Chemical Composition and Ruminal Fermentation Characteristics of Selected Forage Species from Traditional Enclosure Areas in the Central Highlands of Ethiopia

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ABSTRACT

This study aimed to characterize key forage species in terms of their chemical composition and ruminal fermentation characteristics thereby determining their nutritional value for ruminants. A total of 20 plots of 40 m x 40 m were established on pasture land in the highland and mid-highland agro-ecologies. Sixteen quadrats (0.5 m x 0.5 m) were randomly laid per plot and samples of 19 key forage species were collected at 50% flowering stages. Species identification was done on site by using guide books and a trained technician. The harvested pasture was sorted by species to allow the investigation between species variability in terms of nutrient contents and in vitro ruminal fermentation parameters. Forage species evaluated in the current study showed noticeable variation in nutritive values and ruminal fermentation parameters. Principal component (PC) analysis showed that the first three PC (PC1, PC2 and PC3) explained 84.2% of the total variation in the dataset. The key forage species clustered according to their chemical composition and in vitro fermentable parameters into five main groups. Centella asiatica forb (sole group) was superior in gas production at 24 h, fractional gas production rate (c) and effective gas production (EGP) but lower in neutral detergent fiber (NDF) and CH_4 production. Forage legumes were clustered close to each other and had higher crude protein (CP), 'c', and EGP production compared to forage grass. Forage grasses except Eragrostis tenuifolia were characterized by higher CH_4 production compared to others. Therefore, appropriate management practices that maintain balanced proportions of herbs, forage legumes, and grasses may result in improved ruminal fermentation of the available diet while reducing methane emissions by ruminants fed on native pasture.

***Keywords**: chemical composition, crop-livestock, fermentation, forage, nutritive value, rumen*

INRODUCTION

Livestock production plays an important role in the economy of Ethiopia serving a wide variety of functions (Duressa et al., 2014). Despite the high livestock population, the current livestock contribution is below its potential which may affect the sustainability of crop-livestock production systems due to complex and interrelated factors such as feed shortage and disease (Selamawit et al., 2017). In the mixed crop-livestock farming system, the feed requirement for livestock is mainly derived from natural pasture, crop residues and stubble grazing (Tolera, 2009; Duressa et al., 2014; Selamawit et al., 2017). Feed resources derived from natural pastures have declined in the highlands because of shrinking pastureland due to the expansion of cropping as the expense of human population pressure. Moreover pasture productivity reduced associated with overutilization the pasture land and land degradation (Melaku et al., 2010).). Feed shortage is more intense in the highlands of the country where more than 75% of both human and livestock populations are concentrated (CSA, 2013). I The increasing pressure on available pasture lands as well as newly emerging climatic phenomena are affecting the botanical composition, biomass yield and nutritive value of pastures (Denekew et al., 2005; Kitabe and Tamir, 2006; Tessema et al., 2010). This in turn affects animal productivity and reproduction performances and hence the livelihoods of smallholder farmers (Melaku et al., 2010). Therefore, it is important to understand the dynamics of natural pastures thoroughly by exploring the changes in terms of botanical composition and nutritional parameters. Such information could be important to devise appropriate grazing land management practices and livestock feeding systems.

It is well known that some of the nutrients such, as the protein content of the grasses and herbaceous plants, decline during the dry season leading to prolonged periods of under-nutrition of livestock reared under such environmental conditions (Yayneshet et al., 2009). Even though natural pasture is the first and most important source of feed for livestock in the highland and mid-altitude agro-ecologies (Alemu et al., 2019), with the exception of some improved forages (Kebede et al., 2016) and fodder trees (Assefa et al., 2015), there is little information on its chemical composition and *in vitro* ruminal fermentation characteristics of the different species. Animal feed characterization works were mainly focused on feed resource assessment and determination of chemical composition based on a few proximate parameters such as DM, CP, NDF, ADF and ADL contents of key species (Geremew et al., 2017; Hussien, 2017). This information does not provide sufficient information to determine the true nutritive values and information that complements the farmers' indigenous knowledge. To evaluate the nutritive value of forage at a relatively minimum cost, there are a number of *in vitro* techniques available so far (Ginger-Reverdin et al., 2002). According to Abdalla et al. (2012), *in vitro* gas production can be used as a rapid evaluation tool to assess the nutritional quality of forages. It also helps to quantify nutrient utilization, and its accuracy in describing digestibility in animals has been validated in numerous experiments (Getachew et al., 2004). In addition, many driving forces such as human population growth and climatic change that alter the biophysical settings of the available grazing lands in the highland area resulted in a loss of soil fertility and thus land degradation which necessitated the need for regular monitoring of pasture composition, productivity, and quality.

Therefore, the objectives of this study were (i) to evaluate the chemical composition and *in vitro* ruminal fermentation parameters of the key forage species from central highlands of Ethiopia and (ii) to determine the relationships between chemical compositions and *in vitro* ruminal fermentation parameters of the key forage species from the study area. Such information is necessary to develop appropriate forage-based feeding systems and enhance animal productivity while minimizing the environmental damage caused by animal grazing on native pasture.

MATERIALS AND METHODS

Experimental Site

Forage collection was done in the Kofele district of West Arsi Zone of Oromia Regional State, Ethiopia, and located at $7^0$00'-$7^0$07' N and $38^0$48'-$39^0$00' E. The district is found within 2200-3200 masl and receives an average rainfall of 1800 mm per annum and has an average temperature of 19.5 ^{0}C. It has a bi-modal rainfall distribution with short rains starting from March to May and the main rainy season extending from June to September/October. The area is characterized as high potential for crop-livestock farming in which cattle and sheep are the most predominant livestock species (Hussien, 2017). Farmers in the study area grow crops such as barely (*Hordeum vulgare*), wheat (*Triticum aestivum*), maize (*Zea mays*) and *enset* (*Ensete ventricosum*) as food crops and potato (*Solanum tuberosum*), head cabbage (*Brassica oleracea*), beetroot (*Beta vulgaris*) and carrot (*Daucus carota*) as cash crops (Hussein 2017).

Forage Sample Collection and Preparation

Forage sample collection was conducted as described by (Wegi et al., 2020). The pasture land was enclosed for five months from May 2018 to September 2018. A total of 20 plots of 40 m x 40 m were established on pastureland (locally described as '*Kalo*'). In a single plot 16 quadrats (0.5 m x 0.5 m) were randomly laid for forage sample collection. Forage samples were collected at 50% flowering stages by mowing the pasture at 5 cm aboveground from the area of 0.25 m^2 quadrats. The harvested sample was sorted by species and pooled to obtain enough quantities of the individual species. Sample collection was done purposely to coincide with the period of utilization of the enclosure area (*kelo*) by farmers as per the information collected from elderly farmers in the study area. Species identification was made on-site by using guidebooks (Benget and Persson, 1974 and others) and a trained technician. For plant species that were difficult to identify at the field, their local names were recorded and herbarium specimens were collected, pressed and dried properly using a plant presser and identified and confirmed at the national herbarium, Addis Ababa University, Ethiopia. A total of 34 species were identified in the process out of which 19 were selected for the present study based on their rank in frequency, dominance, abundance, important value index (IVI) and elderly farmers' judgments, as reported by (Wegi et al., 2020). Samples of composite forage species were taken and oven-dried to a constant weight at 60 ^{0}C for 48 hours and then ground in a Willey mill to pass through a 1 mm sieve to use for chemical composition analysis.

Chemical Analysis

All analyses were done in triplicate, and analysis for DM, ash, and nitrogen (N) contents were done according to AOAC (2006). Nitrogen content was determined by using a standard Kjeldahl method and CP was calculated as N x 6.25. Neutral detergent fiber content was determined according to the method of Van Soest et al. (1991), in contrast acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined according to the method of Van Soest and Robertson (1985). Heat-stable amylase was not added to NDF, and both NDF and ADF were expressed including of residual ash. Organic matter digestibility (OMD) and Metabolizable energy (ME) content of the experimental feeds were estimated using the equation of Menke and Steingass (1988).

***In Vitro* Ruminal Total Gas Production Measurement**

The *in vitro* gas production was determined according to Menke and Steingass (1988). Three rumen-cannulated male sheep were used as rumen inoculum donors, and rumen fluid was collected from the sheep prior to morning feeding into a thermos flask that had been pre-warmed to a temperature of 39 °C and pooled and filtered through cheese-cloth. Partially oven-dried and ground forage samples (200 mg) were accurately weighed and carefully dropped into 100 ml glass syringes. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution, and two blanks containing 30 ml of medium (inoculums and buffer) only were incubated at the same time. The syringe was tapped and pushed upward by the piston to eliminate air in the inoculum. The silicon tube in the syringe was then tightened by a metal clip to prevent the escape of gas. The syringes were placed in a rotor inside the incubator at 39 °C, and the samples were incubated once in a triplicate, since the data were meant to be used for multivariate analysis. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. The volume of a gas produced was recorded at 3, 6, 12, 24, 48, 72 and 96 h and the average of the volume of gas produced by blank against each incubation time was deducted from the recorded values from each sample of incubated bottles.

Methane Gas Production Measurement

A parallel experiment was conducted simultaneously for methane measurement, as it was not possible to measure gas production thereafter due to the addition of sodium hydroxide to the incubated materials. At the end of the 48 h incubation period and recording the final gas volume in the syringe, 4 ml of (10 M) sodium hydroxide was introduced from the latter into the incubated contents, thereby avoiding gas escape. Sodium hydroxide was added to absorb CO_2 that was produced during the process of fermentation and the remaining volume of gas was recorded as methane (Fievez et al., 2005). The average volume of gas produced from the blanks was deducted. The *in vitro* ruminal gas production work was carried out at the Animal Feed and Nutrition laboratory of Hawassa University, Ethiopia and the care, handling and maintenance of cannulated sheep were done following animal welfare regulations of the animal ethics committee of the University of Pretoria (NAS086/2019). The post-incubation parameters such as ME (MJ/kg DM) and OMD (%) of the incubated samples were estimated according to the equation of Menke and Steingass (1988) by using 48 h gas production data, while short-chain fatty acid (SCFA, μmo) at 24 h post incubation was computed from gas volume using the linear equation by Makkar (2005).

$$ME\ (MJ/kg\ DM) = 2.2 + 0.136\ GV + 0.0057\ CP$$

$$OMD\ (\%) = 14.88 + 0.889\ GV + 0.45\ CP + 0.651\ ash$$

$$SCFA\ (\mu mol) = 0.0222GV - 0.00425$$

Gas Production Characteristics

Gas production characteristics were determined by the non-linear model developed by Ørskov and Mcdonald (1979), as modified by Osuji et al. (1993):

$$Y = b\ (1 - e^{-ct})$$

Where Y = the cumulative volume of gas produced at time t; b = the asymptotic gas volume; c = fractional fermentation rate; t = incubation time and e = base of the natural logarithm. The intercept is not included in the model as there is no gas production from unfermented feed, as explained by Osuji et al. (1993). Effective gas production (EGP) is calculated using the equation:

$EGP = (bc/(c+k))$, where k is the outflow rate from the rumen, assumed to be 5%/h.

Statistical Analysis

Values for chemical composition and *in vitro* gas production were presented in a graph and table for visual comparison. Multivariate analysis was performed using the chemical composition and *in vitro* fermentable parameters data to characterize the key forage species in terms of chemical composition and gas production characteristics and systematically group them into different groups to investigate variations between and within the group. The correlation matrix was used for the calculation after the data was mean-centered and standardized for principal component (PC) analysis. The first three principal components (PC1 to PC3) that explain the greater amount of variation were plotted graphically to identify forage species with similar characteristics. Points displayed close to each other on the graph represent species that are similar in their nutrient content and *in vitro* fermentation parameters. Data for PCA were analysed using SAS software, and for cluster analysis, the Paleontological Statistics test (PAST) was used

(Hammer et al., 2001). Correlations between the characteristics were computed on the mean values of the forage species during cluster analysis.

RESULTS

Chemical Composition of Selected Pasture Species

The chemical composition of the selected forage species from the central highlands of Ethiopia is shown in Table 1. Crude protein (g kg^{-1} DM) content of the grass species varied widely from 45 to 99 with the lowest for *Eragrostis tenuifolia* and the highest for *Cynodon dactylon.* The CP content for forage legumes ranged from 174 to 202 (g kg^{-1} DM) and was comparable except for *Trifolium simense* (139 g kg^{-1} DM), which was the lowest in CP content among others. The CP content for sedge and forb ranged from 83 to 137 and a higher CP content was observed for *Centella asiatica* forb.

Table 1. Mean chemical composition of selected forage species from the central high lands of Ethiopia

Forage species	IVI	DM	Ash	CP	NDF	ADF	ADL
	(%)[a]	g kg^{-1}	g kg^{-1} DM				
Grasses							
Andropogon amethystinus	57.0	921	122	67	608	334	56
Cynodon dactylon	3.8	915	138	99	592	300	38
Eleusine floccifolia	122.6	922	83	45	698	454	74
Eragrostis botryodes	2.1	914	64	50	654	383	56
Eragrostis tenuifolia	1.6	902	98	45	459	330	77
Helictotrichon elongatum	2.2	923	89	62	642	373	54
Pennisetum sphacelatum	2.4	927	104	48	682	446	68
Pennisetum thunbergii	51.7	924	114	51	624	343	49
Poa leptoclada	10.2	915	75	67	637	356	54
Sporobolus pyramidalis	9.2	919	75	58	632	374	57
Legumes							
Trifolium cryptopodium	4.5	913	140	202	537	348	86
Trifolium mattirolianum	2.6	898	121	194	491	328	77
Trifolium rueppellianum	2.0	904	97	174	491	352	70
Trifolium simense	5.0	901	111	139	488	392	97
Trifolium tembense	12.7	902	139	195	487	316	82
Others (sedge and forbs)							
Cyperus rigidifolius	16.5	909	74	118	664	394	67
Cerastium octandrum	5.0	921	109	83	622	351	50
Uebelinia abyssinica	14.1	913	136	102	606	337	62
Centella asiatica	18.3	894	139	137	343	257	49

ADF=acid detergent fibre; ADL=acid detergent lignin; CP=crude protein; DM=dry matter; NDF=neutral detergent fibre; IVI=important value index; [a]Average important value index (IVI) obtained from highland and mid-highland agro-ecologies from enclosure and open access grazing land for each species as observed by Wegi et al. (2020).

The NDF (g kg^{-1} DM) for the grass species in the current study varied from 592 to 698 except for *Eragrostis tenuifolia* (459), while those for legumes varied from 487 to 537 with the lowest for *Trifolium tembense* and the highest for *Trifolium cryptopodium*. The NDF contents of sedge and forbs varied from 606 to 664 except for *Centella asiatica* forb (343). Acid detergent fiber (g kg^{-1} DM) varied from 300 to 454, 316 to 392 and 257 to 394 and ADL (g kg^{-1} DM) varied from 38 to 77, 70 to 97 and 49 to 67 for grasses, legumes, and others (sedge and forbs), respectively. In general, grasses contained higher NDF and ADF components and lower ash and CP contents than legumes. Higher IVI for the selected forage species such as *Eleusine floccifolia*, *Andropogon amethystinus* and *Pennisetum thunbergii* were observed in that order, as indicated in Table 1.

In Vitro Fermentation Parameters and Gas Production Characteristics

Total gas and methane production and predicted parameters are shown in Table 2. For all studied species, cumulative gas production increased during incubation. The volume of gas produced by the grass species varied widely from species to species and the differences were not consistent for different incubation times. Gas produced by the grass species at 24 h (ml/g DM) ranged from 27.3 to 155.7 with the lowest for *Pennisetum sphacelatum* and the highest for *Poa leptoclada.* On the other side, gas produced by legumes (ml/g DM) at 24 h varied from 145.1 to 210.3 with the lowest for *Trifolium cryptopodium* and the highest for *Trifolium rueppellianum.* Gas produced by *Centella asiatica* forb at 24 h of incubation (237.8 ml/g DM) was the highest from other species studied. The average gas produced from legumes (179.9 ml/g DM at 24) was higher than gas produced from the grass species (99.4 ml/g DM at 24). Fractional gas production rate (c) and EGP showed wide variability for the evaluated forage species regardless of their botanical composition. The fractional gas production rate (c) varied from 0.001 to 0.025 h^{-1} for grasses, and 0.027 to 0.044 h^{-1} for legumes. The volume of EGP ranged from 19.4 ml/g DM for *Pennisetum sphacelatum* to 125.7 ml/g DM for *Poa leptoclada*. The highest EGP volume of 190.9 ml/g DM was observed for *Centella asiatica* forb. The gas production patterns for the key grass species are shown in Figure 1 while for legumes and others (sedge and forbs), it is shown in Figure 2 and Figure 3, respectively.

Methane produced (ml/g DM) by the grass species at 48 h of the incubation period varied from 27.8 to 130.3, with the lowest for *Eragrostis tenuifolia* and the highest for *Eleusine floccifolia* (Table 2). Methane produced (ml/g DM) by forage legumes ranged from 33.0 to 49.8, and sedge and forbs ranged from 16.5 to 33.5. The lowest methane production was recorded from *Cyperus rigidifolius* sedge, which is the second dominant species in the highland area during the short rainy season next to *Centella asiatica* forb. On average, grasses produced 43.9% higher methane than legumes in the current study. Short-chain fatty acid (SCFA) produced (μmol) at 24 h from the species studied varied widely from 0.59 to 5.26 with the lowest from *Pennisetum sphacelatum* grass and the highest from *Centella asiatica* forb. The *in vitro* OMD in the grass species ranged from 47.4 to 59.1%, from 56.5 to 60.4% in legumes and from 55.7 to 66.4% in sedge and forb species. Metabolizable energy (MJ/kg DM) concentrations in the grasses ranged from 7.1 to 8.7, in legumes from 7.4 to 8.4 and from 7.8 to 9.5 in sedges and forbs.

Table 2. *In vitro* total gas and gas production characteristics (ml/g DM), methane (CH_4, ml/g DM), SCFA (µmol) production, OMD (%) and ME (MJ/kg DM) contents of forage species from the central highlands of Ethiopia

Forage species	Total gas		Gas production characteristics		CH_4	SCFA	OMD	ME
	24 h	96 h	c	EGP	48 h			
Grasses								
Andropogon amethystinus	32.6	122.2	0.001	24.6	73.3	0.70	52.4	7.6
Cynodon dactylon	112.0	270.4	0.017	90.9	60.3	2.46	56.5	7.9
Eleusine floccifolia	132.8	325.3	0.013	97.7	130.3	2.93	48.4	7.2
Eragrostis botryodes	95.7	287.1	0.005	72.8	123.0	2.10	54.7	8.4
Eragrostis tenuifolia	133.1	277.3	0.025	107.2	27.8	2.93	59.1	8.7
Helictotrichon elongatum	120.2	363.2	0.012	110.1	43.5	2.65	50.9	7.4
Pennisetum sphacelatum	27.3	79.3	0.002	19.4	95.8	0.59	47.4	7.1
Pennisetum thunbergii	54.1	248.9	0.002	80.5	70.3	1.18	52.5	7.8
Poa leptoclada	155.7	311.5	0.025	125.7	71.0	3.44	53.3	7.8
Sporobolus pyramidalis	130.5	326.3	0.016	107.0	57.3	2.88	51.0	7.6
Legumes								
Trifolium cryptopodium	145.1	295.8	0.027	121.5	33.0	3.20	60.3	8.0
Trifolium mattirolianum	194.9	300.7	0.041	148.6	41.8	4.31	60.4	8.2
Trifolium rueppellianum	210.3	312.7	0.043	162.2	49.8	4.65	59.7	8.4
Trifolium simense	183.1	269.2	0.044	139.2	39.0	4.04	56.5	7.9
Trifolium tembense	166.4	260.6	0.04	130.4	47.3	3.67	57.5	7.4
Others (forb and sedges)								
Cyperus rigidifolius	79.8	291.5	0.002	60.0	16.5	1.75	55.7	7.8
Cerastium octandrum	59.7	190.1	0.005	47.3	24.3	1.30	56.8	8.3
Uebelinia abyssinica	43.8	246.5	0.002	39.5	33.0	0.95	56.5	8.1
Centella asiatica	237.8	293.7	0.059	190.9	33.5	5.26	66.4	9.5

DM= dry matter; ME= metabolizable energy; OMD= organic matter digestibility; SCFA= short chain fatty acid; c= fractional gas production rate (%/h). EGP is the effective gas production calculated using the equation EGP = (bc/(c+k)), where k is outflow rate from the rumen, assumed to be 5%/h.

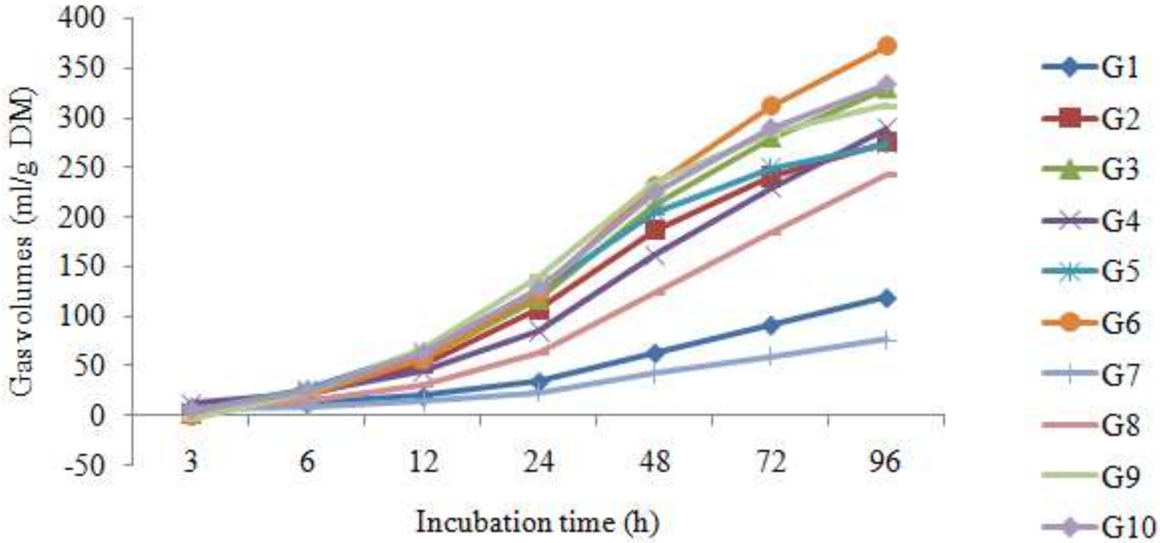


Figure 1. Gas production patterns for the tropical grasses.

G1=*Andropogon amethystinus*; G2=*Cynodon dactylon*; G3=*Eleusine floccifolia*; G4=*Eragrostis botryodes*; G5=*Eragrostis tenuifolia*; G6=*Helictotrichon elongatum*; G7=*Pennisetum sphacelatum*; G8= *Pennisetum thunbergii*; G9=*Poa leptoclada*; G10= *Sporobolus spyramidalis.*

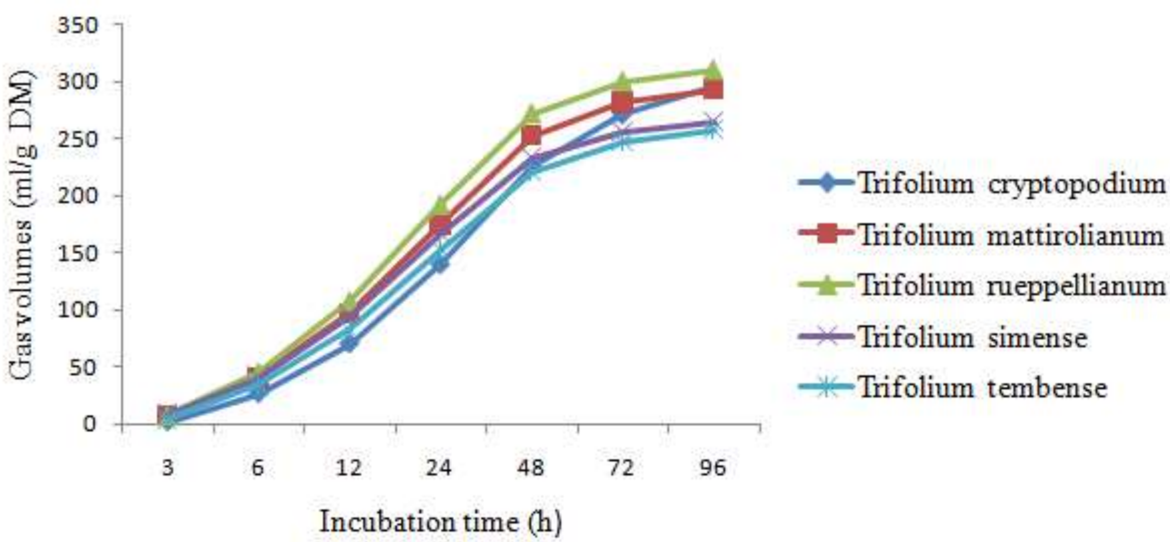


Figure 2**.** Gas production patterns for forage legumes

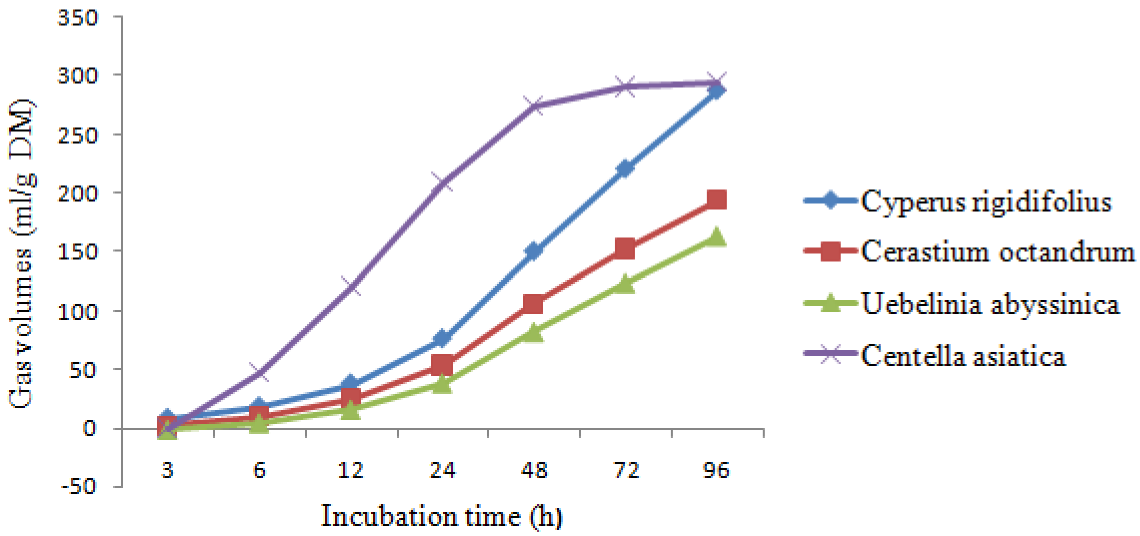


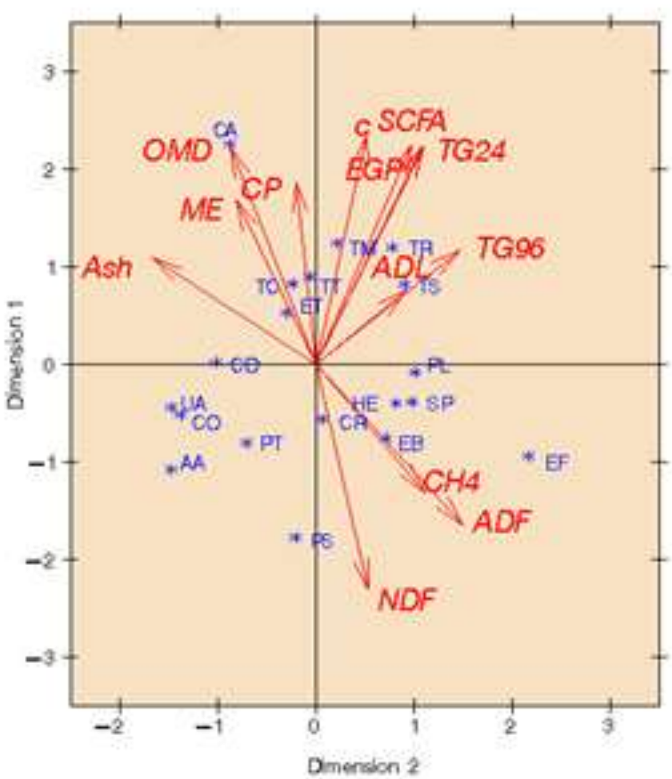
Figure 3. Gas production pattern for sedge and forb species

Principal component analysis

From the PCA correlation variance, the first three principal components (PC1 to PC3) had an eigenvalue greater than 1 (Table 3), and these three components explained 84.2% of the total variation of the data. In particular, the first principal component (PC1), which explained 54.7% of the total variation, was positively associated with CP, OMD, ME, fractional gas production rate (c), EGP and total gas production at 24 h, whereas it was negatively correlated with NDF, ADF and CH_4 production. The second PC (PC2), which explained 17.9% of the total variation, was positively associated with ADF and total gas production at 96 h, while it was negatively associated with ash, OMD and ME content. The third PC (PC3), which explained 11.6% of the total variation was positively correlated with CP and ADL, and was negatively correlated with ME and total gas production at 96 h. The data structure of forage species in the two-dimensional space was shown in Figure 4 for PC1 vs. PC2 and Figure 5 for PC1 vs. PC3. Forage species with higher values for PC1 (*Centella asiatica* forb and all legume species evaluated) were characterized by higher CP content, fractional gas production rate (c), EGP, and total gas production at 24 h, but had lower fiber contents and CH_4 production. Similarly, forage grasses that had higher values for PC2 (*Eleusine floccifolia*, *Eragrostis botryodes*, *Pennisetum sphacelatum* and *Poa leptoclada*) were characterized by higher ADF and CH4 production. Legume species such as *Trifolium simense* and *Trifolium rueppellianum* had greater total gas production along PC2. *Pennisetum sphacelatum* grass was characterized by higher ADF, and forage legumes such as *Trifolium tembense*, *Trifolium cryptopodium* and *Trifolium simense* were characterized by higher CP and lignin content and these had higher correlation values with PC3.

Table 3. Eigenvector coefficient of 13 chemical composition and *in vitro* fermentable parameters for the first three principal components with Eigenvalue of the total variance.

Variables	Principal Component		
	First	Second	Third
Ash	0.163	−0.435	0.309
Crude Protein	0.28	−0.05	0.402
Neutral detergent fibre	−0.346	0.14	−0.045
Acid detergent fibre	−0.248	0.393	0.251
Acid detergent lignin	0.112	0.244	0.638
Total gas production at 24 h	0.333	0.282	−0.051
Total gas production at 96 h	0.175	0.384	−0.305
Methane production	−0.198	0.291	−0.1
Short chain fatty acid	0.333	0.286	−0.052
Organic matter digestibility	0.333	−0.227	−0.065
Metabolizable energy	0.251	−0.213	−0.378
Fractional gas production rate (c)	0.351	0.136	0.079
Effective gas production	0.336	0.256	−0.093
Eigenvalue	7.108	2.331	1.51



AA= *Andropogon amethystinus*;
CD= *Cynodon dactylon*;
EF= *Eleusine floccifolia;*
EB= *Eragrostis botryodes*;
ET= *Eragrostis tenuifolia*;
HE= *Helictotrichon elongatum*;
PS= *Pennisetum sphacelatum*;
PT= *Pennisetum thunbergii*;
PL= *Poa leptoclada*;
SP= *Sporobolus pyramidalis*;
TC= *Trifolium cryptopodium*;
TM= *Trifolium mattirolianum*;
TR= *Trifolium rueppellianum*;
TS= *Trifolium simense*;
TT= *Trifolium tembense*;
CR= *Cyperus* rigidifolius;
CO= *Cerastium octandrum*;
UA= *Uebelinia abyssinica*;
CA= *Centella asiatica.*

Figure 4. Principal component analysis for PC1 vs. PC2 showing the grouping and separation of forage species based on chemical composition and *in vitro* fermentation parameters.

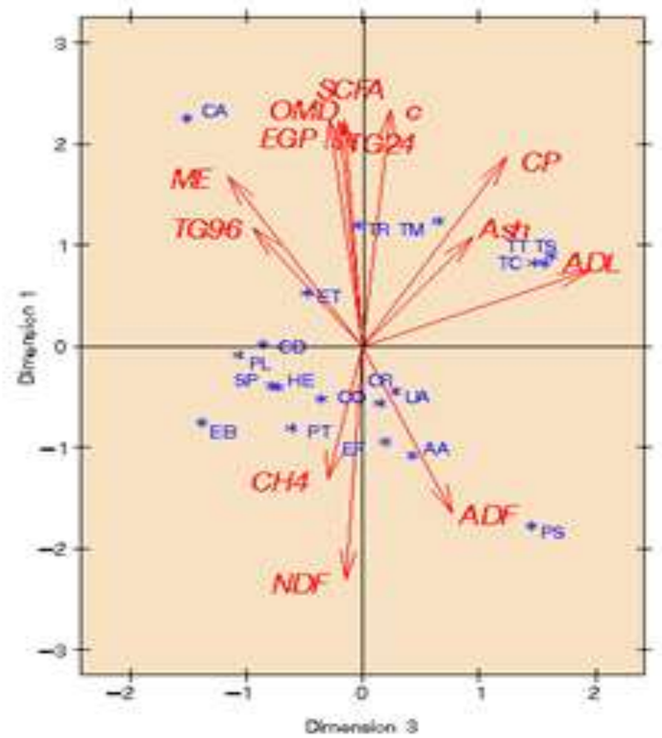


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Figure 5. Principal component analysis for PC1 vs. PC3 showing the grouping and separation of forage species based on chemical composition and *in vitro* fermentation parameters.

Cluster Analysis

The results of the cluster analysis are shown in Figure 6. The chart shows that the key forage species were classified into five groups with the possibility to divide group IV into two sub groups (IVa and IVb). Group I included *Centella asiatica* forb which had higher gas production at 24 h, higher fractional gas production rate (c) and higher EGP, but lower in NDF and CH_4 production compared to the rest of groups. Group II is represented solely by *Eragrostis tenuifolia* grass and group III contains all legumes, which differed from groups IV and V than had all grass species except *Eragrostis tenuifolia.* Group III which had all the legumes have on average higher CP content, higher fractional rate of fermentation (c) and higher EGP, but lower CH_4 production compared to grasses in groups IV and V. Group II, which contained *Eragrostis tenuifolia* grass differed from group III (all the legume species) having lower CP content and lower CH_4 production and lower fractional rate of fermentation (c) compared to average values of group III. Group IV (most of the grass species) differed from group V (*Eleusine floccifolia* and *Pennisetum sphacelatum* grasses) by producing lower CH_4 production than the average values recorded for group V.

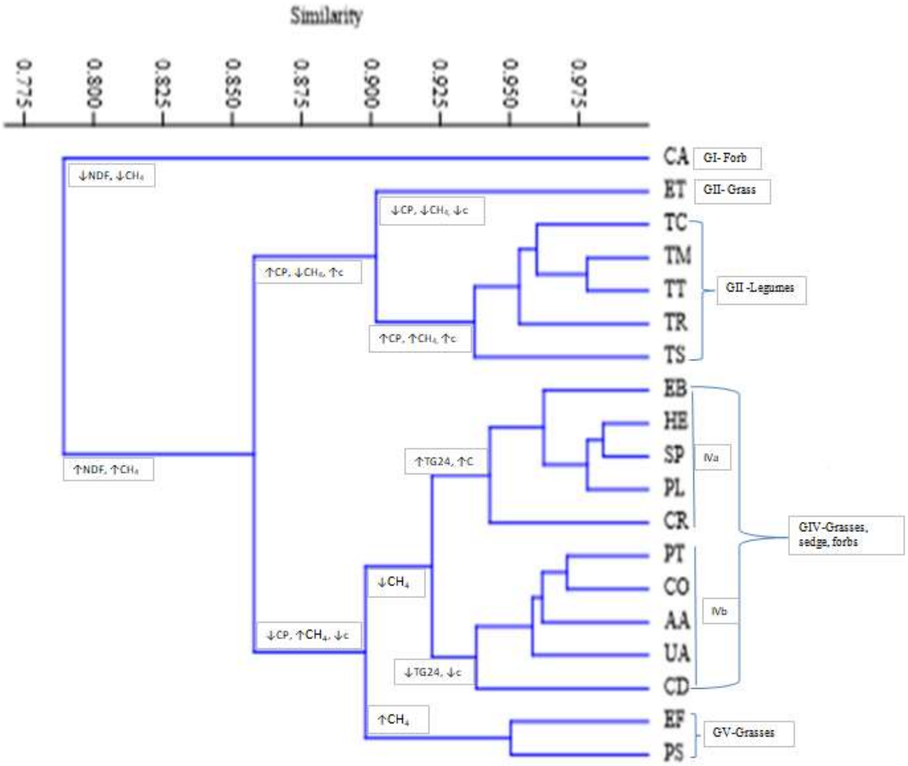

Figure 6. Multivariate cluster analysis plot showing groups of different forage species based on similarities in chemical composition and *in vitro* fermentable parameters.

Grasses: AA= *Andropogon amethystinus*; CD= *Cynodon dactylon*; EF= *Eleusine floccifolia*; EB= *Eragrostis botryodes*; ET= *Eragrostis tenuifolia*; HE= *Helictotrichon elongatum*; PS= *Pennisetum sphacelatum*; PT= *Pennisetum thunbergii*; PL= *Poa leptoclada*; SP= *Sporobolus pyramidalis*,

Legumes: TC= *Trifolium cryptopodium*; TM= *Trifolium mattirolianum*; TR= *Trifolium rueppellianum*; TS= *Trifolium simense*; TT= *Trifolium tembense*,

Sedge: CR= *Cyperus rigidifolius*, forbs: CO= *Cerastium octandrum*; UA= *Uebelinia abyssinica*; CA= *Centella asiatica*,

c= fractional gas production rate; CP= crude protein; G= group; NDF= neutral detergent fiber; TG24= gas production at 24 h.

DISCUSSION

Chemical Composition of Selected Pasture Species

The nutritive value of a ruminant feed is determined by the concentration of its chemical components, as well as the rate and extent of digestion. Except for *Cynodon dactylon* which had 99 g/kg DM, the CP contents of the grass species from the current study was below 70 g/kg DM. It is below the minimum level required for normal rumen functioning (Van Soest, 1994). On the other side, the CP contents of most legume species under study were above 150 g/kg DM, a minimum of CP contents for lactation and growth (Norton, 1982). The CP contents for sedge (*Cyperus rigidifolius*) and forb species in this study were well above 70 g/kg DM, a minimum level for rumen functioning as indicated by Van Soest (1994). As the proportions of the grass components was greater than 80% (Wegi et al., 2020), increasing the proportions of forage legumes in the pasture by using different grazing land management practices could be necessary to increase animal productivity and reducing methane emission simultaneously.

Animal feeds with more than 650 g/kg DM NDF were classified as low-quality roughages (Sigh and Oosting, 1992). All legumes and forbs and 70% of the grass species in the current study contained NDF below this threshold level (650 g/kg DM). Lower NDF contents were observed for grasses in the present study compared to 613 to 877 NDF for the key grass species from natural pasture in crop-livestock mixed farming system of Southern Ethiopia (Gumiyo et al., 2013), and similarly lower fibre contents for the grass species were observed compared to sub-tropical grass species in transitional rangeland of South Africa (Du Toit et al., 2018). Such variation might be observed due to the stage of maturity of the grass species at harvest, as higher fiber contents resulted in increased forage species age (Mero and Udén, 1998). The moderate quality of fiber contents from the evaluated forage species might be due to the harvesting stages, as the forage sample collection was done at the flowering stage when farmers in the study area utilize the conserved grazing area known traditionally as "*Kalo*".

In Vitro Fermentation and Multivariate Analysis

Gas production reflects differences in the chemical composition of feedstuffs and has applications in predicting their nutritional value (Anele et al., 2016). Although gases produced during the rumen fermentation process are waste products and no nutritive value to the ruminant, it provides a useful basis from which ME, OMD and SCFA can be predicted (Babayemi et al., 2006). Gas produced by legume species at 24 h in the current study was slightly lower than the 213 to 257 mg/g DM gas produced at the same incubation period for legume hays grown in Turkey (Suha Uslu et al., 2018). The highest gas production was recorded for *Centella asiatica* forb at 24 h of incubation period with higher CP and OMD along PC1 (Table 1 and Figure 4) compared to other forbs and grass species. This is in line with Du Toit et al. (2018), who found the highest total gas volume after 24 h of incubation for species with higher *in vitro* organic matter digestibility (IVOMD) and CP concentrations. The higher gas production for legume species compared to grass species in the current study agrees with Singh et al. (2012), who found more gas production by legumes than by cereals or grasses. This is clearly observed from cluster analysis in the present study (Figure 6) that all legume species clustered close, and from PCA analysis (Figure 4), they had higher total gas production at 24 h and higher EGP which was highly correlated with PC1.

Methane production is associated with energy loss and contributes to global warming (Babayemi and Bamikole, 2006). The variation in methane production between grass species in the current study may be attributed to variations in the chemical composition, fiber components in particular (Table 1). From PCA, forage grasses such as *Eleusine floccifolia*, *Pennisetum sphacelatum* and *Eragrostis botryodes* were greater in NDF, ADF and CH_4 production strongly associated with PC1, and as a result, *Eleusine floccifolia* and *Pennisetum sphacelatum* were clustered close to each other in cluster analysis (Figure 6). This provides an opportunity for selecting low methane emissions for grass species by targeting their fiber contents. Similarly, Gemeda and Hassen (2014) found a positive correlation between methane with fibre components. The higher methane production for grass species compared to legume species agrees with previous researches (Navarro-Villa et al., 2011; Melesse et al., 2017), which found higher methane production for grass species than leguminous forages. The lower methane production in legumes compared to grasses might be attributed to the less extensive *in vitro* rumen fermentation of legumes as suggested by Navarro-Villa et al. (2011). The higher SCFA from legume species and *Centella asiatica* forb in the current study might be due to their lower fiber fractions (Table 1) compared to grasses and sedges. The current is in line with the discovery by Van Soest (1994) of greater SCFA for species containing lower fiber fractions. This is observed from PCA that forage legumes and *Centella asiatica* forb had lower fiber components (NDF and ADF) along PC1. Gas production is also a reflection of the formation of SCFA and the synthesis of microbial biomass (Getachew et al., 1998). Similarly, as indicated by (Ajayi and Babayemi, 2008), SCFA is a reflection of energy availability in a feedstuff. This is a possible explanation why forage legumes and *Centella asiatica* forb in the current study had higher EGP compared to forage grasses.

All grass species in the current study contained lower OMD than the range of OMD values of 56.1 to 79.6% reported by Melesse et al. (2017) for some common grass species grown in Ethiopia. This was clearly observed from PCA (Figure 4) in which all grass species evaluated (except *Eragrostis tenuifolia*) had negative values and association with PC1. On the contrary, all grass species studied had a value above 22.5 to 42.2% OMD reported by Du Toit et al. (2018) for selected sub-tropical grass species. Forage legumes evaluated in this study had lower OMD than the report of Melesse et al. (2017) for some common legumes grown in Ethiopia and by Suha Uslu et al. (2018) for some legume plants grown in Turkey. As reported by Meissner et al. (2000), forage species having an OMD of 70% or more are considered to be of high quality. Thus all forage species in the current study, which had a value below 70% OMD were assessed to be low-quality forage. The ME in the grass species in the current study were well above 3.0 to 5.7 MJ/kg DM reported by Du Toit et al. (2018) for selected sub-tropical grass species and in the range of ME reported for common tropical grass forages in Ethiopia (Melesse et al., 2017). On the other side, the values of ME obtained for legumes and sedge and forbs were below 8.9 to 10.3 MJ/kg DM reported by Melesse et al. (2017) for common tropical legume forages in Ethiopia except for *Centella asiatica* forb recorded 9.5 MJ/kg DM.

CONCLUSIONS

The key forage species characterized in the current study showed noticeable variation in terms of chemical composition and *in vitro* fermentable parameters such as gas and CH_4 production, EGP and fractional gas production rate (c). Multivariate analysis clustered the forage species into five main groups according to their chemical composition (NDF, CP) and *in vitro* fermentable parameters (c, CH4 and total gas at 24 h). The PCA revealed that the fiber components were positively correlated with CH_4 production. At the same time the cluster analysis showed that forage grasses which had higher fiber contents and higher CH_4 production, were clustered close to each other as compared to other species. The implication is that early grazing of native pasture dominated by forage grass species is important and will likely enhance the nutritional value of the forage and subsequently improve livestock productivity while simultaneously reducing CH_4 emission by ruminants. Lower methane emission, in turn, minimizes the environmental damage caused by livestock species. Similarly, the evaluation of forage species included in this study in terms of chemical composition and *in vitro* fermentable parameters will serve as a source of information to improve the existing knowledge pool which helps to design appropriate pasture-based feeding systems and grazing land management practices. Furthermore, *Centella asiatica* forb was superior in most of the nutritive value parameters included in this study. Hence, further studies should be conducted regarding its yield, anti-nutritional and actual feeding values to recommend this forb as a possible forage crop in the feeding system of ruminants in the central highlands of Ethiopia and similar agro-ecologies elsewhere.

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