and the chromatogram was developed using two solvents. An assay for catechin, epicatechin and condensed tannins with Vanillin-HCl spray showed that all the seed fractions contain high molecular weight condensed tannins, which did not move from the origin. Other sprays used in this study were the ferric ion reagent (detects phenolic compounds), potassium iodate reagent (detects gallic acid and its esters) and sodium nitrite reagent (detects ellagic acid and its esters).

In vitro gas production and degradation of the pods was estimated using the Reading Pressure Technique (RPT) (Mauricio *et al*., 1999). The separated fruit components were incubated with and without PEG-4000, as described by Makkar *et al* (1995) except that the PEG was dissolved in the buffer solution to give a concentration of 1g PEG per gram dry matter (DM) of sample. Pressure readings were taken at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 95 hours post-incubation. Samples with and without PEG were incubated in triplicate for each of the withdrawal periods (24, 48 and 95 hours) to determine organic matter degradation (OMD). After incubation samples were filtered through pre-weighed glass-sintered crucibles. The residue was oven-dried (100°C) for 24 hours and weighed before being ashed at 500°C overnight to determine OMD.

Results and Discussion

Chemical composition

Table 1 shows preliminary data of the chemical composition of fruits and their components. The seed to pod ratios (by weight) were 46:54, 26:74, 44:56 and 30:70 for A. *erioloba*, A. *nilotica*, A. *erubiscens* and P. *thoningii*, respectively. The seed fraction has the highest nitrogen content in all tree species, ranging from 25.3 (A. *nilotica*) to 58.5 g/kg DM (A. *erubiscens*) (Table 1). The seed fraction contains the lowest amount of ADIN compared to other pod fractions. The proportion of fibre bound nitrogen (ADIN) is highest in the hull fraction with 64.5% of total nitrogen in A. *nilotica* hulls being ADIN. The ADL fraction is also lowest in the seed fractions of all the tree species. However, accurate measurement of ADL is dependent on complete oxidation of the fibre components, a factor that will be checked in later experiments. Neutral detergent fibre and ADF levels were low in the seed fractions with the exception of A. *nilotica* seeds, whose NDF and ADF content is higher ($P<0.05$) than that of the hull fraction. This is consistent with the findings of Tanner and co-workers (1990) for A. *nilotica* fruits.

The seed contains most of the protein of tree fruits. For tree species with small sized seeds, the proportion of seeds passing through the alimentary tract undigested is high (Tanner *et al*., 1990), thereby reducing nitrogen availability to the animal. It is, therefore, important to quantify the proportion, by weight, of the seeds to the whole fruit as well as estimating the proportion of undigested seeds passing through the alimentary tract for each tree species. This information will assist in the identification of tree pods that may need to be ground before being fed to goats.

The highest level of total phenolics (TP), expressed as gallic acid equivalents (GE), was found in the pod and hull fractions of A. *nilotica* (11.63 and 10.68g GE/mg DM respectively). Generally, pods and hulls of all species had the higher levels of total phenolics compared to the seed, with the exception of A. *erubiscens*. This could be because the hulls protect the seed from microbial attack and herbivory. The high levels of phenolic compounds might also be the cause of the high levels of ADIN values found in hulls. It is possible that tannin-protein complexes form a significant part of ADF in the hull fraction and thus contribute to the relatively high nitrogen in the ADF fraction. P*iliostigma thonigii* gave the highest absorbency readings from CT and NDF-CT. With the exception of A. *erubiscens* seed, all other seed fractions had the lowest CT levels. However, the seed fractions, except that of P. *thonigii*, had the highest level of NDF-CT indicating that a higher proportion of the CT in the seed fraction could be fibre bound or is present in the seed coat.

Results of TLC revealed catechin and epicatechin in the fruit and hull fractions of A. *erioloba* and A. *nilotica*. A*cacia erubiscens* hulls gave negative reactions with all sprays suggesting either no, or very low, polyphenolic content (Table 2). A*cacia erubiscens* fruit and A. *erioloba* seeds gave a negative reaction with the potassium iodate spray showing that these two fractions do not contain gallic acid. The thin layer chromatograms of P. *thonigii* showed a positive reaction only at the origin with Vanillin-HCl and Ferric ion sprays. This indicates the presence of high molecular weight tannins in this species.

In vitro gas production bioassay

Treatment with PEG increased ($P < 0.05$) the amount of gas produced by most fruit components (Table 3). Addition of PEG, however, did not affect ($P > 0.05$) gas production from A. *erubiscens* fruit and hull fractions, which also had the lowest levels of phenolics (Table 1). A significant ($P < 0.05$) species by fraction interaction showed that the extent to which PEG treatment improved gas production in the fruit fractions largely depends on the tree species.

The effects of PEG on OMD are shown in Table 3. A*cacia erubiscens* and D. *cinerea* fruits, seeds and hulls had low degradabilities, possibly due to high NDF, ADF and ADL levels. Slightly larger differences in OMD of PEG treated and untreated samples were observed with 24h degradation values for most species. At 95h, OMD values did not differ greatly between the treated and untreated samples. However, the degradation values of PEG treated samples are probably an underestimate. This is because PEG-tannin complexes are insoluble in boiling water, most organic solvents, neutral and acid detergent solutions (Makkar *et al.*, 1995), and may have been retained in the crucible as undegraded matter. This may have resulted in artificially low degradability values from samples to which PEG was added.

Future Work

The effect of tannins on the *in vitro* fermentation characteristics of tree pods was established through the use of PEG. Generally, inactivation of tannins resulted in increased gas production and OM degradation. However, inactivation of tannins with PEG cannot be used by smallholder farmers for improving the nutritive value of tree fruits due to its high cost. The next step, therefore, is to find alternative and cheaper ways of inactivating the tannins, hence the experiments outlined below.

- Tree pods will be soaked in water or sodium hydroxide solution in an attempt to inactivate the phenolic compounds, thus making the protein contained in the pod more readily available to the animal. Assuming a response the approach will be developed to use sodic soil and wood ash solutions
- A determination of *in vitro* improvement in the nutritive value of pods by comparing the effects of PEG, alkaline solution (determined above) and water treatments on *in vitro* gas production and degradation characteristics of ground pods
- I*n vivo* estimation of the nutritive value of treated and untreated pods, as protein supplements for goats.

Table 1: Total carbohydrates (TCHOS), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), Nitrogen (N) and Acid Detergent Insoluble Nitrogen (ADIN) content (g/kg DM), total phenolics (gallic acid equivalent mg/mg DM), total condensed tannins (CT) (AU_{550nm}/10mg DM) and fibre-bound tannins (NDF-CT) (AU_{550nm}/10mg NDF) content of separated fruit components

Species	Fraction	TCHOS	N	ADIN	NDF	ADF	ADL	TP	CT	NDF-CT
Dichrostachys cinerea	Fruit	3.47	19.85	5.67	441.28	230.63	65.80	6.56	1.68	0.25
Acacia erioloba	Fruit	13.46	21.25	3.85	415.07	248.19	38.20	5.63	0.37	0.06
	Hulls	15.76	9.40	2.90	499.22	315.92	53.05	6.09	0.36	0.05
	seeds	6.21	35.80	7.65	262.59	157.82	34.61	1.70	0.21	0.11
A. *erubiscens*	fruit	2.49	27.10	6.68	542.63	326.12	101.84	1.35	0.24	0.10
	hulls	1.93	14.05	5.28	659.06	394.07	115.00	0.60	0.14	0.0
	seeds	3.32	58.50	10.71	246.41	132.09	33.96	1.58	0.56	0.14
A. *nilotica*	fruit	22.82	14.65	7.81	236.42	150.58	41.50	11.64	0.60	0.07
	hulls	27.86	11.90	7.68	227.29	149.47	45.22	10.68	0.65	0.05
	seeds	4.25	25.30	6.57	356.16	193.05	44.09	2.33	0.39	0.05
Piliostrigma. Thoningii	fruit	9.70	13.45	4.18	493.43	284.33	81.21	6.43	2.05	1.29
	hulls	11.30	10.10	6.22	501.59	296.86	87.84	5.06	1.85	1.39
	seeds	4.35	31.70	9.50	377.70	129.57	44.72	5.20	1.61	0.38
S.E.M		**0.32**	**1.05**	**0.60**	**7.59**	**3.12**	**2.94**	**0.33**	**0.02**	**0.03**

Table 2: Detection of phenolic compounds in separated fruit fractions using different spray reagents on thin layer chromatograms

Species	Fraction	Spray reagents			
		Vanillin-HCl **(Condensed tannins)**	Ferric ion **(Phenolics)**	Potassium iodate **(Gallotannins)**	Sodium Nitrite **(Ellagitannins)**
Dichrostachys cinerea	fruit	+[1]	+	+	+
Acacia erioloba					
	fruit hulls	+	+	+	+
	seeds	+	+	+	+
A. *erubiscens*				+	
	fruit hulls	+	+	-[2]	+
	seeds	–	+	–	+ –
A. *nilotica*					
	fruit shulls	+	+	+	+
	seeds	+	+	+	+
Piliostigma thoningii					
	fruit hulls	+	+	+	+
	seeds	+	+	+	+

[1,2]*+ indicates positive reaction to spray reagent, - indicates negative reaction*

Table 3: Cumulative gas production (ml) (95-*h* Gas Volume), percent increase in cumulative gas volume (% increase) and organic matter degradability (OMD, *g/g*) of PEG treated and untreated separated fruit fractions at 24, 48 and 95 hours post-incubation

Species	Fraction	95-h Gas Volume			24-h OMD		48-h OMD		95-h OMD	
		Without PEG	**With PEG**	**% increase**	**Without PEG**	**With PEG**	**Without PEG**	**With PEG**	**Without PEG**	**With PEG**
Dichrostachys cinerea	Fruit	47.98	155.77	224.66	0.34	0.39	0.37	0.39	0.39	0.48
Acacia erioloba	Fruit	130.42	163.83	25.62	0.42	0.52	0.54	0.59	0.55	0.65
	Hulls	110.32	136.56	23.79	0.36	0.46	0.49	0.48	0.51	0.51
	Seeds	111.37	166.30	49.32	0.47	0.51	0.59	0.70	0.70	0.83
A. *erubiscens*	Fruit	101.84	115.19	13.11	0.42	0.44	0.49	0.50	0.49	0.49
	Hulls	79.45	79.31	0	0.30	0.28	0.33	0.31	0.35	0.33
	Seeds	129.72	174.03	34.16	0.70	0.79	0.84	0.82	0.86	0.83
A. *nilotica*	Fruit	78.79	149.47	89.71	0.60	0.68	0.62	0.76	0.69	0.79
	Hulls	53.56	139.16	159.82	0.66	0.68	0.67	0.74	0.70	0.77
	Seeds	118.88	154.67	30.11	0.43	0.56	0.62	0.74	0.74	0.84
Piliostigma thoningii	Fruit	143.28	192.59	34.42	0.44	0.47	0.56	0.50	0.56	0.51
	Hulls	157.54	202.07	28.27	0.45	0.46	0.57	0.49	0.58	0.52
	Seeds	106.17	201.18	89.49	0.30	0.62	0.42	0.68	0.52	0.71
S.E.M		**4.68**						**0.01**	**0.01**	**0.01**

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Questions and Answers

What were the reasons for the high kid mortality?

During the dry season in the communal areas the animals get very weak. A supplement is now added post-partum.

Discussion took place about the role of supplements in addition to acacia. It was pointed out that in the unsupplemented trial reported here goats appeared to be doing as well as the control ones.

What are the benefits for the farmers?

The farmers already know that pods are good for the goats and that A. *tortilis* is the best. If farmers want to use other pods it involves a lot of work. It might be better for them to pick lots of tortilis and sell it.

Some farmers were already aware of the benefits of pods. However, pod production appears more variable than originally thought. To rely on one species, especially A. *tortilis*, is not advisable.

One of the objectives of R7351 is to reduce kid mortality: what actions was the project taking to address the reduction? Was poor nutrition the main cause of mortality?

By supplementing does before and after birth so that the quality and quantity of their milk was improved, kid mortality would decrease. There might be a link between climatic stress and nutritional status.

Was housing for goats had been looked at during the data collection, and if not could it now be included?

The kraals are open but when goats kid they are housed inside when the weather is hot. Earlier work at Matopos suggests that housing should be part of a management package.

Is access to milk or the birth rate the constraining factor with kid mortality, e.g. if a doe produced triplets would two be sacrificed in order to feed the third? Is survival related to birth-rate?

It is a combination of birth rate and nutrition. When a doe gives birth to triplets most die anyway, those weighing less than 2kg being most likely to die.

Is the common practice for farmers to sacrifice kids?

At Matopos farmers were asked to pay more attention to triplet births to give the kids more chance of surviving.

One of the important issues raised at the previous workshop was the yield of pods per hectare in the communal areas. The browse project (R6984) is collaborating with R7351 to estimate pod yield; could the project team provide any data?

It was difficult to obtain this data from communal areas and the estimates needed further work.

Professor Illius suggested a survey[1] that would have found measurements of pod yield and tree density in a non-invasive way. He stated that it was important to collect this data. Mr Sikosana said that this had been a very time-consuming exercise and some measurements had already been taken. Indeed, some data had been collected by enthusiastic farmers involved in the project.

It was suggested that as the trees would be of different ages, tree biomass should also be measured. Fresh leaves would be of better quality than older leaves.

Clarification of what had been measured in R7351 was requested.

For the purpose of the project it was only the pods that had been looked at. The Project Leader said that as far as he was aware, the major published work on tree pod yield was from the 1950s (O. West).

The older leaves would have different nutrient qualities to younger pods. The pods are a distinct group because you collect enough from the trees. The possible collection method is to fence off the tree and collect them.

In an average year in the project's area, there is always enough food for goats to eat as they are able to eat a wider variety of substances than cattle. When people talk about 'insufficient nutrients' for goats maybe this is due to goats having insufficient time to browse and, therefore, the management of the goats also needs to be studied.

This would not totally apply to the communal areas as often there is no browse available in the dry season.

Had the project team discovered any ethnoveterinary uses of pods during their PRA exercises?

None of the farmers in the survey had mentioned any ethnoveterinary practices. Sometimes people will indicate that a tree is valuable but do not give a reason for it; you are only likely to know this information if you have lived in the area for a long time. Pods are said to be good for treating ringworm but the project team was unaware of any documentation on this. Farmers use the leaves to treat internal parasites.

Was the information gathered during the PRA exercises documented as ethnoveterinary medicine is now a big issue for researchers/development agencies etc?

As the older generation is dying and younger people move away from farming this knowledge is becoming less well known. The Forestry Commission was suggested as the best source of information on ethnoveterinary issues.

Alternative uses of pods: if all pods are harvested for goats, will other animals suffer? (this concern was also expressed last year)

No, total pod yield is many times in excess of that needed to supplement the current goat herd. However, some communities may need to restrict outsiders collecting pods from their areas.

A representative of the farmers asked if it was possible to get breding stock from Matopos.

This is not possible because most of the animals are government property, but when the station needs to sell them this is done at auction. However the station is now getting interested in exchanging male stock and plans to raise males and exchange them with the farmers. The station has two breds of goats; Mashone (small) and Matabele, but there could be different strains within the two. The FAO have a programme to characterise the goats.

[1] Professor Illius agreed to give details of the research methods to Mr Sikosana