Effects of tropical browse plants on *in vitro* rumen protein degradability

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Abstract

Leaves of Multi-purpose trees (MPT) provide a very important ecosystem service in the form of feed for ruminant livestock in most tropical countries. They are often fed to ruminants as supplements due to their appreciable levels of crude protein and less concentration of recalcitrant cell wall fiber.The presence of condensed tannins in tropical MPT could minimise protein degradation in the rumen, reduce rumen ammonia concentration and ultimately reduce overall dry matter digestibility. In this study, eight MPT (*Ceiba pentandra, Khaya senegalensis, Senna siamea, Ficus gnaphalocarpa, Pterocarpus erinaceus, Albizia lebbeck, Azadirachta indica* and *Gmelina arborea*) were incubated with two levels of nitrogen to test the effect of condensed tannin on *in vitro* ammonia nitrogen concentration. The MPT's in another experiment were incubated *in sacco* to determine the extent of protein degradability. The crude protein (CP) of the MPT was in the range of 92.2 to 229 g/kg DM. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were in the range of 216-307 g/kg DM, 163.4 to 291 g/kg DM and 94.8-282 g/kg DM respectively. The highest condensed tannin (CT) was obtained in *F gnaphalocarpa* with no CT detected in *A Lebbeck*. There was no effect of the MPT and nitrogen buffer interaction on the *in vitro* gas production parameters at 72 h. There was no difference in *in vitro* dry matter digestibility (IVDMD) between the two nitrogen (N) buffered rumen fluids. Leaves of MPT incubated in nitrogen sufficient (NSf) buffer consistently had a higher NH_3N concentration than the nitrogen deficient (ND). However, the NH_3N concentration from the ND buffer remained within levels that ensured IVDMD was not different from that of NSf. The *in sacco* CP degradation characteristics differed among the leaves at 72 h. *A. indica* had a higher proportion of its protein content being soluble. The MPT with CT had lower proportion of their protein being degraded particularly in C. *pentandra.* Ammonia nitrogen concentration was maintained within limits that did not negatively affect IVDMD when the leaves of the MPT were incubated in ND and Nsf.

Key words: *ammonia, browse plants, condensed tannins, in vitro digestibility, in sacco digestibility*

Introduction

The dry season in most tropical savannah is usually characterized by minimal crop cultivation which gives animals' unrestricted access to grazing lands and crop residues from cultivated fields. The quality of forage is usually low during this period with high levels of recalcitrant cell wall fiber leading to a lower feed intake and poor digestibility (Crowder and Chheda, 1982). Rampant bush fires in the savannah zone also contributes to the loss of biomass on grazing lands in the dry season resulting in a reduction in forage availability

Multi-purpose savanna tree species provide serval ecosystem services to the communities in which they are found. They provide services such as shade, carbon sequestration and removal of air pollutants (Nowak et al 2008). The use of pods, fruits and leaves of several indigenous savanna MPT as feed supplement for livestock is gaining significance among small holder farmers in the savannah zone of Ghana particularly in the dry season (Ansah and Nagbila 2011).

Leaves of MPT have been reported to maintain a high amount of crude protein and low fibre even in the dry season (Sarkwa et al 2011; Le Houérou 1980; Pellew 1980). They also contain varying levels of plant secondary metabolites such as condensed tannins (CT) that may inhibit their levels of inclusion in ruminant diet. This is due to the tendency of CT to reduce rumen protein degradation which affects the supply of adequate ammonia nitrogen to fiber degrading microbes (Makkar and Becker 1998; Shayo and Uden 1999; Aganga and Tshwenyane 2003). Inadequate supply of rumen ammonia nitrogen has been found to affect the growth of rumen micro-organisms which could affect dry matter digestibility (Leng 1991). Despite the role of CT in reducing dietary protein degradation in the rumen, it may increase the supply of by-pass protein for digestion and absorption in the small intestine (McNabb et al 1996). Information on the effect of the leaves of these indigenous MPT on rumen ammonia concentration, pH and crude protein degradation is limited.

The *in vitro* gas production and *in sacco* degradability techniques have over the years been proven to be the most reliable, simple and efficient way of evaluating fodder trees and shrubs for their potential in the animal industry (Ørskov et al 1980; Menke and Steingass 1988; Theodorou et al 1994). This study was therefore carried out to investigate the extent of protein degradation, ammonia nitrogen supply and *in vitro* dry matter digestibility of tropical browse plants from the savannah zone of Ghana.

Materials and methods

Source of experimental material and processing

Branches of eight MPT (*Ficus gnaphalocarpa, Ceiba pentandra, Khaya senegalensis, Azadirachta indica, Gmelina arborea, Pterocarpus erinaceus, Senna siamea* and *Albizia Lebbeck*) were harvested from natural grazing lands within Nyankpala during the early dry season (September to December, 2011). A total of ten plants of each tree species were randomly harvested, after which the leaves were separated from the branches and bulked for each plant species. About 3 kg leaves of each plant was sampled from the bulk and used for the experiment. These plants were selected based on some interaction with livestock farmers within the zone and also from literature (Ansah and Nagbila, 2011). Nyankpala is located in the Tolon District and falls within the guinea savannah zone of Ghana (9°54'N, 0°59'W). It has a unimodal rainfall pattern with an annual mean rainfall of 1,043 mm and at an altitude of

183 m above sea level. The mean annual temperature is between 28˚C to 30˚C whilst the mean annual day relative humidity is 54%.

Chemical composition

About 200 g of fresh leaves were sampled and oven dried (60˚C) for 48 h for dry matter determination. Approximately 2.8 kg of the fresh leaves were air dried to a constant weight and packaged into plastic bags for transportation to the Harper Adams University, UK. The samples were milled (1-2 mm) using a hammer mill (IKA MF, 10, UK).

The nitrogen concentration of the leaves was determined using the Leco nitrogen analyser (Leco FP-528-UK) after which the crude protein was calculated by multiplying the nitrogen content by 6.25 (AOAC, 2000). The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined in accordance to Goering and Van Soest (1970). Condensed tannin (CT) was estimated according to the method of Porter et al (1986). The ammonia nitrogen of the filtrate from each treatment in the *in vitro* gas study was determined following the procedure of Watson and Galliher (2001) and was used on the Foss Kjeltac analyser (Unit 2300, UK).

***In vitro* gas study**

The method described by Theodorou et al (1994) was used for the *in vitro* gas production. An 8*2 factorial in completely randomised design was used for the study. The factors were the eight MPT and the two-nitrogen buffer (nitrogen sufficient (NSf) and nitrogen deficient (ND)) and were incubated five times. In each incubation, the samples were replicated three times. The NSf buffer contained 4 g of NH_4HCO_3/l following the procedure of Theodorou et al (1994) whilst the ND buffer had all the chemicals used in NSf except the NH_4HCO_3. This was done to test the ability of the proteolytic microbes to degrade the protein and supply ammonia nitrogen to the microbial population in the rumen in the presence of CT. Rumen fluid was obtained from four rams fitted with a fistula 4 h after morning feeding. The rams had an average live weight of 95 kg and were fed *ad libitum* on wheat straw and concentrate (Wynnstay ram master coarse mix, UK) at a rate of 1.1 x maintenance (AFRC 1993).

Approximately 2.0 g of each milled MPT was weighed into a 250 ml bottle, oven dried (39^{o}C) overnight and about 200 ml of buffered rumen fluid (Media + rumen fluid) dispensed into the bottles using a peristaltic pump. Gas production from each bottle was measured using a pressure transducer (T443; Bailey and MacKay Ltd., Birmingham UK) and the content of each bottle was agitated at each time of pressure reading (Theodorou et al 1994).

The pressure readings were taken at 3, 6, 12, 18, 24, 36, 48, 60, 72 h in psi and converted to volume (ml/g DM). After 72 h, the seals of the bottles were removed and the pH of the content taken. The content of each bottle was then filtered under vacuum through a dried, pre-weighed sintered glass crucible (50 ml, porosity P1, Pyrex). About 20 ml of filtrate from each bottle was stored in plastic tubes and frozen (-4^{o}C) for ammonia nitrogen analysis. The residue after filtering was then placed in an oven for drying at a temperature of 105^{o}C for 4 h for *in vitro* dry matter digestibility (IVDMD) determination. The gas readings were then fitted to the exponential equation $Y = b (1 - e^{-ct})$ of Ørskov and McDonald (1979) without an intercept using sigma Plot 10th edition (Systat Software Inc. 2006).

Where Y = gas volume at time t (ml)

b = asymptotic gas production (ml/g DM)

t = time (h)

c = fractional rate of gas production (ml/h)

In sacco study

The method described by Sinclair et al (1993) was adopted for this study. The wether rams (4) used for the *in sacco* study were fitted with permanent rumen cannulae and housed under continuous lighting with free access to water and mineral lick. The rams were fed a basal diet containing straw and concentrate (Ram master coarse mix). Approximately 5.0 g of each of the MPT were weighed into synthetic fibre bags with a pore size of 42 *um*. The synthetic fiber bags were removed from the rumen at 4, 8, 16, 24, 48 and 72 h and a new set replaced following the complete exchange method of Paine et al (1982). The retrieved bags were placed in a bucket of cold water to remove rumen debris, prior to being washed for 40 min in a domestic washing machine. In addition, zero hour time points were determined by washing the bags filled with 5.0 g of the MPT samples in the washing machine without incubation. The bags and contents were oven dried (105ºC) to a constant weight. The contents of the bags after drying was analysed for nitrogen using the Leco nitrogen analyser.

The CP degradability was determined by fitting the data to the exponential equation ($P = a + b (1 – e^{-c(t-L)})$ of McDonald (1981) in sigma Plot 10^{th} edition (Systat Software Inc. 2006).

Where 'P' is the total ruminal CP digested at time t; 'a' is the immediately soluble protein; 'b' is the slowly degradable protein and 'c' is the rate of degradation of fraction b at time t. L represents the lag time.

Statistical analysis

In vitro gas study: Data from this study was analysed using two-way ANOVA at 5 % significant level in Genstat version 12.1 (2009), VSN International Ltd. The variation among the treatment means was determined using Fisher's unprotected least significant difference test.

In sacco study: Data was analysed as a randomised block design using Genstat version 12.1 (2009), VSN International Ltd. The animal effect was used as the block. The variation among the treatment means was determined using Fisher's unprotected least significant difference test.

The Pearson correlation was used to explore if there was any relationship between the nutrient composition and *in vitro* gas production as well as *in sacco* degradability parameters.

The hierarchical cluster analysis was carried out to show similarities among the browse plants using the ammonia nitrogen concentration, crude protein, degradability, crude protein concentration and *in vitro* dry matter digestibility.

Results

Dry matter and Chemical composition

The dry matter (DM) and chemical composition of the leaves are shown in Table 1. The average DM content of the MPT ranged from 345.4 to 448.3 g/kg DM with the highest and least obtained in *S. siamea* and *F. gnaphalocarpa* respectively.

The highest CP content was reported in *A. lebbeck* (229.2 g/kg DM) whilst *A. senegalensis* (92.2 g/kg DM) had the least. The highest average NDF was recorded in *P. erinaceus* with F. *gnaphalocarpa* having the least (Table 1). The ADL ranged from 94.8 to 282.3 with the highest recorded in *G. arborea*. Condensed tannin (CT) was present in all the MPT except *A. lebbeck.*

Table 1. Mean (g/kg±SD) dry matter and chemical composition of leaves of MPT

MPT	DM	CP	NDF	ADF	ADL	CT
A . indica	399±0.6	154±1.0	237±3.2	183±1.0	134±0.2	35±3.1.2
A. lebbeck	394±0.6	229±3.6	296±2.2	193±4.5	196±0.1	0.00
C. pentandra	365±1.2	126±2.7	271±1.2	291±0.4	200±0.5	102±1.7
S. siamea	448±0.3	175±2.2	262±2.2	256±0.6	111±0.5	2±0.2
F. gnaphalocarpa	345±0.6	93±0.2	216±0.8	274±0.7	109±0.1	114±1.2
G. arbórea	374±0.6	151±0.5	248±1.0	163±3.9	282±3.0	4±0.2
K. senegalensis	434±0.2	92±1.1	250±0.9	281±0.6	94±1.0	66±2.9
P. erinaceus	383±0.5	136±2.9	307±2.4	205±3.3	105±0.3	14±0.1

CT condensed tannin, DM dry matter, CP crude protein, NDF neutral detergent fiber, ADF acid detergent fiber,
ADL acid detergent lignin.

The highest CT was observed in *F. gnaphalocarpa* (115 g/kg) and decreased in the order of *F. gnaphalocarpa*< C. *pentandra* < *K. senegalensis* < *A. indica* <*G. arborea* < *P. erinaceus* < *S. siamea* (1.8 g/kg DM).

***In vitro* gas study**

The interaction between the MPT and nitrogen buffer did not differ for all the fermentation parameters (Table 2). There was no effect of the nitrogen buffer on the asymptote gas production, fractional rate of gas production and IVDMD. Ammonia nitrogen concentration and pH were not affected by the nitrogen level in the buffer (Table 2).

The asymptote gas production (b), fractional rate of gas production (c) and cumulative *in vitro* gas production (IVGP) did not differ among MPT.

Table 2. Mean cumulative gas production and fermentation characteristics of nitrogen buffer and MPT

	Buffer	b	c	pH	NH_3N (mg/l)	IVDMD (g/g)	IVGP ml/g DM (72h)
A . lebbec k	ND	358	0.08	6.7	296.5	0.831	371
	NSf	351	0.07	6.7	454.8	0.508	360
C. pentandra	ND	262	0.06	6.7	187.7	0.599	262
	NSf	361	0.06	6.7	277.4	0.527	356
K. senegalensis	ND	365	0.08	6.6	219.6	0.448	377

S. siamea	NSf	341	0.05	6.7	277.4	0.311	340
	ND	316	0.07	6.6	293.4	0.647	321
F. gnaphalocarpa	NSf	324	0.08	6.6	402.4	0.695	330
	ND	275	0.06	6.6	167.9	0.598	279
P. erinaceus	NSf	344	0.06	6.7	257.1	0.632	349
	ND	336	0.08	6.7	281.8	0.651	342
A. indica	NSf	369	0.07	6.7	437.7	0.676	374
	ND	285	0.07	6.6	146.4	0.603	261
G. arborea	NSf	286	0.07	6.6	290.5	0.631	292
	ND	389	0.06	6.6	243.7	0.469	389
	NSf	378	0.08	6.7	324.0	0.491	381
SED		56.7	0.01	0.05	40.10	0.13	59.8
P (MPT)		56.7	0.01	0.05	40.10	0.13	59.8
P . (Buffer)		0.28	0.60	0.16	<.001	0.038	0.20
P . (MPT*buffer)		0.29	0.94	0.01	<.001	0.324	0.28

ND nitrogen deficient, NSf nitrogen sufficient, NS not significant, b asymptotic gas production, c fractional rate of gas production, IVDMD in vitro dry matter digestibility, IVGP in vitro gas production

The mean NH_3N concentration was higher in the low CT-containing MPT especially in *A. lebbeck*. The highest average IVDMD was found in S. *siamea* and the least in *G. arborea*. The pH recorded at 72 h for all the MPT was above 6.

***In sacco* study**

The protein solubility (a), protein degradability (b) and total ruminal digestibility of CP (P) differed among the MPT leaves (Table 3). The fraction of protein immediately soluble (a) in the rumen was highest in *A. indica* and least in *F. gnaphalocarpa*. The lowest degradable protein (b) fraction and total ruminal digestibility (P) of the crude protein was found in *C. pentandra*.

Table 3. Mean *in sacco* crude protein degradation characteristics (g/kg)

	p	**a**	**b**	**c**
A. lebbec k	787	470	317	0.05
C. pentandra	393	377	15.9	0.07
K. senegalensis	598	469	129	0.06
S. siamea	671	418	253	0.05
F. gnaphalocarpa	425	360	65.4	0.06
P. erinaceus	420	390	29.2	0.07
A. indica	595	504	91.0	0.06
G. arborea	544	457	86.6	0.07
SED	33.1	7.38	31.4	0.01
P-value	0.001	0.001	0.001	0.074

a immediately soluble protein, b slowly degradable protein, P the total ruminal CP digested, c rate of degradation of

fraction b Lag time missing, please indicate how to estimate P (the total ruminal CP digested)

The CP content of the MPT was positively correlated to the slowly degradable CP (b) and total ruminal CP degradability (P) (Table 4). There was no correlation between NDF, ADF and CT and *in sacco* degradation characteristics.

Table 4. Pearson correlation between crude protein (CP) neutral detergent fiber (NDF), acid detergent fiber (ADF), Condensed tannin (CT) and constants of *in sacco* CP degradability, in vitro gas production and in vitro NH_3N concentration of MPT

Constants	**CP**	**NDF**	**ADF**	**CT**
CP degradation				
a	0.414	-0.033	-0.574	-0.535
b	0.753*	0.236	-0.166	-0.566
c	-0.362	0.298	-0.346	0.224
a+b	0.744*	0.172	-0.044	-0.644
Gas production				
b	0.084	0.371	-0.259	-0.413
c	0.840**	0.500	-0.566	-0.882**
Ammonia nitrogen	0.712*	0.810*	-0.366	-0.786*

**P<0.05, **P<0.01*

In the gas production study, CP was positively correlated to the fractional rate of degradation (c) and NH_3N level. The correlation between NDF and NH_3N was positive. The CT content of MPT was negatively correlated with the rate of gas production (c) and NH_3N. In the hierarchical cluster analysis of the MPT, *Ceiba pentandra* and *Ficus gnaphalocarpa* showed most similarity followed by *A. lebbeck* and *S. siamea (*Figure 1).

Figure 1. Dendrogram of the hierarchical cluster analysis showing the similarities between the browse plants using ammonia nitrogen concentration, crude protein degradability, crude protein concentration and *in vitro* dry matter digestibility

Discussion

The DM content of the browse plants reported in this study compared favourably with what was reported by Ouédraogo-Koné et al (2008) for browse plants harvested during the periods of October to December in the South Sudanian zone of Burkina Faso. The similarity in results

is attributable to the time when the plants were harvested. This period is often characterised by the sprouting of fresh leaves which have very high moisture content. The CP concentration of the browse plants was above the minimum CP (60-80 g/kg) required for sustenance of microbial growth (Van Soest 1982). Rumen microbial stability is very important for cell wall degradation and carbohydrate fermentation and it is expected that any feed used as supplement would be able to ensure this stability. The three plants (*C. pentandra, F. gnaphalocarpa* and *K. senegalensis*) with CT above 60 g/kg all had higher ADF than NDF. This agrees with the results of Getachew et al (2000) who reported higher ADF than NDF in some CT-containing plants and attributed this to the insolubility of the CT-protein complex and CT-fibre complex when digested with the acid detergent solution. Ammonia nitrogen concentration in the rumen is a balance between the degradation of feed protein and uptake of ammonia for synthesis of microbial protein (Hariadi and Santoso 2010). The presence of CT in the browse plants influenced the concentration of NH_3N in the *in vitro* gas study. This was reflected in the relatively low NH_3N concentration reported in the leaves with higher CT concentration.

In the *in sacco* study, the MPT with higher concentration of CT had a lower proportion of their protein being soluble with the degradable fraction also being low. This could be an indication that most of the NH_3N reported in the ND buffer in the *in vitro* gas study was emanating from the immediately soluble protein with very little from the degradable fraction of the protein. This finding agrees with the report by Oh et al (2008) who found that diets containing high concentration of soluble protein resulted in increased rumen ammonium concentration. The lower proportion of the protein degraded in the CT-containing leaves especially, *C. pentandra* can be attributed to the reaction between CT and protein. Condensed tannin has been reported to bind with protein in the rumen especially when the pH of the of the rumen is high (Hariadi and Santoso 2010; Getachew et al 2000).

Conclusion

- The leaves of the MPT studied have potential to be used as dry season feed supplement for small ruminants based on their nutrient composition and ability to supply the needed NH_3N without compromising rumen pH.

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