

Short Chain Fatty Acid Production, Organic Matter Digestibility and Metabolisable Energy Content of Indigenous Browses from Ethiopian Rift Valley

Amsalu Sisay^{*}, Tegene Negesse and Ajebu Nurfeta

School of Animal and Range Sciences, College of Agriculture, Hawassa University, Ethiopia
Correspondence author: Amsalu Sisay

Abstract: This study was conducted to estimate the organic matter digestibility, metabolisable energy and short chain fatty acid production of indigenous browses from rift valley of Ethiopia using *in vitro* gas production technique. Leaves of *Acacia seyal*, *Acacia senegal*, *Acacia tortilis*, *Prosopis juliflora*, *Milletia ferruginea*, *Vernonia amygdalina*, *Croton macrostachyus* and *Cordia africana* were collected, oven-dried, ground and analyzed for their chemical composition and *in vitro* gas production characteristics. General linear model procedure of SAS, Version 9.2 was used for statistical analysis. The highest ($P<0.05$) rate of gas production (c) and potential gas production ($a+b$) were observed for *Acacia seyal* and the least were for *Cordia Africana*. The highest ($P<0.05$) organic matter digestibility was observed for *Acacia seyal* and *Vernonia amygdalina* while the lowest ($P<0.05$) was for *Cordia africana*. The highest ($P<0.05$) metabolisable energy (ME) value was for *Acacia senegal*, *Vernonia amygdalina* and *Acacia seyal* while the lowest ($P<0.05$) was for *Cordia africana*. Short chained fatty acid (SCFA) was highest for *Acacia seyal* and lowest for *Cordia africana*. Crude protein (CP) was highest ($P<0.05$) for *Acacia senegal* and lowest for *Acacia seyal*. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were highest for *Cordia africana* and lowest for *Acacia seyal*. The rate of gas production (c) and potential gas production ($a+b$) had positive and significant ($P<0.001$) correlation with CP and negative correlation with fiber (NDF, ADF) and condensed tannin (CT) contents of browses. All browse species studied had high CP contents, sufficient to be considered as high protein forages that can be used as supplements for low quality roughages. The high potential gas production ($a+ b$), highest metabolisable energy (ME) and SCFA production of *Acacia seyal*, coupled with its fastest rate of fermentation (c) would make the browse potential supplement of low quality roughages.

Key words: indigenous browses; *in vitro* gas production; fermentation characteristics; gas production kinetics.

Date of Submission: 03-01-2018

Date of acceptance: 20-01-2018

I. Introduction

Tropical browses are believed to have high protein content, which makes them promising supplements to crop residues and poor quality natural pasture based diets [1]. The relevance of evaluating the nutritional value of forage makes an important contribution to the protein and energy intake of grazing animals [1]. This is particularly important in arid and semi arid regions where availability and quality of forage may be severely limited during the dry season. Traditionally, the energy value of forage consumed by grazing animals is estimated from *in vitro* organic matter digestibility or *in situ* organic matter degradability [2]. However, these methods are laborious, expensive and time consuming [3]. Menke and Steingass [4] developed the *in vitro* gas production technique to evaluate the nutritive value of forages and to estimate the rate and extent of DM degradation indirectly using the gas production during the fermentation. Determination of *in vitro* gas production characteristics is essential because it provides information on fermentation kinetics of forages consumed by ruminants, which is dependent on the rate of passage and the degradation rate [5]. The rate and extent of DM fermentation in the rumen are crucial determinants of nutrients utilized by ruminants [6]. *In vitro* gas production method has numerous advantages over *in vitro* and *in vivo* digestibility and degradability methods. The gas production method is less animal dependent, appropriate for characterizing the soluble and insoluble but fermentable fractions of the feeds [7]. This technique simulates the digestive processes generated by microbial activity and it helps us to understand feed fermentation and degradability as a function of nutritional quality and nutrient availability for the bacteria [8]. The *in vitro* gas production has been widely used to estimate the nutritive quality of cereal straws [9] as well as in improved forages [10]. However, there is little information about energy value of leaves of indigenous browses in Ethiopia. Hence, the aim of this study was to evaluate the nutritional quality of Ethiopian indigenous browses using the *in vitro* gas production technique.

II. Materials And Methods

2.1 Description of the study area

The study area is located at the center of the Ethiopian Great Rift Valley extending from 7° 05' to 8° 00' N and from 38° 20' to 38° 50' E at an elevation ranging between 1500 - 1700m above sea level. The area is generally characterized by semi-arid and sub-humid climate with mean annual rainfall of 700 – 1000 mm and range of temperature varying between 20 °C and 26 °C. The long rainy season is from June to September and short rainy season from April to May.

2.2 Sample collection

Leaves and twigs (<3mm) of dominant browse species like *Acacia seyal*, *Acacia senegal*, *Acacia tortilis*, *Prosopis juliflora* from the bottom land and *Millettia ferruginea*, *Vernonia amygdalina*, *Croton macrostachyus* and *Cordia africana* from the top land of the Rift Valley were hand plucked at the end of long rainy season (end of September, 2015). The harvested samples were pooled for each individual species, shed dried and composite samples were taken, oven dried at 60 °C for 48h and ground using 1.0 mm sieve size for chemical analysis and *in vitro* gas production study.

2.3 Chemical Analysis

All chemical analysis was conducted in triplicates. Leaves of the browses were analyzed for dry matter (DM), ether extract (EE), and ash according to AOAC [11]. The NDF, ADF and ADL were determined according to Van Soest *et al.* [12]. Nitrogen was determined by Kjeldhal procedure and CP calculated as N x 6.25. Condensed tannin (CT) was determined using the method described by Makkar [13]. The CT (% in dry matter) as leucocyanidin equivalent was calculated by the formula: % CT = (A 550 nm x 78.26 x dilution factor) / (% dry matter)

2.4 *In vitro* gas production study

In vitro gas production (GP) was measured according to the procedure described by Menke and Steingass [4]. The rumen fluid was collected from three rumen fistulated Arsi-Bale sheep fed with *Chloris gayana* grass hay *ad lib* and supplemented with 200 gm of concentrate (70% wheat bran and 30% linseed cake) daily. The rumen fluid was collected before morning feed and then prepared and purged with CO₂ to maintain anaerobic conditions [14]. The rumen fluid was transferred to a large glass beaker inside a 39°C water bath being continuously purged with CO₂ and continuously stirred as recommended by Goering and Van Soest [15]. The media solutions (buffer solution, macro and micro mineral solution) were prepared and utilized as described in Goering and Van Soest [15].

Each sample weighing about 200 mg was put into 100ml calibrated glass syringe together with 30ml rumen liquid and culture media solution on a 1:2 ratios. Syringes were incubated in a water bath at 39°C, where a transparent plastic lid with holes held the syringes upright. Two blank syringes with the rumen liquid and culture media solution were also incubated. The incubation period was 96 hours. The volume of the gas was recorded after 3, 6, 12, 24, 48, 72 and 96 hours. Gas production (ml/200 mg) at t hours was calculated as: $G_t = [(V_t - V_0 - G_0) \times 200] / W_s$

Where: G_t = gas production value (ml/200 mg) at t hours, G_0 = gas production of blank syringes (ml), V_0 = volume in ml at begin, V_t = volume in ml at t hours, W_s = weight of dried sample in mg. *In vitro* organic matter digestibility (OMD) at 24-hours was calculated from the equation: $OMD (\%) = 14.88 + 0.889 * GP + (0.45 * CP \%) + (0.651 * Ash \%)$ [4]. Where: OMD = organic matter digestibility, CP = crude protein content of feed samples, GP = gas produce at 24-hrs. Metabolisable energy (ME) was calculated from equation: $ME (KJ/kgDM) = 2.20 + 0.136 * GP + 0.057 * CP$ [4]. Where: GP = gas production over 24-hrs of incubation, CP = crude protein content of feed sample. Short-chained fatty acids (SCFA) were estimated as: $SCFA (mmol) = 0.0239 * GP - 0.0601$ [16]. Where: GP = net gas volume at 24-hrs of incubation. The kinetics of the gas production was estimated using the following equation: $Y = a + b(1 - e^{-ct})$. Where: Y = the volume of gas produced with time (t), a = the gas produced from soluble fraction (ml), b = the gas produced from insoluble but fermentable fraction (ml), (a + b) = the potential gas production, c = the gas production rate, t = time.

2.5 Statistical analysis

Data were analyzed using general linear model (GLM) procedure of SAS, Version 9.2. Single-factor analysis of variance (ANOVA) was used to assess the effects of browses on nutrient composition, *in vitro* gas production, OMD, ME and SCFA with the following model; $Y_{ij} = \mu + B_i + e_{ij}$. Where: Y_{ij} is an observation, μ is the overall mean, B_i is the effect of browses and e_{ij} is the experimental error. Multiple correlation analysis was used to establish the relationship between chemical compositions and *in vitro* gas production parameters. The means were separated by Duncan multiple range test. Differences between means were considered statistically significant if $P < 0.05$.

III. Results

3.1 Chemical composition

The chemical compositions of browses are given in Table 1. All browses used in the current study had a CP content above 13% DM. *Acacia senegal* had the highest ($P<0.05$) CP content while *Acacia seyal* had the lowest ($P<0.05$). The NDF and ADF of browses varied from 13.98 to 48.22 and 9.39 to 28.98% of DM, respectively. *Cordia africana* had the highest ($P<0.05$) NDF, ADF and ADL concentration while *Acacia seyal* and *Acacia tortilis* had the lowest ($P<0.05$). The lowest ($P<0.05$) ash contents were observed in *Acacia seyal* and *Acacia tortilis*. The CT concentration of the browses ranged from 0.30 to 3.40% of DM. The CT content of *Acacia tortilis*, *Acacia seyal* and *Millettia ferruginea* was significantly higher than the remaining browse species.

Table 1: Chemical composition (% DM) of indigenous browses from Ethiopian Rift Valley

3.2 In vitro gas production

Values and trends of gas production during the incubation periods are given in Table 2. Gas production for all browses, except *Acacia tortilis* and *Prosopis juliflora*, increased with time up to 96 hours of incubation period. Gas production for *Acacia tortilis* and *Prosopis juliflora* increased up to 24 hours of incubation and after that it remains constant. *Acacia seyal* produced significantly ($P<0.05$) the highest gas volume at all incubation times followed by *Acacia senegal*. The lowest ($P<0.05$) gas volume was produced by *Cordia Africana* at all incubation time. Gas production of all other browses was statistically similar ($P>0.05$) up to 12 hours of incubation periods. After 12 hours of incubation significant ($P<0.05$) differences among all browses was observed. Rate of gas production from *Cordia Africana*, *Prosopis juliflora* and *Acacia tortilis* was slower during early incubation period.

Table 2: In vitro gas production (ml/200 mg DM) of indigenous browses from Ethiopian Rift Valley

3.3 Kinetics of gas production

The *in vitro* gas production kinetics of browses is given in Table 3. There was a significant ($P<0.05$) difference in potential gas production (a + b), rate of gas production (c), gas production from soluble (a) and insoluble (b) fractions among browse species. Gas production from both soluble (a) and insoluble (b) fractions for *Acacia seyal* was the highest ($P<0.05$) of all browse species. The rate of gas production (c) and potential gas production (a + b) for *Acacia seyal* were also highest ($P<0.05$) of all browse species. The least ($P<0.05$) gas production from insoluble fraction (b), least rate of gas production (c) and least potential gas production (a + b) were observed from *Cordia Africana*. Gas production from soluble fraction of *Cordia Africana* was significantly ($P<0.05$) lower than that of all browses except *Acacia tortilis*. The shortest ($P<0.05$) lag time, fastest fermentation rate and highest ($P<0.05$) gas production potential were observed for *Acacia seyal* and *Acacia senegal*. The longest ($P<0.05$) lag time was observed for *Cordia Africana* followed by *Acacia tortilis*.

Table 3: In vitro gas production kinetic parameters of indigenous browses from Ethiopian Rift Valley

3.4 Short chain fatty acids, metabolisable energy and organic matter digestibility

The estimated values of short chained fatty acids (SCFA), metabolisable energy (ME) and organic matter digestibility (OMD) of browses after 24 hours of incubation are given in Table 4. Browse species significantly ($P<0.05$) differed in their SCFA, ME, OMD and gas production. Short chained fatty acid (SCFA) was highest for *Acacia seyal* while the lowest value was for *Cordia africana*. The highest ($P<0.05$) OMD was observed for *Acacia seyal* and *Vernonia amygdalina* while the lowest ($P<0.05$) was for *Cordia africana*. Metabolisable energy of browses ranged from 4.59 to 7.20% DM. *Acacia Senegal*, *Vernonia amygdalina* and *Acacia seyal* had the highest ($P<0.05$) ME value while *Cordia africana* had the lowest. Gas production at 24 hours ranged from 9.83 to 30.30 ml 200 mg⁻¹ DM.

Table 4: Organic matter digestibility, Metabolisable energy and Short chained fatty acids of browses at 24 hours post incubation

3.5 Correlation between gas production estimates and chemical composition

The correlation coefficient (r) of gas production characteristics with chemical composition is given in Table 5. Gas production from soluble fractions (a) had a positive and significant ($P<0.001$) correlation with CP and negative correlation with cell wall (NDF, ADF and ADL) and CT contents. A negative and significant ($P<0.001$) correlation was observed between gas production from insoluble fractions (b) and ADF contents of the browses. Rate of gas production (c) and potential gas production (a+b) had positive and significant ($P<0.001$) correlation with CP and significant ($P<0.001$) negative correlation with ADF contents. In general all gas production estimates had positive and significant ($P<0.001$) correlation with CP and significant ($P<0.001$) negative correlation with ADF contents.

Table 5: correlation coefficient (r) of gas production characteristics with chemical composition of browses

IV. Discussion

4.1 Chemical composition

The range of CP contents of the browses in the current study is in line with the findings of Njidda and Nasiru [17] and Theart *et al.* [18] who reported 14 to 21% and 13 to 25%, respectively. It has been indicated that most tropical browses are high in CP and can be used to supplement poor quality roughages to increase productivity of ruminant livestock in the tropics [17 - 20] which is consistent with the results obtained in the current experiment.

In this study, the NDF and ADF of browses varied from 14 to 48 and 9 to 29% DM, respectively, which is comparable with findings of Fadel Elseed *et al.* [21] and Kaitho *et al.* [22]. The low NDF, ADF and ADL contents in *Acacia seyal* and *Acacia tortilis* in this study indicate that these browses have better dry matter intake and high digestibility. On the other hand, the high NDF and ADF contents of *Cordia africana* could be associated with poor dry matter intake and digestibility. This is because Schroeder [23] reported the negative correlation of NDF and ADF with dry matter intake and digestibility of browses. The high gas production and OMD values of *Acacia seyal* and the low gas production and OMD values of *Cordia africana* observed in Table 2 of this study supports the above arguments.

The range of CT concentration in the current study is in line with the findings of Fadel Elseed *et al.* [21] who reported values ranging from 0.2 to 6 % of DM for similar browses from Sudan. The CT concentrations of *Acacia tortilis*, *Acacia seyal* and *Acacia senegal* in the current study were comparable with the findings of Mahala and Elseed [24] who reported a CT content of 5, 3 and 3 % DM for *Acacia tortilis*, *Acacia seyal* and *Acacia senegal* respectively. Kechero and Janssens [25] reported CT content of *Milletia ferruginea*, *Vernonia amygdalina*, *Croton macrostachyus* and *Cordia africana* as 4, 0.2, 0.1 and 0.1 % DM, respectively, which are similar to the current results. The CT concentrations of browses in this study are less than 4% of DM which are not high enough to reduce intake and digestibility of nutrients. Barry and Duncan [26] reported that plant species with low (< 5 of %DM) content of CT do not affect voluntary feed intake and nutrient digestibility. Hence, these browses in the current study can be good supplements to low quality roughages.

4.2 In vitro gas production

In the current study the cumulative volume of gas production increased with increasing time of incubation. The gas production pattern in this study indicated that some browses (*Acacia tortilis*, *Prosopis juliflora*, *Vernonia amygdalina* and *Cordia africana*) terminated fermentation before 72 h of incubation while more fermentation of dry matter were still possible beyond 96 hours for other remaining browses. Gas production after 96 hours of incubation ranged between 12 and 56 ml/200mg DM and this was comparable with the finding of Mahala and Fadel Elseed [24], who reported a range between 21 and 55 ml/200mg DM. The highest gas volume and fastest rate of production throughout the incubation period from *Acacia seyal* could be attributed to its low cell wall (NDF, ADF and ADL) contents and highest proportion of soluble carbohydrate (Table 1). Generally, gas production is a function of fermentable carbohydrate and hence, the volume and rate depends on the nature of carbohydrates [27,28]. The gas production from *Vernonia amygdalina* increased at an increasing rate up to 24 h of incubation time and after that it did not increase. The possible reason for this production trend could be the high CP concentration and low fermentable carbohydrates or high non-fermentable cell wall (ADL and ash) contents of the browse (Table 1). The incubation pattern of protein-rich feeds is usually characterized by initial fast fermentation and reach maximum after 20 h of incubation and after 46 h of incubation protein content is likely fully fermented [29].

4.3 Kinetics of gas production

Ranges of gas production parameters (a, b, c) reported in this study are consistent with previous reports on tropical browses [30-32]. The fastest rate of fermentation (c) and highest potential gas production (a + b) for *Acacia seyal* could be due to its lowest cell wall contents (NDF, ADF ADL) which indicates better nutrient availability for rumen microorganisms (Table 1.). This result is in line with findings of Kamalak [33]. The potential gas production (a + b) is associated with degradability of feeds [34]. Ndlovu and Nherera [35] found that gas production rate (c) was negatively correlated with NDF and ADF. Maheri-Sis *et al.* [36] found that low NDF and ADF contents of feedstuffs clearly resulted in higher *in vitro* gas production. The shorter lag time, faster rate of fermentation (c) and higher gas production potential (a + b) of *Acacia seyal* and *Acacia senegal* suggests that rumen microbes were able to utilize the feed better probably due to a higher content of fermentable nutrient. A higher potential gas production can contribute significantly to energy supply via short chain fatty acid production [37]. The longest lag time for *Cordia africana* and *Acacia tortilis* could be due to their negative value of the gas production from soluble fraction (a). Lag time is indicative of the time taken for microbes to attach themselves to the substrates, and microbial attachment to insoluble substrate has been reported to be a pre-condition for digestion to proceed [38].

4.4 Short chained fatty acids, metabolisable energy and organic matter digestibility

The range of SCFA in this study was in line with the findings of Omoniyi *et al.* [39] who reported a range of 0.19 to 0.35 mmol for indigenous browses from Nigeria. The highest SCFA and ME for *Acacia seyal* in the current study could be due to its highest gas production after 24 h of incubation time. A higher gas production can contribute significantly to energy supply via short chain fatty acid (SCFA) production [37]. The highest gas production for *Acacia seyal* could in turn be due to the lowest cell wall (NDF, ADF and ADL) contents (Table 1). Gas production at 24 hours of incubation in the current study ranged from 9.83 to 30.30 ml/200mg DM, which is in line with the findings of Brenda *et al.* [40] who reported a range of 18.4 to 30.5 ml/200mg DM for multipurpose tropical tree leaves. Similarly Aderinboye *et al.* [41] reported a range from 22 to 30.6 ml/200mg DM for leaves of browsers of Nigeria. In the current study OMD and ME ranged from 40.3 to 56.86% DM and from 4.59 to 7.20 MJ/kg DM respectively. This result is in line with the findings of Njidda and Nasiru [17] who reported similar range of 30.64 to 55.44% DM and from 3.33 to 6.23 MJ/kg DM for OMD and ME of tannin containing tropical browses, respectively. The highest value of OMD and ME for *Acacia senegal* in the current study could be attributed due to its highest CP content (Table 1). This result is in agreement with the findings of Karabulut *et al.* [42], Parissi *et al.* [43] and Tolera *et al.* [44] who found positive correlation between CP and ME and OMD for tropical browses with high CP content.

4.5 Correlation between gas production estimates and chemical composition

The positive and significant correlation between CP and all fractions of gas estimates (a, b, c and a+b) in the current study agrees with the findings of Ahmed *et al.* [45] and Kamalak *et al.* [46]. The negative and significant correlation between ADF and insoluble fractions (b), potential gas production (a+b) and rate of production (c) is in line with the findings of Ahmed *et al.* [45], Abdulrazak *et al.* [47] and Kamalak *et al.* [46]. In the current study all fractions of gas estimates (a, b, c and a+b) were negatively correlated with CT contents of the browses and this was consistent with the findings of Frutos *et al.* [48]. The negative correlation between potential gas production and ADF and CT may be due to the reduction of microbial activity from increasingly adverse environmental conditions as incubation time progress [49].

The positive significant correlation between gas production from soluble fractions (a) and CP in the current study could indicate the major contribution of CP to the rapidly fermentable fractions of the total dry matter. The negative significant correlation between gas production from insoluble fractions (b) and ADF could indicate that fermentation of insoluble fraction are affected more by poorly digestible cell wall components. In the current study all gas production estimates had positive and significant correlation with CP and negative correlation with cell wall (NDF, ADF ADL) contents and this is an indication of good quality feed. It is well accepted that forage degradation in the rumen is mainly affected by the cell wall content and its lignifications [17]. Theart *et al.* [18] reported negative correlation of NDF, ADF and lignin with *in vitro* digestibility. It is well established that low contents of poorly digestible cell wall (ADF and ADL) and a high CP content are indicators of good forage quality [50].

V. Conclusion

All browse species studied had high CP contents, sufficient to be considered as high protein forages that can be used as supplements for low quality roughages. Browse species with higher crude protein and lower cell wall contents showed better potential for gas production and *in vitro* organic matter digestibility. The high potential gas and SCFA production of *Acacia seyal*, coupled with its fastest rate of fermentation would make the browse potential supplement of low quality roughages.

Acknowledgements

This research project was fully supported by the research fund granted by the Vice President for Research and Technology Transfer of the Hawassa University for which the authors are highly grateful. The support received from Mr. Tadesse Bekore in analysing the feed nutrient composition is highly acknowledged.

References

- [1]. Cline HJ, Neville BW, Lardy GP, Caton JS. Influence of advancing season on dietary composition, intake, site of digestion and microbial efficiency in beef steers grazing a native range in western North Dakota. *J. Anim. Sci.* 2010; 8: 2812-2824.
- [2]. Waterman RC, Grings EE, Geary TW, Roberts AJ, Alexander LJ, MacNeil MD. Influence of seasonal forage quality on glucose kinetics of young beef cows. *J. Anim. Sci.* 2007; 85: 2582-2595.
- [3]. France J, Lopez S, Kebread E, Bannink A, Dhanoa MS, Dijkstra J. A general
- [4]. Compartmental model for interpreting gas production profiles. *Anim. Feed Sci. Technol.* 2005; 123-124: 473-485.
- [5]. Menke KH and Steingass H. Estimation of the Energetic Feed Value Obtained From Chemical Analysis and In Vitro Gas Production Using Rumen Fluid. *Animal Research and Development.* 1988; 28.
- [6]. Mould FL, Morgan R, Kliem KE, Krysstallidou E. A review and simplification of the *in vitro* incubation medium. *Anim Feed Sci. Technol.* 2005; 124: 155-172.

- [7]. Jancík F, Koukolova V, Homolka P. Ruminant degradability of dry matter and neutral detergent fiber of grass. Czech.J. Anim. Sci. 2010; 9:359-371
- [8]. Adesogan AT, Krueger N K, Kim S C. A novel wireless automated system for measuring fermentation gas production kinetics of feeds and its application to feed characterization. Anim.Feed Sci. Technol. 2005; 123-124: 211-223.
- [9]. Murillo M, Herrera E, Reyes O, Gurrola J N and Gutiere E. Use in vitro gas production technique for assessment of nutritional quality of diets by range steers. African Journal of Agricultural Research. 2011; 6: 2522-2526.
- [10]. Valizadeh R, Sobhanirad S, Mojtahedi M. Chemical composition, ruminal degradability and in vitro gas production of wheat straw inoculated by *Pleurotus ostreatus* mushrooms. J. Anim. Vet. Adv. 2010; 7: 1506-1510.
- [11]. Njidda AA. In vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production of semi-arid browses of north-eastern Nigeria. Global Vet. 2010; 4: 292-298.
- [12]. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 14th ed. Washington D. C. USA; 2005.
- [13]. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 1991; 74: 3583–3597.
- [14]. Makkar HPS. Quantification of Tannins in Trees and Shrubs Foliage. IAEA. Kluwer. Academic Publisher. Dordrecht, the Netherlands. 2003.
- [15]. Grant RJ and Mertens DR. Influence of buffer pH and raw corn starch addition on *in vitro* fiber digestion kinetics. Journal of Dairy Science. 1992; 75:2762-8.
- [16]. Goering HK, Van Soest PJ. Forage fiber analysis. In: Agricultural Handbook No. 379. Agricultural Research Service, US Department of Agriculture, Washington, D.C., USA.1970.
- [17]. Getachew G, Makkar H P S and Becker K. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acids and *in Vitro* gas production. The Journal of Agricultural Science. 2002; 139: 341-352
- [18]. Njidda AA and Nasiru A. In Vitro gas production and dry matter digestibility of Tannin-containing Forages of Semi-Arid Region of North-Eastern Nigeria. Pakistan Journal of Nutrition 2010; 9: 60-66.
- [19]. Theart JJF, Hassen A, van Niekerk WA, Gameda BS. In vitro screening of Kalahari browse species for rumen methane mitigation. Science Agriculture. 2015; 72:478-483.
- [20]. Makkar HPS and Becker K. Do tannins in leaves of trees and shrubs from Africa and Himalayan regions differ in level and activity? Agro-forestry System. 1998; 40: 59-68.
- [21]. Njidda AA, Ikhimioya I, Abbator FI and Ngoshe AA. Proximate chemical Composition and Some antnutritional constituents of selected browses of Semi arid region of Nigeria. Proc. Of 34th Ann. NSAP Con.March 15 -18 2009. University of Uyo, Nigeria, 2009; pp: 633-635.
- [22]. Fadel Elseed AMA, Amin AE, Khadiga A, Abdel Ati J, Sekine M, Hishinuma M and Hamana K. Nutritive evaluation of some fodder tree species during the dry season in Central Sudan. Asian-Australian Journal of Animal Sciences. 2002; 15: 844-850.
- [23]. Kaitho R, Nsahlai I, Williams B, Umunna N, Tamminga S and Jvan Bruchem. Preference, rumen degradability gas production and chemical composition of browses. Agro forestry Systems. 1997; 39:129-14.
- [24]. Schroeder JW. Forage Nutrition for Ruminants. North Dakota State University Cooperative Extension. 2004. Retrieved from <http://www.ag.ndsu.edu/pubs/ansci/dairy/as1252w.htm>.
- [25]. Mahala AG and Fadel Elseed ANMA. Chemical Composition and *In Vitro* Gas Production Characteristics of Six Fodder Trees Leaves and Seeds. Research Journal of Agriculture and Biological Sciences 2007; 3:983-986.
- [26]. Kechero Y and Janssens GPJ. Evaluation of nutritive value of leaves of Tropical tanniferous trees and shrubs. Livestock Research for Rural Development. 2013; 25: 2013.
- [27]. Barry TN and Duncan SJ. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. Voluntary intake. British Journal of Nutrition.1984; 51:485-491.
- [28]. Demeyer DI and Van Nevel CI. Methanogenesis, an integrated part of carbohydrate fermentation and its control. In: Digestion and metabolism in the ruminant. McDonald, L.W and A.C.I.Warner, (Eds). Armidale, N.S.W., Australia: The University of New England Publishing Unit. 1975; pp: 366-382.
- [29]. Blummel M, Steingass H and Becker K. The relationship between in vitro gas production, in vitro microbial biomass yield and N-15 incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutr. 1997b; 77:911-921.
- [30]. Cone JW and Van Gelder AH. Influence of protein fermentation on gas production profiles. Animal Feed Science and Technology.1999; 76: 251-264.
- [31]. Larbi A, Smith JW, Kurdi IO, Adekunle IO, Raji AM and Ladipo DO. Chemical composition, rumen degradation and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry seasons in the humid tropics. Anim. Feed Sci. Tech. 1998; 72:81-96.
- [32]. Siaw D E KA, Osuji P O, Nsahlai I V. Evaluation of multipurpose tree germplasm: the use of gas production and rumen degradation characteristics. J. Agric. Sci. Camb. 1993 120: 319–330.
- [33]. Nsahlai I V, Siaw DEK and Osuji PO. The relationships between gas production and chemical composition of 23 browses of the genus *Sesbania*. Anim. Sci. 1994; 65: 13-20.
- [34]. Kamalak A. Determination of nutritive value of leaves of a native grown shrub, *Glycyrrhiza glabra* L using in vitro and in situ measurements. In: Small Ruminant Research. 2006; 64. p. 268-278.
- [35]. Khazaal K A, Dentinho MT, Ribeiro R and Ørskov ER. A comparison of gas production during incubation with rumen contents in vitro and nylon bag degradability as predictors of the apparent digestibility in vivo and voluntary intake of hays. Animal Production.1993; 57:105-112.
- [36]. Ndlovu LR and Nherera FV. Chemical composition and relationship to in vitro gas production of Zimbabwean browsable indigenous tree species. In: Anim. Feed Sci. Technol. 1997; 69. p. 121-129
- [37]. Maheri-Sis N, Chamani M, Sadeghi AA, MirzaAghazadeh and Aghajanzadeh-Golshani A. Nutritional evaluation of kabuli and desi type chickpeas (*cicer arietinum* L.) for ruminants using in vitro gas production technique. Afr. J. Biotechnology. 2008; 7: 2946-2951.
- [38]. Remesy C, Demigne C, Morand C. Metabolism of short-chain fatty acids in the liver. In: Cummings, J. H., Rombeau, J. L., Sakata, T., (Eds), Physiological and clinical aspects of short-chain fatty acids, (Cambridge University Press, Cambridge). 1995; p. 171–190.
- [39]. McAllister T A, Bae H D, Jones G A and Cheng KJ. Microbial Attachment and Feed Digestion in the Rumen. J. Anim. Sci. 1994; 72:3004-3018
- [40]. Omoniyi LA, Isah OA, Taiwo O O, Afolabi B AD, and Fernandez AJ. Assessment of Nutritive Value of some Indigenous Plants Consumed by Ruminants in the Humid and Sub-Humid Region of Nigeria using In Vitro Technique. The Pacific Journal of Science and Technology 2013, 14: 1.

- [41]. Brenda K, Nguyen VL, Preston T R and Orskov ER. Nutritive value of leaves from tropical trees and shrubs: In vitro gas production and in Sacco rumen degradability. *Livest. Res. Rural Dev.* 1997; 9: 4-8
- [42]. Aderinboye R Y, Akinlolu A O, Adeleke M A, Najeeem G O, Ojo V O A, Isah O A, Babayemi O.J. In Vitro gas Production and Dry Matter Degradation of Four Browse Leaves Using Cattle, Sheep and Goat Inocula. *Slovak J. Anim. Sci.* 2016; 49: 32–43.
- [43]. Karabulut A, Onder Canbolat, Hatice Kalkan, Fatmagul Gurbuzol, Ekin Sucu and Ismail Filya. Comparison of In vitro Gas Production, Metabolizable Energy, Organic Matter Digestibility and Microbial Protein Production of Some Legume Hays. *Asian-Aust. J. Anim. Sci.* 2007; 20: 517 – 522.
- [44]. Parissi Z M, Papachristou T G and Nastis A S. Effect of drying method on estimated nutritive value of browse species using an in vitro gas production technique. *Anim. Feed Sci. Technol.* 2005; 30:119-128.
- [45]. Tolera A, Khazaal K, Ørskov E R. Nutritive evaluation of some browses species. *Anim. Feed Sci. and Technol.* 1997; 67: 181-195.
- [46]. Ahmed Gofoon Mahala and Abdel Nasir MA Fadel Elseed. Chemical Composition and in Vitro Gas Production Characteristics of Six Fodder Trees Leaves and Seeds. *Research Journal of Agriculture and Biological Sciences* 2007; 3: 983-98.
- [47]. Kamalak A, Canbolat O, Gurbuz Y, Ozay O and Ozkose E. Chemical composition and its relationship to in vitro gas production of several tannin containing tree and shrubs leaves. *Asian-Australian Journal of Animal Science* 2005; 18: 203-208.
- [48]. Abdulrazak SA, Fujihara T, Ondilek J K, Orskov ER. Nutritive evaluation of some Acacia tree leaves from Kenya. *Anim. Feed Sci. Technol.* 2000. 85: 89–98.
- [49]. Frutos P, Hervas G, Ramos G, Giraldez FJ, Mantecon A R. Condensed tannin content of several shrub species from a mountain area in northern Spain, and its relationships to various indicators of nutritive value. *Anim. Feed Sci. Technol.* 2002; 95: 215–226.
- [50]. Abreub JM F and Bruno-Soares AM. Chemical composition, organic matter digestibility and gas production of nine legume grains. *Animal Feed Science Technology* 1998; 70:49-57
- [51]. Van Soest PJ. *Nutritional Ecology of the Ruminant*, second edition. Cornell University press, Ithaca, NY, USA. 1994.

Table 1: Chemical composition (% DM) of indigenous browses from Ethiopian Rift Valley

	Ash	EE	CP	NDF	ADF	ADL	CT
<i>Acacia tortilis</i>	7.22 ^b	3.09 ^{ab}	21.76 ^c	19.48 ^f	12.63 ^e	1.79 ^{bc}	3.0 ^a
<i>Acacia seyal</i>	6.50 ^b	3.30 ^{ab}	13.03 ^e	13.98 ^g	9.39 ^f	0.80 ^c	3.4 ^a
<i>Acacia senegal</i>	10.63 ^a	4.38 ^a	28.81 ^a	22.04 ^e	12.07 ^e	1.79 ^{bc}	1.3 ^b
<i>Prosopis juliflora</i>	12.95 ^a	3.07 ^{ab}	21.33 ^c	30.86 ^c	21.13 ^b	3.16 ^{bc}	1.0 ^b
<i>Millettia ferruginea</i>	10.62 ^a	3.49 ^{ab}	22.46 ^c	40.77 ^b	22.42 ^b	7.44 ^a	3.0 ^a
<i>Vernonia amygdalina</i>	12.6 ^a	2.86 ^b	22.51 ^c	22.08 ^e	14.35 ^d	3.65 ^b	0.4 ^b
<i>Croton macrostachyus</i>	12.54 ^a	2.92 ^{ab}	25.94 ^b	27.72 ^d	16.51 ^c	2.97 ^{bc}	0.3 ^b
<i>Cordia africana</i>	12.97 ^a	1.23 ^c	18.44 ^d	48.22 ^a	28.98 ^a	9.66 ^a	0.3 ^b
SE	0.50	0.29	0.25	0.31	0.29	0.57	0.2

Means in the same column with different superscript differ significantly ($P < 0.05$). EE = Ether Extract, CP = crud protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, CT = Condensed Tannin.

Table 2: In vitro gas production (ml/200 mg DM) of indigenous browses from Ethiopian Rift Valley

Browse species	Incubation time (hrs)						
	3	6	12	24	48	72	96
<i>Acacia tortilis</i>	2.84 ^{de}	7.57 ^{cd}	11.37 ^e	15.15 ^d	15.15 ^d	15.15 ^e	16.10 ^d
<i>Acacia seyal</i>	10.75 ^a	13.68 ^a	20.52 ^a	30.30 ^a	47.89 ^a	53.75 ^a	55.71 ^a
<i>Acacia senegal</i>	7.91 ^b	10.88 ^b	16.82 ^c	24.73 ^b	30.67 ^b	34.62 ^b	37.59 ^b
<i>Prosopis juliflora</i>	3.95 ^{cd}	5.92 ^d	8.39 ^f	11.85 ^e	11.85 ^e	11.85 ^f	11.85 ^e
<i>Millettia ferruginea</i>	3.95 ^{cd}	9.89 ^{bc}	14.84 ^d	20.77 ^c	24.73 ^c	24.73 ^d	27.70 ^c
<i>Vernonia amygdalina</i>	5.92 ^{bc}	11.9 ^{ab}	18.27 ^b	26.67 ^b	28.64 ^b	29.63 ^c	29.63 ^c
<i>Croton macrostachyus</i>	4.90 ^{cd}	7.84 ^{cd}	12.74 ^e	19.61 ^c	25.49 ^c	30.39 ^c	35.29 ^b
<i>Cordia africana</i>	0.98 ^e	1.97 ^e	5.41 ^g	9.83 ^e	11.80 ^e	11.80 ^f	11.80 ^e
SE	0.6	0.5	0.3	0.5	0.5	0.5	0.5

Means in the same column with different superscript differ significantly ($P < 0.05$). Gp = gas production, GP24/96 = ratio between gas production at 24 hrs and 96 h, GP48/96 = ratio between gas production at 48 h and 96 h.

Table 3: In vitro gas production kinetic parameters of indigenous browses from Ethiopian Rift Valley

	a	b	a + b	c	Lag time
<i>Acacia tortilis</i>	-3.30 ^f	18.80 ^f	15.50 ^e	0.04 ^e	1.40 ^b
<i>Acacia seyal</i>	5.50 ^a	55.40 ^a	60.90 ^a	0.14 ^a	0.00 ^e
<i>Acacia senegal</i>	4.70 ^a	32.90 ^c	37.60 ^b	0.11 ^b	0.00 ^e
<i>Prosopis juliflora</i>	0.80 ^c	11.20 ^h	12.00 ^f	0.02 ^f	0.00 ^e
<i>Millettia ferruginea</i>	-0.20 ^{cd}	26.30 ^e	26.10 ^d	0.07 ^d	0.10 ^d
<i>Vernonia amygdalina</i>	-1.20 ^{de}	30.80 ^d	29.60 ^c	0.07 ^d	0.40 ^c
<i>Croton macrostachyus</i>	3.30 ^b	34.10 ^b	37.40 ^b	0.09 ^c	0.00 ^e
<i>Cordia africana</i>	-2.20 ^e	14.30 ^g	12.10 ^f	0.03 ^f	2.50 ^a
SE	0.2	0.2	0.4	0.001	0.004

Means in the same column with different superscript differ significantly ($P < 0.05$). a = gas production from soluble fraction (ml), b = gas production from insoluble fraction (ml), $a + b$ = potential gas production (ml), c = rate of gas production (fraction/h) and lag time (h).

Table 4: Organic matter digestibility, Metabolisable energy and Short chained fatty acids of browses at 24 hours post incubation

	GP24	OMD	ME	SCFA
<i>Acacia tortilis</i>	15.15 ^d	42.80 ^c	5.50 ^c	0.30 ^d
<i>Acacia. Seyal</i>	30.30 ^a	51.85 ^b	7.06 ^a	0.66 ^a
<i>Acacia senegal</i>	24.73 ^b	56.68 ^a	7.20 ^a	0.53 ^b
<i>Prosopis juliflora</i>	11.84 ^e	43.39 ^c	5.03 ^d	0.22 ^e
<i>Millettia ferruginea</i>	20.77 ^c	50.32 ^b	6.3 ^b	0.44 ^c
<i>Vernonia amygdalina</i>	26.67 ^b	56.86 ^a	7.11 ^a	0.58 ^b
<i>Croton macrostachyus</i>	19.61 ^c	52.08 ^b	6.35 ^b	0.41 ^c
<i>Cordia africana</i>	9.83 ^e	40.3 ^d	4.59 ^e	0.17 ^e
SE	0.5	0.5	0.6	0.1

Means in the same column with different superscript differ significantly ($P < 0.05$). GP = gas production (ml/200mg DM), OMD = organic matter digestibility (%), ME = metabolisable energy (MJ/kg DM), SCFA = Short chained fatty acids (mmol).

Table 5: correlation coefficient (r) of gas production parameters with chemical composition of browses

Chemical composition	a	b	a + b	c
Ash	0.33	0.22	0.10	0.05
Crude protein	0.86 ^{**}	0.79 ^{**}	0.88 ^{**}	0.90 ^{**}
Neutral detergent fiber	-0.23	-0.43	-0.41	-0.39
Acid detergent fiber	-0.31	-0.58 ^{**}	-0.56 ^{**}	-0.56 ^{**}
Acid detergent lignin	-0.38	-0.33	-0.37	-0.35
Condensed tannin	-0.28	-0.13	-0.17	-0.10

a = gas production from soluble fraction, b = gas production from insoluble fraction, $a + b$ = potential gas production, c = rate of gas production. * = ($P < 0.05$) and ** = ($P < 0.001$)

Amsalu Sisay "Short Chain Fatty Acid Production, Organic Matter Digestibility and Metabolisable Energy Content of Indigenous Browses from Ethiopian Rift Valley." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.1 (2018): 61-68.