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Full Length Research Paper

Assessing the feeding values of leaves, seeds and seeds-removed pods of *Moringa stenopetala* using *in vitro* gas production technique

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This study presents the feeding values of leaves, seeds and seeds-removed pods of *Moringa stenopetala* for ruminants as evaluated by *in vitro* gas production technique. Feed samples were analyzed for proximate nutrients using official methods. Each feed samples (200 mg) were incubated in buffered rumen fluid for 96 h and fermentation characteristics were estimated using established *in vitro* gas production models. Metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) were calculated from corrected 24 h gas production data. The gross energy content (MJ/kg DM) was 24.9, 18.6 and 16.8 for seeds, leaves and seeds-removed pods, respectively. The crude protein ranged from 11.2% in seeds-removed pods to 39.5% in seeds, the average being 16.8%. The sugar content was 10.7 and 7.3% for leaves and seeds respectively. The OMD value was highest in leaves (74.3%) followed by seeds (52.3%) and seeds-removed pods (38.1%). The highest SCFA (103 mmol) was obtained from leaves and the lowest from seeds-removed pods (31 mmol). The ME (MJ/kg DM) ranged from 4.15 in seeds-removed pods to 9.94 in leaves. The average gas production from insoluble but with time fermentable fraction was 53.0, 32.4, and 26.1 ml/200 mg DM for leaves, seeds and seeds-removed pods, respectively. *In vitro* gas volumes at various incubation times were positively correlated with ash, sugar, nitrogen free extract and non-fiber carbohydrate; while they were negatively correlated with cellulose, hemicelluloses, neutral and acid detergent fibers. In conclusion, *Moringa stenopetala* leaves can be used as potential sources of protein and energy supplements to tropical livestock feeding system by replacing expensive conventional feed resources.

Key words: Chemical compositions, feeding values, *in vitro* gas production, leaves, *M. stenopetala*, seeds, seeds-removed pods.

INTRODUCTION

Foliages from drought tolerant multi-purpose trees could be used as alternative protein and energy sources during drought periods of tropical countries. Among multi-purpose tree foliages, Moringa tree foliages are known for better biomass yield, nutrient composition and drought tolerant in tropical and sub-tropical climates (Sanchez et al., 2006).The genus Moringaceae is represented by 14 species to which *Moringa stenopetala (M. stenopetala)* belongs. *M. stenopetala*, often referred to as the African Moringa tree, is a multipurpose tree native to Ethiopia, northern Kenya and eastern Somalia, which has a wide range of adaptation from the arid to humid climates. It has been cultivated for both human food and animal feed in southern Ethiopia and recently distributed to other regions of the country beyond its origin places. *M. stenopetala* is a contingency crop in frequently drought-affected lowland areas with its high yielding capacity under drought condition and harvested all round the year. It has the potential as alternative animal feed resources during dry periods. Recent studies indicated that leaves of *M. stenopetala* have been successfully used in poultry

Abbreviations: ME, Metabolizable energy; **OMD,** organic matter digestibility; **SCFA,** short chain fatty acids.

ration to substitute soybean meal (Melesse et al., 2011). In another study conducted by Gebregiorgis et al. (2011) supplementation of sheep with dried *M. stenopetala* leaves has improved the growth performance parameters. There is apparently information gap on the feeding values of *M. stenopetala* tree parts to ruminant animals in east Africa. The main objective of this study was to assess the feeding values of leaves, seeds and seeds-removed pods of *M. stenopetala* using *in vitro* gas production technique.

MATERIALS AND METHODS

Sample collection

Samples of fresh leaves and dry pods of *M. stenopetala* were collected at Hawassa nursery site of Southern Agricultural Research Institute located at Hawassa district of Sidama administrative zone, Ethiopia. Each sample was randomly collected from four different trees aged 3 years old. The location where samples were collected is situated at an altitude of 1700 m having an annual rainfall of 900-1000 mm. The seeds were separated from the dry pod by hand to obtain both seeds and seeds-removed pods separately. Samples of green leaves and seeds-removed pods were then dried at 65°C over 48 h and ground to pass through a 1 mm sieve and the seeds were ground using coffee grinder before analysis. Ground feed samples were labeled and kept in air-tight plastic containers until analysis.

Chemical analysis

Dietary concentrations of crude nutrients were analyzed according to the VDLUFA official methods (Naumann and Bassler, 2004). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were expressed with residual ash. Nitrogen was determined by Kjeldahl method using Kjeltec auto sampler system (Kjeltec 2300, Foss Tecator AB, Sweden). Energy content was determined by using bomb calorimeter (IKA-Calorimeter C7000 isoperibolic, Janke and Kunkel IKA Analysentechnik, Staufen, Germany). Minerals were determined from filtered ash solutions using an Inductively Coupled Plasma spectrometer (ICP-OES) as reported by (Rodehutscord and Dieckmann, 2005). Cellulose and hemicellulose were computed as ADF minus ADL and NDF minus ADF, respectively. Non-fiber carbohydrate (NFC) content was calculated as 100 – (NDF + CP + crude fat + ash) according to NRC (2001). Nitrogen free extract (NFE) was computed by difference of organic matter and the sum of CF, EE and CP. All chemical analyses were conducted in duplicate on each individual sample.

In vitro fermentation procedures

Gas production was determined according to the procedure of VDLUFA official method (Naumann and Bassler, 2004) and Menke and Steingass (1988). About 200 mg of feed sample from each Moringa tree parts was weighed in four replications and transferred into pre-warmed 100 ml calibrated glass syringes, fitted with Vaseline lubricated pistons. Rumen fluid was collected before morning feeding from 2 to 3 ruminally canulated sheep. Rumen fluid was collected from the rumen with manually operated vacuum pump and transferred into pre-warmed thermos flasks, transported to the laboratory, filtered through eight layers of cheesecloth and flushed with CO_2. Rumen fluid was added to the buffered mineral solution with constant stirring, while maintained in a water bath at 39°C. A total of 30 ml incubation medium consisting of 10 ml rumen fluid, 5 ml of bicarbonate buffer, 5 ml of macro-mineral solution and 10 ml of distilled water was then dispensed into pre-warmed glass syringes containing the feed samples. All handling was under continuous flushing with CO_2. After closing the clips on the silicon tube at the syringe tip, syringes were gently shaken and the clips were opened to remove gas by pushing the piston upwards to achieve complete gas removal.

After closing the clip, the initial volume was recorded and the syringes were placed in a temperature controlled incubation rotor set at 39°C.

Three blanks containing 30 ml of medium as well as triplicate samples of reference hay and concentrate feed of known gas production parameters were also included as standards. Incubation was completed in duplicate within each run and runs were replicated yielding four observations per sample.

The gas volume was recorded at 2, 4, 6, 8, 10, 12, 14, 18, 24, 30, 36, 48, 60, 72 and 96 h of incubation according to time pattern of Blümmel and Becker (1997).

The gas produced due to fermentation of substrate was corrected by the blank syringes (containing no substrate). The volume of gas produced (means of two runs) was plotted against the incubation time and fermentation kinetics were described by using the exponential equation $Y = a + b (1 - e^{-ct})$ as described by Blümmel and Ørskov (1993), where, Y is the volume of gas produced at time 't' (ml); a is the intercept gas produced from soluble fractions; b is the gas produced from insoluble but with time fermentable fraction; c is the rate of fermentation (ml/hr); and t is the time at measurement (h).

The gas produced by test substrates was corrected by the blank syringes (containing no substrate), and 24 h gas production was corrected by the standards for the estimation of organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acids (SCFA).

The ME and OMD were estimated according to Menke et al. (1979) and Menke and Steingass (1988) and SCFA by using the equation of Blümmel et al. (1999):

$$ME\ (MJ/kg\ DM) = 2.20 + (0.136*Gv) + (0.0057*CP) + (0.00029*EE);$$

$$OMD\ (\%) = 14.88 + (0.889*Gv) + (0.45*CP) + (0.651*XA);$$

$$SCFA\ (mmol/l) = (0.0239*Gv) – 0.0601$$

Where, Gv, CP, EE and XA are corrected 24 h gas volume (ml/200 mg DM), crude protein, ether extract and ash (g/kg DM) of the incubated samples, respectively.

Statistical analysis

The model parameters were estimated by a non-linear regression procedure using the computer software GraphPad Prism (2004, Version 4.0, GraphPad Software, San Diego California USA). Results on chemical compositions including calculated and estimated gas production parameters were subjected to one-way ANOVA analysis by using SAS software package (SAS, 2004) and differences of means were separated by Duncan multiple range test. Moreover, Pearson correlation procedures were performed with the SAS software package.

RESULTS

Crude nutrients

The average values of crude nutrient compositions of *M.*

Table 1. Average crude nutrient compositions (%, in DM basis) and gross energy contents (MJ/kg DM) of leaves, seeds and seeds-removed pods of *M. stenopetala.*

Crude nutrient	Leaves	Seed	Seeds-removed pod	Pooled S.E.M
Ash	9.54[a]	5.71[b]	5.80[b]	0.254
Crude protein	28.4[b]	39.5[a]	11.2[c]	0.331
Crude fat	8.38[b]	33.4[a]	1.62[c]	0.497
Crude fiber	9.60[b]	9.47[b]	53.9[a]	0.812
Nitrogen free extract	41.1[a]	9.01[c]	22.9[b]	0.763
Sugar	10.7[a]	7.34[b]	-	1.602
Gross energy	18.6[b]	24.9[a]	16.8[c]	0.143
Neutral detergent fiber	13.6[b]	14.4[b]	71.1[a]	0.546
Acid detergent fiber	12.5[b]	11.5[c]	64.5[a]	0.767
Acid detergent lignin	3.20[b]	4.27[b]	22.5[a]	0.517
Cellulose	9.30[b]	7.29[c]	41.9[a]	0.466
Hemicelluloses	1.05[c]	2.85[b]	6.68[a]	0.506
Non fiber carbohydrate	40.4[a]	6.93[c]	10.5[b]	0.945

[a-c] Means between tree parts within a column with no common superscript differ significantly (P<0.05); S.E.M= standard error of mean

Table 2. Least square means of mineral contents in leaves, seeds and seeds-removed pods of *M. stenopetala*

Mineral	Leaves	Seed	Seeds-removed pod	Pooled S.E.M	P
Major minerals (g/kg DM)					
Calcium (Ca)	18.6[a]	1.91[b]	3.92[b]	0.647	***
Phosphorus (P)	3.78[b]	10.6[a]	2.08[c]	0.162	***
Ca : P	4.95[a]	0.18[c]	1.91[b]	0.310	***
Magnesium (Mg)	4.60[a]	4.98[a]	1.93[b]	0.333	***
Potassium (K)	16.6[b]	15.4[b]	23.0[a]	0.710	***
Sodium (Na)	5.38[a]	2.60[b]	9.63[b]	0.396	***
Trace minerals (mg/kg DM)					
Zinc (Zn)	28.5[b]	35.9[a]	23.0[b]	2.076	**
Manganese (Mn)	86.1[a]	16.1[c]	26.2[b]	2.207	***
Copper (Cu)	12.4[b]	17.4[a]	11.0[b]	0.729	***

[a-c]Means between tree parts within a column with no common superscript differ significantly (P < 0.05); **= P < 0.01; *** P<0.001; S.E.M = Standard error of mean.

stenopetala tree parts are shown in Table 1. In general, significant differences in chemical compositions were observed between the investigated tree parts. In seeds, the contents of CP, fat and gross energy were significantly (P < 0.05) higher than those of leaves and seeds-removed pods. On the other hand, leaves contained significantly (P < 0.05) higher ash, NFE, sugar and NFC values than seeds and seeds-removed pods. In general, seeds-removed pods had significantly (P < 0.05) higher CF, NDF, ADF, ADL, cellulose and hemicellose contents than leaves and seeds-removed pods.

Minerals

As shown in Table 2, a highly significant (P < 0.001) difference was observed between Moringa tree parts in mineral contents. Accordingly, the contents of Ca, Ca : P, Na and Mn in leaves were significantly (P < 0.001) higher than those of seeds and seeds-removed pods. However, seeds contained significantly (P < 0.01) higher Zn and Cu contents than leaves and seeds-removed pods. The contents of Mg and K in seeds and leaves were comparable. Seeds-removed pods had significantly (P < 0.001) higher levels of K than leaves and seeds.

In vitro gas production, calculated and estimated parameters

The *in vitro* gas volume, calculated values of meta-bolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) for *M. stenopetala*

Table 3. Least square means of 24 h gas volume, calculated metabolizable energy, organic matter digestibility and short chain fatty acids in leaves, seeds and seeds-removed pods of *M. stenopetala.*

Feed materials	Gv24 (ml/200 mg DM)	ME (MJ/kg DM)	OMD (%)	SCFA (mmol)
Leaves	45.6[a]	9.94[a]	74.3[a]	103 [a]
Seeds	15.5[c]	7.22[b]	52.2[b]	36.6[b]
Seeds-removed pods	17.8[b]	4.15[c]	37.4[c]	31.1[c]
Overall means	26.3	7.05	54.6	56.9
Pooled S.E.M	0.618	0.084	0.563	1.455
P	***	***	***	***

[a-c]Means within a column with no common superscripts differ significantly (P < 0.05). Gv24 = corrected gas volume over 24 h; ME = Metabolizable energy, OMD= Organic matter digestibility, SCFA = Short chain fatty acids; *** = P<0.001; S.E.M = standard error of mean

tree parts are presented in Table 3. The *in vitro* gas, ME, OMD and SCFA values between tree parts were significantly different (P < 0.001; R2 = 0.99). Accordingly, the gas volume and calculated values for gas production, ME, OMD and SCFA in leaves were significantly (P < 0.001) higher than those of seeds and seeds-removed pods. Similarly, seeds had significantly (P < 0.001) larger values of ME, OMD and SCFA than seeds-removed pods. However, the corrected gas volume in seeds-removed pods was significantly (P < 0.01) higher than that of seeds.

As presented in Table 4, there was no significant difference in gas produced from soluble fractions (parameter a) between Moringa tree parts. However, a highly significant (P < 0.01) difference was noted in gas production from the insoluble but with time fermentable fraction (parameter b) and rate of gas production (parameter c) among the investigated Moringa tree parts. The overall mean of gas produced from soluble fractions (parameter a) for leaves, seeds and seeds-removed pods was generally positive. The average gas production from soluble fraction (parameter a) was 3.39, 2.65 and 1.73 ml/200 mg DM for leaves, seeds-removed pods and seeds, respectively. The gas production from the insoluble but with time fermentable fraction (parameter b) for leaves ranged from 50.6 to 58.4 the average being 53.0 ml/200 mg DM. The average gas volume from insoluble but with time fermentable fraction (parameter b) for seeds varied between 31.7 and 32.7, the average being 32.4 ml/200 mg DM. The corresponding values for seeds-removed pods were 26.1 ml/200 mg DM with a range of 25.1 to 27.4. Accordingly, the gas production parameters for the leaves were always higher than seeds and seeds-removed pods and the differences were highly significant (P < 0.001; R2 = 0.99). The rate of gas production (parameter c) for leaves varied between 0.093 and 0.097 ml/h with the mean of 0.095 and was significantly (P < 0.001; R2 = 0.99) higher than the rates observed for seeds and seeds-removed pods. The gas production rate for seeds ranged from 0.039 to 0.042, the average being 0.04 ml/h while for that of seeds-removed pods varied between 0.045 and 0.047 with an average of 0.046. As a result, leaves fermented at a faster rate (nearly + 100%) than both seeds and seeds-removed pods, while fermentation rates of the latter two feed samples were more or less similar

Associations between *in vitro* gas production and chemical compositions

As presented in Table 5, a highly significant (P < 0.001) positive correlation was observed in gas production at various incubation times as well as estimated parameters (b and c) with ash, sugar, NFE and NFC. On the other hand, CF inversely correlated (P < 0.001) with gas production and estimated parameters “b” and “c”. Similarly, Pearson correlation coefficient showed inverse relationships of gas volumes at various incubation times as well as estimated parameters “b” and “c” with CF, NDF, ADF, ADL, cellulose and hemicelluloses. Nevertheless, no significant relationships were observed for these parameters with CP, crude fat and gross energy contents. Moreover, no significant correlations were found between chemical compositions and parameter “a”.

DISCUSSION

Chemical and mineral compositions

The average CP content of leaves in the present study is comparable with that reported by Gebregiorgis et al. (2011) for leaves of *M. stenopetala (27.9%)*; but slightly lower than those reported by Moyo et al. (30.3%; 2011) for *M. oleifera* leaves. Conversely, CP values reported by Negesse et al. (2009) and Melesse et al. (2011) for *M. stenopetala* leaves were generally higher than those obtained from the same Moringa specie of the present study. The observed variations in CP contents in the literature may be due to the age of leaves at harvest, soil type and fertility, as well as agro-ecology in which the trees were growing.

The NFE in Moringa leaves of the present study is

Table 4. Least square means of estimated parameters a, b, and c (ml/200 mg DM) in the *in vitro* gas production model Y=a + b (1 - e^{-ct}) when applied to 200 mg *M. stenopetala* feed samples over 96 h incubation.

Feed material	a	b	c	R^2	Sy. x
Leaves	3.39[a]	53.0[a]	0.10[a]	0.96	2.875
Seed	1.73[a]	32.4[b]	0.04[b]	0.99	1.163
Seeds-removed pods	2.65[a]	26.1[c]	0.05[c]	0.98	1.026
Overall means	2.59	37.2	0.06	-	-
Pooled S.E.M	0.571	0.147	0.164	-	-

[a-c]Means within a column with no common superscripts differ significantly (P < 0.05). ME = Metabolizable energy; OMD = Organic matter digestibility; SCFA = Short chain fatty acids; a= intercept, gas produced from soluble fractions; b = gas produced from insoluble but with time fermentable fraction; c = rate of fermentation (ml/hr); S.E.M = standard error of mean

Table 5. Pearson correlation coefficients of *in vitro* gas volumes and estimated parameters with chemical compositions of *M. stenopetala* tree parts.

Chemical composition[(2)]	Gv at various incubation times[(1)]			Estimated parameters[(3)]		
	Gv24	Gv48	Gv96	a	b	c
Ash	0.96***	0.95***	0.95***	0.46 ns	0.92***	0.97***
Crude protein	0.18 ns	0.27 ns	0.22 ns	-0.17 ns	0.32 ns	0.02 ns
Crude fiber	-0.76**	-0.81**	-0.78**	-0.12 ns	-0.84***	-0.64*
Crude fat	-0.25 ns	-0.17 ns	-0.22 ns	-0.32 ns	-0.11 ns	-0.40 ns
Sugar	0.99***	0.99***	0.99***	0.34 ns	0.99***	0.98***
NFE	0.99***	0.99***	0.99***	0.13 ns	0.99***	0.98***
Gross energy	-0.24 ns	-0.16 ns	-0.21 ns	-0.33 ns	-0.10 ns	-0.39 ns
NDF	-0.58*	-0.64*	-0.60*	0.01 ns	-0.68*	-0.44 ns
ADF	-0.53 ns	-0.60*	-0.56 ns	0.03 ns	-0.65*	-0.39 ns
ADL	-0.59*	-0.66*	-0.62*	0.001 ns	-0.70*	-0.46 ns
Cellulose	-0.51 ns	-0.58*	-0.54 ns	0.04 ns	-0.63 ns	-0.34 ns
Hemicellulose	-0.74**	-0.79**	-0.76**	-0.12 ns	-0.85**	-0.58*
NFC	0.98***	0.96***	0.97***	0.34 ns	0.94***	0.98***

*P 0.05; **P < 0.01; ***P<0.001; ns = not significant. [(1)] Gv24 = gas volume at 24 h; Gv48 = gas volume at 48h; Gv96= gas volume at 96 h. [(2)] NFE = Nitrogen free extract; NDF = neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; NFC = non-fiber carbohydrate. [(3)]a = intercept, gas produced from soluble fractions; b = gas produced from insoluble but with time fermentable fraction; c = rate of fermentation (ml/h).

slightly higher than that of alfalfa hay (40.0%), but comparable to that reported by Abas et al. (2005) for grass hay (44.8%). The range of NFC contents of leaves indicated that they can be easily degraded or fermented as NFC is a crude estimate of the carbohydrate pool that differ in digestibility from NDF.

The CP content in seeds-removed pods is slightly higher than that of grass hay (9.5%), but superior to that of wheat straw (4.3%) as reported by Abas et al. (2005). Both NDF and ADF contents in leaves are comparable to those reported by Gebregiorgis et al. (2011) for *M. stenopetala* leaves but lower than the values reported by Sanchez et al. (2006) for *M. oleifera* leaves. However, the NDF, ADF and ADL values reported for *M. oleifera* leaves by Moyo et al. (2011) were slightly lower than those found in the present study. The ADF and ADL values found in seeds-removed pods are considerably higher than those reported for rice straw by Sallam et al. (2007). The high contents of fibrous substances in seeds-removed pods, especially high ADF and ADL contents, may affect the dry matter intake by ruminants. The fact that seeds-removed pods contained >50% NDF indicate that they may have only fair proportions of soluble carbohydrate which is helpful to maintain a proper rumen function (Oni et al., 2008).

The contents of phosphorous, magnesium, potassium, zinc and manganese found in *M. stenopetala* leaves of the present study are generally comparable to those of *M. oleifera* leaves reported by Moyo et al. (2011). However, the calcium content of *M. stenopetala* leaves is numerically lower than those reported for *M. oleifera* leaves by Moyo et al. (2011); but considerably higher

than those reported for *Ensete ventricosum* parts (leaves lamina, leaves midrib, pseudostem and corm) by Nurfeta et al. (2008).

In vitro fermentation, calculated and estimated parameters

The gas volumes reported by Abas et al. (2005) for wheat bran (46.2 ml/200 mg DM) and alfalfa hay (41.2 ml/200 mg DM) were comparable to those observed in leaves. The gas production from leaves is slightly higher than that reported by Mirzaei-Aghsaghali et al. (2011) for tomato pomace (an industrial by-product of tomato processing). However, the *in vitro* gas obtained from leaves in the current study was considerably higher than that reported by Oni et al. (2010) for four Cassava varieties.

Values of gas produced from soluble fractions (parameter a) from leaves, seeds and seeds-removed pods was generally positive being higher than those reported for tomato pomace by Mirzaei-Aghsaghali et al. (2011). The value of “a” for leaves was comparatively higher than seeds and seeds-removed pods suggesting an early onset of fermentation and microbial attachment to this particular feed material. The average gas production from insoluble but with time fermentable fraction (parameter b) for leaves was consistent with the findings of Mirzaei-Aghsaghali et al. (2011) for tomato pomace (53 ml) and comparable with the findings of Sallam et al. (2007) for berseem hay (50.9 ml). Rate of fermentation (parameter c) obtained from Moringa leaves in the present study is in good agreement with the findings of Mirzaei-Aghsaghali et al. (2011). High fermentation rates indicates high nutrient availability for ruminal microorganisms; while lower fermentation rate values may be the result of greater NDF content, whose chemical components could slow down substrate fermentation speed (Fievez et al., 2005).

Gas volume produced at 24 h from seeds-removed pods was lower than those reported by Abas et al. (2005) for wheat straw and by Oni et al. (2010) for four varieties of Cassava leaves. Seeds-removed pods had thus low fermentation, probably because of high lignifications as suggested by Rubanza et al. (2003), which can limit the attachment of microorganisms on substrates. Although, the CP and fat contents were higher than leaves, the gas volume obtained from seeds was considerably low. Akinfemi et al. (2009) suggested that gas production from protein fermentation is relatively small as compared to carbohydrate fermentation; while contribution of fat to gas production is negligible. In agreement with the present findings for leaves, Kiran and Krishnamoorthy (2007) reported an average calculated ME of 10.2 MJ/kg DM for common protein supplements. Similarly, consistent with current results for leaves, Anele et al. (2009) reported ME values of 9.56 - 10.6 MJ/kg DM for leaves of tropical multi-purpose trees. The ME values reported for potato pomace by Mirzaei-Aghsaghali et al. (2011) are also in good agreement with the present findings of Moringa leaves. A high relationship (r=0.98) was determined between *in vivo* OMD and *in vitro* gas volume at 24 h (Menke et al., 1979). The OMD values obtained from leaves in the current study are consistent with the findings of Murillo et al. (2011). The calculated OMD values for alfalfa (66.3%) and grass (55.7%) hays reported by Abas et al. (2005) were lower than those of leaves in the present study. The *in vitro* predicated OMD values from seeds in the current study were comparable with those of fish meal reported by Palizdar et al. (2011). The OMD for seeds-removed pods was somewhat lower than those reported by Sallam et al. (2007) for rice straw (41.5%) and Abas et al. (2005) for wheat straw (45.2%).

Since the SCFA content is an indicator of the energy value of diets, its prediction from *in vitro* gas measurements becomes apparently useful under circumstances where laboratories lack gas chromatographic equipment, more importantly in developing countries. Getachew et al. (2002) reported a close association between SCFA and gas production *in vitro* and use of this relationship to estimate the SCFA production from *in vitro* gas values. The SCFA for leaves was higher than reported by Babayemi (2007) for different forage species. Higher production of gas in leaves and the eventual predominance of SCFA could probably describe an increased proportion of acetate and butyrate but a decrease in propionate production as suggested by Babayemi et al. (2004). *In vitro* estimated SCFA for leaves of African multipurpose trees ranged from 87 to 101mmol/L, the average being 94 (Anele et al., 2009) and is in line with the values of leaves in the current study. In general, values of OMD and SCFA in leaves were higher than those of seeds and seeds-removed pods possibly because leaves contain more fermentable carbohydrate, which is a vital substrate for growth of ruminal microorganisms.

Associations of *in vitro* gas production with chemical compositions

The presence of correlations between nutrient compositions and *in vitro* gas production characteristics is in good agreement with the findings of Getachew et al. (2003), and Cerrillo and Juarez (2004). Although not significant, CP content showed a trend of positive correlation with gas production at various incubation times. Consistent with the current findings, Gasmi-Boubaker et al. (2005) reported a positive correlation of CP with gas production for tropical browse species. Similarly, Oni et al. (2010) reported a significant positive correlation of CP contents of Cassava leaves with gas volumes measured at 48 h of incubation. Contrary to the present findings, Getachew et al. (2004) observed a

negative correlation between the volume of gas produced and the CP content of the feed samples incubated *in vitro*.

A highly significant ($P < 0.001$) positive correlation of gas production and estimated parameters (parameters b and c) was observed with ash, sugar, NFE and NFC. Getachew et al. (2004) and De Boever et al. (2005) reported that NFC was positively correlated with potential gas production and gas production at 6, 24 and 48 h of incubation. Consistent with the findings of Blümmel et al. (1999), there was no significant relationship of fat with gas volumes at various incubation times. This could be explained by the fact that gas production from fat fermentation is relatively small as compared to carbohydrate fermentation (Blümmel et al., 1999).

The negative relationships of CF, NDF, ADF and ADL contents with *in vitro* gas production at various incubation times is consistent with the reports of Gasmi-Boubaker et al. (2005) and Sallam et al. (2007). Similarly, Parissi et al. (2005) and De Boever et al. (2005) reported a negative relationship of gas production with NDF, ADF and ADL contents. These feed constituents are known to be less degradable than soluble carbohydrates and therefore reduce gas production. In contrary, Oni et al. (2010) reported a positive correlation of ADF contents of Cassava leaves with *in vitro* gas production. In agreement with the reports of Garcia et al. (2005), no significant correlations were observed between gas production from soluble fraction (parameter a) and chemical compositions. In contrary, Parissi et al. (2005) reported a positive correlation of parameter “a” with NDF and ADL contents. Consistent with the current findings, Parissi et al. (2005) found a negative association of gas production from insoluble but with time fermentable fraction (parameter b) with CF, NDF and ADL contents. The absence of a significant correlation of gas production rate (parameter c) with NDF, ADF and ADL contents agrees with the findings of Getachew et al. (2004) and Parissi et al. (2005). In conclusion, the chemical compositions, *in vitro* gas production parameters and calculated metabolizable energy, organic matter digestibility and short chain fatty acids indicated that leaves can be used as potential supplement sources of protein, energy and mineral in ruminant and non-ruminant feeding in the tropics. Moreover, *in vitro* gas technique could be employed as alternative cheap and effective feed evaluation system to predict the digestibility and energy content of ruminant feedstuffs in the tropics.

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