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CHEMICAL COMPOSITION, RUMINAL DEGRADATION KINETICS AND METHANE PRODUCTION (*IN VITRO*) POTENTIAL OF LOCAL AND EXOTIC GRASS SPECIES GROWN IN PESHAWAR

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Abstract

Livestock production, and small scale and extensive grazing livestock production systems in Pakistan lack long-term sustainability due to declining quantity and quality of green forages and pastures. Information on the nutritional value of range/pasture and cultivated grass species is required to design proper strategies not only for nutritional management of grazing animals, but also for development of good quality forage resources. Therefore, the current study was planned to: (i) analyze the chemical profile of traditional and novel grasses grown in Peshawar; (ii) quantify the methane emission potential of the grass species; and (iii) quantify the differences among species in their nutritive value and methane emission. Ten grass species, namely, *Sudex (Sorghum × sudangrass)*, *Jumbo grass* (*Sorghum bicolour× Sorghum sudanefe*), *Sorghum almum, Pennisetum purpureum, Vetiveria zizanioides, Panicum colaratum, Cynodon dactylon, Bothriochloa pertusa, Splenda setaria* and *Desmostachya bipinnata* were evaluated under uniform agronomic and environmental conditions. The results showed that the contents of all measured chemical components, mineral profile (except Zn), *In vitro* digestibility of dry matter (DMD), and *In vitro* gas (GP) and methane-production had large variation among the grass species. Among the grasses, Jumbo grass had greater CP (11.9% DM) content and *In vitro* DMD (65.9% DM), and produced greater amount of total gas, that contained lowest proportion of methane. In contrast, *D. bipinnata* had lowest contents of CP (6.3% DM) and *In vitro* DMD (43.7% DM), and produced lower amount of total gas, with highest proportion methane in total GP. Next to *D. bipinnata*, *V. zizanioides* had lower degradability/GP and highest proportion of methane in total gas. The large variation in chemical composition, DMD and methane-emission potential of the summer grass species presents a prospect to select and further develop grass species that have lower methane-emission potential and high nutritional value. Further research is needed to investigate the changes in chemical profile, DMD and methane-emission of forage species between seasons and with maturity.

Key words: Tropical grass species, Nutritive value, Methane emission, Rumen fermentation, *In vitro* gas production.

Introduction

Livestock production, and the small-holding and the extensive grazing (transhumant, nomadic and sedentary farming) livestock production systems in Pakistan lack long-term sustainability, due to declining quantity and quality green forages and pastures, particularly during extended summer and winter dry periods (Khan and Habib, 2012; Khan *et al*., 2015b). Moreover, the conventional concentrates such as oilseeds and cereal grain byproducts are increasingly becoming scarce and expensive, and most of the smallholder subsistence farmers could not afford to feed high-cost commercial concentrates to their animals for a longer period (Habib *et al*., 2016a, b). Therefore, development of good quality forage and pasture resources for the entire year is the major issue to be researched and addressed in the livestock sector of the country.

Forages and pastures are the cheapest, natural and often the major source of nutrients for ruminant livestock and ensuring the availability of good quality forage year-round can significantly improve the productivity, product quality (healthiness) and profitability of dairy and fattening animals. Rangeland grasses in Pakistan sustain the feeding requirements of millions of grazing animals (Habib *et al*., 2016a). However, the productivity of the grazing livestock has remained low, mainly because of inadequate quantity and quality of the grazed pasture, due to recurrent droughts with concomitant overgrazing, and lack of range improvement practices (Khan *et al*., 2009a). The lower crude protein (CP) contents and digestibility of range grasses in the tropics have been characterized as the major limitations in dry-matter and energy intakes, and as such in the productivity of the grazing animals (Bezabih *et al*., 2014a). Preliminary investigations also show that the range grasses can be deficient in some essential minerals such as Na and Cu (Khan *et al*., 2009b). Recent research shows that information on chemical composition, digestibility and methane emission potential of pasture species is needed for the sustainable and knowledge-based improvement of grazing livestock production (Bezabih *et al*., 2014b).

Methane emission from the enteric fermentation of ruminants has been a major constraint to sustainable livestock production and human development in the tropics, because of its major contribution (18%) to global anthropogenic greenhouse gases and to the loss of useful dietary energy (5-7%) (Hristov *et al*., 2013). Optimizing animal productivity and reducing methane emission is therefore the central focus of livestock feed research in the tropics (Defries and Rosenzweig, 2010). Forages with higher rate and extent of degradation in the rumen are expected to increase the productivity of animal and reduce the emission of methane to the environment (Bezabih *et al*., 2014a). Conventionally, various *In vivo* and *in sacco* methods have been used for determination of the rate and extent of feed digestibility (Habib *et al*., 2013). However, these methods are expensive, laborious, require fistulated animals and large quantities of feed, thereby making them unsuitable for routine feed evaluation. The fully automated *In vitro* gas production (IVGP) is an advanced alternative technique that can be used to directly quantify the rate and extent of ruminal degradation of relatively large number of samples in small time through time series (every 20 min) measurements of cumulative gas production over 72 h (Khan *et al*., 2015a).

We hypothesized that there is large variation in the nutritional value methane-production potential among the traditional and novel grass species during summer season, which can be further exploited to improve pasture quality while also contributing to environmental sustainability. This study was therefore designed with the following aim: (i) to investigate the nutrient composition, mineral profile and *In vitro* dry matter digestibility (DMD) of selected traditional and novel exotic grass species grown in Peshawar; (ii) measure the *In vitro* total gas (GP) methane emission of the grass species; and (iii) to quantify the differences among grass species in their methane emission and nutritional values.

Material and Methods

Experimental site and grass management: Ten grass species, including six range grass species and five cultivated grass species, were examined (Table 1). Replicate (n = 4) plots (10 m × 15 m) of already established range grasses at the range research station of Pakistan Forest Institute Peshawar (71°49′ E longitude, 34°02′ N latitude and 350 m above sea level) were used. Each of the cultivated grass species was sown in replicate (n = 4) plots (10 m × 15 m) at the fodder research fields (71°48′ E longitude, 34°02′ N latitude and 347 m above sea level) of The University of Agriculture, Peshawar. Based on soil nutrient profile, all plots were fertilized at the rate of 30 kg N, 36 kg K_2O and 130 kg P_2O_5 per hectare at sowing. Fourteen days after regrowth/ emergence, the grasses were fertilised at the rate of 120 kg N per hectare.

Table 1. Description of the range and cultivated summer grass species.

Botanical name	Common name
Range grasses	
Sorghum almum	Columbus grass
Vetiveria zizanioides	Khus grass
Cynodon dactylon	Bermuda grass
Desmostachya bipinnata	Drab/Halfa
Bothriochloa pertusa	Palwan
Panicum colaratum	Buffalo grass
Cultivated grasses	
Pennisetum purpureum	Mot grass
Sudex (sorghum × sudangrass)	Sada bahar
Jumbo grass (*sorghum bicolour× sorghum sudanefe*)	Sada bahar (J)
Splenda setaria	Australian grass

Sampling: From all plots representative samples were collected from second regrowth, four weeks after the previous cut, representing normal maturity for feeding of these grasses. The harvest date was projected from the established knowledge of the maturity of these grasses at the respective stations. Four randomly selected spots (1 m^2 each) were sampled from each replicate plot. Grass samples were manually harvested at approximately 5 cm above the ground. After harvesting the four samples of each replicate plot were pooled, mixed, and a representative subsample (~1.5 kg) was shifted to pre-labelled, polythene zip-bags and subsequently transported in cooling boxes to the Animal Nutrition laboratory. After arrival in the laboratory, each subsample was portioned into two equal parts; one part was dried at 60ºC till constant weight for chemical analyses, while the second part was immediately frozen as a backup.

Chemical analysis: The dried samples were ground to 1 mm particle size using in Thomas–Willey laboratory mill for chemical analysis and *In vitro* DMD. For *In vitro* GP and methane production analysis the samples were ground to 2 mm particle size. The content of dry matter (DM) was analysed by oven drying the samples at 103°C till constant weight (International Organization for Standardization (ISO), method 6496). The content of CP (N × 6.25) was analysed according to Kjeldal method (ISO, method 5983) using a Kjeltec-2400 Autoanalyzer (Foss Analytical A/S, Hillerød, Denmark); ash content was measured by incinerating the samples in muffle furnace at 550ºC for 4 h (ISO, method 5984); and ether extract (EE) was analysed by extraction of samples with petroleum ether for 6 h (40/60, v/v) (ISO, method 6492). The content of neutral detergent fiber (NDF) was analysed according to method of Van Soest *et al*., (1991), while the contents of acid detergent fiber (ADF, method 973.18) and acid detergent lignin (ADL; method 973.18 followed by 3 h extraction with 72% H_2SO_4) were analysed according to Anon., (1995). The two-step *In vitro* procedure of Tilley and Terry (1963) was used for analysis of *In vitro* DMD. Atomic absorption spectrophotometer (Buck Scientific 240VGP, Milan, Italy) was used for analyses of macro and micro minerals in the pre-digested samples with a mixture of nitric, per-chloric and sulphuric acids according to Anon., (1995).

***In vitro* total gas and methane production analysis:** The *In vitro* GP and methane production analysis of the grass samples were performed at the Animal Nutrition Laboratory of Wageningen University, The Netherlands. A fully automated gas production apparatus (Cone *et al*., 1996) was used to measure cumulative GP. All samples were incubated in duplicate in buffer rumen fluid for 72 h. The gas produced during incubation was automatically measured every 20 min for 72 h incubation, and the data was corrected for blank gas productions (i.e. by the buffered rumen fluid without sample). The methane concentration was measured by sampling small aliquots of gas (10 µl) from the top of bottles using a gas tight syringe (Hamilton 1701N, point style 5 needles, 51 mm; Hamilton, Bonaduz, Switzerland) at 0, 2, 4, 6, 8, 10, 12, 24, 30, 48, 56, and 72 h. The gas samples were immediately analysed for methane content by gas chromatography (TRACE GC Ultra; Thermo Electron Corp., Waltham, MA).

Statistical analysis

The fixed effect of the pasture species on the nutrient and mineral composition, IVDMD, cumulative IVGP and CH_4 production was determined using PROC MIXED Procedure (Littell *et al*., 2006) of Statistical Analysis System (SAS, 2003). The replication (samples from replicate plots of each grass) were considered as random effect. The model used was:

$$Y_{ij} = \mu + PS_i + \text{Є}_{ij}$$

where, Y_{ij} is the response variables, μ is the overall mean, PS_i is the fixed effect of pasture species, and Є_{ij}, is the random error. If response was significant ($p<0.05$), then post-hoc analysis was performed to determine pair-wise differences among pasture species, using Tukey-Kramer test.

Results

Large variation ($p<0.001$) was observed in the contents of all chemical components, *In vitro* DMD and total methane emission during 72 h incubation in buffer rumen fluid among the grass species (Table 2). The content of CP was higher ($p<0.05$) in *jumbo grass* (11.9% DM) and lowest in *D. bipinnata* (6.3% DM). Next to *jumbo grass*, *C. dactylon, S. setaria* and *Sudex* had CP content of >10%, which could support moderate level of productivity in ruminants. Among the grass species, *Jumbo grass* had the lowest ($p<0.05$) content of NDF (62% DM), ADL (4.9% DM) and methane production (55 mL/g organic matter (OM)), and highest ($p<0.05$) content of *In vitro* DMD (65.9% DM). In contrast, the highest content of NDF (66.7% DM), ADL (15.8% DM) and methane production (73 mL/g OM) and lowest *In vitro* DMD were observed for *D. bipinnata.* These observations show a negative relationship between NDF, ADF and ADL contents with *In vitro* DMD and a positive relationship with methane production. The NDF contents of *V. zizanioides*, *D. bipinnata* and *B. pertusa* were more than 60% DM, and as such they can negatively affect the intake, feed digestibility and animal productivity.

Figure 1 shows the rate and extent of *In vitro* total GP during 72 h rumen incubation in buffer rumen fluid, that reflects the rate and extent of feed fermentation in the rumen of animals. There was a large variation in the rate and extent of total GP of the grass species, the highest total gas production was observed for *jumbo grass* and lowest total gas production was observed for *D. bipinnata.* On the other hand, *jumbo grass* had the lowest proportion of methane in total GP, and *D. bipinnata* had the highest proportion of methane in total GP (Fig. 2). Next to *jumbo grass*, Sudex, *P. purpureum* and *S. almum* had higher *In vitro* DMD and produced higher amount to total GP, respectively (Fig. 1). Concomitantly, next to *jumbo grass,* Sudex, *P. purpureum* and *S. almum* had lower proportion of methane in total GP (Fig. 2).

The macro- and micro-mineral contents of the grass species are shown in Table 3. Except Zn, the content of all macro- and micro-minerals varied among the grass species. Notably, *S. setaria* had higher ($p<0.05$) contents of Ca (1.15% DM), P (1.29% DM), Mg (0.84% DM) and Fe (0.20 mg/g). While, *D. bipinnata* had the lowest ($p<0.05$) content of Ca and P, but highest ($p<0.05$) of Mg among the grass species. Compared to macro-minerals, the variation in micro-mineral profile was relatively small.

Table 2. Dry matter (DM) contents at harvest, nutrient composition, DM digestibility (DMD, *In vitro*) and cumulative methane production of the summer grasses.

Grass species	DM (g/100 g FM)	Chemical composition (g/100 DM)								methane mL/g OM (72 h)	*In vitro* DMD (g/100 g DM)
		Ash	CP	EE	NDF	CHO	ADF	ADL	Cellulose		
Range grasses											
Sorghum almum	23.9^{d}	8.2^{fg}	9.2^{cd}	6.1^{b}	56.5^{e}	73.5	26.7^{c}	8.3^{de}	29.8^{b}	55^{f}	58.8^{cd}
Vetiveria zizanioides	26.4^{cd}	9.8^{de}	8.0^{ef}	3.3^{e}	66.5^{a}	75.8	30.6^{b}	12.2^{b}	35.9a	71^{a}	43.3^{g}
Cynodon dactylon	28.8^{abc}	11.1^{bc}	11.7^{a}	5.3^{bc}	58.7^{cd}	68.6	23.7^{d}	8.6^{d}	35.0a	62^{d}	58.7^{d}
Desmostachya bipinnata	32.1^{a}	9.2^{ef}	6.3^{g}	1.9^{f}	66.7^{a}	71.7	31.9^{a}	15.8^{a}	34.7^{b}	73^{a}	43.7^{g}
Bothriochloa pertusa	31.6^{ab}	10.3^{cd}	7.7^{f}	7.2^{a}	61.9^{b}	74.6	31.9^{a}	11.4^{b}	29.9^{b}	69^{b}	47.1^{f}
Panicum colaratum	28.3^{bc}	8.6^{g}	8.7^{de}	3.1^{e}	58.7^{cd}	77.1	23.9^{d}	10.6^{bc}	36.4^{a}	72^{a}	47.1^{f}
Cultivated grasses											
Pennisetum purpureum	24.0^{d}	10.9^{bc}	9.7^{bc}	4.3^{d}	55.7^{e}	71.9	29.4^{b}	6.6^{ef}	26.3^{c}	57^{e}	61.5^{bc}
Sudex$^{\#}$	16.8^{e}	8.7^{f}	10.4^{b}	4.7^{cd}	53.7^{f}	67.7	27.2^{c}	5.7^{f}	26.5^{c}	56^{ef}	63.1^{b}
Jumbo grass$^{\#}$	18.9^{e}	12.7^{a}	11.9^{a}	5.1^{cd}	52.2^{f}	72.5	27.1^{c}	4.9^{f}	25.0^{c}	55^{f}	65.9^{a}
Splenda setaria	24.2^{d}	11.7^{b}	10.0^{b}	6.0^{b}	57.3^{de}	73.2	29.9^{b}	9.1^{cd}	27.4^{c}	65^{c}	52.8^{e}
Significance											
SEM	0.629	0.17	0.14	0.14	0.11	0.20	0.26	0.32	0.416	0.30	0.48
Significance	***	***	***	***	***	***	***	***	***	***	***

Means in the same column with different letters differ at $p<0.05$

FM, fresh matter; EE, ether extract; CP, crude protein; NDF, neutral detergent fiber; ADL, acid detergent lignin; ADF, acid detergent fiber; CHO, carbohydrates

SEM, standard error of the mean; ***, $p<0.001$

$^{\#}$, Sudex, *sorghum* × *sudangrass*; Jumbo grass, *sorghum bicolour* × *sorghum sudanefe*

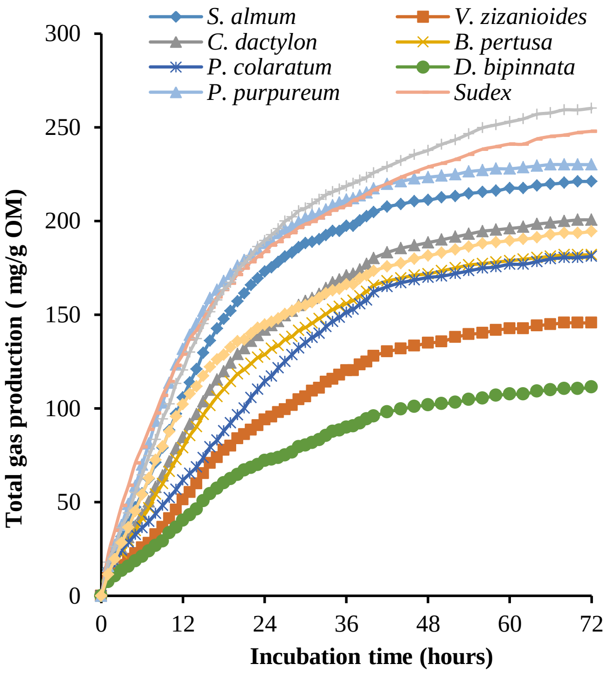

Fig. 1. Cumulative gas volume of the summer grass species during 72 h rumen incubation.

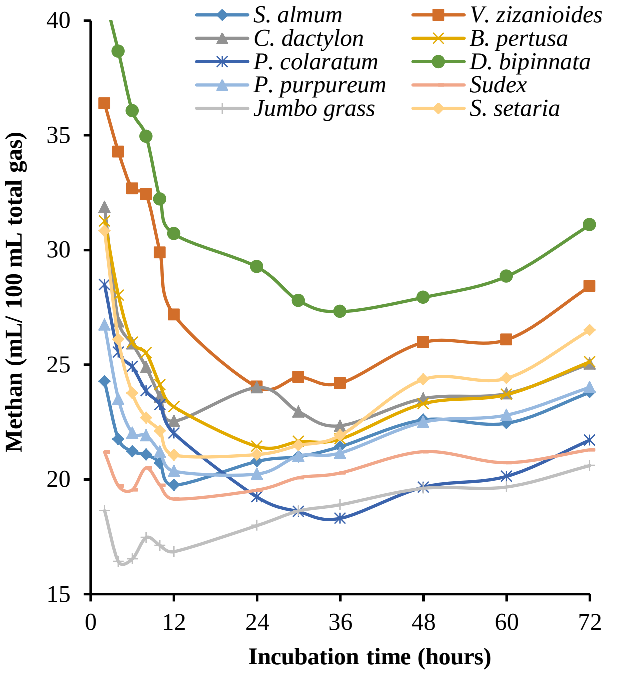

Fig. 2. Proportion of methane in total gas produced during incubation of the summer grass species in buffer-rumen fluid for 72 h.

Table 3. Macro and micro minerals contents of the range and cultivated summer grass species.

Sample name	Macro minerals (g/ 100 g DM) #			Magnesium	Micro minerals (ppm)†			
	Calcium	Sodium	Phosphorus		Iron	Copper	Zinc	Manganese
Range grasses								
Sorghum almum	0.80^{b}	0.15d	0.33^{cd}	0.78^{a}	0.19^{a}	0.07^{ab}	0.11	0.09^{bc}
Vetiveria zizanioides	0.40^{de}	0.17^{d}	0.27^{d}	0.80^{a}	0.07^{c}	0.01^{b}	0.14	0.09^{bc}
Cynodon dactylon	0.90^{b}	0.18^{d}	0.72^{a}	0.55^{c}	0.04^{c}	0.09^{ab}	0.15	0.20^{a}
Desmostachya bipinnata	0.35^{e}	0.16^{d}	0.39^{cd}	0.81^{a}	0.12^{b}	0.01^{b}	0.12	0.08^{bc}
Bothriochloa pertusa	0.60c	0.26^{c}	0.56^{b}	0.72^{ab}	0.17^{a}	0.08^{ab}	0.13	0.11^{bc}
Panicum colaratum	0.35e	0.27^{c}	0.37^{c}	0.65^{b}	0.03^{c}	0.01^{b}	0.11	0.07^{bc}
Cultivated grasses								
Pennisetum purpureum	0.40^{de}	0.26^{c}	0.26^{d}	0.79^{a}	0.04^{c}	0.11^{a}	0.12	0.16^{a}
Sudex	0.50^{cd}	0.68^{b}	0.26^{d}	0.75^{ab}	0.14^{b}	0.09^{ab}	0.13	0.08^{b}
Jumbo grass	0.52^{cd}	0.73^{b}	0.28^{d}	0.75^{ab}	0.12^{b}	0.17^{a}	0.14	0.04^{c}
Splenda setaria	1.50^{a}	1.29^{a}	0.12^{e}	0.74^{ab}	0.18^{a}	0.05^{b}	0.14	0.06^{c}
SEM	0.006	0.007	0.0006	0.018	0.002	0.014	0.20	0.007
Species	***	***	***	***	**	***	NS	**

Mean with different letters in the same column are different at $p<0.05$

NS, not significant ($p>0.05$); ***, $p<0.001$; **, $p<0.01$; SEM, standard error of the mean

Discussions

The results of this study present the first comprehensive dataset on the chemical composition, mineral profile, *In vitro* DMD, *In vitro* total gas (rate and extent) and methane production of ten summer grass species, grown under uniform agronomic conditions and harvested at similar stage of maturity. The dataset produced from the well replicated experimental design using standard protocols and advanced analytical techniques can be used by animal nutritionist for diet formulation for the grazing animals. At the same time the large variability in chemical composition, digestibility and methane production among the grass species present an opportunity for plant breeders and forage/pasture development organization to select and further develop grass species with higher nutritional value, digestibility and lower methane production.

Chemical composition of pasture species: Many factors such as genotypes, maturity stage at harvest, growing condition and post-harvest management influence the chemical composition of forages (Bezabih *et al*., 2014a; Khan *et al*., 2015b). In this study, the grass species were grown in four replicate plots under uniform agronomic conditions and samples were randomly collected at similar stage of maturity that provided good power for studying between-species variations in chemical composition and feeding value. Except, *D. bipinnata*, all grass species had CP contents above the minimum recommended level of 7% for normal rumen functioning (Bezabih *et al*., 2014b). However, none of the grass species had CP content of more than 15% DM required for optimum growth and milk production (Anon., 2001). Tropical grasses generally contain moderate to lower CP contents due to rapid growth and maturity. In this study 50% of the grass species has moderate level (>10%) of CP, and these could be used to support livestock productivity, particularly during the dry periods. Next to CP content, plant cell walls (NDF) content largely influence the nutritional value of tropical grasses. For example, grasses with higher NDF content (60-65% DM), can negatively influence the DM intake and productivity of animals (Bezabih *et al*., 2014b). Except *V. zizanioides*, *D. bipinnata* and *B. pertusa* all other grass species contained NDF levels below this threshold, and had a CP content ≥8.7%, indicating that the grass biomass of these species during the sampling period (main growing season) is of moderate quality.

In agreement with previous findings (Khan *et al*., 2009b), the Na contents of the grass species were lower than the recommended level of 0.1-0.4% DM, except *Sudex*, *jumbo grass* and *S. setaria*. Similarly, except *jumbo grass,* all other investigated grass species had Cu content lower than the recommended level (0.8 to1.4 mg/g DM). The biomass of all grass species was also deficient in Fe, Zn and Mn. Supplementation of the deficient (micro)-mineral to grazing animals, particularly to lactating and growing animals, will be required. On the other hand, the Mg content (minimum recommended 0.2% DM) of all grass species and Ca (minimum recommended level is 0.4%DM) contents of most of the grass species were more than recommended for diary animals (Anon., 2001). The results of the present study are important for proper mineral supplementation of the animals, as feeding of too high levels of minerals can negatively affect animal productivity and health.

***In vitro* cumulative gas and methane production:** The rate and extent of rumen fermentation largely influence the nutritional value of grasses. Because, the rate and extent of ruminal fermentation is the major determinant of forage digestibility, dry matter intake, feed efficiency and animal productivity (Mertens, 2009). The *In vitro* total GP data measured through the fully automated gas production system over 72-hour incubation of grass species in buffer rumen fluid (Fig. 2), reflects the difference in ruminal fermentation (rate and extent) among the grass species (Cone *et al*., 1996; He *et al*., 2018). The GP is mainly influenced by the chemical composition of the grasses, particularly the CP and NDF content, but also by NDF composition such as the content of lignin (He *et al*., 2018). The NDF is least digestible (40 to 70%) component of forages, whereas the digestibility of non-NDF components is very high (> 90%) and less variable. Therefore, NDF content is the major determinant of forage digestibility (Mertens, 2009). In this study forage species with higher content of CP and lower content of NDF had higher rate and extent of total GP. These results are in line with the findings of Van Soest *et al*., (1991) and Bezabih *et al*., (2014b), who reported that the NDF and CP contents of forages influence the degradability and feeding value of forages. Grass species with lower content of NDF and higher content CP such as *jumbo grass*, Sudex, *P. purpureum* and *S. almum* produced the highest gas volume, whereas those with the higher NDF and lower CP such as D. bipinnata content produced lower gas volumes. This result is consistent with findings of Kamalak *et al*., (2005). Abdulrazak *et al*., (2000) and Bezabih *et al*., (2014b) reported that the GP kinetics and *In vitro* DMD were negatively correlated with NDF, ADF and ADL contents of forages, and positively correlated with CP. These findings demonstrate that grasses with higher CP content and lower fiber content will support higher rate and extent of rumen fermentation of OM.

Grasses with a higher degradation rate are expected to produce lower amount of methane than those with a lower rate and extent of degradation (Waghorn *et al*., 2002). The proportion of methane in the total gas is a vital index for devising strategies for methane mitigation based on per unit organic matter degradation in the rumen. The variability observed in the methane to total gas production for the investigated grass species, highlights the potential opportunity to screen the grass species for low methane-production. The relative position of grass species with respect to the ratio of methane in total gas (Fig. 2) altered with the progress in incubation time, highlighting the fact that continuous (time series) gas production and composition data collection method such as with the GP apparatus, provides more reliable understandings of the kinetics of methane production than the batch system. *jumbo grass,* Sudex, *P. purpureum* and *S. almum* had lower proportion of methane in total GP, which make them good candidate species for further selection and evaluation.

Conclusions

The chemical composition, mineral profile, *In vitro* DMD, *In vitro* GP (rate and extent) and *In vitro* methane production during 72 h incubation of the grasses showed huge variation, presenting a strong prospect for selection of grass species with high nutritional value and minimum methane-emission potential. Most of the studied grasses had moderate nutritional quality, in terms of supporting the productivity of grazing animals. Among the grasses, Jumbo grass had greater CP (11.9% DM) content and *In vitro* DMD (65.9% DM), and produced greater amount of total gas, that contained lowest proportion of methane, highlighting its potential of animal feeding and for reducing the environment foot-print of livestock under small holding production systems. However, the dynamics of changes in chemical composition and feeding values throughout the season and with maturity needs further systematic research.

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