**NUTRIENT CONTENT, *IN VITRO* GAS PRODUCTION AND POST INCUBATION PARAMETERS OF INDIGENOUS BROWSE FODDERS IN DIFFERENT AGRO-ECOLOGICAL ZONES OF NORTHWEST ETHIOPIA[[1]](#footnote-1)**

**[CONTENIDO DE NUTRIENTES, PRODUCCIÓN DE GAS IN VITRO Y PARÁMETROS POST-INCUBACIÓN DE FORRAJES NATIVOS EN DIFERENTES ZONAS AGROECOLÓGICAS DEL NOROESTE DE ETIOPÍA]**

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**SUMMARY**

**Background:** In Ethiopia indigenous browse species have the potential to serve as a sustainable supplement for poor quality feeds and enhance ruminant livestock production. **Objective:** To analyze the nutrient content, in vitro gas production, and post incubation parameters of indigenous browse species in northwestern Ethiopia. **Methodology:** Eight indigenous browse fodder trees were collected from each agroecological zone for this experiment. Leaf and pod samples of the fodder trees were collected, dried and ground for laboratory analysis. Analysis of variance was carried out for nutrient content, in vitro gas, and methane production of the samples within agroecological zones using standard analytical procedures. The statistical design used to analyze the data was Completely Randomized Design (CRD).  **Results:** The highest CP content was recorded from *Vernonia amygdalina* (223.4 g/kg DM)*,* *Dodonaea viscosa* (207.7 g/kg DM), and *Acacia abyssinica* (216.0 g/kg DM) in the highland, midland and lowland agro-ecology respectively. In lowland area, NDF, ADF and ADL contents were highest for *Albezia amara* leaves and lowest for *Acacia brevispica* respectively. *V. amygdalina*, *Stereospermum kunthianum* and *Ficus vasta* had the highest gas volume at 24-hour incubation time in high, mid and lowland, respectively. The estimated organic matter digestibility, metabolizable energy and short chain fatty acid at 24 hour of incubation was the highest for *V. amygdalina*, *S kunthianum* and *F. vasta* in high, mid and lowland, respectively. *Ficus sycomorus* and *Myrica salicifolia* had the lowest methane production among the species in the low, mid and highland study areas, respectively. **Implications:** The present study provides a valuable resource for selecting suitable feed options for livestock in different agroecological zones. High CP and digestible browse species like *V. amygdalina,* *S. kunthianum*, and *F. vasta* could be prioritized for livestock feed. Low methane-producing species like *F. sycomorus* and *M. salicifolia* could be incorporated into diets to reduce enteric methane emissions. **Conclusion**: Based on their nutrient composition and in vitro gas production potential, these feed resources are of better quality to supplement grazing livestock during the seasons of critical feed shortage as the main feed resources are either limited in availability or lower in nutrient composition and digestibility in the study areas.

**Key words*:*** Browse species; digestibility; gas production; Nutrient content; Methaneproduction.

**RESUMEN**

**Antecedentes:** En Etiopía, las especies autóctonas de ramoneo tienen el potencial de servir como complemento sostenible para piensos de mala calidad y mejorar la producción ganadera de rumiantes. **Objetivo:** Analizar el contenido de nutrientes, la producción de gas *in vitro* y los parámetros post-incubación de especies de ramoneo autóctonas en el noroeste de Etiopía. **Metodología:** Para este experimento se recolectaron ocho plantas forrajeras autóctonas de cada zona agroecológica. Se recolectaron, secaron y molieron muestras de hojas y vainas de los árboles forrajeros para análisis de laboratorio. Se llevó a cabo un análisis de varianza para el contenido de nutrientes, gas *in vitro* y producción de metano de las muestras para cada zona agroecológica utilizando procedimientos analíticos estándar. El diseño estadístico utilizado para analizar los datos fue Diseño Completamente Aleatorio (CRD). **Resultados:** El mayor contenido de PB se registró en *Vernonia amygdalina* (223.4 g/kg MS), *Dodonaea viscosa* (207.7 g/kg MS) y *Acacia abyssinica* (216.0 g/kg MS) en tierras altas, medias y bajas. respectivamente. En las tierras bajas, los contenidos de NDF, ADF y ADL fueron más altos para las hojas de *Albezia amara* y más bajos para *Acacia brevispica*, respectivamente. *V. amygdalina, Stereospermum kunthianum* y *Ficus* *vasta* tuvieron el mayor volumen de gas en un tiempo de incubación de 24 horas en las tierras altas, medias y bajas, respectivamente. La digestibilidad de la materia orgánica, la energía metabolizable y los ácidos grasos de cadena corta estimados a las 24 horas de incubación fueron los más altos para *V. amygdalina, S. kunthianum* y *F.* *vasta* en zona de tierras altas, medias y bajas, respectivamente. *F. sycomorus* y *M. salicifolia* tuvieron la menor producción de metano entre las especies en las áreas de estudio bajas, medias y altas, respectivamente. **Implicaciones:** El presente estudio proporciona un recurso valioso para seleccionar opciones de alimentación adecuadas para el ganado en diferentes zonas agroecológicas. Se podría dar prioridad a las especies de ramoneo de alto contenido de CP y digestibilidad como *V. amygdalina*, *S. kunthianum* y *F. vasta* para la alimentación del ganado. Especies con bajo nivel de producción de metano, como *F. sycomorus* y *M. salicifolia*, podrían incorporarse a las dietas para reducir las emisiones entéricas de metano. **Conclusión:** Sobre la base de su composición de nutrientes y su potencial de producción de gas *in vitro*, estos recursos alimenticios son de mejor calidad para complementar el pastoreo del ganado durante las temporadas de escasez crítica de alimentos, ya que los principales recursos alimenticios tienen una disponibilidad limitada o una composición de nutrientes y digestibilidad más bajos en las áreas de estudio.

**Palabras clave:** Explorar especies; digestibilidad; producción de gas; Contenido nutritivo; Producción de metano.

**INTRODUCTION**

Poor animal nutrition is one of the main constraint to enhance livestock productivity in Sub-Saharan region (Balehegn *et al.*, 2020; Matovu and Alçiçek 2023). In Ethiopia ruminant animals depend mainly on natural pastures and crop residues feed resources. However, the utilization of natural pasture and crop residues for enhancing livestock performance is constrained by their inherent low nutritional value such as protein, mineral, carbohydrates and vitamins (Mengistu *et al.*, 2017). This suggests that improving the performance of ruminant livestock that are fed low-quality feed resources requires the application of various mechanisms to enhance feed quality.

Indigenous browse species have the potential to serve as a sustainable supplement for poor quality feeds and enhance ruminant livestock production (Shenkute *et al.*, 2012). For livestock production in the Sub-Saharan areas, including Ethiopia, indigenous fodder tree species have long been considered essential for livestock nutrition, particularly where the quantity and quality of feedstuff is low for long period (Derero and Kitaw 2018; Humbelani *et al.*, 2021). Browse fodder produces a considerable quantity of forage biomass as leaves, pods and fruits that are rich in nutrients (Abraham *et al.*, 2023; Amad and Zentek 2023) However, their nutritional quality differs among species due to the variation in chemical composition and associated characteristics, which originate from intrinsic and environmental factors (Dereje *et al.*, 2021; Ravetto Enri *et al.*, 2020; Sebolai 2018).

Poor forage digestibility in ruminants is a concern, as it compromises animal productivity and contributes to greenhouse gas emissions (GHG) (Abraham *et al.*, 2023; Palangi *et al.*, 2022). According to the report of Gerber et al (2013), the contribution of livestock to the anthropogenic GHG emissions is about 14.5% considering the emission report of IPCC (2006). Methane (CH4) gas from livestock production activities is a significant source of greenhouse gas emissions which have been shown to influence climate change (Kumar *et al.*, 2018; Min *et al.*, 2020). Ruminant production systems contribute between 18% and 33% of methane emissions (Canul-solis *et al.*, 2020). Enteric CH4 emissions, which represent energy loss from ruminant animals, are problematic due to their significant impact on animal performance and their contribution to climate change. According to FAO (2022), 12% of a ruminant’s energy intake is usually lost as methane through the enteric fermentation process. Due to this, there has been growing interest in searching of feeds that could help mitigating methane production in the rumen. There are reports which claim the inclusion of browse fodder parts in poor-quality fiber-based diets significantly enhanced the digestibility and hence mitigated enteric CH4 emissions (Abraham *et al.*, 2023; Badgery *et al.*, 2022; Bayssa *et al.*, 2016; Berhanu *et al.*, 2019) However, there is no adequate information on in vitrogas production and methane mitigation potential of indigenous browse species in northwest Ethiopia, particularly in the south Gondar zone where many indigenous browse species potentially exist but not yet fully exploited as livestock feed. Therefore, this study was carried out to evaluate the nutrient content, digestibility, kinetics of gas production, and CH4 production potential of indigenous browse species.

**MATERIAL AND METHODS**

**Description and selection of the study area**

Indigenous browse samples were collected from south Gondar zone of Amhara National Regional State, Northwestern Ethiopia. The three districts were selected purposively on the basis of accessibility and representativeness of different agro-ecologies. The districts were named *Ebinat* (representing low land), *Libo Kemkem* (mid land) and *Farta* (high land).

*Ebinat* district is located between 11o and 12o north latitude, and 37o and 38o east longitude. It is located 122 km from Bahir Dar the capital of the region and 714 km from Addis Ababa. Its altitude ranges from 1800m to 2150m above sea level. Annual temperature and rainfall ranges from 25°C to 30°C and from 500 to 900mm respectively. The district has a total livestock population of 562,040 of these, 131,505 are indigenous goats. The district has mainly kolla (lowland) and woina dega (midland) agro-ecological zones which accounted for 45.1 % and 35.5 % respectively which is suitable for sorghum and other low land crop production.

*Libo Kemkem* district separates from Fogera Woreda on the West by Lake Tana, on the North by the Central Gondar Zone and, on the East by Ebinat Woreda. It covers an area of 9,514 km2 and located at 120 04.351‟N - 120 10.926‟N and 370 44.266‟E – 370 50.057‟E. The district far from Bahir Dar and Addis Ababa is 88.71km and 656 km respectively. The altitude varies from 1800 to 2850 meter above sea level. Crop farming and animal husbandry, is the basis of livelihoods of the people of the district and it is characterized by rain fed, oxen driven, small scale subsistence oriented and labor-intensive activities. The number of goats raised in the district were 61,770. In the district various types of crops such as teff (*Eragrotis teff*), beans (*Phaseolus vulgaris L*.), wheat (*Triticum aestivum L*.), and barley (*Hordeum vulgare L*) are grown.

The *Farta* district is located at 11°40' N latitude and 38 ° E longitude and located at about 97 km north east of Bahir Dar and 667 km from Addis Ababa. It lies within an altitude range of 1920-4135 above sea level. The district receives an average annual rain fall of 900-1099 mm and a mean temperature in the range of 9-25°C. The livestock population of the district is estimated to be 168,307 cattle, 80,792 sheep, 32,667 goats, 28,849 equines and 186,861 poultry. Agroforestry is the common practice in which trees and shrubs are an integral component of the farming system that complements the function of livestock feed uses and improve productivity.

**Foliage sample collection and preparation**

The samples of the indigenous browse species were collected from sampling sites in Ebinat, Libo Kemkem and Farta districts of the south Gondar zone from August to September 2022. Eight (8) indigenous browse fodder plants were collected from each agroecology for laboratory analysis. Samples were randomly harvested and bulked to form representative samples from 10 plants of each species, collected from each agroecology. Samples were oven-dried at 55oC for 72 h for constant weight to determine the dry matter (DM) and ground using a Wiley mill to pass through a 1mm sieve size. The ground feed samples were kept in airtight plastic containers until the chemical composition analysis and in vitro gas production was measured.

**Chemical analysis of selected indigenous browse plants**

The chemical analyses of the forage samples were determined using standard analytical methods. The dry matter and ash content of the sample were determined according to the procedures of AOAC (1990). The Kjeldahl method was used to determine total nitrogen (N) and crude protein (CP) was calculated as N x 6.25 (AOAC 1990). The neutral detergent fiber (NDF) was analyzed using the detergent extraction method as described by Van Soest *et al.* (1991) and acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to Van Soest and Robertson (1985).

***In vitro* Dry Matter Digestibility**

The*in vitro* dry matter digestibility (IVDMD) was determined according to Tilley and Terry (1963) as modified by Van Soest and Robertson (1985), in which the second stage (pepsin digestion) is substituted with a neutral detergent solution. This treatment removes all indigestible microbial matter and leaves as a residue of the undigested plant cell wall, and the values represent true digestibility.

***In Vitro* Gas Production**

The *in vitro* gas production was carried out by using the gas production technique of Menke and Stengass (1988). Rumen fluid was obtained from three rumen canulated Arsi-Bale sheep by suction tube fed natural pasture hay as a basal feed and supplemented with 200g of concentrate mixture (64% wheat bran, 35% noug seed cake, and 1% salt) per sheep/day. The animals had free access to clean drinking water and mineral licks. Rumen fluid was collected before morning feeding and then prepared and purged with CO2 to maintain anaerobic conditions (Makkar 2003).

The incubation of sampled species was carried out according to Menke and Stengass (1988) 120 ml in calibrated syringes in three batches at 39 °C. Each sample weighing about 200 mg was placed in the syringe and 30ml of inoculum was added that contained cheese cloth strained rumen liquor and buffer solution under continuous flushing with CO2. The volume of gas production was measured at 3, 6, 12, 24, 48 and 72h. The gas production characteristics were estimated using the Orskov and Mcdonald (1979) equation:

Y = a + b (1 – ect)

Where

Y = volume of gas produced at time ‘t’,

a = intercept (gas produced from the fermentable fraction),

b = gas production from the non-fermentable fraction,

(a+b) = total gas production,

c = gas production rate constant for the non-fermentable fraction (b), t = incubation time

**Post-incubation parameters**

Post-incubation parameters such as metabolizable energy (ME, MJ/ Kg DM), organic matter digestibility (OMD%) and short-chain fatty acids (SCFA, mmol/L) were estimated at 24 h of incubation time Menke and Stengass (1988). At the end of 24 h of incubation, 4 ml of 1N Na (OH)2 was added to the substrate in each syringe to determine the production of methane (Fievez *et al.* 2005). *In vitro* organic matter digestibility (IVOMD) was calculated from the equation: IVOMD (%) = 18.53 + 0. 9239GP (at 24hr) + 0.0540 CP. Where: IVOMD = *in vitro* organic matter digestibility at 24 hours and CP = crude protein (%). Metabolizable energy (ME) was calculated from the equation: ME (MJ/g DM) = 2.20 +0.136GP+0.0057CP. Where: GP=Gas production over 24 hr of incubation; CP=Crude protein content of feed samples. Short-chain fatty acids (SCFA) were calculated from the equation: SCFA = 0.0239\*GP – 0.0601.

**Statistical analysis**

Analysis of variance (ANOVA) was carried out on chemical composition, volume of *in vitro* gas production, *in vitro* gas production characteristics, and post-incubation parameters using the General linear model (GLM) procedure of Statistical Package for Social Science (SPSS version 26). The statistical design used to analyze the data was Completely Randomized Design (CRD). Means were separated using Tukey’s comparison test. The Model was:

Yij = µ+Ai + eij

Where, Yij = response variable, µ = overall mean, Ai = species effect and eij= random error

**RESULTS**

**Nutrient content of indigenous browse species**

The nutrient content of selected indigenous browse species is presented in Table 1. The DM content of the browse fodder in the lowland area ranged between 878.9 g/kg DM and 921.1 g/kg DM whereas in the midland between 899.7 g/kg DM and 926.4 g/kg DM. In the lowlands, *Ficus vasta* and *Albezia amara* pods had the highest DM content among the leaves and pods of indigenous browse fodder species, whereas the leaves of *Albezia amara* and *Ficus sycomorus* had the lowest, respectively. *Cordia Africana* had the highest DM in the midland whereas, *F. sycomorus* leaves had the lowest.

The crude protein (CP) content of indigenous browse fodder species (IBFS) for the highland, midland, and lowland ranged from 89.0 to 223.4 g/kg DM, 130.3 to 207.7 g/kg DM and 102.1 to 216.0 g/kg DM, respectively. In the highlands, the highest CP content was recorded from *V. amygdalina* followed by *Cordia africana* and the lowest was recorded S*yzygium giuneens*. In the midlands, *Dodonaea viscosa* had the highest CP content followed by *Acacia abyssinica*. In the lowlands, the highest CP content was recorded for *Acacia abyssinica*.

The ash content of indigenous browse species in the highlands ranged from 44.7 to 157.7 g/kg DM, where *Syzygium giuneens* and *Myrica salicifolia* exhibited the lowest while *Cordia Africana* exhibited the highest. The ash content of IBFS varied from 62.7 to 138.6 g/kg DM, where *A. amara* pods and *F. sycomorus* revealed the lowest and the highest value in the lowland respectively.

**Table 1. The nutrient compositions of selected indigenous browse fodder species (g/kg DM).**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Browse species** | **DM** | **Ash** | **CP** | **NDF** | **ADF** | **ADL** | **IVDM** |
| **Lowland** |  |  |  |  |  |  |  |
| *Ficus vasta* | 921.1 | 114.2 | 181.5 | 411.2 | 182.0 | 44.4 | 619.4 |
| *Acacia abyssinica* | 911.5 | 74.3 | 216 | 449.4 | 184.5 | 58.9 | 566.6 |
| *Acacia brevispica* | 904 | 72.9 | 102.1 | 218.8 | 95.9 | 26.4 | 553.1 |
| *Ficus sycomorus* | 897.5 | 138.6 | 166.4 | 390 | 185.3 | 47.8 | 536.5 |
| *Cordia africana* | 910.7 | 128.9 | 208.1 | 534.1 | 234.0 | 72.6 | 547.6 |
| *Terminalia brownii* | 916.3 | 77.4 | 150.8 | 386.3 | 169.6 | 33.5 | 561.4 |
| *Albezia amara* (pod) | 916.5 | 62.7 | 154.6 | 589.2 | 228.1 | 63.6 | 563.1 |
| *Albezia amara* (leaves) | 878.9 | 71.5 | 198.1 | 773.7 | 330.5 | 101.4 | 505.1 |
| **Midland** |  |  |  |  |  |  |  |
| *Ficus vasta* | 907.8 | 128.7 | 139.6 | 438.6 | 186.3 | 59.2 | 570.5 |
| *Acacia abyssinica* | 914.8 | 59.5 | 178.5 | 356.8 | 148.7 | 28.7 | 594.0 |
| *Cordia africana* | 926.4 | 147.2 | 151.3 | 638.6 | 243.3 | 81.1 | 513.1 |
| *Ficus sycomorus* | 899.7 | 183.9 | 130.3 | 413.7 | 204.7 | 49.9 | 523.3 |
| *Ficus thonnigii* | 902.6 | 153.1 | 160.5 | 476.0 | 191.2 | 49.0 | 546.1 |
| *Dodonaea viscosa* | 919.9 | 77.0 | 207.7 | 359.2 | 137.1 | 30.1 | 677.2 |
| *Syzygium giuneens* | 916.4 | 82.9 | 135.4 | 489.3 | 238.2 | 75.5 | 518.5 |
| *Stereospermum kunthianum* | 917 | 84.1 | 137.9 | 277.4 | 142.8 | 33.1 | 638.8 |
| **Highland** |  |  |  |  |  |  |  |
| *Ficus vasta* | 898.1 | 145.4 | 123.1 | 460.3 | 209.0 | 48.2 | 522.8 |
| *Acacia abyssinica* | 909.2 | 60.6 | 209.3 | 436.4 | 132.5 | 50.2 | 577.2 |
| *Ficus thonnigii* | 905.9 | 157.2 | 197.3 | 402.0 | 150.4 | 50.7 | 582.6 |
| *Cordia africana* | 909.2 | 157.7 | 219.9 | 503.5 | 200.7 | 52.7 | 558.3 |
| *Vernonia amygdalina* | 913.3 | 113.8 | 223.4 | 378.6 | 177.1 | 47.1 | 664.5 |
| *Olea africana* | 940.5 | 66.5 | 187.3 | 454.2 | 207.6 | 44.5 | 610.9 |
| *Syzygium giuneens* | 903.5 | 44.7 | 89.0 | 582.0 | 299.6 | 45.2 | 484.3 |
| *Myrica salicifolia* | 898 | 58.2 | 139.4 | 479.7 | 201.6 | 69.9 | 540.3 |

Note: AEZ = Agroecological zone; DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; IVDM = In vitro dry matter digestibility

The NDF and ADF content of indigenous browse species ranged from 378.6 to 582.0 g/kg DM and 132.5 to 299.6 g/kg DM in the highlands, respectively. In the midlands, the NDF content ranged from 277.4 to 638.6 g/kg of DM and the ADF content from 137.1 to 243.3 g/kg DM. *S. giuneens* had the highest NDF and ADF content, and *V. amygdalina* had the lowest NDF in highland agroecology. While, *C. africana* had the highest NDF and ADF content in the midlands. In the lowlands, *A. amara* pod and *C. africana* had the highest NDF content, *A. amara* leaves had the highest and *A. brevispica* had the lowest ADF content in the lowlands, respectively.

In the highlands, the ADL content of indigenous browse species ranged between 44.5 and 69.9 g/kg of DM. *Olea africana* contained the lowest, whereas *M. salicifolia* had the highest ADL content. The ADL content of indigenous browse species in the midlands ranged from 28.7 to 81.1 g/kg DM. The lowest ADL content was found in *Acacia abyssinica* and the highest was in *C. africana*. In the lowland, the ADL content ranged from 26.4 to 101.1 g/kg of DM. The leave*s of A. amara* had the highest ADL, while *A. brevispica* had the lowest in lowland agroecology.

***In vitro* dry matter digestibility of indigenous browse species**

The *in vitro* dry matter digestibility (IVDMD) contents varied among the studied indigenous browse species. The IVDMD of IBFS in the highland ranged between 484.3 and 664.5 g/kg DM. *V. amygdalina* had the highest IVDMD, whereas *S. giuneens* had the lowest IVDMD. In the midland, the IVDMD of IBFS ranged from 513.1 to 677.2 g/kg DM. The lowest IVDMD was found in *C. Africana* and the highest values of IVDMD were recorded for *D. viscosa*. In the lowland area *Ficus vasta* (619.4 g/kg) DM had the highest while the lowest values were obtained in *A. amara* leaves (505.1 g/Kg) DM.

***In vitro* gas production of indigenous browse fodder species**

*In vitro* gas production of IBFS from the study area are presented in Table 2. The browse species samples showed variability in the total volume of gas produced. The volume of gas released from fermented samples increased with increasing incubation time throughout 72 h incubation time. Among the samples from the highland areas the lowest gas volume was recorded for *S. giuneens* (12.99 ml/200 mg) while *V. amygdalina* (40.66 ml/200 mg) produced a higher gas volume than the other species at 24 h of the fermentation period. At 24 h of incubation, the gas production volume ranged from 12.32 ml/200 mg (S. *giuneens*) to 47.61 ml/200 mg (*S. kunthianum*) in the midland area. All the *in vitro* gas volume production of the IBFS exhibited significant differences among species in the lowland. At 24 h of incubation, *A. amara* leaves produced lowest (3.33 ml/200 mg) and *F. vasta* (36.95 ml/200 mg) were higher gas volume.

**Table 2. In vitro gas production (ml/200 mg DM) of selected indigenous browse fodders.**

| Incubation period | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Species | 3hr | 6hr | 9hr | 12hr | 24hr | 48hr | 72hr |
| **Lowland** |  |  |  |  |  |  |  |
| *Ficus vasta* | 4.96ab | 9.32ab | 15.65a | 23.98a | 36.95a | 44.94a | 46.94b |
| *Acacia abyssinica* | 1.65de | 3.00ef | 5.00d | 6.33e | 11.99e | 16.99g | 19.49f |
| *Acacia brevispica* | 7.32a | 11.33a | 14.65a | 17.99b | 32.97b | 41.47b | 50.46a |
| *Ficus sycomorus* | 3.99bc | 5.99cd | 7.99c | 10.98d | 17.97d | 29.47e | 33.47d |
| *Cordia africana* | 3.56bcd | 5.32de | 8.32c | 12.31d | 26.28c | 34.97c | 36.47c |
| *Terminalia brownii* | 3.66bcd | 5.66cd | 8.33c | 10.99d | 16.65d | 26.48f | 28.48e |
| *Albezia amara* (leaves) | 1.33e | 1.96f | 1.33e | 1.67f | 3.33f | 5.49h | 6.49g |
| *Albezia amara* (pods) | 4.99ab | 7.97bc | 10.64b | 14.64c | 23.62c | 31.42d | 33.41d |
| Mean | 3.93 | 6.32 | 8.98 | 12.36 | 21.22 | 28.9 | 31.9 |
| SD | 0.78 | 0.68 | 0.68 | 0.61 | 0.8 | 12.28 | 13.56 |
| CV | 19.8 | 10.7 | 7.6 | 5.0 | 3.8 | 1.06 | 3.2 |
| **Midland** |  |  |  |  |  |  |  |
| *Ficus vasta* | 6.66b | 8.99bc | 7.99c | 12.32c | 17.98de | 29.47c | 32.46c |
| *Acacia abyssinica* | 6.00b | 11.99b | 15.66b | 18.99b | 26.98b | 37.98b | 39.98b |
| *Ficus sycomorus* | 3.33b | 3.66c | 5.66c | 7.99c | 15.31de | 26.95cd | 29.95d |
| *Cordia africana* | 5.00b | 6.99bc | 8.99c | 11.66c | 20.31cd | 28.47d | 30.97cd |
| *Ficus thonnigii* | 4.00b | 5.33c | 6.33c | 8.32c | 16.32de | 24.48d | 27.47e |
| *Dodonaea viscosa* | 5.65b | 7.98bc | 9.65bc | 12.64c | 22.62bc | 39.39b | 41.39b |
| *Syzygium giuneens* | 5.66b | 6.99bc | 7.99c | 9.99c | 12.32e | 15.48e | 16.48f |
| *Stereospermum kunthianum* | 12.32a | 22.97a | 31.96a | 40.61a | 47.61a | 57.93a | 59.93a |
| Mean | 6.07 | 9.36 | 11.78 | 15.31 | 22.43 | 32.52 | 34.83 |
| SD | 1.69 | 1.84 | 2.14 | 1.68 | 2.46 | 0.82 | 0.76 |
| CV | 27.8 | 19.6 | 18.2 | 10.9 | 11 | 2.5 | 2.2 |
| **Highland** |  |  |  |  |  |  |  |
| *Ficus vasta* | 3.00d | 5.00e | 6.99c | 8.99d | 16.65de | 23.48de | 24.98e |
| *Acacia abyssinica* | 5.66bc | 9.98bc | 13.99b | 16.65bc | 24.31bc | 31.97bc | 34.47c |
| *Cordia africana* | 3.66cd | 6.33de | 9.66c | 12.99cd | 22.64cd | 26.98bc | 29.47d |
| *Ficus thonnigii* | 3.66cd | 8.33cd | 13.99b | 19.65b | 26.64bc | 34.97bc | 34.97c |
| *Vernonia amygdalina* | 8.32a | 16.65a | 25.64a | 33.30a | 40.66a | 50.44a | 52.94a |
| *Olea Africana* | 5.99b | 10.99b | 16.65b | 20.98b | 30.97b | 36.96b | 38.96b |
| *Syzygium giuneens* | 3.00d | 5.33e | 6.99c | 9.33d | 12.99e | 20.48e | 21.48f |
| *Myrica salicifolia* | 2.66d | 4.99e | 7.99c | 10.99cd | 13.65e | 18.98e | 16.53g |
| Mean | 4.5 | 8.45 | 12.73 | 16.6 | 23.56 | 30.53 ± | 31.72 |
| SD | 0.56 | 0.94 | 1.29 | 2.09 | 2.36 | 1.86 | 0.63 |
| CV | 12.4 | 10.9 | 10.2 | 12.6 | 10.02 | 6.1 | 1.99 |

Note: Superscripted a, b ,c, d e: *Means in the same column with different superscript differ significantly (P<0.05) (P<0.05).*

Abbreviation: SD = standard deviation; CV = Coefficient of variation.

***In vitro* gas production characteristics of indigenous browse species**

The *in vitro* gas production characteristics of IBFS are presented in Table 3. In the lowland, the immediate fermented values (“a”) of browse species ranged from 30.8% for *A. amara* leaves to 35.7% for *F. vasta*. The lowest slowly degradable fraction (“b”) was obtained from *A. amara* leaves(31.4%) and highest in *A. abyssinica* (62.9%). Potential gas production (a + b) ranged from 62.2% for *A. amara* leaves to 98.7% for *A. abyssinica*. The rate of gas production (“c”) ranged from 0.004% /h for *A. amara* leavesto 0.025 %/ h for *F. vasta.*

Among the browse fodder species in the midland, the gas production from immediately soluble portion “a” value ranged from 29.6% (*S. kunthianum*)to 37.5% in (*A. abyssinica*). The gas production from water-insoluble but degradable portion “b” fraction was highest in *S. kunthianum* (70.1 %) and lowest 32.5% in S. *giuneens*. The rate of gas production “c” ranged from 0.006% /h in S. *giuneens* to 0.761% /h in *S. kunthianum* leaves. The potential gas production (a+b) values ranged from 67.3 % in *Ficus thonnigii* to 99.7 % in *S. kunthianum* leaves.

In the highland species, the rapidly soluble fraction (“a”) value varied from 26.9% for *V. amygdalina* to 38.8% for *Olea africana*. Slowly degradable fraction (“b”) value ranged from 26.4% for *M. salicifolia* to 65.6% for *V. amygdalina.* Potential gas production (*a* + b) ranged from 62% for *M. salicifolia* to 92.5% for *V. amygdalina.* The rate of gas production (“c”) ranged from 0.013%/h for *M. salicifolia* to 0.073%/h for *V. amygdalina.* The difference in values of “a”, “b” and “c” between species might be attributed to the concentration of carbohydrates in each browse species.

**Table 3. In vitro gas production characteristics of selected indigenous browse fodder.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Gas production characteristics | | | | | |  |
| Species of browse plants | a (%) | b (%) | a + b (%) | c (hr-1) | LT (hr) | RSD |
| **Lowland** | | | | | | |
| *Ficus vasta* | 35.7 | 51.8 | 87.5 | 0.025 | 28.4 | 5.768 |
| *Acacia abyssinica* | 35.8 | 62.9 | 98.7 | 0.018 | 30.3 | 2.643 |
| *Acacia brevispica* | 32.5 | 35.7 | 68.2 | 0.017 | 176.5 | 1.635 |
| *Ficus sycomorus* | 33.5 | 50.1 | 83.6 | 0.015 | 59.9 | 1.386 |
| *Cordia Africana* | 33.4 | 47.8 | 81.2 | 0.019 | 48.2 | 3.937 |
| *Terminalia brownii* | 34.1 | 44.9 | 79 | 0.013 | 79.2 | 2.11 |
| *Albezia amara (leaves)* | 30.8 | 31.4 | 62.2 | 0.004 | - | 0.787 |
| *Albezia amara (pods)* | 35.1 | 43.4 | 78.5 | 0.016 | 63.6 | 3.252 |
| **Midland** | | | | | | |
| *Ficus vasta* | 33.8 | 40.8 | 74.6 | 0.018 | 68.8 | 1.788 |
| *Acacia abyssinica* | 37.5 | 53.6 | 91.1 | 0.024 | 26.4 | 3.712 |
| *Ficus sycomorus* | 30.5 | 51.4 | 81.9 | 0.012 | 78.8 | 1.875 |
| *Cordia Africana* | 34.3 | 46.4 | 80.7 | 0.014 | 68.6 | 2.447 |
| *Ficus thonnigii* | 30.9 | 36.4 | 67.3 | 0.019 | 107.2 | 1.364 |
| *Dodonaea viscosa* | 32.2 | 58.9 | 91.1 | 0.016 | 44.5 | 2.671 |
| *Syzygium giuneens* | 36.4 | 32.5 | 68.9 | 0.006 | 301.2 | 1.678 |
| *Stereospermum kunthianum* | 29.6 | 70.1 | 99.7 | 0.761 | 8.4 | 1.636 |
| **Highland** | | | | | | |
| *Ficus vasta* | 33.5 | 41.7 | 75.2 | 0.014 | 83.7 | 1.935 |
| *Acacia abyssinica* | 37.7 | 44.9 | 82.6 | 0.017 | 47.9 | 2.752 |
| *Cordia Africana* | 34.9 | 44.1 | 79 | 0.016 | 63.4 | 3.239 |
| *Ficus thonnigii* | 36.9 | 55.2 | 92.1 | 0.019 | 32.3 | 6.115 |
| *Vernonia amygdalina* | 26.9 | 65.6 | 92.5 | 0.073 | 10.9 | 1.693 |
| *Olea Africana* | 38.8 | 52.2 | 91 | 0.016 | 37.1 | 4.808 |
| *Syzygium giuneens* | 34.6 | 43.3 | 77.9 | 0.015 | 68 | 2.826 |
| *Myrica salicifolia* | 35.6 | 26.4 | 62 | 0.013 | - | 2.463 |

Note: a = gas production from the soluble fraction; b = gas production from the insoluble but degradable fraction; a + b = potential gas production; c = the rate constant of gas production of b; RSD= residual standard deviation.

**The post incubation parameters of indigenous browse species**

The organic matter digestibility (OMD), short-chain fatty acid (SCFA), metabolizable energy (ME) and methane production of selected browse species are shown in Table 4. The post-incubation parameters OMD, ME, SCFA and methane production of IBFS exhibited significant differences among the three agroecologies. In highland area, OMD at 24 h incubation time ranged from 33.34% in *S. giuneens* to 68.48% in *V. amygdalina*. The OMD values of browse species in the midland of the study area at 24hr ranged between 37.32% in S*yzygium giuneens* to 68.88% in *S. kunthianum.* At 24 h incubation time, the OMD of *Ficus vasta* (63.33%) was highest and the value of *A. amara* (31.41%) was lowest in lowland agroecology of the study area. Low OMD in this study may be due to the high NDF content of the species. The NDF value has a negative effect on OMD.

**Table 4. Post incubation parameters of selected indigenous browse fodder species.**

| Post incubation parameters | | | |  |  |
| --- | --- | --- | --- | --- | --- |
| Species name | OMD (%) | ME(MJ/kg) DM | SCFA (mmol/L) | Methane (ml/200mg) | MT/GP at 24 h |
| **Lowland** |  |  |  |  |  |
| *Ficus vasta* | 63.33a | 8.26a | 0.82a | 4.67a | 0.07ab |
| *Acacia abyssinica* | 40.09d | 5.06d | 0.23e | 1.33cd | 0.03bc |
| *Acacia brevispica* | 53.53b | 7.27b | 0.73b | 2.66bc | 0.04ab |
| *Ficus sycomorus* | 47.37c | 5.59c | 0.37d | 0.67d | 0.02c |
| *Cordia africana* | 55.99b | 6.96b | 0.57c | 2.67bc | 0.05ab |
| *Terminalia brownii* | 41.50d | 5.32c | 0.34d | 2.66bc | 0.05ab |
| *Albezia amara leaf* | 31.41e | 3.78e | 0.02f | 0.33d | 0.01c |
| *Albezia amara pod* | 46.92c | 6.29cd | 0.50c | 3.66ab | 0.07a |
| Mean  SD | 47.52  0.95 | 6.07  0.11 | 0.45  0.07 | 2.33  0.58 | 0.042  0.01 |
| CV | 2 | 1.7 | 14.3 | 24.8 | 29.2 |
| **Midland** |  |  |  |  |  |
| *Ficus vasta* | 45.52c | 5.44b | 0.37cd | 2.66cd | 0.05abc |
| *Acacia abyssinica* | 50.77b | 6.89ab | 0.59b | 6.66b | 0.10a |
| *Ficus sycomorus* | 46.32c | 5.02b | 0.31cd | 0.23e | 0.01d |
| *Cordia africana* | 49.33c | 5.83b | 0.43bc | 3.66c | 0.07abc |
| *Ficus thonnigii* | 46.58c | 6.91ab | 0.33cd | 3.67c | 0.08ab |
| *Dodonaea viscosa* | 49.34c | 6.46b | 0.48bc | 3.67c | 0.07abc |
| *Syzygium giuneens* | 37.32d | 4.65b | 0.23d | 1.67d | 0.04d |
| *Stereospermum kunthianum* | 68.88a | 9.46a | 1.07a | 8.66a | 0.10a |
| Mean  SD | 49.26  2.19 | 6.33  0.66 | 0.476  0.06 | 3.79  0.57 | 0.065  0.012 |
| CV | 4.4 | 10.4 | 12.4 | 15.2 | 18.6 |
| **Highland** |  |  |  |  |  |
| *Ficus vasta* | 44.69de | 5.17c | 0.34cd | 3.67cd | 0.08bc |
| *Acacia abyssinica* | 49.85cd | 6.69b | 0.52bc | 6.67b | 0.12a |
| *Cordia africana* | 55.17bc | 6.53b | 0.48bc | 6.66b | 0.13a |
| *Ficus thonnigii* | 57.68b | 6.95b | 0.58b | 7.66b | 0.12a |
| *Vernonia amygdalina* | 68.48a | 9.01a | 0.91a | 9.66a | 0.12a |
| *Olea Africana* | 55.17bc` | 7.48b | 0.68b | 6.68b | 0.11ab |
| *Syzygium giuneens* | 33.34f | 4.48c | 0.25e | 4.65c | 0.09abc |
| *Myrica salicifolia* | 37.08ef | 4.85c | 0.27e | 2.66d | 0.06c |
| Mean  SD | 47.52  0.72 | 6.39  0.12 | 0.50  0.02 | 6.04  0.58 | 0.103  0.011 |
| CV | 15.1 | 1.7 | 3.9 | 9.6 | 10.7 |

Means in the same column with different superscript differ significantly (P<0.05)

Note: ME = metabolizable energy; OMD = organic matterdigestibility; SCFA = Short Chain Fatty Acids; MT/GP = methane to gas ratio; SD = standard deviation.

The SCFA values (mmol/L) in the highland area ranged from 0.25 mmol/L for *S. giuneens* to 0.91 mmol/L for *V. amygdalina*. *S. kunthianum* (1.07 mmol/L) has the highest while S. *giuneens* (0.23 mmol/L)recorded the lowest SCFA production amongst the studied browse species in the midland area**.** In lowland browse species,SCFA was the highest in *Ficus vasta* (0.82 mmol/L) and the lowest in *A. amara* leaf(0.02 mmol/L). The metabolizable energy (ME) value of browse species in the highland ranged from 4.48 MJ/kg in *S. giuneens* to 9.01 MJ/kg DM in *V. amygdalina*. The metabolizable energy content of browse species observed in the midland ranged from 4.65 MJ/kg DM for *S. giuneens* to 9.46 MJ/kg DM for *S. kunthianum.*

Methane production among indigenous browse species showed significant differences in the lowland, midland and highland study areas. Among all species *F. vasta* (4.67 ml/200 mg), *S. kunthianum* (8.66 ml/200 mg) and *V. amygdalina* (9.6 ml/200 mg) had the highest CH4 production, whereas *A. amara* (0.33 ml/200 mg), *F. sycomorus* (0.23 ml/200 mg) and *M. salicifolia* (2.66 ml/200 mg) produced the least in the low, mid and highland study areas respectively.

**DISCUSSION**

**Nutrient content of indigenous browse species**

The nutritive values of indigenous browse fodder in the present finding are within the range reported for most browse species in the tropics (Yisehak and Janssens 2013). The value of CP (89 g/kg DM to 223.4 g/kg DM) contents of the browses in the current study is in line with the findings of Shenkute *et al.* (2012) who reported that the crude protein (CP) contents of the browse species ranged from 89 to 209 g/kg DM in mid rift valley of Ethiopia. Similarly Desalegn *et al.* (2016) reported that the CP content of indigenous browse fodder varied between 105.3 and 233.4 g/kg DM in northwestern Ethiopia. The CP content of all selected indigenous browse fodder species in the study areas was above the minimum critical levels of CP content (80 g/kg DM) required for normal function of rumen microorganisms (NRC 2001). It has been indicated that most tropical browses are high in CP and can be used to supplement poor quality roughages to increase the productivity of ruminant livestock (Abraham *et al.*, 2023; Aynalem *et al.*, 2020; Derero and Kitaw 2018; Njidda *et al.*, 2022) which is consistent with the results obtained in the current experiment.

The NDF and ADF of browse fodder varied from 218.8 to 773.7 g/kg DM and 95.9 to 330.5 which is comparable with the findings of Almaz *et al.* (2021) who showed that NDF and ADF content of multipurpose browse species ranged from 226 to 497.5 and 185 to 282 g/kg DM respectively in the highland of Awi zone northwest Ethiopia. According to the report of Sisay *et al.* (2018), the neutral detergent fiber, acid detergent fiber and acid detergent lignin were highest for *C. africana*.

Voluntary DM intake and DM digestibility are dependent on the cell wall constituents (fiber components) of the forages. In current study the NDF content except S. *giuneens* in the highland, all species have an NDF of less than 55%, which was lower than the concentration suggested as a limit which could affect intake and digestibility in ruminants (Van Soest and Robertson 1985). On the other hand, all the browse species in all agro-ecologies had ADF content < 40% which is believed to have high quality feed (Kellems and Church 1998). Browse fodder species that have high content of ADF might have lower digestibility since the digestibility of feeds and ADF content is negatively correlated (Mokoboki *et al.*, 2019; Sisay *et al.*, 2018). The fiber composition of the species was variable among the browse species and varied between low to moderate categories. Since that forages are the main source of ruminant animals (Chebli *et al.*, 2021). Variability in the nutrient content of browse species might be due to plant species, soil types and locations. These factors influence chemical composition, digestibility and nutrient utilization by animals.

The *in vitro* dry matter digestibility in the current study varied from 484.3 g/kg DM in *S. giuneens* to 677.2 g/kg DM in *D. viscosa*. This variation could be attributed to factors like species type and location (Cheema *et al.*, 2014; Melaku *et al.*, 2010). The current finding is in agreement with a previous report (Misrak, 2023) who investigated that the IVDMD value varied between 639.2 g/kg DM in *O. africana* to 462.8 g/kg DM in *C. africana*. The result reported by (Bayssa *et al.* 2016; Feyisa *et al.*, 2022; Ravetto Enri *et al.*, 2020) also indicated that indigenous browse species in various locations had high digestibility percentage.

***In vitro* gas volume and production characteristics**

The study found significant differences in the volume of gas production and gas production characteristics among the browse species. In this investigation, *V. amygdalina* (40.66 ml/200 mg*, S. kunthianum* (47.61 ml/200 mg and *F. vasta* (36.9 ml/200 mg*)* had the highest gas and, *S. giuneens* (12.99 ml/200 mgand12.32 ml/200 mg),and *A. amara* (3.33 ml/200 mg) had the lowest gas volume at 24 h after incubation of the feed samples in high, mid and lowland respectively. Gas production after 72 h of incubation ranged between 6.49 and 56.9 ml/200 mg DM and this was comparable with the finding of (Sisay *et al.*, 2018) who showed that a range between 11.85 and 53.73 ml/ 200 mg DM of different indigenous browse species from Ethiopia rift valley. On the other hand the above result was lower than the report of Abraham *et al.* (2023),who stated a range between 23 and 71 ml/200 mg DM of indigenous legume trees and shrubs in the semi-humid condition of southern Ethiopia. The highest gas volume at 24 h of incubation had positive effects on the concentration of SCFA and ME production from the browse species. The variation in OMD and gas production among browse species is the reflection of the chemical composition of the forage species especially the amount of NDF in the browse species. It was reported by (Terrill *et al.*, 1992) that the presence of high NDF levels negatively affects OMD of the feed samples.

The highest insoluble but rumen degradable (‘b’) fraction was observed for *V. amygdalina, S. kunthianum* and *A. abyssinica* species in high, mid and lowland respectively. These reflected a superior index of microbial degradation of the browse and high fermentation rates of the feed samples in the rumen. On the other hand, the highest lag time (LT) in *F. vasta, S. giuneens* and *A. brevispica* reflected the delay in the fermentation rate of these species which was an implication of the effect of either high tannin rumen microbial fermentation or high level of fiber fraction (NDF) in the species that would hinder the penetration of rumen microorganisms to the soluble carbohydrate fraction for rapid fermentation rate. Mutai *et al.* (2022) and Yisehak *et al.* (2014) investigated that, *A. brevispica* (16.474 mg/g DM) and *S. giuneens (*172g/kg DM)leaves contained high levels of condensed tannins which might be resulted in lower gas production due to reduced rumen microbial activity

**The post incubation parameters of indigenous browse species**

The estimated OMD, ME and SCFA from the gas volume at 24 h of incubation was the highest for *V. amygdalina, S. kunthianum* and *F. vasta* in high, mid and lowland respectively*.* The least for *S. giunees* in the highland and midland and, *A. amara* species in the lowlands. The amount of gas released when browses were incubated has been reported to be closely related to digestibility of feeds for ruminants (Krishnamoorthy *et al.*, 1995). Thus, gas volume can be considered a good reflection of substrate fermented to volatile fatty acids and an estimate of potential digestibility in the rumen (Getachew *et al.*, 2000). The OMD, ME and SCFA values of the studied browse species estimated in the experiments were within the range reported for most browse trees and shrub species native to the tropics (Abraham *et al.*, 2023; Amanzougarene and Fondevila, 2020; Bayssa *et al.*, 2016; Sisay *et al.*, 2018).

Methane production, which is the major loss from ruminants during rumen fermentation of feeds was observed to be high in *F. vasta*, *A. amara* pods, *S. kunthianum* and *A. abyssinica* species. But a lower volume of methane gas was produced from *A. amara* leaves and *F. sycomorus* species*.* This showed high conversion rate of carbohydrate fermentation products to acetate instead of CH4. The presence of a high concentration of polyphenolics might be the cause of the browse species indicated above's lower CH4 production. Depending on the chemical makeup of the phenolic compounds found in different browse species, the effect of phenolic compounds on methanogenesis might be attributed to the direct suppression of methanogenic archaea (Patra and Saxena 2010).

Hassan Sallamab *et al.* (2010) suggested that the methane reduction potential of any feed stuff can be estimated from the percentage of in vitromethane gas production and the feed stuff can be arbitrarily classified in three categories; low reduction potential (% CH4 in gas, 11-14%); medium potential (% CH4 in gas, 6-11%) and high potential (% CH4 in gas, 0-6%) with respect to standard grass hay. In the current study, the browse fodder under investigation were classified as low to high methane reduction potential since the percent methane production in the current study ranged from 1% to 13% of the total gas. The differences in CH4 among the species reflect the observed variations in fiber fraction.

**CONCLUSIONS**

In this experiment the indigenous browse fodder species yielded moderate to high CP values. Leaves of *Acacia abyssinica, Dodonaea viscosa, Vernonia amygdalina* in lowland, midlands and highland respectivelyhave been identified as alternative sources of protein, suggesting their potential in supplementing low quality forages. NDF, ADF and ADL content also varied among different species, with some species showing higher fiber content compared to others. Based on the result indigenous browse species produced substantial gas volume, OMD, ME and SCFA and CH4 however, varied among species. The study also found that certain browse species such as *Albezia amara, Ficus sycomorus* and *Myrica salicifoliain* from lowland, midlands and highlands respectivelywere identified as potential candidates in mitigating CH4 production. This indicates that these browse species have a higher conversion rate of carbohydrate fermentation products to acetate instead of methane. The classification of the browse fodder species into low to high methane reduction potential categories based on their methane production percentages provides valuable information for livestock producers looking to reduce methane emissions from ruminant. In conclusion, all browse species had moderate to high CP contents that can be used as a supplement for low-quality roughages to improve feed quality and reduce methane production.Further research in this area could help in promoting the use of browse species with better nutritional quality and lower methane emission from livestock production.

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**Compliance with ethical standards.** There is no university committee to approve the protocol to sample rumen liquor. However, all procedures were performed to ensure minimum distress to animals and to maintain animal welfare.

**Data availability.** Data are available with the corresponding author upon reasonable request. Berihun2947@gmail.com

**Author contribution statement (CRediT). B. Kibret –** Conceptualization; Investigation; Methodology; Data curation; Formal analysis; Writing original draft; Writing review and editing. **B. Asmare --** Conceptualization; Investigation; Methodology; Validation; Supervision; Writing review and editing. **L. Yeheyis --** Conceptualization; Investigation; Methodology; Validation; Supervision; Writing review and editing. **M. Bayssa --** Methodology; Data curation; Formal analysis;Validation.

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