Assessment of the feeding value of five shrub legumes of local interest

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Abstract

The intake and digestibility of tree and shrub foliage by ruminants are limited due to the presence of undesirable secondary plant metabolites. In particular, tannins have been implicated in the failure of ruminants to consume some forage legumes. Therefore, the aim of the present experiment was to determine the extractable condensed tannin (CT) contents of five leguminous shrubs and to examine the relationship of CT with in vivo dry matter digestibility (IVDMD). The presence of CT, which ranged from 6.92 (A. auriculiformis) to 0.78% (G. sepium), affected the nutritive value of these plants to different degrees. The shrubs used in this study can be classified according to their CT contents (determined by butanol–HCl method) as follows. CT free, traces (<1%), low (1-3%), medium (4-6%) and high (>6%) CT levels found in Erythrina indica, Gliricidia sepium, Leucaena leucocephala, Calliandra calothyrsus and Acacia auriculiformis respectively. In contrast, Erythrina indica was free of condensed tannins. In vivo DMD ranged from 49% in C. calothyrsus to 59.6% in E. indica. Inverse relationships between concentrations of condensed tannins and IVDMD (r=-0.925) and crude protein (r=0.7359) indicated negative effects of CT. However, CT correlated positively with NDF (r=0.49) and ADF (r=0.75) fractions. Therefore, it could be assumed that CT bound with fibre fractions reduces digestibility and CP. CT contents of tender leaves and stems (0-30 cm from tip) and mature leaves and stems (30-60 cm from tip) in A. auriculiformis and C. calothyrsus were 7.39%, 6.05% respectively and 5.46%, 4.38% respectively, which may be due to the chemical defense mechanism found in the younger plant parts of browse leguminous plants.

Compared with other species, E. indica with a low ADF, lowest ADL and CT and having the highest IVDMD with a high CP content was quantitatively the best. However, feeding strategies should be developed to improve the synergistic effects of feeding C. calothyrsus and Acacia auriculiformis. It is advisable to mix tender and mature leaves as much as possible when used under cut and fed systems for ruminants.

Keywords: Condensed tannins, feeding value, shrub legumes, ruminants, plant metabolites

Introduction

A major cause of low productivity of livestock in tropical regions is the inadequate amount and poor quality of feeds. Forage from trees and shrubs are important sources of feed for ruminants in these areas, particularly during the dry periods, where the quality and quantity of feeds are limited. However, the use of tree foliage is limited due to the presence of significant amount of secondary plant compounds such as tannins. Tannins are a special type of phenolic compounds of plant origin and there are two chemically distinct types called hydrolysable and condensed tannins.

The effects of tannins on ruminant animals range from beneficial to toxic including death. The toxic effect of condensed tannins are less well understood but generally binding to plant proteins and cell wall carbohydrates, decrease the digestibility of usually protein and sometimes fibre (Van Soest *et al*, 1986). With a better understanding of tannin properties, mechanism of tannin action and proper management of the forage browses could become an invaluable source of protein for strategic supplementation.

Therefore the objectives of the present study were to determine the extractable condensed tannin (CT) contents of five leguminous shrubs and to examine the relationship of CT with *in vivo* dry matter digestibility.

Materials and Methods

Collection and drying of samples

The investigation consisted of two parts. Initially the following locally available five leguminous shrubs near Mapalana were selected (Table 1).

Table 1. Common, and botanical name of 5 leguminous forage species tested

Common name	Scientific name
Acacia	*Acacia auriculiformis*
Ipil ipil	*Leucaena leucocephala*
Gliricidia	*Gliricidia sepium*
Calliandra	*Calliandra calothyrsus*
Erabadu	*Erythrina indica*

Edible parts (tip to 60cm-tender stems and leaves) weighing approximately 500g of fresh matter from each spp. of five the different forages were collected. Subsequently the following two species that were found to be high in tannin content were selected for further investigation.

- *Acacia auriculiformis*
- *Calliandra calothyrsus*

Edible parts of the two species were divided into tender stems and leaves (tip to 20cm) and mature stems and leaves (20-60cm). Samples were carried in a cool box to the laboratory and oven dried at 50°C for three days for determination of dry matter content (IAEA TECDOC).

Grinding and storage of samples

Approximately 300g each of dried samples were ground and passed through a 2mm screen. All the ground material including those parts inside the mill were taken, mixed well and approximately 200g of each sample were ground to pass through 0.5mm screen. Then they were stored in dark bottles, labeled and kept in a refrigerator.

Chemical analysis

Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) of edible parts of five spp. were determined following the method of Van Soest *et al.* (1991). Nitrogen was determined by Kjeldhal procedure. Crude protein (CP) content was calculated according to the formula N X 6.25. *In vivo* dry matter digestibility (nylon bag technique) values obtained from a previous study done by Seresinhe *et al.* were adopted. Harvesting, drying and grinding of samples were done similarly.

Analysis of condensed tannins

Extraction of tannins

Ground plant material (200mg) was taken in to a 25ml beaker, mixed with 25ml 70% aqueous acetone, suspended in an ultrasonic water bath and subjected to ultrasonic treatment for 20 minutes at room temperature and centrifuged for 10 minutes at 3000g at 4°C. Then the supernatant was collected and kept on ice. The pellet left in the centrifuge tube was transferred to the beaker using 10ml 70% aqueous acetone, then subjected to ultrasonic treatment for 20 min. and again centrifuged and supernatant was collected as above.

Preparation of reagents

(1) Butanol-HCl reagent (butanol-HCl 95:5 v/v): n-butanol (950ml was mixed with 50ml of concentrated HCl (37%).
(2) Ferric reagent (2% ferric ammonium sulfate in 2N HCl): Concentrated HCl (16.6ml) was made up to 100ml with distilled water and 2g of ferric ammonium sulfate were dissolved in that volume.

Determination of condensed tannins

Tannin containing extract (0.5ml) was taken into a test tube, butanol-HCl (0.1ml), ferric reagent (0.1ml) were added and the tube was vortexed. Then the tube was kept in a boiling water bath for 60 min., cooled to room temperature and the absorbance was recorded at 550nm. The absorbance of a blank (unheated mixture) was also recorded.

Condensed tannins (% in dry matter) as leucocyanidine were calculated using the following formula (Porter *et al.*, 1986). (A 550nm x 78.26 x Dilution factor) / (% dry matter).

Treatment effects were analyzed using SAS procedure ANOVA (SAS/STAT, 1999) and differences among mean values were investigated by the Duncan multiple range test. The level of significance was fixed at $P<0.05$.

Results

Composition of the five leguminous shrub species used in this experiment is presented in Table 2.

Table 2. Composition of five leguminous shrub species

Species	DM	CP	NDF	ADF	ADL
Acacia auriculiformis	29.8	17.8	49.00	40.75	8.10
	(1.98)	(1.4)	(0.53)	(0.95)	(0.42)
Leucaena leucocephala	29.4	26.8	33.53	23.64	8.45
	(0.85)	(1.56)	(0.78)	(0.86)	(0.43)
Gliricidia sepium	24.2	20.0	35.10	35.90	5.10
	(1.98)	(1.33)	(1.2)	(0.88)	(0.67)
Calliandra calothyrsus	39.5	19.7	37.30	40.70	4.50
	(2.12)	(1.45)	(0.92)	0.97)	(0.69)
Erythrina indica	15.7	26.3	44.54	26.51	0.00
	(0.99)	(1.33)	(0.76)	(0.82)	(0.55)

Data are mean values of three replicates (fifteen samples)
Values in parentheses are standard deviations

The dry matter content of five leguminous species ranged from 15.7 to 39.5%. Highest (P<0.05) DM content was observed in *C. calothyrsus* followed by *A. auriculiformis* (29.8%), while the lowest (P<0.05) DM content was found in *E. indica*. All the species had desirable CP levels (17.8-26.8%). The NDF contents ranged from 49-33.53%. *A. auriculoformis* had highest NDF and ADF fractions (49% and 40.75%) while lowest ADF content was observed with *L. leucocephala* (23.64%). ADL contents did not differ among *A. auriculiformis* and *L. leucocephala* and *C. calothyrsus* and *G. sepium* considerably. *E indica* was free of ADL.

The contents (% in dry matter) of condensed tannins and IVDMD of five leguminous shrubs are presented in Table 3.

Table 3. Contents (% in dry matter) of condensed tannins and IVDMD of five leguminous shrubs

Species	CT	IVDMD
Acacia auriculiformis	6.92 (002)	50.0 (0.33)
Leucaena leucocephala	1.58 (0.06)	57.0 (0.56)
Gliricidia sepium	0.78 (0.21)	58.0 (0.23)
Calliandra calothyrsus	4.60 (0.03)	49.0 (0.46)
Erythrina indica	0.00 (0.00)	59.6 (0.42)

Data are mean values of three replicates (fifteen samples)
Values in parentheses are standard deviations

A. auriculiformis had the highest CT content (6.92%) followed by *C. calothyrsus* (4.6%) and the lowest CT content was found in *G sepium*. *E. indica* was free of CT while having the highest IVDMD (59.6%). The lowest IVDMD was observed with *C. calothyrsus* (49%).

Percentages of dry matter (DM) and condensed tannin (CT) contents of the five shrub legumes at two maturity stages are presented in Table 4.

Table 4. Percentages of dry matter (DM) and condensed tannin (CT) contents of the five shrub legumes at two maturity stage

Species	Maturity	DM	CT
A. auriculiformis	Tender leaves and stems (0-30 cm from the tip)	29 (0.40)	7.39 (0.56)
	Mature leaves and stems (30-60 cm from the tip)	37 (0.32)	5.46 (0.38)
C calothyrsus	Tender leaves and stems (0-30 cm from the tip)	30 (0.05)	6.05 (0.67)
	Mature leaves and stems (30-60 cm from the tip)	37 (1.41)	4.38 (0.41)

Data are mean values of three replicates (twelve samples)
Values in parentheses are standard deviations

Higher DM contents were observed in mature leaves and stems with lower DM in tender leaves and stems of both species. Tender parts had higher CT contents than mature parts of both species.

Correlation coefficients (r) between condensed tannin of five leguminous shrub legumes with CP, fibre and IVDMD contents are presented in Table 5.

Table 5. Correlation coefficients (r) between CT of five leguminous shrub legumes with CP, fibre and IVDMD

	IVDMD	CP	NDF	ADF	ADL
CT	-0.9253	-0.7359	0.4865	0.7497	0.1943
	0.032	0.763	0.005	0.154	0.78

Pooled data for 5 browses (N=15)

Upper values are co-relation co-efficeints

Lower values are significant levels

IVDMD (In vitro dry matter digestibility), CP (Crude protein), NDF (Neutral detergent fibre), ADF (Acid detergent fibre), ADL (Acid detergent lignin)

CT was negatively correlated with IVDMD and CP and positively correlated with NDF, ADF and ADL.

Discussion

High DM contents of all shrub legumes tested in this experiment, in association with moderate to high CP contents (Table 1) suggest their potential use, especially as dry season feed supplements for ruminants.

The CT contents of *G. sepium* and *C. calothyrsus* observed in this study were comparable with the CT values reported by previous workers (Balogan *et al.* 1998; Seresinhe and Iben 2003). CT content found for *A. auriculiformis* in this study was slightly higher than that of the values obtained for *A. currassavica* (Balogun *et al.* 1998) but comparable with the findings of Hagerman and Butler (1978) for *A. auriculiformis* (Pepsin precipitation method).

In this study, it was found that *E. indica* was free of CT and ADL and having the highest IVDMD value together with the second highest CP content which confirms its better quality as a livestock feed. Results of previous work of Seresinhe *et al.* (1998), confirmed that *E. indica* also had higher IVDMD than *L. leucocephala* and *G. sepium*. In fact, it is the only species used for limited human consumption. It was reported that the dry matter yield of *E. Indica* was lower than the other tested species due to frequent leaf shedding (Seresinhe *et al.* 1998). Although the quality parameters were superior, low biomass yield could be a limitation with *E. indica*. *G. sepium* and *L. leucocephala* were low in CT levels (<2%). Tannins at >2% in the diet can may be beneficial as they protect the leaf protein against degradation in the rumen but the protein remains digestible (Barry 1983).
The forages used in this study can be classified on the basis of their contents of CT according to the findings of Balogun *et al.* (1998). *G. sepium* contained traces of CT (<1%); *L. leucocephala* had a low level of CT (1-3%); *C. calothyrsus* had medium level (4-6%) and *A. auriculiformis* had a high level of CT (>6%).

According to the results, it is also evident that, tender leaves and stems of the two species tested contained high levels of CT as compared with mature parts. According to the investigations of Teague (1989), higher tannin content was observed in young *Acacia karoo* leaves, according to the chemical defense mechanism found in young growing parts of browse leguminous plants (Teague, 1989).

Conclusions

Common shrub legume species exhibited a wide range in the content of CT. Higher CT contents in tender leaves and stems in shrub legumes seemed to offer a defense mechanism against browsing. Increasing levels of CT in association with higher levels of cell wall contents tend to reduce IVDMD. However, a negative correlation in between CT with CP may have positive effects on the utilization of CP by ruminants. Studies on the intake and ruminant performance will help to substitute the conclusions drawn in this study.

References

Balogun, R. O., Jones, R. J.and Holmes, J. H. G., (1998). Anim. Feed Sci. Technol. 76,77.

Barry T. M.; 1983: Br. J. Nutr., 54, 221.

Hagerman, A. E. and Butler, L. G., (1978). Protein precipitation methods and standards for assaying tannin: J. of Chem. Ecol., 15: 1795-1810.

Porter L. J., Hirstich C. N. and Chan B. G., (1986). Phytochemistry, 25, 223.

SAS/ STAT, (1999). User's Guide, SAS Institute, Cary, NC, USA.

Seresinhe, R.T. and Iben, C., (2003). In vitro assessment of two tropical shrub legumes in relation to their extractable tannin contents: J. Ani. Physiol. A. Anim. Nutr. 87, 109-115.

Teague, W. R., (1989). Patterns of selection of Acacia karroo by goats and changes in tannin levels and in vitro digestibility following defoliation: J. Grassl. Sos, South Afr., 6(4), 230-235.

Technical document of the first research coordination meeting of the FAO/ IAEA coordinated research project on use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage, (1999). IAEA, Vienna.

Van soest, P. J., Conklin, N. L. and Horvath, P. J., (1986). Tannins in foods and feeds: Proceedings of the Cornell Nutrition Conference for Feed Manufactures, 115-122.

Van Soest, P. J., Robertson, J. B. and Lewis, B. A., (1991). J. Dairy Sci., 74, 3583.