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Research Note

In vitro organic matter disappearance of tanniferous browse using rumen liquid from goats ingesting grass versus browse

Caroline C Wambui[1], James P Muir[2]*, John Githiori[3] and Barry D Lambert[2]

[1] *Egerton University, PO Box 536, Nakuru Town 20115, Kenya*
[2] *Texas A&M AgriLife Research, 1229 North US Highway 281, Stephenville, TX 76401, USA*
[3] *International Livestock Research Institute, PO Box 30709, Nairobi 00100, Kenya*
* *Corresponding author, e-mail: j-muir@tamu.edu*

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***In vitro* organic matter disappearance (IVOMD) of African browse species often indicates artificially low nutritive value due to the presence of condensed tannins (CT). Diet of the rumen liquor donor may change this if it contains CT. Leaves from 11 browse species were evaluated for their IVOMD of nitrogen, fibre fractions and CT concentrations. Crude protein (CP) ranged from 14% to 25%, whereas neutral detergent fibre (NDF) varied from 20% to 40%. The CT fractions differed ($P \leq 0.05$) among the browse species with most CT either soluble or bound to protein. *Acacia brevispica* and *Berchemia discolor* had the greatest concentrations of total CT, whereas *Balanites aegyptiaca* and *Prosopis juliflora* had the least. Rumen liquid from goats on grass diet (no CT) or browsing species with CT resulted in differences in IVOMD of NDF ($P \leq 0.05$); 37% less ($P = 0.11$) fibre-bound CT IVOMD resulted when rumen liquid donors consumed browse containing CT. This indicates that (1) during IVOMD assays, rumen fluid donor diets should reflect, as closely as possible, those of the target animals and their diets, and (2) rumen liquid donor diet composition, especially forage CT concentrations, should be documented when evaluating results of IVOMD trials that use rumen liquor donors consuming CT.**

Keywords: condensed tannins, IVOMD, NDF, nitrogen, phenolic compounds, rumen liquid

Leguminous trees are an important feed resource in the arid and semi-arid areas that constitute the majority of Africa's landmass, approximately 75% in the case of Kenya (FAO 2013a). Camels, sheep, goats, and cattle are the main economic enterprise in these areas (FAO 2010). Indigenous browse species that cattle select in drier regions of East Africa include *Acacia tortilis*, *Acacia brevispica*, *Berchemia discolor*, *Grewia bicolor*, *Terminalia brownii*, *Balanites aegyptiaca* and *Ziziphus mucronata* (Dharani 2011). *Prosopis juliflora*, native to the Americas, was introduced to East Africa in the early 1980s as a means to reclaim degraded lands (Maghembe et al. 1983), but has since become an invasive weed there and in many other parts of the world, including Kenya (Mwangi et al. 2008). *Pithecellobium dulce*, *Gliricidia sepium* and *Leucaena leucocephala* are multipurpose tree legumes introduced from Central America and naturalised along the coastal lowlands of Africa. Several authors have documented improved animal performance when these are used as supplements to roughage-based diets. Greater average daily gains, milk yield and reduced weight loss during early lactation were recorded compared to control unsupplemented diets (Muinga et al. 1992, Abdulrazak et al. 1997, Kahindi et al. 2007).

Tropical browse species often contain plant secondary metabolites that limit their utilisation as forage (Aerts et al. 1999). Many plants produce chemicals that are not directly involved in the process of plant growth (secondary compounds) but act as deterrents to insect, ruminant and fungal attack (Feeny 1976). The roles of plant condensed tannins (CT; phenolic compounds), one of these secondary compounds, in the ruminant environment are myriad (Muir 2011) but are most commonly thought to be beneficial in reducing microbial degradation of plant protein to ammonia production in the rumen, thus enhancing the flow of undegraded plant protein to the intestines (Waghorn et al. 1987). Bloat prevention and anthelmintic properties are among other benefits of CT in ruminant diets (Norton 2000, Niezen et al. 1995). The use of plant CT as a natural anthelmintic is increasingly important as gastrointestinal nematodes become resistant to artificial anthelmintics (van Wyk et al. 1999). However, at levels above 5% in ruminant diets, CT can cause a decrease in feed intake, diet digestibility or may even be toxic (Wang et al. 1996).

Condensed tannins may play an important role in determining laboratory predictors of forage nutritive potential (Terrill et al. 2010). The extent of fibre degradation or protein availability, for example, can be influenced by the

presence of CT and the degree to which these are bound to plant fibre or protein (Terrill et al. 1994). Condensed tannins have been known to interfere with laboratory fibre analysis, hence limiting the understanding of the role of CT-fibre interaction in plants on fibre utilisation in ruminant animals (Terrill et al. 1994). It is relevant, therefore, to evaluate the extent of browse fibre and protein degradation during *in vitro* rumen liquor incubation to more accurately estimate nutrient availability *in vivo*. The diet of the animal donating that liquor may also affect the extent of degradation in the presence of CT.

The objective of our study was to detect differences in *in vitro* disappearance of fibre fractions, nitrogen (N) and CT in Kenyan browse species when incubated to rumen liquor from goats consuming grass with no CT or browse with CT. Our hypotheses were: (1) there are differences in CT, N and *in vitro* organic matter disappearance (IVOMD) concentrations among selected Kenyan browse species and (2) rumen liquor from goats consuming browse with CT will result in different IVOMD compared to that measured using rumen from goats consuming only grass.

Leaves from 10 individuals of *A. tortilis*, *A. brevispica*, *B. discolor*, *G. bicolor*, *T. brownii*, *B. aegyptiaca* and *Z. mucronata* were collected in April 2008 from naturally growing mature trees at Chemeron Field Station of Egerton University in Marigat Baringo District, Kenya. *Prosopis juliflora* leaves and pods were also collected at Baringo. Leaves of naturalised introduced species (*P. dulce*, *G. sepium* and *L. leucocephala*) were collected from Kilifi District, Kenya. These areas are located in eastern Kenya with semi-arid climates of less than 510 mm y^{-1}, sandy to loam soils and 6–8 months of annual dry season (FAO 2013b). Two distinct populations, separated by distance (1000 m) and ecosystem (upland and riverine), were sampled at each location and these were considered replications. The collected plant material was spread on a mat in a room to dry under prevailing ambient room temperatures and stored pending laboratory analyses. The composite of leaves from 10 trees in each replication was considered the experimental unit.

After drying in a forced-air oven at 65 °C until weight loss ceased, plant material was ground through a 1 mm sieve before the analyses were conducted. ANKOM F57 filter bags (ANKOM Technologies, Macedon, NY, USA) were used for the study. Samples were weighed into preweighed filter bags and heat sealed using an impulse bag sealer. As per ANKOM protocol, samples for determination of neutral detergent fibre (NDF) were subsequently used to run acid detergent fibre (ADF) and acid detergent lignin (ADL) (sequential analysis) in a modified version of methods originally described by van Soest and Robertson (1980). A maximum of 24 bags were placed into the bag suspender of the ANKOM^{200} Fiber Analyzer for each run. Sodium sulphite was added in the NDF procedure to cleave the bond between fibre and CT, hence allowing proper determination of the NDF fraction in those samples that contained CT. Alpha amylase was also added to digest soluble starch/carbohydrates in the samples. Nitrogen concentrations were determined from samples digested by a Dumas method (AOAC 1990) using an Elementar vario Macro N analyser (Elementar Americas, Mt Laurel, NJ, USA).

Condensed tannin standards were prepared from the plant material for each species (Wolfe et al. 2008). Values reported, therefore, are true concentrations rather than relative to a universal standard. Individual plant sample CT analyses were determined according to methodology described by Terrill et al. (1992). This method assays and quantifies protein-bound (PBCT), fibre-bound (FBCT) and extractable (ECT) CT fractions.

Six rumen-cannulated wether goats (Boer × Spanish) were used for the experiment. The goats were divided into two groups of three goats and subjected to two different feeding regimens that constituted the treatments. Prior to the start of the experiment, goats were subjected to an adaptation period of two weeks to allow development of necessary rumen microorganisms as influenced by diet. All goats had access to water and mineral licks ad-libitum. Feed regimen 'Hay' consisted of feeding penned wethers bermudagrass (*Cynodon dactylon* (L.) Pers. 'Coastal') hay (Table 1). The 'Browse' regimen consisted of allowing goats to browse in a native Stephenville, Texas, USA, wooded rangeland dominated by the perennial browse species listed in Table 1. Nitrogen concentrations were fairly similar between the hay and the browse diets; however, the hay had greater ADF concentrations than some browse species and only a fraction of the ADL concentration. Rumen fluid was collected from all three animals on each diet and mixed prior to IVOMD.

The IVOMD concentrations were determined for 48 h using an ANKOM Daisy II Incubator (ANKOM Technologies, Macedon NY, USA) inoculated with rumen liquid extracted from rumen-fistulated wethers on hay or browse diets. This ANKOM system emulates the Tilley and Terry (1963) two-stage *in vitro* digestibility technique (Coblentz et al. 1997) but washes the sample in a neutral detergent (van Soest et al. 1991) instead of a pepsin solution. Residues were corrected for residual ash. The IVOMD residues were then subjected to N, fibre and CT analyses to determine the proportion of each plant component that disappeared during the *in vitro* fermentation. These IVOMD were repeated on three different days (different rumen liquor collections) but these were not considered replications for IVOMD comparisons (Huang et al. 2010); the original leaf collections were considered replications for interspecific comparisons. All assays were duplicated and averages of these were used as data points. The equation used to determine these proportions of N, fibre and CT factored in the concentrations before IVOMD, the IVOMD itself, and concentrations post-IVOMD:

$$100 * (1 - [(100 - \text{IVOMD}) * \text{post IVOMD}] / \text{pre IVOMD})$$

The data were subjected to analyses of variance. Batched tree leaf samples from each location were considered experimental units. Browse analysis was a single-factor experiment in which species was the independent variable, whereas the IVOMD trial was a two-factorial experiment in which species and rumen liquid source were the two independent variables. If differences ($P \leq 0.05$ except for FBCT and PBCT disappearance during the IVOMD assay) were detected, the means were separated by least significant difference (LSD; $P \leq 0.05$).

Ethical guidelines of the Texas A&M University System Institutional Animal Use Committee were followed for all animals involved in this trial.

Results of the chemical analysis for the Kenyan browse species (Table 2) were similar to those reported by various authors (Kahindi et al. 2007, Osuga et al. 2008). The greatest N concentration was found in *A. brevispica* (4.1% N equivalent to 25.6% CP), 44% greater than *T. brownii* at 2.3% N, equivalent to 14.3% CP. Most species had NDF concentrations over 30% and ADF values over 20%, although some, notably *B. discolor*, had fibre fraction concentrations below 20%. Those species with greater NDF and ADF concentrations also tended to have greater ADL concentrations, up to 13.4% for *B. aegyptiaca*; *T. brownii* was a notable exception with very low ADL concentrations despite having among the greatest concentrations of NDF and ADF. In Kenya, low-quality feed resources (native grasses or crop residues) are characterised by low CP levels (<7%) and high NDF (>60%) (Norton 1994, Abdulrazak et al. 1997, Wambui et al. 2006), so the use of some of these high-N and low-fibre browse species as dry season supplements to ruminants is promising.

Total condensed tannin concentrations ranged up to 11.9% and were greatest in *A. tortilis* and *G. bicolor* (Table 3). *Acacia tortilis*, *A. brevispica* and *G. bicolor* had the greatest concentrations of ECT, which ranged up to 6.6%. *Berchemia discolor* had greater PBCT (5.1%) than any other species, whereas *B. discolor* and *A. tortilis* had greater FBCT concentrations. This fraction generally had the lowest CT concentration of the three fractions measured. Method of processing sample influences the relative CT fraction concentrations (Wolfe et al. 2008). Drying of plant samples at room temperature, as in the case of the present study, may have increased polymerisation of CT and their binding with other cellular constituents (Terrill et al. 1994). Accurate estimation of the nutritive value of forages with high concentrations of CT is influenced by the relationship between CT and detergent fibre analyses.

Unlike N, ADF and ADL, NDF disappearance during IVOMD was influenced by an interaction of plant species and the diet of the animals that provided the rumen liquor (Table 4). In the case of *Z. mucronata*, *A. brevispica*, *G. sepium* and *T. brownii*, sourcing rumen fluid from goats consuming browse decreased NDF disappearance during *in vitro* digestion; in all other species, rumen liquid source had no effect. The IVOMD of species with high (*G. bicolor* and *A. tortilis*) as well as those with low (*B. aegyptica* and *P. juliflora*) CT concentrations relative to the medium concentrations were not affected. Four species whose IVOMD were affected had intermediate concentrations (3.2–7.4% total condensed tannin concentrations) but others with intermediate concentrations were not affected by rumen source, indicating that something other than CT concentration may be at play. Condensed tannins are known to reduce the digestibility of feed by inhibiting rumen microbe activity (Woodward and Reed 1995) but this may be mitigated by rumen microbial population shifts if animals are habituated to browse containing CT. Our results indicate that adjusting rumen microorganism populations to the presence of CT did not result in similar NDF disappearance during *in vitro* degradation as measured using rumen liquid from animals whose rumen was not adjusted to browse. We hypothesise that CT in the rumen

Table 1: Nitrogen (N), acid detergent fibre (ADF), acid detergent lignin (ADL) and total condensed tannin concentrations (TCT) (reported as a percentage of organic matter) of bermudagrass hay and leaves from browse species consumed by goats that supplied rumen liquor for the trial in Texas, USA

Browse species	N	ADF	ADL	TCT
Celtis spp.	1.98 ± 0.2	21.5 ± 2.2	6.5 ± 0.9	2.8 ± 0.2
Cynodon dactylon	1.95 ± 0.2	34.3 ± 1.2	3.2 ± 0.3	0.0
Quercus spp.	1.70 ± 0.2	33.3 ± 0.8	12.8 ± 0.5	4.3 ± 0.4
Smilax spp.	2.08 ± 0.3	32.7 ± 1.6	12.8 ± 1.6	3.4 ± 0.6
Ulmus spp.	1.68 ± 0.1	18.7 ± 1.6	8.4 ± 0.4	5.2 ± 0.3

Table 2: Nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) concentration of leaf organic matter collected from browse species in East Africa. Means within a column with dissimilar letters differ ($P \leq 0.05$) according to a least significant difference multiple mean separation. SEM = Standard error of the mean

Browse species	N	NDF	ADF	ADL
Acacia brevispica	4.1^{a}	32.2^{abc}	22.3	9.4^{abc}
Acacia tortilis	3.0^{de}	25.5^{cd}	20.8	9.5^{abc}
Balanites aegyptiaca	2.8^{def}	31.0^{abc}	23.4	13.4^{a}
Berchemia discolor	3.8^{abc}	19.1^{d}	17.9	5.6^{cd}
Gliricidia sepium	3.9^{ab}	26.5^{bcd}	19.7	8.7^{abcd}
Grewia bicolor	3.4^{bcd}	35.9^{a}	23.1	7.1^{bcd}
Leucaena leucocephala	3.8^{abc}	29.9^{abc}	19.2	8.1^{bcd}
Pithecellobium dulce	3.6^{abc}	35.5^{a}	24.2	11.1^{ab}
Prosopis juliflora (leaves)	3.5^{abcd}	31.9^{abc}	23.6	10.8^{ab}
P. juliflora (pods)	2.2^{f}	35.0^{ab}	27.1	4.5^{cd}
Terminalia brownii	2.3^{ef}	33.1^{abc}	27.5	3.9^{d}
Ziciphus mucronata	3.2^{cd}	29.9^{abc}	14.8	4.9^{cd}
SEM	0.3	2.8	2.4	1.6

Table 3: Extractible (ECT), protein-bound (PBCT), fibre-bound (FBCT) and total (TCT) condensed tannins in leaves collected from browse species in East Africa. Means within a column with dissimilar superscript letters differ ($P \leq 0.05$). SEM = Standard error of the mean

Browse species	ECT	PBCT	FBCT	TCT
Acacia brevispica	4.1^{ab}	1.8^{bc}	1.5^{bcd}	7.4^{bc}
Acacia tortilis	5.8^{a}	3.0^{b}	3.1^{a}	11.9^{a}
Balanites aegyptiaca	0.0^{c}	0.0^{c}	0.1^{e}	0.2^{e}
Berchemia discolor	0.5^{c}	5.1^{a}	2.2^{ab}	7.8^{bc}
Gliricidia sepium	0.0^{c}	3.0^{b}	1.9^{bc}	4.9^{cd}
Grewia bicolor	6.6^{a}	2.9^{b}	1.2^{cde}	10.7^{ab}
Leucaena leucocephala	1.9^{bc}	2.5^{b}	2.0^{bc}	6.4^{cd}
Pithecellobium dulce	1.0^{c}	1.5^{bc}	0.7^{de}	3.2^{de}
Prosopis juliflora (leaves)	0.0^{c}	0.2^{c}	0.2^{e}	0.4^{e}
P. juliflora (pods)	0.1^{c}	0.4^{c}	0.2^{e}	0.7^{e}
Terminalia brownii	1.2^{c}	1.3^{bc}	0.6^{de}	3.2^{de}
Ziziphus mucronata	1.0^{c}	1.6^{bc}	0.7^{de}	3.3^{de}
Effect of species	0.001	0.004	0.0009	<0.0001
SEM	0.89	0.64	0.35	1.1

Table 4: Percentage of *in vitro* organic matter disappearance (IVOMD), nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) that disappeared in leaf organic matter of the residue of East African browse species after 48 h *in vitro* incubation in rumen liquor from wethers fed bermudagrass hay or browsing in a Texas wooded rangeland. LSD = Least significant difference, SEM = standard error of the mean

Browse species	Treatment	IVOMD	N[a]	NDF[a]	ADF[a]	ADL[a]
Species × diet *P*-value			0.80	<0.001	0.99	0.96
Acacia brevispica	Hay	91.5	79.4	96.4	94.6	89.3
	Browse	84.6	90.2	88.2	93.9	94.2
Acacia tortilis	Hay	67.3	75.8	72.3	71.2	59.8
	Browse	65.4	62.5	69.1	69.3	70.8
Balanites aegyptiaca	Hay	81.3	87.9	89.3	86.4	82.8
	Browse	78.9	89.8	86.4	85.8	85.3
Berchemia discolor	Hay	73.0	89.0	78.4	74.9	71.5
	Browse	71.6	87.3	76.1	73.3	69.2
Gliricidia sepium	Hay	84.8	86.1	91.9	87.9	83.4
	Browse	77.9	88.2	82.8	85.9	85.3
Grewia bicolor	Hay	79.6	81.1	88.4	85.4	82.6
	Browse	75.6	87.2	83.1	83.5	83.6
Leucaena leucocephala	Hay	79.8	88.0	87.6	82.9	76.1
	Browse	75.7	79.4	82.1	81.8	79.5
Pithecellobium dulce	Hay	78.2	87.4	86.3	83.0	77.0
	Browse	75.2	91.3	82.3	80.9	79.9
Prosopis juliflora (leaves)	Hay	85.4	85.9	93.2	89.4	81.0
	Browse	84.6	86.1	92.5	88.7	81.3
P. juliflora (pods)	Hay	75.5	79.2	84.6	84.6	75.5
	Browse	76.1	92.0	85.4	83.9	74.3
Terminalia brownii	Hay	78.4	80.1	82.9	80.7	75.7
	Browse	71.2	67.1	70.0	76.4	80.7
Ziziphus mucronata	Hay	74.5	78.7	81.9	79.4	74.7
	Browse	69.2	85.1	73.4	77.0	73.1
LSD among species for hay		–	–	4.1	–	–
LSD among species for browse		–	–	7.4	–	–
LSD within species for diet		–	–	5.6	–	–
Effect of species (Hay)		–	0.95	<0.0001	<0.0001	0.002
SEM		–	7.5	1.3	1.7	3.1
Effect of species (Browse)			0.0003	0.0003	<0.0001	0.17
SEM		–	3.1	2.4	1.6	5.3

[a] 100 * (1 − [(100 − IVOMD) * post IVOMD] / pre IVOMD)

liquid of goats consuming browse high in CT caused this reduction in the four plant species and, in ruminants, would likely result in greater NDF bypass in the rumen. It may be that the rumen microbial population of goats left to browse freely had insufficient time to adapt to the presence of CT in diets. The presence of CT in the rumen liquor therefore reduced NDF disappearance *in vitro* in some leaf materials, whereas in rumen liquid from animals whose diets included only hay, this may not have occurred. It may also be that rumen microorganism populations adjusted to some forms (Muir 2011) of CT but not to those present in the four species that exhibited lower NDF disappearance when placed in rumen liquor from animals consuming browse species containing CT.

There were no species by rumen donor diet interactions for PBCT and FBCT disappearance during *in vitro* incubation (Table 5). For most species, PBCT disappearance percentages were above 75%, ranging up to 100% for *B. aegyptiaca*; the only exception was *P. juliflora* leaf PBCT, of which only 6% disappeared *in vitro*. There were no differences between hay and browse diet disappearance percentages of PBCT. This was not the case for FBCT, which had 37% greater ($P = 0.11$) disappearance percentage during incubation with rumen liquor from animals eating grass hay compared to animals browsing plants containing CT. Among the 11 species, FBCT disappearance ($P = 0.11$) was likewise variable with a similar range as that observed for PBCT. With few exceptions, PBCT disappearance levels were greater than FBCT.

Our data support the following conclusions in response to our original hypotheses: (1) there are differences in CT, N and IVOMD concentrations among the East African browse species and (2) rumen liquor from goats consuming browse with CT will result in different fibre IVOMD compared to that measured using rumen from goats consuming only grass.

The 11 Kenyan browse species varied considerably in their CT concentrations, fractionation as well as disappearance rates of fibre and CT *in vitro*. Because of this high variability, general conclusions that cover all species are difficult to make. One point is clear, however: the presence of browse in goat rumen fluid donor diets affects NDF and FBCT disappearance *in vitro*. This leads us to conclude that (1) during IVOMD assays, rumen fluid donor diets should, as closely as possible, reflect those of the target animals

Table 5: Proportion of protein-bound (PBCT) and fibre-bound (FBCT) condensed tannin that disappeared in leaf organic matter of 11 East African browse trees after *in vitro* incubation for 48 h in rumen liquor from wethers fed bermudagrass hay or browsing Texas wooded rangeland browse. Because there were no species × diet interactions ($P \geq 0.20$) species data were the average of two diets and diet data were average of 11 species.

Browse species	PBCT [a]	FBCT
Species *P*-value	0.0003	0.11
LSD among species	0.53	0.69
Diet *P*-value	0.71	0.11
LSD between diets	ns	0.28
Disappearance (%)		
Acacia brevispica	75.9	67.0
Acacia tortilis	78.7	47.6
Balanites aegyptiaca	100.0	77.1
Berchemia discolor	90.8	91.2
Gliricidia sepium	78.4	64.5
Grewia bicolor	91.2	75.5
Leucaena leucocephala	92.8	82.5
Pithecellobium dulce	94.2	89.4
Prosopis juliflora (leaves)	6.0	7.0
P. juliflora (pods)	92.1	76.7
Terminalia brownii	85.3	72.7
Ziziphus mucronata	89.0	45.5
Hay		76.8
Browse		56.0

[a] 100 * (1 − [(100 − IVOMD) * post IVOMD] / pre IVOMD)

in the field and (2) diet composition, especially forage CT concentrations, should be carefully documented when publishing results of IVOMD trials that use rumen liquor donors consuming CT.

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