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In-vitro screening of Kalahari browse species for rumen methane mitigation

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ABSTRACT: The nutritional value of browse foliage from the Thorny Kalahari Dune Bush veld of South Africa is not characterized. Most of this browse species is rich in tannin, but still palatable, and is consumed by ruminants during the dry season, as well as having a role to play in mitigating enteric methane emission from ruminants. In this study, the rumen methane mitigation potential of 19 browse species foliage collected from the Thorny Kalahari Dune Bush veld, was analyzed in terms of chemical composition, *in vitro* fermentation, digestibility and methane production. *In vitro* gas and methane production and organic matter digestibility (IVOMD) were determined by using rumen fluid collected, strained and anaerobically prepared. A semi-automated system was used to measure gas production (GP) from each browse species by incubating 400 mg samples in a shaking incubator at 39 °C with or without inclusion of 400 mg of polyethylene glycol (PEG). Data for all the parameters collected were statistically analyzed using the SAS (9.0) general linear model (GLM) procedure, and differences between foliage species were determined using Duncan's multiple-range test. *Acacia luederitzii* and *Monechma incanum* showed the best potential for decreasing methane production by up to 90 % after 48 h of incubation. The secondary components (mainly tannins) of the browse species appeared to have a significant effect on volatile fatty acids (VFA), methane and gas production as judged by the comparison of samples incubated with or without PEG inclusion. The substantial amount of crude protein (CP) content coupled with their anti-methanogenic effect during fermentation would make these browses a potential mitigation option for small scale farmers and pastoralists in sub-Sahara Africa. However, it is also very important that systematic and strategic supplementation in a mixed diet should be looked at as the way forward in terms of best utilization.

Keywords: *in vitro* fermentation, browse foliage, digestibility, tannin

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Introduction

Methane is among the most important greenhouse gases responsible for a significant loss of energy by the ruminant through enteric methane (CH_4) fermentation (Jayanegara et al., 2011). Livestock, especially ruminants, contribute to CH_4 emissions through enteric fermentation and fermentation also taking place in the manure. This is especially the case when feeding highly fibrous diets which are prevalent in the tropics. In this regard, inclusion of feeds containing plant secondary metabolites, such as tannin in diets of ruminants, seems promising as a nutritional strategy to reduce CH_4 emissions from ruminants (Sebata et al., 2011; Sultan et al., 2012).

Tannins are polyphenolic compounds which bind to protein and act as chemical additives for protecting and decreasing ruminal degradation of proteins in ruminant feeds. Earlier studies suggested that feeding forages that contain tannins for ruminants generally effectively inhibit CH_4 produced during enteric fermentation (Puchala et al., 2005). In this regard, tannins of lower molecular weights were found to be more effective against methanogens than their monomeric precursors or tannins of higher molecular weight (Tavendale et al., 2005). However, there is huge diversity in tannin structure and concentration, and other chemical constituents among the browses, which may affect variably, the observed response in rumen fermentation, digestibility of nutrients and methanogen activity (Mueller-Harvey, 2006). This indicates that tannin from different plants might show a different response in gas production, true digestibility and methane production. The objective of this study was to investigate variations among palatable browse species foliage collected from the Kalahari bush dune veld in South Africa in terms of gas production characteristics, forage digestibility, VFA, ammonia-nitrogen (NH_3-N) and CH_4 production. The single effect of tannin was also studied and quantified for each browse species by incubating the browse plants with or without polyethylene glycol (PEG).

Materials and Methods

Study area description

Browse foliage sample collection for this study was carried out in the North West Province of South Africa in the Thorny Kalahari Dune Bush veld with an altitude of about 900 to 1100 m above sea level. The soil is sandy to sandy loam, and has excessive drain with high base status dunes that contain elevated concentrations of copper, which are bound in the soil in the form of secondary copper hydroxyl mineral atacamite ($Cu_2(OH)_3Cl$). The area has highly erratic rainfall that ranges from 150 to 350 mm per year; however, it has barely exceeded 150 mm per year for the last two years and during the study period. The wettest months are usually from January to April and the extreme temperatures range from a winter low reaching -10.3 °C to summer highs of up to 45.4 °C (Van Rooyen, 2001).

Sample collection, preparation and chemical analysis

Samples of nineteen tannin rich browse plants (Table 1) were collected during the summer rainy season (Feb 2012). In this study, a shrub was considered as a small to medium-sized woody plant and distinguished from a tree by its multiple stems and shorter height, usually under 6 m tall. The browses were harvested at the same time when they were at the same maturity stage; almost at early vegetative stage. Fresh foliages (leaves and stems < 3 mm diameter) were harvested from at least five randomly selected and tagged representative plants of each species. During the sampling, fresh plant leaves and twigs (less than 3 mm in stem diameter) were hand plucked from 19 browse species to be used. Samples were dried at 55 °C for 48 h in a forced oven and ground to pass through a 1 mm sieve in a Wiley mill and used for chemical analysis. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined using 200/220 Fiber Analyzer according to the methodology described by van Soest et al. (1991). Sodium sulphite and heat-stable amylase were used in an analysis of NDF. Nitrogen was analyzed following the method described in AOAC (2002) in a Nitrogen and Protein analyzer, and crude protein (CP) was calculated as N × 6.25. Determinations of total phenols (TP) and total tannins (TT) were made following the procedure described by Makkar (2003).

***In vitro* fermentation, digestibility and methane production**

The rumen fluid was collected from three rumen cannulated Merino sheep fed *ad libitum* alfalfa hay. It was prepared and purged with CO_2 to maintain anaerobic conditions (Grant and Mertens, 1992). After blending, the rumen fluid was transferred to a large glass beaker inside a 39 °C water bath being continuously purged with CO_2 and continuously stirred as recommended by Goering and van Soest (1970). The buffer solution, macro and micro mineral solution were prepared in large volumes and utilized as needed as described in Goering and van Soest (1970).

Gas production was determined as described by Theodorou et al. (1994). Four hundred mg of the ground browse/shrub samples with and without PEG (molecular weight, 6000, analytical grade Sigma-Aldrich) were incubated with 40 mL of diluted rumen fluid (15 mL rumen fluid + 25 mL culture medium) in serum bottles in a CO_2 atmosphere. Two serum bottles containing rumen fluid inoculum were incubated as controls and used to account for gas production in the absence of the substrate. All serum bottles were returned to the incubator and the rotary shaker was turned on at 120 revolutions per minute (rpm). Two replicates of the same browse and four differ-

Table 1 – Mean chemical and phenolic composition of browse plants (g kg^{-1} DM) used in the study (n = 3).

Species name	OM	CP	NDF	ADF	ADL	Total phenol	Total tannin
Trees							
Acacia erioloba	939.8^{c}	169.8^{b}	456.3^{c}	391.8^{d}	324.8^{a}	182.4^{d}	145.4^{d}
Boscia albitrunca	924.4^{e}	253.3^{a}	369.2^{f}	292.2^{g}	128.5^{e}	24.7^{h}	9.1^{h}
Acacia haematoxylon	932.8^{d}	131.2^{e}	533.4^{a}	431.0^{b}	275.7^{b}	204.1^{c}	163.4^{c}
Olea europaea	939.1^{c}	74.9^{j}	355.6^{g}	327.8^{f}	131.3^{e}	154.9^{f}	73.0^{g}
Ziziphus mucronata	908.9^{f}	157.3^{d}	364.6^{gf}	291.3^{g}	166.6^{c}	136.2^{g}	79.4^{g}
Terminalia sericea	948.7^{b}	97.4^{i}	520.1^{b}	445.7^{a}	105.3^{f}	223.4^{b}	106.8^{f}
Rhus lancea	951.0^{a}	120.3^{h}	454.0^{c}	404.5^{c}	95.0^{g}	226.6b	197.0^{b}
Acacia karroo	930.9^{d}	124.7^{g}	427.2^{d}	351.8^{e}	133.3^{ed}	217.2^{b}	200.7^{b}
Prosopis glandulosa	937.8^{c}	163.5^{c}	403.6^{e}	347.8^{e}	101.8^{f}	171.2^{e}	132.0^{e}
Acacia luederitzii	939.8^{c}	130.6^{f}	460.3^{c}	385.8^{d}	138.8^{d}	314.5^{a}	298.8^{a}
Mean	*935.3*	*142.3*	*434.4*	*366.9*	*160.1*	*185.5*	*140.6*
SEM	*0.728*	*0.01*	*0.413*	*0.386*	*0.197*	*3.4*	*3.14*
Shrubs							
Acacia mellifera	915.9^{gh}	203.4^{c}	373.6^{f}	302.1^{f}	80.1^{h}	54.8^{f}	30.6^{f}
Acacia hebeclada	937.1^{dc}	233.0^{b}	401.2^{e}	390.0^{dc}	97.4^{f}	30.5^{h}	16.1^{g}
Grewia flava	929.8^{de}	125.1^{h}	443.9^{d}	399.5^{c}	105.3^{e}	233.7^{c}	171.3^{c}
Dichrostachys cinera	959.5^{a}	141.5^{e}	508.9^{a}	376.8^{d}	227.8^{d}	386.5^{a}	319.8^{a}
Hermannia burchelli	924.7^{fe}	177.2^{d}	369.7^{f}	353.2^{e}	69.9^{i}	189.3^{d}	148.3^{d}
Lycium cinereum	783.7^{i}	328.9^{a}	363.0^{f}	277.2^{g}	85.4^{g}	24.3^{h}	12.8^{g}
Monechma genistifolium	790.5^{i}	83.8^{i}	494.2^{b}	465.1^{a}	266.2^{b}	44.9^{g}	28.2^{f}
Hermanniato mentosa	915.0^{fe}	136.2^{f}	474.9^{c}	426.4^{b}	290.3^{a}	97.3^{e}	72.1^{e}
Monechma incanum	919.4^{gf}	130.6^{g}	449.1^{d}	350.1^{e}	232.1^{c}	307.6^{b}	282.5^{b}
Mean	*892.8*	*173.3*	*430.9*	*371.2*	*161.6*	*152.1*	*120.2*
SEM	*2.5*	*0.01*	*0.356*	*0.499*	*0.14*	*3.08*	*2.9*

Means with different superscript (letters) across the column for each parameter are significantly ($p < 0.05$) different; OM = organic matter; CP = crude protein; ADF = acid detergent fibre; NDF = neutral detergent fibre; ADL = acid detergent lignin.

ent cycles were executed for every browse sample studied. Fermentation was terminated after 48 h by removing the serum bottles from the incubator and placed on ice. Supernatants were taken immediately, pipetted and stored at -20 °C until analyzed for ammonia nitrogen (NH_3-N) (McDonald et al., 1960) and volatile fatty acid (VFA) (Ottenstein and Bartley, 1971). The *in vitro* organic matter digestibility (IVOMD) was done according to Tilley and Terry (1963), as modified Engels and van der Merwe (1967).

Methane production was measured from duplicate bottles incubated for each browse by taking gas samples at 2, 12, 24, and 48 h and analyzing the methane concentration using gas chromatography (GC) equipped with a stainless steel column packed with Porapak-Q and a Flame Ionization Detector. The GC was calibrated with standard CH_4 before injection of the sample. Gas produced from each bottle at various times was recorded and samples of the gas were taken using a Hamilton syringe. One mL of sampled gas produced was injected manually (pull and push method of sample injection) into the GC.

Data were statistically analyzed using the general linear model (GLM) procedure from SAS (Statistical Analysis System, Version 9.2), considering each incubation run as a block for species. The average of the two bottles was considered as data from an experimental unit. The differences between means were determined using Duncan's multiple range test.

Results

Chemical composition

The browse studied showed significant variations in chemical and phenolic composition (Table 1). In this study, forage from shrub species had a higher mean value of crude protein concentration (173.3 g kg^{-1} DM) than tree species (142.3 g kg^{-1} DM). Among the tree forage species, *Boscia albitrunca* had the highest and *Olea europaea* the lowest CP concentrations. Within the shrub species, *Lycium cinereum* had the highest CP concentration while *Monechma genistifolium* had the lowest CP concentration. *Acacia haematoxylon* had the highest NDF and ADF concentrations while *Acacia erioloba* had the highest ADL concentrations among the tree species. The highest concentrations for NDF, ADF and ADL were found in *Dichrostachys cinerea*, *Monechma genistifolium*, and *Hermannia tomentosa* among shrubs, respectively.

Acacia luederitzii and *Dichrostachys cinerea* had the highest concentration of total phenols and total tannins among the tree and shrub species, respectively. Among the tree species, *Boscia albitrunca* had the lowest phenolic concentration, while *Lycium cinereum* had the lowest concentration of total phenols and total tannins.

In vitro gas and methane production

The *in vitro* gas and methane production of browse species studied at 12 and 24 h of incubation (± PEG) are shown in Table 2. Both in the presence and absence of PEG there was a significant ($p < 0.05$) variation amongst browse species in terms of gas and methane production. Inclusion of PEG in fermentation of tropical browses resulted in a significant ($p < 0.001$) increase in gas and methane profile particularly in browse forage species with a high secondary phenolic content. However, no effects of PEG on the gas and methane profiles of lower concentration tannin plants, such as *Acacia mellifera*, *Acacia hebeclada* and *Lycium cinereum* were detected. In the current study, the volume of methane produced at different incubation periods differed significantly ($p < 0.001$) between the browse species, depending on the concentration of tannin. In the presence of tannins, *Rhus lancea* and *Acacia luederitzii* showed significantly low methane volumes among tree browses, while *Lycium cinereum* and *Monechma incanum* presented significantly low methane volumes among shrub browses.

Feed digestibility and certain rumen related parameters

The IVOMD, total VFA and methane expressed in mass are shown in Table 3. The IVOMD differed ($p < 0.05$) within trees and shrubs both in the presence and absence of PEG. As expected, browses with high tannin concentrations were found to be low in digestibility; however, inclusion of PEG significantly improved their digestibility. Among the tree foliage, *Boscia albitrunca* and *Ziziphus mucronata* have the highest IVOMD values both in the presence and absence of PEG. Among shrubs, *Lycium cinereum* showed the highest value of IVOMD both in the presence and absence of PEG.

The VFA and NH_3-N concentrations differed significantly ($p \leq 0.001$) between trees and shrubs species. The absence of tannin significantly increased the concentration of VFA. *Ziziphus mucronata* showed the highest concentration of VFA in both the presence and absence of PEG among tree browses, while *Hermannia mentosa* showed the highest VFA concentration among shrub browses both in the presence and absence of PEG.

The recorded NH_3-N concentrations were the highest for *Boscia albitrunca* of the tree browses and for the shrubs, *Acacia mellifera* without PEG. *Acacia karroo* and *Monechma incanum* had the lowest NH_3-N concentrations among trees and shrubs, respectively, with PEG. With the inclusion of PEG, *Acacia karroo* and *Lycium cinereum* produced the highest concentrations of NH_3-N among trees and shrubs, respectively.

The correlation coefficients of ruminal fermentation parameters (IVOMD, CH_4) with the chemical composition and phenolic compounds of the trees and shrubs are presented in Table 4. Significantly ($p < 0.05$), positive correlations were observed between IVOMD and nitrogen and NFC content. There were significant ($p < 0.001$) negative correlations of IVOMD with fiber components (ADF, cellulose, hemi-cellulose) and phenolic compounds (total phenol, total tannin, condensed tannin and hydrolysable tannin). Methane production showed a significant ($p < 0.001$) negative correlation with phenolic compounds (TP, TT, CT and HT) and positive correlation with fiber components.

Table 2 – Mean gas production and methane production (mL g^{-1} DM) of browse plants studied (n = 8).

Scientific name	Gas volume				Methane gas			
	12 h		24 h		12 h		24 h	
	PEG +	PEG -	PEG +	PEG -	PEG +	PEG -	PEG +	PEG -
Trees								
Acacia erioloba	116.8^{d}_{1}	84.50^{d}_{2}	135.5^{dc}_{1}	106.5^{e}_{2}	17.25^{c}_{1}	9.45^{d}_{2}	39.5^{cbd}_{1}	28.25^{d}_{2}
Boscia albitrunca	150.0^{b}_{1}	120.0^{b}_{2}	182.0^{a}_{1}	154.3^{b}_{2}	22.58^{b}_{1}	19.50^{b}_{1}	64.5^{a}_{1}	63.25^{a}_{1}
Acacia haematoxylon	96.00^{f}_{1}	60.25^{f}_{2}	115.5^{f}_{1}	71.75^{g}_{2}	15.33^{c}_{1}	8.00^{e}_{2}	34.0^{ced}_{1}	20.00^{e}_{2}
Olea europaea	101.5^{f}_{1}	101.3^{e}_{1}	140.5^{dc}_{1}	137.8^{c}_{1}	8.83^{d}_{2}	17.20^{c}_{1}	25.5^{e}_{2}	50.0^{b}_{1}
Ziziphus mucronata	167.8^{a}_{1}	139.3^{a}_{2}	188.3^{a}_{1}	172.3^{a}_{2}	31.25^{a}_{1}	25.75^{a}_{1}	64.00^{a}_{1}	63.75^{a}_{1}
Terminalia sericea	96.00^{f}_{1}	55.50^{f}_{2}	117.5^{f}_{1}	68.25^{g}_{2}	15.70^{c}_{1}	6.58^{f}_{2}	33.50^{ced}_{1}	17.95^{e}_{2}
Rhus lancea	125.8^{dc}_{1}	36.75^{g}_{2}	157.5^{b}_{1}	54.50^{h}_{2}	18.63^{cb}_{1}	1.63^{h}_{2}	43.50^{cb}_{1}	7.08^{g}_{2}
Acacia karroo	130.0^{c}_{1}	72.00^{e}_{2}	145.3^{c}_{1}	92.00^{f}_{2}	23.00^{b}_{1}	3.88^{g}_{2}	45.00^{b}_{1}	12.25^{f}_{2}
Prosopis glandulosa	106.5^{fe}_{1}	103.8^{c}_{1}	121.0^{fe}_{1}	120.3^{d}_{1}	17.70^{c}_{1}	16.20^{c}_{1}	40.50^{ed}_{1}	30.5^{c}_{2}
Acacia luederitzii	116.3^{de}_{1}	36.00^{g}_{2}	130.8^{de}_{1}	44.75^{i}_{2}	22.00^{b}_{1}	0.13^{i}_{2}	43.25^{cb}_{1}	1.58^{h}_{2}
Mean	120.5	81.00	143.5	102.3	19.23	10.83	43.75	39.5
SEM	3.30	2.88	3.90	3.03	0.58	0.18	1.30	0.30
Shrubs								
Acacia mellifera	75.30^{e}_{2}	92.25^{d}	122.5^{d}_{1}	104.3^{c}_{2}	130.8^{e}_{1}	9.38^{c}_{2}	35.25^{e}_{1}	24.08^{de}_{2}
Acacia hebeclada	138.3^{a}_{1}	134.5^{a}_{2}	161.3^{a}_{1}	158.5^{a}_{1}	27.25^{c}_{1}	24.20^{b}_{1}	63.0^{b}_{1}	51.5^{b}_{2}
Grewia flava	108.5^{c}_{1}	55.50^{f}_{2}	141.8^{c}_{1}	85.75^{e}_{2}	19.63^{d}_{1}	8.25^{d}_{2}	48.25^{d}_{1}	28.25^{d}_{2}
Dichrostachys cinera	109.3^{c}_{1}	58.25^{f}_{2}	134.3^{c}_{1}	71.75^{f}_{2}	20.75^{d}_{1}	2.58^{e}_{2}	45.25^{de}_{1}	7.45^{e}_{2}
Hermannia burchelli	139.0^{a}_{1}	119.3^{b}_{2}	162.0^{a}_{1}	152.3^{a}_{2}	31.50^{b}_{1}	26.0^{a}_{2}	65.75^{b}_{1}	64.00^{a}_{1}
Lycium cinereum	69.00^{f}_{1}	68.75^{e}	87.75^{e}_{1}	79.50^{e}_{2}	1.08^{f}_{1}	0.50^{f}_{2}	1.58^{f}_{1}	1.825^{f}_{1}
Monechma genistifolium	92.50^{d}_{1}	86.00^{d}_{2}	105.8^{d}_{1}	103.8^{d}_{1}	25.25^{c}_{1}	24.63^{b}_{1}	52.25^{c}_{1}	42.00^{c}_{2}
Hermanniato mentosa	126.5^{b}_{1}	100.5^{c}_{2}	151.5^{b}_{1}	132.8^{b}_{2}	35.75^{a}_{1}	24.95^{b}_{2}	77.25^{a}_{1}	58.0^{ba}_{2}
Monechma incanum	134.8^{a}_{1}	59.75^{f}_{2}	164.0^{a}_{1}	73.25^{f}_{2}	36.75^{a}_{1}	1.50^{fe}_{2}	77.00^{a}_{1}	4.45^{fe}_{2}
Mean	110.3	86.00	136.8	106.8	23.45	13.55	51.75	31.25
	2.8	2.58	2.93	3.13	0.15	0.40	0.36	1.063

Means with different superscript (a,b,c,d,e,f,g,h) across the column for each parameter are significantly ($p < 0.05$) different; PEG = Polyethylene glycol; PEG (-) denotes presence of tannin, PEG (+) denotes absence of tannin.

Discussion

The Thorny Kalahari Dune Bush veldt is characterized by irregular rainfall all through the year and intense water stress, which results in limited herbaceous flora existing in this part of Africa for most of the year. Thus, browse tree and shrub foliages are the main feed sources for ruminants. In the current study, except for *Olea europaea*, *Terminalia sericea* and *Monechma genistifolium,* the CP values observed were within the optimal range of 110-160 g CP kg^{-1} DM recommended by the NRC (2001) for maintenance requirements of small ruminants. In addition, results obtained in this study for browse species, phenolic components were similar to the results reported by Jayanegara et al. (2011). Although the phenolic concentrations were slightly high, these browse species can be a potential supplement during times of drought. Addition of PEG could overcome adverse effects of high tannins on nutrient availability, as indicated by cumulative gas production, IVOMD, VFA and NH_3-N concentrations. As observed in this study for *Rhus lancea*, *Acacia luederitzii* and *Monechma incanum*, inclusion of PEG during the incubation of tannin-rich plants led to an increase in gas production of 100 % and this has the potential to increase rumen fermentation. Feed fermentation in the rumen and digestibility determine nutritive values of browse and these parameters are further influenced by chemical and phenolic compositions. High gas production indicated more fermentation which supported high rumen microbial production (van Soest, 1994). In the current study, the main factors affecting gas production and IVOMD of browse were high fiber and tannin concentrations. In agreement with current findings, studies done on different tropical browses have showed negative effects of plant phenolic compounds on the fermentation rate and level of digestion (Bhatta et al., 2009; Sebata et al., 2011). The negative effect of tannins on fermentation and digestion could be related to the formation of tannin-carbohydrate and tannin-protein complexes that are less degradable or to its toxicity to rumen microbes (Bhatta et al., 2009). Moreover, it acted as a toxicant to methanogens, reduced acetate and butyrate production (i.e. reduced fiber degradation) or caused a decline in organic matter (OM) digestion (Patra and Saxena, 2011).

Production of methane is a sink for hydrogen in the rumen during the process of utilization of feed energy. However, with fermentation of tannin rich plants, its bacteriocidal and bacteriostatic effects on the rumen microbes, and inactivation of its enzymes, greatly suppresses fermentation and this could result in a decrease of CH_4 production (Patra and Saxena, 2011). In the current study, the presence of tannin decreased methane production in

Table 3 – Mean volatile fatty acid (mmol L^{-1}) production, ammonia N (mg L^{-1}), *in vitro* organic matter digestibility (g kg^{-1} DM) and methane (CH4) production in mass (g kg^{-1} IVOMD) from the browse plants studied after 24 h of incubation.

Species names	Total VFA		NH_3-N		IVOMD		CH4	
	PEG +	PEG -	PEG +	PEG -	PEG +	PEG -	PEG +	PEG -
Trees								
Acacia erioloba	99.6^{i}_{1}	95.0^{h}_{2}	20.5^{f}_{2}	33.3^{b}_{1}	44.0^{d}_{1}	44.6^{c}_{1}	21.0^{b}_{1}	10.5^{fe}_{2}
Boscia albitrunca	113.9^{c}_{1}	106.7^{c}_{2}	22.8^{b}_{2}	36.6^{a}_{1}	62.2^{a}_{1}	62.1^{a}_{1}	17.2^{ed}_{1}	15.4^{ba}_{2}
Acacia haematoxylon	100.1^{h}_{1}	97.2^{g}_{1}	21.7^{c}_{2}	25.1^{e}_{1}	31.0^{f}_{1}	30.9^{d}_{1}	25.3^{a}_{1}	14.0^{bc}_{2}
Olea europaea	109.7^{d}_{1}	106.5^{d}_{1}	19.0^{j}_{2}	22.3^{g}_{1}	53.2^{b}_{1}	49.2^{bc}_{2}	11.9^{f}_{2}	13.1^{dc}_{1}
Ziziphus mucronata	156.3^{a}_{1}	119.7^{a}_{2}	20.7^{e}_{2}	29.2^{c}_{1}	63.2^{a}_{1}	64.7^{a}_{1}	16.1^{e}_{1}	12.4^{d}_{2}
Terminalia sericea	103.0^{g}_{1}	82.1^{j}_{2}	20.2^{g}_{2}	22.8^{f}_{1}	38.5^{e}_{1}	24.9^{d}_{2}	19.2^{cbd}_{1}	15.7^{a}_{2}
Rhus lancea	115.5^{b}_{1}	104.9^{e}_{2}	21.0^{d}_{1}	18.8^{h}_{2}	36.7^{e}_{1}	23.0^{d}_{2}	18.6^{cd}_{1}	11.8^{de}_{2}
Acacia karroo	108.1^{f}_{1}	98.6^{f}_{2}	26.9^{a}_{1}	16.1^{j}_{2}	48.8^{c}_{2}	56.1^{ba}_{1}	19.7^{cb}_{1}	4.72^{g}_{2}
Prosopis glandulosa	108.5^{e}_{1}	110.9^{b}_{1}	19.4^{i}_{2}	25.7^{d}_{1}	55.4^{b}_{1}	56.5^{ba}_{1}	13.8^{f}_{1}	10.1^{f}_{2}
Acacia luederitzii	99.0^{j}_{1}	84.0^{i}_{2}	19.9^{h}_{1}	17.2^{i}_{2}	43.3^{d}_{1}	44.9^{c}_{1}	17.5^{ced}_{1}	4.04^{g}_{2}
Mean	111.4	100.6	2	24.7	47.6	45.7	18.1	11.2
SEM	1.14	1.58	0.51	0.72	0.45	0.58	0.12	0.09
Shrubs								
Acacia mellifera	104.3^{h}_{1}	107.0^{e}_{1}	21.5^{g}_{2}	29.9^{a}_{1}	57.1^{c}_{2}	59.2^{cd}_{1}	12.2^{d}_{1}	12.7^{b}_{2}
Acacia hebeclada	118.0^{b}_{1}	115.6^{b}_{1}	22.4^{b}_{2}	26.2^{c}_{1}	61.8^{b}_{1}	61.1^{cb}_{1}	13.0^{d}_{1}	10.0^{c}_{2}
Grewia flava	106.7^{g}_{1}	101.7^{h}_{1}	22.0^{d}_{2}	28.5^{b}_{1}	32.5^{g}_{1}	30.9^{g}_{2}	22.0^{b}_{1}	18.3^{a}_{2}
Dichrostachys cinera	111.8^{d}_{1}	83.5^{i}_{2}	21.2^{h}_{1}	20.0^{h}_{1}	33.5^{g}_{1}	31.0^{g}_{2}	25.7^{a}_{1}	7.21^{d}_{2}
Hermannia burchelli	107.0^{f}_{1}	107.7^{d}_{1}	21.5^{f}_{1}	22.3^{f}_{1}	56.2^{c}	63.5^{b}_{1}	17.5^{c}_{1}	12.1^{b}_{2}
Lycium cinereum	107.9^{e}_{1}	105.7^{f}_{1}	22.7^{a}_{2}	25.3^{d}_{1}	77.1^{a}	81.1^{a}_{1}	3.54^{e}_{1}	2.16^{f}_{2}
Monechma genistifolium	103.5^{i}_{1}	103.1^{g}_{1}	16.4^{i}_{2}	21.5^{g}_{1}	$52.5d_{2}$	57.1^{d}_{1}	18.0^{c}_{1}	12.9^{b}_{2}
Hermanniato mentosa	133.1^{a}_{1}	116.2^{a}_{2}	22.3^{c}_{2}	24.0^{e}_{1}	40.9^{f}_{2}	47.3^{e}_{1}	25.6^{a}_{1}	17.4^{a}_{2}
Monechma incanum	114.8^{c}_{1}	114.0^{c}_{1}	21.9^{f}_{1}	19.9^{i}_{2}	47.6^{e}_{1}	38.7^{f}_{2}	20.7^{b}_{1}	5.42^{e}_{2}
Mean	111.9	106.0	2.13	24.7	51.0	52.2	17.6	10.9
SEM	3.54	5.12	0.57	0.68	0.58	0.36	0.17	0.089

Means with different superscript (letters) within a column for each parameter are significantly ($p < 0.05$) different; PEG = Polyethylene glycol; PEG (-) denotes presence of tannin, PEG (+) denotes absence of tannin. VFA = volatile fatty acids; IVOMD = *in vitro* organic matter digestibility.

Table 4 – Correlation coefficients (r) between *in vitro* fermentation parameters with chemical composition and phenolic compounds of browse plants studied.

	IVOMD	TVFA	NH_3-N	GP_{24}	CH_4
Chemical composition					
Ash	0.00043	0.40475	0.30581	0.43446	0.38066
OM	0.00043	0.40475	0.30581	0.43446	0.38066
CP	0.66639*	0.28331	0.50042*	0.47026	0.08677
NDFN	0.0054	-0.31476	-0.2924	-0.3473	-0.46133
NDF	-0.74652*	-0.5932*	-0.5304	-0.67893*	-0.3723
ADF	-0.63965*	-0.38326	-0.34233	-0.50149*	-0.07653
ADL	-0.27028	-0.0922	0.04748	-0.18242	-0.00323
NFC	0.05584	0.01108	-0.01605	0.06941	-0.11949
Phenolic components					
TP	-0.69493*	-0.5631*	-0.55093*	-0.73452*	-0.63429*
TT	-0.60308*	-0.4968*	-0.58449*	-0.7401*	-0.67445*
CT	-0.56258*	-0.47298*	-0.58643*	-0.73431*	-0.67601*
HT	-0.61957*	-0.50323*	-0.56955*	-0.72738*	-0.65773*

OM = organic matter; CP = crud protein; NDFN= Nitrogen in neutral detergent fiber; NDF = neutral detergent fiber; ADF = acid detergent fibre; ADL = acid detergent lignin; NFC = non-fibre carbohydrates; TP = total phenols; TT = total tannins; CT = condensed tannins; HT = hydrolysed tannins; IVOMD = *in vitro* organic matter digestibility; TVFA = total volatile fatty acids; NH_3-N- rumen ammonia; GP_{24}- gas production; CH_4- methane.

all the browse species. The decrease in CH_4 production with the inclusion of tannin in ruminant diets was similarly reported by several researchers (Sebata et al., 2011; Sultan et al., 2012). In addition, with an increase in tannin concentration in browse species, a reduction effect on methane production was found. The impact of tannins on methane production varies with tannin chemical structure (plant origin) as well as with its concentration.

In tropical ruminant production systems, it is also very important that systematic and strategic supplementation of these forages in a mixed diet should be the way forward in their utilization. In such a way it is possible to attain a maximal depressing effect on enteric CH_4 production with minimal detrimental effect on rumen fermentation characteristics of poor quality roughage based diets. This can also insure the best option of utilization of these browses in all systems of livestock production.

Conclusion

From the results obtained in this study, variations were observed in the fermentation of trees and shrubs as well as in methane gas production. For example, tree forage (*Rhus lancea* and *Acacia luederitzii*) and shrub forage (*Dichrostachys cinera* and *Monechma incanum*) decreased methane and gas production significantly and it seems that *Acacia luederitzii* and *Monechma incanum* have a prolonged effect on CH_4 production. The substantial amount of CP content of certain species coupled with anti-methanogenic effect during fermentation, would offer farmers and pastoralists farming in sub-Sahara Africa an opportunity to use some of the browses for reducing CH_4 production. However, further studies need to be undertaken to determine the variation in the efficacy of the browse samples collected in different seasons in reducing CH_4 and mechanisms to integrate these browses to complement low quality feeds for different ruminant groups.

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