Chemical composition, in vitro dry matter digestibility and Gas production of four browse species and their combinations used as feed for small ruminants

Article *in* West African Journal of Applied Ecology · December 2020

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Chemical composition, *in vitro* dry matter digestibility and Gas production of four browse species and their combinations used as feed for small ruminants

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Abstract

Browse species as complete feed for ruminants is uncommon. This may be due to low dry matter (DM) and high condensed tannins (CT) contents limiting its potential to influence weight gain. Drying however improves DM content and reduces CT levels and its astringency. The objective of this study was to determine chemical composition, *in vitro* parameters and to evaluate the potential benefits of feeding small ruminants on dried browse leaves and their combinations. The browse species were *Albizzia lebbek*, *Gliricidia sepium*, *Moringa oleifera* and *Millettia thoningii*. Rumen fluid was obtained from two fistulated forest type wethers for the *in vitro* evaluation. The DM, crude protein (CP), ash, CT, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose and lignin were 866-916 g/kg, 101-303 g/kg DM, 74.7-200 g/kg DM, 0.9-1.3 g/kg DM, 202-552 g/kg DM, 205-520 g/kg DM, 94-381 g/kg DM and 105-192 g/kg DM respectively. The organic matter digestibility (OMD), *in vitro* dry matter digestibility (IVDMD), *in vitro* gas production (IVGP), metabolisable energy (ME) and short chain fatty acids (SCFA) of the four browse species and their combinations recorded were 314.9-721.6 g/kg DM, 515.4-721 ml/g DM, 139-602 ml/g DM, 314.6-1406.9 ml/g DM and 3.1-14.4 ml/g DM respectively. There were positive associative effects shown by the combined browse leaves between IVDMD and IVGP. The regression analyses revealed that relationships between IVGP and CP and IVDMD and CT and all relationships between OMD, SCFA, ME and IVGP were significant ($p<0.05$). All the browse species and their combinations had IVDMD values of more than 500 g/kg DM and low gas production. The high CP and ash contents, low CTs, low to moderate fibre components, moderate to high IVDMD and low IVGP of the four browse species and their combinations make them potentially valuable as feed resources for small ruminant production.

Introduction

The production of gas parameters of browse species might indicate variations in their nutritional content that may be closely related to their chemical components (Cerrillo and Juarez, 2004).The *in vitro* gas production (IVGP) technique has become an important tool to assess potential rumen digestibility of ruminants diets (Getachew et al. 2002; Salem et al., 2007). This technique permits the calculation of the fraction degraded which may either be fermented to produce volatile fatty acids (VFAs) or incorporated into the biomass of microbes and energy levels of browse species (Blummel et al. 2003; Salem et al., 2007). Additionally, IVGP technique is valuable when evaluating the potential of browse species containing tannins in nutrition of ruminants (Salem et al. 2007; Norman et al., 2010). The physicochemical components of feeds influence the dynamics of fermentation in the rumen (Tedeschi et al., 2009).

The use of browse species as a supplement to roughage or fibrous feeds is common (Annan and Tuah, 1999; Fleischer et al. 2000; Sarkwa, 2008; Idan, 2014; Adjorlolo et al., 2020) but the use of browse species as a complete feed is not. This may be due to low dry matter (DM) limiting its potential to influence live weight gain and high CT (Condensed tannins) (> 40 g/kg DM) content which reduces its intake, availability of metabolisable energy (ME) and absorption of amino acid by animals (McSweeney et al., 2001; Basha et al., 2012). Drying of browse species may improve DM content and reduce CT levels and its astringency. However, the chemical composition, *in vitro* dry matter digestibility (IVDMD), IVGP, SCFA (Short chain fatty acids), OMD (Organic matter Digestibility) and ME of dried forms (48 hours sun dried) of the four browse species and their combinations and the samples taken in both wet and dry seasons and bulked to reveal the quality of these browse leaves throughout the year have not been documented. This gap is what this study sought to address. It was hypothesized that IVDMD will highlight the benefit of combining the dried browse species in feeding sheep and there may be positive associative effects. The objective of this study was to determine the potential benefits of feeding small ruminants on dried leaves of four browse species and their combinations. This was done by evaluating the chemical composition, IVDMD, IVGP, ME, SCFA and OMD of four dried browse species and their combinations.

Materials and Methods

Location

The study was carried out at the Livestock and Poultry Research Centre (LIPREC), University of Ghana, Legon (5° 68'N, 0°10'W). The pattern of rainfall is bimodal with the major rains in June while the minor is in September-October. Total rainfall ranges from 508 mm to 743 mm annually. Temperatures varies between 24ºC and 33 ºC.

Preparation of forage samples, procedure for data collection and estimation of outputs

Fresh samples of *Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) were collected monthly in May, June and July for wet season and December, January and February for dry season to represent early, mid and late collections for both seasons. All collected samples were sun dried for 48 hours and ground. Both wet and dry season samples were bulked for the analyses (early wet and dry: Replicate 1; mid wet and dry: Replicate 2; late wet and dry: Replicate 3) and were mixed thoroughly for each browse leaves. The same principle of bulking early wet and dry season as Replicate 1, mid wet and dry season as Replicate 2 and late wet and dry season were used to formulate the combinations of the browse leaves. This sample collection was done to have a good representation for the whole year. Rice straw was chopped and treated with urea (Fleischer et al. 2000). Urea treated rice straw (UT) was added to this study because it is one of the dry season feeding strategies (Fleischer et al., 2000; Amaning-Kwarteng et al., 2010) just as browse leaves was used as control. The browse species used in this study were *Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO), *Millettia thonningii* (MT), AL+MO+MT, AL+GS+MT, AL+GS+MO+MT, AL+MO+GS, AL+MO, AL+MT, AL+GS, GS+MT, MO+GS, MO+GS+MT, MO+MT and urea treated rice straw. Proximate, fibre components and CT were determined using the methods of A.O.A.C (2016), Goering and Van Soest (1970) and butanol-HCl method as described by Iqbal et al. (2011) respectively.

The IVDMD was analysed by the method of Menke and Steingass (1988). Rumen liquor for the *in vitro* work was obtained from two forest type fistulated wethers of average weight 23kg. The fistulation was carried out by using the Required Surgical Procedures for fistulating the gastrointestinal tract as documented by the Department of Primary

Industries of New South Wales Government, Australia (NSM Government, 2012). Modified version of rumen cannulae as described by Elices et al. (2010) was used. The fistulated animals were fed fresh *Panicum maximum* (Average daily Energy intake was 21.88 MJ/ kg DM) and provided water on *ad lib* basis. There was two weeks feed adjustment period before collection of rumen fluid. The rumen content was collected through the cannulae between 8am- 9am before feeding. The rumen content was strained through four layers of cheese cloth to obtain the rumen liquor. The use of fistulated animals was approved by Noguchi Institutional Animal Care and Use Committee of University of Ghana, Legon (Protocol number 2017-02-2R).

Half gram each of the samples were weighed in duplicates and placed into a labelled 50 ml centrifuge tube. Twenty eight millilitres (28 ml) of McDougall's solution was poured into each of the centrifuge tubes and prewarmed in water bath at 39 ºC. A buffer and ruminal fluid solution (in a ratio of 4 to one) of 7 ml was added. The tubes were flushed with carbon dioxide and the caps were placed on the tube. The tubes were inverted several times to suspend the samples and placed on a rack. Four blank tubes containing no sample and 35 ml McDougall's to rumen fluid mixture were also placed on the rack. Then, the rack was placed into a water bath at 39 ºC. The tubes were incubated for 48 hours and inverted 2, 4, 8, 20 and 28 hours after the start of incubation to suspend the samples. The tubes were removed from the water bath after 48 hours of incubation and were centrifuged for 15 minutes at 2000 xg. The liquid was suctioned off by vacuum and the samples were frozen until pepsin digestion was carried out. Thirty five millilitres (35 ml) of pepsin solution was poured into each tube and incubated for 48 hours in a water bath at 39 ºC. The samples were shaken at 2, 4 and 6 hours after pepsin solution was added. At the end of the pepsin digestion, the samples were filtered using the modified Buchner funnel and ashless filter paper. The filter paper containing the samples were placed in an aluminium pan and dried in an oven at 105 ºC for 24 hours. The dried samples (DS) were weighed and the difference between the initial weight (IW) and the DS was noted as degraded weight (DW). IVDMD (%) was calculated as follows:

$$\mathbf{IW - DS = DW \ldots\ldots\ldots\ldots (i)}$$

$$\mathbf{IVDMD\ (\%) = DW/\ IW \times 100\ \% \ldots\ldots (ii)}$$

Rumen liquor was obtained from two fistulated forest type wethers of average weight 23kg for the IVGP. The IVGP was carried out by the procedure of Menke and Steingass (1988) as validated by Anele et al. (2009). The pistons were greased with vaseline and 200 mg of each of the samples were weighed into the syringes (Triplicate per each sample). Rumen fluid was collected from two fistulated forest type wethers and was strained through two layers of cheese cloth into a warm two litres flask filled with carbon dioxide. A medium comprising of water, micro element solution, buffer solution, resazurin and reduction solution was prepared. Two parts of the prepared medium was mixed with one part of rumen fluid and kept under carbon dioxide in a water bath at 39 ºC and stirred using magnetic stirrer. Thirty millilitres (30 ml) of rumen fluid-medium mixture was added to the piston glass syringe and pre-warmed at 39 ºC. Any gas bubbles found in the syringe was removed and the plastic clip on the rubber tube was closed. The syringe was placed in a Hohenheim gas tester at 39 ±0.5 ºC. The syringes were shaken automatically in the Hohenheim gas tester. Production of gas was noted at 3, 12 and 24 hours after incubation. Maize starch and *Panicum maximum* hay were used as standards for the IVGP work. Fifty seven syringes were used per cycle (15 browse + 1 UT (Urea treated straw) + 1 Starch + 1 Starch and *Panicum maximum* hay + 1 Blank = 19; 19 X 3 = 57). Gas production (Gp) was calculated as follows:

$$\mathbf{G_p = (V_{24}\, V_0 - G_{p0})\,(F_H + F_{HS})\,/\,2 \ldots (iii)}$$

$V_{24}\, V_0$ is the total increase in volume of gas or gas produced at 24 hours for sample;

G_{p0} is volume of gas (ml) produced at 24 hours for blank (mixture of rumen fluid and medium);
F_H is gas production for *Panicum maximum* hay (200 mg) at 24 hours;
F_{HS} is gas production for *Panicum maximum* hay (140 mg) and maize starch (60 mg).
Menke and Steingass (1988) equations were used to estimate predicted SCFA, ME and OMD as follows:

SCFA = 0.0239IVGP (ml/g DM;24 hours) 0.060 ....(iv)

ME = 2.20 + 0.136 IVGP (ml/g DM; 24 hours) + 0.00574 CP…......................…(v)

OMD=14.88 + 0.889 IVGP (ml/g DM; 24hours) + 0.45CP + 0.651 Ash ………(vi)

This IVDMD and IVGP procedures were done in three different cycles.

Experimental Design and Statistical Analyses

The data were analysed using a completely randomised design and subjected to analyses of variance (ANOVA) using GenStat (2009) version 12.1 according to the model as follows:

Yij = μ + Ti + Eij ………………..(vii)

Yij is the response variable such as DM, CT, Ash, CP, NDF, ADF, Cellulose and lignin, IVDMD, IVGP, ME, OMD and predicted SCFA; μ is the overall mean;

Ti is the different browse leaves; Eij is the residual error.

Significant differences were separated using Student Newman Keuls Test. Relationships between chemical composition, IVDMD, IVGP, SCFA, OMD and ME were determined by regression analyses.

Results

Chemical Composition and In Vitro Dry Matter Digestibility

TABLE 1
Proximate, Fibre and Condensed Tannins Content (g/kg DM) of Four Browse Species and their Combinations Sun Dried for 48 Hours

Species	DM	CP	Ash	NDF	ADF	Cellulose	Lignin	CT
AL	900[a]	287[bcd]	74.7[a]	453[h]	354.9[g]	156.[fg]	192[g]	1.2[a]
GS	866[a]	288[bcd]	115[cd]	325[d]	257[c]	115.[bc]	139[cd]	0.9[a]
MO	873[a]	330[e]	157[f]	202[a]	205[a]	94.[a]	105a	1.0[a]
MT	894[a]	234[a]	109[c]	534[j]	391[h]	235.6[k]	139[cd]	1.1[a]
AL + GS + MT	889[a]	270[bc]	102[bc]	493[i]	336[f]	187[i]	155[ef]	1.1[a]
AL + GS + MO + MT	888[a]	289[bcd]	130[e]	398[f]	303[e]	151[f]	131[bc]	1.3[a]
AL + MO + GS	887[a]	302[bcd]	139[e]	324[d]	268[c]	125[c]	149[de]	1.1[a]
AL + MO + MT	889[a]	287[bcd]	117[cd]	419[g]	302[e]	167[gh]	143[cde]	1.2[a]
AL + MO	885[a]	304[cd]	127[de]	320[d]	261[c]	125[d]	148d[e]	1.1[a]
AL + MT	895[a]	266[b]	94.6[d]	476[i]	351[g]	198[j]	162[f]	1.2[a]
AL + GS	886[a]	284[bcd]	104[bc]	364[e]	305[e]	139.[e]	164[f]	1.1[a]
GS + MT	887[a]	267[b]	110[c]	394[f]	327[f]	178[h]	139[cd]	1.0[a]
MO + GS	877[a]	309[de]	141[e]	263[b]	236[b]	107[b]	138[cd]	1.1[a]
MO+ GS + MT	877[a]	290[bcd]	135[e]	354[e]	289[d]	157[fg]	131[bc]	1.1[a]
MO + MT	886[a]	286[bcd]	136[e]	287[c]	288[d]	168[gh]	124[b]	1.1[a]
UT	916	101	200	552	520	381	191	-

Means with different superscripts in a column are significantly different ($p<0.05$).
DM= dry matter, CP= crude protein, NDF= neutral detergent fibre, ADF= acid detergent fibre, CT= condensed tannins
NB: Chemical composition for UT was not statistically analysed because it was obtained as one bulk feed

TABLE 2

In Vitro Dry Matter Digestibility (IVDMD), Gas Production(IVGP) (ml/g DM), Organic Matter Digestibility (OMD), Short Chain Fatty Acids (SCFA) and Metabolisable Energy (ME) of Four Browse Species and their Combinations Sun Dried for 48 Hours

Species	OMD (g/kg DM)	IVDMD (g/kg DM)	IVGP (0-3hrs)	IVGP (3-12hrs)	IVGP (12-24hrs)	IVGP (24hrs)	SCFA (ml/g DM)	ME (kJ/g DM)
AL	419.0 ^{cde}	554 ^{ab}	101 ^{ef}	42.1 ^{c}	72.0 ^{abcd}	215. ^{cd}	5.1 ^{cd}	504.5 ^{cde}
GS	399.3 ^{bcd}	721 ^{c}	118. ^{g}	16.8 ^{ab}	67.6 ^{abcd}	202 ^{cd}	4.8 ^{bcd}	474.4 ^{bcde}
MO	442.2 ^{de}	631 ^{abc}	61.8 ^{ab}	67.4 ^{d}	69.6 ^{abcd}	199 ^{cd}	4.7 ^{bcd}	466.2 ^{bcde}
MT	314.9 ^{a}	581 ^{ab}	73.0 ^{bcd}	16.9 ^{ab}	55.0 ^{abcd}	139 ^{a}	5.6 ^{d}	364.9 ^{ab}
AL + GS + MT	357.2 ^{b}	555 ^{ab}	84.3 ^{cde}	33.7 ^{abc}	56.0 ^{abcd}	174 $^{ab\ c}$	4.1 ^{abc}	407.9 ^{abcd}
AL + GS + MO + MT	448.1 ^{e}	534 ^{a}	118 ^{fg}	67.4 ^{d}	61.4 ^{abcd}	246 ^{d}	5.8 ^{d}	575.6 ^{e}
AL + MO + GS	382.0 ^{bc}	598 ^{ab}	78.6 ^{bcde}	44.9 ^{c}	37.0 ^{ab}	158 ^{ab}	3.7 ^{ab}	370.3 ^{abc}
AL + MO + MT	315.4 ^{a}	586 ^{ab}	62.2 ^{abc}	27.7 ^{abc}	44.1 ^{abc}	134 ^{a}	3.1 ^{a}	314.6 ^{a}
AL + MO	418.5 ^{cde}	590 ^{ab}	92.7 ^{de}	36.4 ^{abc}	77.9 ^{bcd}	207 ^{cd}	4.9 ^{bcd}	488.9 ^{bcde}
AL + MT	371.5 ^{b}	602 ^{ab}	61.8 ^{abc}	112.3 ^{e}	28.3 ^{a}	202 ^{bcd}	4.8 ^{bcd}	474.9 ^{bcde}
AL + GS	387.8 ^{bc}	586 ^{ab}	89.8 ^{de}	33.7 ^{ac}	93.6 ^{cd}	211 ^{cd}	5.0 ^{bcd}	495.3 ^{bcde}
GS + MT	390.9 ^{bc}	561 ^{ab}	50.5 ^{a}	67.4 ^{d}	88.4 ^{cd}	206 ^{cd}	4.9 ^{bcd}	483.4 ^{bcde}
MO + GS	439.3 ^{de}	676 ^{bc}	84.2 ^{bde}	33.7 ^{abc}	99.7 ^{d}	218 ^{cd}	5.1 ^{cd}	510.4 ^{de}
MO+ GS + MT	423.5 ^{cde}	631 ^{abc}	89.8 ^{de}	44.8 ^{c}	79.2 ^{bcd}	214 ^{cd}	5.1 ^{cd}	501.4 ^{cde}
MO + MT	444.0 ^{de}	658 ^{abc}	123.5 ^{g}	33.7 ^{abc}	80.9 ^{bcd}	238 ^{d}	5.6 ^{d}	557.8 ^{e}
Urea treated straw(UT)	721.6 ^{f}	515.4	140.4 ^{h}	186 ^{f}	276 ^{e}	602 ^{e}	14.4 ^{e}	1406.9 ^{f}

Means with different superscripts in a column are significantly different ($p<0.05$).

NB: IVDMD of UT was not statistically analysed because it was obtained as one bulk feed

The chemical component of the feeds used for the *in vitro* analyses have been presented in Table 1. The IVDMD values recorded range from 534.4-720.5 g/kg DM with the browse leaves, *Gliricidia sepium* (GS) being the highest ($p<0.05$) and the combination of *Albizzia lebbek*, *Gliricidia sepium*, *Moringa oleifera* and *Millettia thoningii* (AL+GS+MO+MT) being the lowest ($p<0.05$) as presented in Table 2. All samples of browse species had more than 500 g/kg DM (Table 2). *In vitro* dry matter digestibility tended to increase with increasing crude protein levels as shown in Table 3.On the contrary, IVDMD declined as ADF, lignin and cellulose contents increased (Table 3). In terms of positive associative effect, the combination of browse leaves over sole browse in IVDMD were as follows: combinations of AL had the highest (AL+GS+MT, AL+MO+GS, AL+MO, AL+MT, AL+GS and AL+MO+MT) followed by combinations of MT (AL+MO+MT,

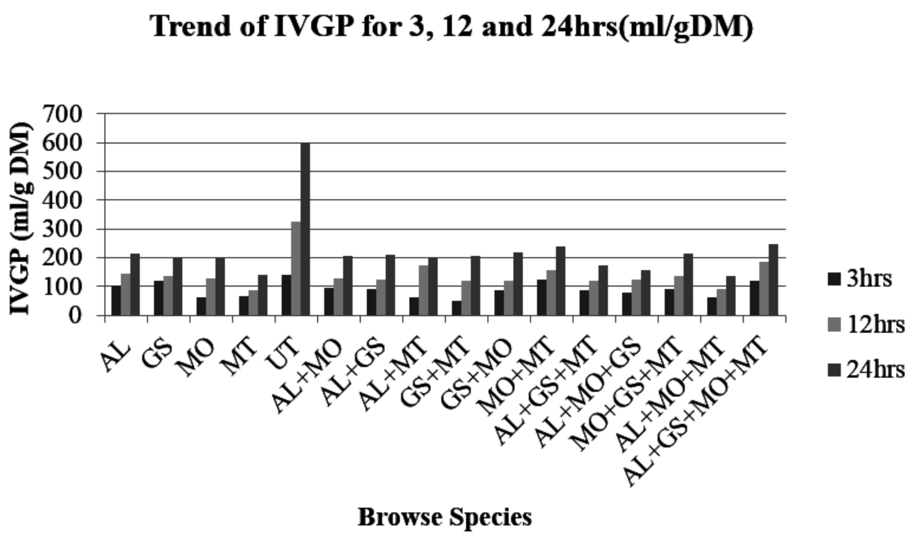

Fig. 1 Trend of *in vitro* gas production (IVGP) (ml/g DM) for 3, 12 and 24 hours of sole and combinations of four browse species

TABLE 3
Relationships between IVDMD, IVGP, SCFA, OMD, ME and Chemical Composition of the Four Browse Species and their Combinations

Relationship between parameters	Equations	Regression
IVDMD & IVGP for four browse species and their combinations	Y= 0.3583x+533.03	0.05 NS
IVDMD & IVGP for four browse species	Y= -0.5277x+721.02	0.06 NS
IVDMD & IVGP for the combinations	Y= 0.7212x+452.7	0.31 NS
IVDMD & CP for four browse species and their combinations	Y= 0.8258x+367.51	0.13 NS
IVDMD & CP for four browse species	Y=0.5849x+454.86	0.10 NS
IVDMD & CP for the combinations	Y=1.4214x+189.95	0.23 NS
IVDMD & ADF for four browse species and their combinations	Y= -0.6171x+787.91	0.35 NS
IVDMD & ADF for four browse species	Y= -0.7338x+788.9	0.46 NS
IVDMD & ADF for the combinations	Y= -0.7502x+820.22	0.34 NS
IVDMD & Lignin for four browse species and their combinations	Y=1.0132x+749.64	0.16 NS
IVDMD & Lignin for four browse species	Y= -0.9942x+764.21	0.24 NS
IVDMD & Lignin for the combinations	Y= -1.0451x+747.94	0.10 NS
IVDMD & cellulose for four browse species and their combinations	Y=0.6427x+702.43	0.22 NS
IVDMD & Cellulose for four Browse Species	Y= -0.6163x+722.73	0.33 NS
IVDMD & Cellulose for the combinations	Y= -0.5593x+683.98	0.14 NS
IVGP & CP for four browse species and their combinations	Y=5.0467x+53.167	0.12 NS
IVGP &CP for four browse species	Y =6.5979x+1.237	0.59 *
IVGP & CP for the combinations	Y =1.3486x+162.03	0.0035 NS
CT & IVDMD for four browse species and their combinations	Y= -0.0013x+1.8815	0.46 NS
IVDMD & CT for four browse species	Y= -0.019x+2.1976	0.99 *
IVDMD & CT for the combinations	Y= -0.0006x+1.4697	0.12 NS
CT & IVGP for four browse species and their combinations	Y= -2.535x+200.37	0.000006 NS
CT & IVGP for four browse species	Y= -38.95x+229.36	0.03 NS
CT & IVGP for the combinations	Y= -21.069x+1.2101	0.44 NS
OMD & SCFA for four browse species and their combinations	Y = 0.0269x-5.9631	0.9391 *
OMD & SCFA for four browse species	Y= 0.0133x-0.7685	0.83 *
SCFA & IVGP for four browse species and their combinations	Y=41.749x+2.946	1 *
SCFA & IVGP for four browse species	Y=41.924x+2.1803	1 *
ME & IVGP for four browse species and their combinations	Y=0.4314-3.5527	0.9986 *
ME & IVGP for four browse species	Y=0.5567x-62.967	0.9959 *
OMD & IVGP for four browse species and their combinations	Y=1.1241x-246.09	0.9395 *
OMD & IVGP for four browse species	Y=0.5561x-30.053	0.8301 *
ME & SCFA for four browse species and their combinations	Y=0.0103x-0.1557	0.9986 *
ME & SCFA for four browse species	Y=0.0133x-1.5527	0.9955 *

NB: NS=Not significant * = p<0.05

AL+MT, MO+GS+MT and MO+MT), then combinations of MO (MO+GS, MO+GS+MT and MO+MT) and GS had none. Apart from the relationship between IVDMD and IVGP for AL, GS, MO and MT samples (Table 3) which was not consistent with the relationship between IVDMD and IVGP for the AL, GS, MO and MT and their combinations (Table 3), the rest were consistent with their corresponding relationships for the four browse species and their combinations (Table 3).

In Vitro Gas Production (IVGP), Short Chain Fatty Acids (SCFA) and Metabolisable Energy (ME)

The IVGP obtained in this study was between 134.0-602.0 ml/g DM with AL+MO+MT producing the lowest ($p<0.05$) and UT yielding the highest ($p<0.05$) as shown in

Table 2 and Figure 1. The UT recorded the highest ($p<0.05$) gas production in all the three periods (Table 2 and Figure 1). In general, the largest volume of gas was produced within the first three hours, followed by the last twelve hours and the least between 3 to 12 hours (Table 2). All the browse species produced less than 100 ml/g DM between 12-24 hours (Table 2). Urea treated rice straw, MO and GS produced more gas as period of incubation increased (Table 2). The SCFA values recorded in this study ranged from 3.14 – 14.35 ml/g DM ($p<0.05$) as shown in Table 2. AL+MO+MT recorded the lowest ($p<0.05$) SCFA level and UT the highest ($p<0.05$). The ME values obtained in this current study was between 314.6 kJ/g DM ($p<0.05$) and 1406.9 kJ/g DM ($p<0.05$) as presented in Table 2. Urea treated rice straw (UT) sample recorded the highest ($p<0.05$) ME value and AL+MO+MT, the lowest ($p<0.05$). The OMD values recorded in this current study was from 314.90 g/kg DM ($P<0.05$) to 721.56 g/kg DM ($P<0.05$) as shown in Table 2. Regarding positive associative effects of IVGP for the combined browse leaves over the sole browse leaves: combinations of AL had the highest (AL+GS+MT, AL+MO+GS, AL+MO+MT, AL+MO, AL+MT and AL+GS), followed by combinations of GS (AL+GS+MT, AL+MO+GS, AL+GS), then combinations of MO (AL+MO+GS, AL+MO+MT) and combination of MT (AL+MO+MT) had the least.

The relationships between IVGP and CP of the four dried browse species and their combinations, IVGP and CP of the four dried browse species and IVGP and CP for combinations of the four dried browse species and all relationships between OMD, SCFA, ME and IVGP were positive (Table 3). Apart from the relationships between IVGP and CP and IVDMD and CT of the four sole browse species and all relationships between OMD, SCFA, ME and IVGP which were significant ($p<0.05$), the rest were not significant ($p>0.05$).

Discussion

Chemical Composition and In Vitro Dry Matter Digestibility (IVDMD)

The CP values recorded in this study were similar to the CP values of browse leaves reported in Ghana by some researchers (Fleischer et al. 2000; Sarkwa et al., 2011). Balogun (1998) worked on CT concentration of 14 browse leaves in Australia including *Gliricidia sepium* and *Albizia lebbek*. *Gliricidia sepium* and *Albizia lebbek* recorded less than 10 g/kg DM which was lower than the threshold of more than 40 g/kg which may have detrimental effect on digestibility (McSweeney et al., 2001). However, the CT values of browse leaves reported by Balogun et al. (1998) were higher than the values recorded in this study (0.9-1.3 g/kg DM). The results obtained indicate that the dried browse species have moderate digestibility and are less fibrous. Therefore, these browse species can be used for supplementing ruminants with limited need for concentrates since constraints to nutrient supply is due to digestion inefficiencies. The results of IVDMD were encouraging since all the browse species had digestibility higher than 500g/kg DM. The IVDMD levels of all browse species and their combinations were far higher than 450 g/kg DM quoted by Youngquist et al. (1990) to be acceptable for weight maintenance of cattle in the tropics. This highlights the potential of using these browse species and their combinations in feeding sheep to obtain good weight gain.

In Vitro Gas Production (IVGP), Short Chain Fatty Acids (SCFA), Organic Matter Digestibility (OMD) and Metabolizable Energy (ME)

This study recorded IVGP of 134-245ml/g DM for the browse species. This is in harmony with 127-271ml/g DM and 200-221ml/g DM reported by Abdala et al. (2012) in some Brazilian browses and Ammar et al. (2004) in some Spanish browses respectively. However, Abdel-Fattah et al. (2005) reported a range of 265-315ml/g DM and Fondevila et al. (2002) reported a range of 306-380 ml/g DM which were higher than the range recorded in this study. On the other hand, Anele et al. (2009) reported a lower range of 36.7-46 ml/g DM.

Hristov et al. (2008) referred to an earlier report by Hungate (1966) and reported that short-term incubations were more suitable for estimating fermentation in the rumen than long-term incubations. The differences in gas production reported by various authors working on browse species (Abdala et al., 2012; Ammar et al., 2004; Abdel-Fattah et al., 2005; Fondevila et al., 2002) is attributable to differences regarding species, chemical composition, condensed tannins content, type of ruminant that the rumen fluid was obtained from for the work and the duration of incubations. Gas production levels from different leaves vary and this could be due to the nature and proportion of fibre present in the leaves (Rubanza et al., 2003).

It is noteworthy that, UT produced majority of the gas during the last twelve hours and this is not in agreement with the report by Sangkhom et al. (2017) which indicated that, most of the gas production occurred within the first twelve hours when the authors used glycerol treated rice straw. The difference in the amount of gas produced for rice straw could be due to the different treatment given to the rice straw which may have altered its chemical composition. According to Abdel-Fattah (2005), rumen liquor from sheep, cattle and buffalo have different fermentation abilities. Therefore, the difference in gas production in the current study and that of Sangkhom et al. (2017) could be due to differences in the rumen liquor used, the current study obtained the liquor from sheep while Sangkhom et al. (2017) obtained it from cattle.

The low IVGP in the browse species samples as compared to that of UT in the present study may be because the browse species have high crude protein contents which may not have been fermented extensively. This in agreement with the findings of Cone and Van Gelder (2000) that protein is not fermented extensively and ammonia produced as a result of fermentation decreases estimated volume of gas produced. This may be in contrast to gas produced from pasture grass whereby Kulivand and Kafilzadeh (2015) found that CP was positively correlated with gas production from eight natural pasture from Iran. The low IVGP may be due to the presence of CT in the browse species. Condensed tannins concentration in these plants ranged from 0.9 to 1.3 g/kg DM. This confirms earlier reports that total IVGP reduced in response to increased CT levels (Huang et al., 2010; Tan et al., 2011). Also, Mbugua et al (2008) and Theodoridou et al. (2011) reported that lower IVGP from tropical forage legumes containing CT may be because of degradation of organic matter but not fermentation. Meale et al. (2012) reported that samples with high IVGP are associated with higher methane production than samples with low IVGP. This is understandable since Kulivand and Kafilzadeh (2015) found that with increasing NDF and ADF, potential gas production and the fermentation rate constant decreased. The role of NDF and ADF in this scenario is important in that, methane production and fibre content are directly proportional (Kirchgessner et al., 1995; Jayanegara et al., 2009), however NDF and ADF concentration in the current study are within acceptable level for stimulating rumen function and saliva production and cannot be considered too high for browse plants. This highlights the potential of using these browse species and their combinations in feeding sheep to produce low and acceptable levels of methane.

According to Van Soest (1994), substrate fermentation to propionate is due to the buffering of acids leading to less gas production. However, high amounts of gases are formed if substrates are fermented to produce butyrate and acetate (Van Soest, 1994). Makkar et al. (1995) reported that the correlations between gas production and SCFA were highly significant. Similar relationship was obtained in this study because regressions between IVGP and SCFA were highly significant. High gas production is associated with high production of SCFA and high OMD (Makkar et al. 1995).

Relationship between IVDMD and IVGP of the four browse species alone was contrary to the relationship between IVDMD and IVGP of the four browse species and their combinations. This confirms the report by Abdel-Fattah (2005) which indicated that the relationships between IVDMD and IVGP are not consistent. The difference in relationships by different authors may be due to the treatment given to the browse species samples prior to the gas production work. For instance, in this study the browse species were subjected to 48 hours sun drying whilst Abdel-Fattah (2005) used the normal oven drying for laboratory work. The relationships between IVDMD and CP and IVGP and CP were positive whilst the relationship between IVDMD and fibre components were negative and this confirms earlier reports by Ammar et al. (2004) and Kulivand and Kafilzadeh (2015). Results of this study corroborates the reports by Evitayani et al. (2004) and Andualem et al. (2016) that there are strong positive relationships between IVGP, OMD and ME.

Conclusion

The dried browse species and their combinations were high in crude protein and ash, low in condensed tannins, low to moderate in fibre components, moderate to high IVDMD and low IVGP. There were positive associative effects in the combined browse leaves with respect to IVDMD and IVGP. From the results, it can be concluded that the four browse species and their combinations are potentially valuable feed resources for small ruminant production.

Acknowledgements

This study was mainly funded by University of Ghana Faculty Development Fund and partially by TRECCAFRICA (European Union). The logistics support of Livestock and Poultry Research Centre, University of Ghana, Legon is highly appreciated. This work is a component of PhD research of the first author at University of Botswana. Authors are very grateful to Dr. R. Ayizanga for assisting with data analyses.

Competing Interest

The authors of this paper declared that they do not have any competing interest for this joint publication.

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