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A survey of nutritional and antinutritional properties of major ruminant feed resources in semi-arid West Africa

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Abstract

Ruminant nutrition in The Gambia, West Africa, relies largely on agricultural by-products such as cereal straws, groundnut hay, or horticultural residues. Feed evaluation of these materials has so far been based mostly on crude nutrient composition. Reports on systematic *in vivo* or *in vitro* trials are scarce, and information on antinutritive components, especially on the tannin content of feed resources is not available at all. Therefore we present a comparative analysis of 18 samples identified as important feed resources for ruminants in The Gambia with a focus on *in vitro* gas production (Hohenheim Gas Test), and tannin content. A differentiated and detailed analysis of tannins was made to distinguish between total phenols determined by Folin- or Ferric Chloride assay, total tannins determined by BSA precipitation, and condensed tannins determined by Butanol-HCl assay. The results illustrate that feed evaluation using one or just a few analytical parameters can be misleading. They underline the usefulness of *in vitro* systems for feed evaluation, and they fill a gap in the description of the regional feed resources. As part of a cooperative research project between the University of Hohenheim, Germany, and the International Trypanotolerance Centre, The Gambia, the data will be used to formulate a tannin free basic diet for cattle adapted to tropical conditions. Supplementation of the basic diet with tannins from various, clearly defined sources will then allow us to study the effects of these compounds on ruminal fermentation.

Introduction

The first option to increase productivity of livestock should be an improvement of the nutritional status of the animals, based on locally available feed resources. Feed evaluation thus becomes a key issue in formulating balanced diets. The International Trypanotolerance Centre, Banjul, The Gambia, has recently committed itself to research on integration of horticultural residues in feeding regimes (Fall et al. 1999; Fall et. al. 1998; Nouala Fonkou 2000). The results are promising, but the availability of these materials is restricted to the Greater Banjul Area. A more widely applicable alternative to upgrade animal diets is the use of shrub and tree leaves, some of which are well

known as dry season feeds. In this study, samples were selected to represent major components of a basic diet as well as potential supplements.
The nutritional value of the samples was estimated based on crude nutrient composition and gas production during *in vitro* fermentation. Tannin contents of the materials were analyzed by common chemical assays, and their biological acitvity assayed by precipitation of protein *in vitro*, and by increase in gas production due to inactivation of the tannins by polyethylene glycol, PEG. This paper presents a description of the selected feeds with respect to both, nutritional and antinutritional properties. Implications for their use in livestock feeding are discussed.

Materials and methods

Plant materials
The 18 samples analysed were: leaves and stems of groundnut hay, the economically most important feed resource in the Gambia; Yafal, a concentrate produced in Senegal, which is based on groundnut cake combined with groundnut husks; six samples of crop residues (leaves and stems of white sorghum, *Sorghum bicolor* stover, leaves and stems of late millet, and early millet stems); young and mature plants of *Andropogon spec*., a grass which is widely available on uncultivated land; four horticultural residues available in the greater Banjul area (maize, two varieties of peas, and green beans); leaves of *Moringa oleifera*, *Pterocarpus eranaceus* (Banneh), and *Guira senegalensis*. The samples were collected in Nov/Dec. 2000, shade dried and stored at room temperature until analysis.

Crude nutrient analysis
Crude nutrients were analysed according to A.O.A.C (1990) and fibre fractions according to Goehring and Van Soest (1970).

In vitro incubation
The Hohenheim Gas Test (HFT) was set up as described by Menke et. al. (1979). Briefly, 200 mg of substrate, ground to 1 mm, were combined with diluted rumen fluid (10 ml inoculum, 20 ml buffer), with and without the addition of 200 mg PEG. Incubations were run in duplicate. Gas production was read from the syringes at regular time intervals up to 96 h. The difference in gas volume induced by PEG (Makkar et. al. 1995) and true digestibility (Blummel and Becker 1997) were determined at 24 h.

Tannin analysis
The plant material was ground to a fine powder and extracted with 80% aqueous methanol. The final ratio after two cycles of sonication and centrifugation was 10 mg dry matter/ml solvent. The extracts were kept at –20°C for ca. 1 week until all the assays were completed.
Total phenols were determided both by the Folin assay (Makkar et. al. 1993) and by the Ferric Chloride assay (Hagerman and Butler 1978) with tannic acid as calibration standard. Total tannins were determined after precipitation with polyvinyl pyrrolidone, PVPP (in combination with the Folin assay) or with bovine serum albumin, BSA, in the Ferric Chloride assay, respectively. Condensed tannins were assayed according to Porter et. al. (1986).

Results

Feed evaluation

The nutritional quality of the samples was evaluated by crude nutrient analysis and *in vitro* fermentation (HFT). The results for crude protein, fibre fractions, and *in vitro* digestibility are shown in table 1.

Table 1: Crude nutrient composition and *in vitro* digestibility of the selected feed samples

		proximate composition				***in vitro* digestibility**	
Material	morphological fraction	CP %	NDF %	ADF %	ADL %	DOM %	TD %
White Sorghum	leaves	9,4	63,5	38,4	8,4	54,9	65,5
Andropogon	mature	7,0	78,9	52,2	11,2	52,4	43,4
Andropogon	young	12,6	71,9	44,9	10,8	51,6	63,6
Groundnut hay	stem	10,0	53,6	44,5	11,2	48,3	65,1
Groundnut hay	leaves	17,7	44,7	34,9	14,3	56,6	76,1
Moringa	leaves	20,7	23,7	17,8	5,5	70,1	85,8
Guira senegalensis	leaves	14,5	62,1	50,2	30,1	43,2	46,4
Banneh	leaves	19,2	49,6	40,7	17,2	71,1	60,4
Yafal Concentrate	mixture	23,0	44,8	25,3	12,0	56,7	75,1
White Sorghum	stem	4,6	78,9	57,3	11,5	51,2	34,7
Late Millet	leaves	6,8	70,6	42,2	9,8	38,8	44,7
Late Millet	stem	3,2	87,1	65,7	13,7	38,1	26,4
Early Millet	stem	5,7	82,8	63,1	15,5	32,8	26,9
Sorghum bicolor	stover	8,5	55,8	36,1	7,3	54,6	67,7
Maize (Baby Corn)	stover	8,5	62,9	40,7	7,3	n.d.	n.d.
Pea (Sugar Snap)	stover	19,7	34,2	24,7	6,2	n.d.	n.d.
Pea (Mange Tout)	stover	12,2	44,1	33,5	6,7	n.d.	n.d.
Bean (Bobby Bean)	stover	11,8	53,0	43,4	15,3	n.d.	n.d.

CP crude protein, NDF neutral detergent fibre, ADF acid detergent fibre, ADL acid detergent lignin, DOM digestible organic matter TD true digestibility; (%) mg/100 mg of dry matter; n.d. not determined. DOM was estimated from gas production, gp_{200} (ml gas produced with 200 mg substrate per 24h), using the formula DOM = 14,88 + (0,889 gp_{200}) + (0,45 CP) + (0,65 CA);

The samples were ranked according to different parameters and divided into a high, medium, and low quality group.

Ranked according to their proximate composition (using CP/NDF as indicator), the bean and pea stovers, groundnut hay, Yafal, and the tree leaves belong to the high quality group. Young *Andropogon*, maize, and leafy sorghum residues form the medium quality group, and mature *Andropogon*, sorghum stems and millet samples have poor nutritional quality. It should be noted, that among the first group, Sugar Snap residue and *Moringa* leaves are even superior to the concentrate feed, Yafal.

The horticultural byproducts were not included in the *in vitro* incubation, but true digestibilities of 60 and 75% have been reported for the Baby Corn and bean and pea stovers, respectively (O.O. Akinbamijo, personal communication). According to *in vitro* digestibility data, the groups are quite similar, except for the *Guira* leaves, that are displaced from the high to the low quality group. Within the medium quality group young *Andropogon* drops slightly and now ranks behind maize and sorghum leaves.

Tannin analysis

Only seven of the 18 tested samples proved to contain phenolic compounds according to at least one of the assays applied. The first indication for the presence of tannins in a

plant can be obtained by a simple colorimetric assay for total phenols (Tab. 2). Two assays that are commonly used for this purpose are the Folin and Ferric Chloride assay.

Table 2: Total phenol content of the selected feed samples

Material	fraction	total phenols Folin %	total phenols FeCl3 %
White Sorghum	leaves	**3,5**	**0,2**
Andropogon	young	1,6	**0,3**
Guira senegalensis	leaves	**16,9**	**2,5**
Banneh	leaves	**4,4**	**0,4**
White Sorghum	stem	1,4	0,0
Late Millet	leaves	2,5	0,0
Late Millet	stem	1,3	0,0

(%) mg tannic acid equivalents/100 mg of dry matter. Positive samples are highlighted by bold figures.

The results of the Folin assay exceed those of the Ferric Chloride assay up to an order of magnitude. The relative difference between the assays persists when total tannins are determined (Tab. 3). Condensed tannins constitute a subfraction of total tannins, which is considered to have pronounced effects in the digestive system of ruminants (Barry and Mcnabb 1999).

Table 3: Total tannincontent of the selected feed samples

Material	fraction	total tannins PVPP/Folin %	total tannins BSA/FeCl3 %	condensed tannins BuOH/HCl %
White Sorghum	leaves	2,4	**0,11**	**0,02**
Andropogon	young	0,6	**0,09**	**0,03**
Guira senegalensis	leaves	**9,6**	**1,74**	**0,42**
Banneh	leaves	3,0	0,00	0,01
White Sorghum	stem	1,5	**0,10**	-0,01
Late Millet	leaves	2,8	0,00	0,01
Late Millet	stem	0,7	0,00	-0,01

(%) mg tannic acid equivalents resp. leucocyanidin equivalents/100 mg of dry matter. Positive samples are highlighted by bold figures.

The biological activity of the tannins can be estimated from the amount of BSA precipitated by the extract, or from the increase in gas production if the plant material is incubated in the presence of PEG, a synthetic polymer that inactivates tannins (Tab. 4).

Table 4: Total phenol content of the selected feed samples

Material	fraction	BSA precipitated (by 500 µl extract) mg	PEG-effect gas prod. increase %
White Sorghum	leaves	**0,30**	5,8
Andropogon	young	**0,46**	**22,4**
Guira senegalensis	leaves	**6,16**	**46,7**
Banneh	leaves	0,06	0,1
White Sorghum	stem	**0,26**	4,6
Late Millet	leaves	**0,23**	**64,4**
Late Millet	stem	0,16	**27,7**

Positive samples are highlighted by bold figures.

Among the tannin containing plants, *Guira senegalensis* has an outstanding position as it contains high amounts of tannins with strong biological activity. Banneh leaves contain significant amounts of phenols, but no active tannins. The sorghum and millet samples display a heterogeneous and sometimes even contradictory picture. White sorghum leaves, and to a lesser degree also the stems, do contain (moderate amounts of) tannins, that are able to precipitate BSA, but they do not show any effect on gas production in the HFT. Vice versa, tannins were barely detectable in the late millet samples, but they exhibited pronounced biological activity, especially in the gas test. Extraction of the millet samples with acetone rather than methanol did not change this result, as the reaction in the Folin-based assays were still negative. In *Andropogon*, finally , the content and activity of tannins seems to depend critically on the vegetation stage, as young plants were clearly positive, but this vanished with age. Groundnut hay, Yafal concentrate, *Moringa* leaves and horticultural residues proved to be free of phenolic compounds.

Discussion

Methodological evaluation of tannin analysis

Both the folin and the ferric chloride assay use a tannic acid standard for calibration. However, if the absorbances are converted into phenol content in percentage of dry mass, the folin assay yields values up to 10 times higher. This is consistent with the results published by other authors who use either one of the assays, and may be due to a profound difference in color yield of the reagents with tannic acid (Mueller-Harvey 2001). In the total tannin assay the folin reagent is used to determine non-tannin phenols after precipitation of tannins by PVPP. Thus, tannins are defined by affinity to this polymer and are quantified indirectly from the difference between total and non-tannin phenols. The ferric chloride assay, on the other hand, allows the direct quantification of tannins precipitated by a model protein, BSA. This is in better agreement with the common definition of tannins as protein binding phenolic compounds. When the BSA in the precipitate is quantified as well, the information generated by this assay is even complemented by an estimation of the biological activity of the tannins.

All of the assays rely on extracted tannins. Extractability, and thereby yield and composition of the tannins in the extract may vary with the solvent and its concentration. We used a standardized extraction with 80% methanol, which is compatible with all the assays performed. Some authors recommend extraction in acetone, but these samples cannot be used in the BSA precipitation assay (Makkar et. al. 1988). If the biological activity of tannins is investigated in the gas test, the major difference is the use of the entire plant material rather than an extract. In case of the late millet samples this assay was the only one that revealed the strong inhibitory effect of millet samples on fermentation. This may be explained by poor extractability of the millet tannins, both in methanol and acetone. On the other hand, the BSA-precipitating activity detected in white sorghum extracts under assay conditions seem to be irrelevant in the rumen, possiby due to a different pH. These observations demonstrate quite well the advantage of the gas test over chemical tannin assays to detect adverse effects of secondary plant compounds in ruminant nutrition.

Integrated feed evaluation

The major difference between the ranking of feed samples according to proximate composition and *in vitro* digestibility occurred for *Guira* and young *Andropogon*, two

plants which proved to contain effective amounts of tannins. Both of these samples responded to all the tannin assays tested, but most convincing in the assays for biological activity. If the *in vitro* fermentation is carried out in parallels with and without PEG, the information on the lower feed value - and the reason for this being tannins – can be obtained in one step.
The excellent results achieved with the *Moringa* leaves indicate that it can be used like the commercially available concentrate and it may be worthwile to harvest and dry the leaves as a regular feed supplement. Banneh leaves have a fairly good quality, no adverse effects, and are available during the dry season. The results for *Andropogon* suggest that there is an optimum stage of maturity, such that a maximum of nutritional value is retaind but as much as possible of the tannins has already been lost when the plant is harvested.

Our future research approach will foster more on *in vitro* studies, in which a tannin-free basic diet is supplemented with tannins from various, clearly defined sources to analyze the effects of these compounds on ruminal fermentation.

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