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Chemical composition and *in vitro* ruminal DM degradability of native pasture grasses and their plant parts

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Abstract
The study was designed to determine the effects of various native grass species *viz*., *Themeda triandra*, *Eragrostis superba*, *Panicum maximum* and *Fingerhuthia africana* harvested at maturity stage and their different parts *viz*., leaves, stems, seed heads and whole plant on chemical composition and *in vitro* ruminal dry matter degradability (DMD). Seed heads of *P. maximum* had the highest (P<0.05) CP content (124.6 g/kg DM) than the other grass species. Leaves of all the grass species had the highest DMD compared to other plant parts. Grass species containing high CP showed an increase in degradability despite a high value of cell wall constituents. Mineral contents of the four grass species studied were below the recommended levels to meet nutrient requirements of cattle and sheep. Therefore, despite the high CP content of these grass species, supplementation is essential for ruminants in high production stages, particularly those fed these grass species as a basal diet.

Keywords: Chemical composition, Dry matter intake, Grasses, *In vitro* ruminal degradation, Rangeland

Introduction

Grass species play an important role as a major source of feed for ruminants in both arid and semi-arid regions. Depending on the season, soil fertility and phenological stage, these grasses may be of sufficient quantity and quality to meet the nutritional requirements of livestock (Rust and Rust, 2013). However, according to Mnisi and Mlambo (2017), these regions experience seasonal fluctuations in quality and quantity of grass forage as a result of prolonged dry periods and erratic rainfall patterns. In addition, a lack of knowledge on the nutritive value of grass species is one of the major challenges faced by many communal resource-poor farmers, which in turn leads to nutrient deficiencies and/or imbalances from incorrect supplementation with unquantified plant parts of most of the grass species that are readily available (Gerrish, 2004).

Despite the long history of livestock grazing in most communal rangelands of the North-West province of South Africa, nutritional composition of grass species growing in these areas has drawn little attention and, as a consequence, there is a gap in literature. Lack of such information impedes the farmers in ration balancing for optimal productivity of ruminant animals (Tainton, 1999). This is because the forage quality is dependent on a delicate balance of two basic factors such as nutrient content and their degradability in animal (Valentine, 1990). Nutrient content and degradability of different parts of grass species are influenced by genetic variation, stage of growth and different growth conditions such as temperature, precipitation and soil composition.

Knowledge on the distribution of chemical constituents to different plant parts of grass species can be considered as one of the keys to a sustainable and an efficient production system, because it assists in their timely utilisation and helps to predict nutrient deficiencies (Dynes *et al*., 2003). There is a need to assess the nutritive value of the grass species in order to guide farmers to meet the nutritional requirements and improve livestock productivity. The study was, therefore, designed to determine the chemical composition and *in vitro* ruminal DMD of grass species and their plant parts harvested at maturity stage.

Materials and Methods

Description of harvesting site: Harvesting of grass samples was conducted at Lokaleng village, a communal grazing area, located in the semi-arid region of Mafikeng (North West Province, South Africa). The geographical coordinates of Lokaleng are 25° 80’ 00" S, 25° 56’ 67" E, with an altitude of 1224 m above sea level. The temperatures around the village range from 3°C to 39°C, with an annual rainfall of 450 mm (SAWS, 2017). The soil type is sandy loam to clay loam and the vegetation type resembles that of a savannah biome. Different kinds of herbivores have unlimited access to this village with no controlled grazing. Soil erosion is high being an open land with free movement of public and livestock at any given time.

Harvesting and processing of grass species: Samples of grass species *viz*., *Themeda triandra*, *Eragrostis superba*, *Panicum maximum* and *Fingerhuthia africana* were clipped randomly using a pair of scissors above 10 cm from the ground level at maturity stage (reproductive stage). The grasses were separated into whole plant, stems, leaves and seed heads. The samples were stored in brown paper bags, labelled and immediately taken to the Animal Science laboratory of the North-West University where they were oven-dried at 60°C until constant weight and thereafter milled to pass through a 2 mm sieve (Polymix PX-MFC 90 D) pending chemical analyses.

Chemical analyses: About 1 g of each sample was placed into pre-weighed crucibles and placed in an oven set at 105°C for 12 hours to estimate dry matter (DM). The loss in weight was measured as moisture content and DM was calculated as the difference between initial sample weight and moisture weight. Organic matter (OM) content was determined by ashing the dried samples in a muffle furnace set at 600°C for 6 hours, and the loss in weight was measured as OM content. Total nitrogen content was determined following the standard macro-Kjeldahl method (AOAC, 2005, method no. 984.13) and was converted to crude protein (CP) by multiplying the percentage N content by a factor of 6.25 and expressed in g/kg DM. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using ANKOM^{2000} Fibre Analyser (M/s ANKOM Technology, New York), according to van Soest *et al*. (1991). A heat-stable bacterial α-amylase was used for the NDF analysis. Acid detergent lignin (ADL) was determined by treating ADF residue in ANKOM F57 bags with 72% sulphuric acid and estimated after drying (105°C) the ADF residue for 12 h. Mineral content was analyzed following the guidelines from Agri-Laboratory Association of Southern Africa (AgriLASA, 1998).

Estimation of potential daily DM intake (DMI): The estimated potential daily DMI was calculated according to Mertens (1987) using the following formula:

$$DMI = \frac{1.2 \text{ X body weight}}{NDF\ (\%)}$$

In vitro ruminal DM degradability: The *in vitro* ruminal DMD of the samples was determined using the ANKOM Daisy^{II} incubator set at 39°C with four rotating jars according to the ANKOM Technology method 3 for *in vitro* true digestibility. The samples were weighed into ANKOM F57 bags (0.45 – 0.5 g), heat-sealed and placed in the digestion jars. Two buffer solutions were prepared and combined at a ratio of 1:5, and 1600 ml of the combined buffer was added to each of the jars and warmed (39°C). Rumen inoculum was collected from a fistulated Bonsmara cow with a live body weight of approximately 550 kg. The donor cow received a ration composed of a mixture of lucerne and blue buffalo grass and had free access to clean fresh water. The animal was cared for according to Institutional Animal Research Ethics Committee (Ethical approval no. NWU-00126-13-A90) and Federation of Animal Science Societies guidelines (FASS, 2010). Rumen fluid was collected into three pre-warmed thermos flasks and immediately taken to the laboratory where it was blended and strained through two layers of warm muslin cloth. Subsequently, 400 ml of rumen inoculum was added to each of the four jars, each of which contained 1600 ml of buffer and grass samples. Throughout the collection period, the digestion jars and the rumen fluid was continuously purged with CO_2 before being closed and placed in the incubation chamber. Filter bags were withdrawn at 12, 24, 48 and 72 h after inoculation and washed with tap water for 20 min using the ANKOM^{2000} Fibre Analyser machine. The washed samples were then dried at 105°C for 12 h before being weighed for *in vitro* ruminal DMD.

Statistical analysis: Chemical composition, estimated DM intake and *in vitro* ruminal DMD data of *T .triandra*, *E. superba*, *P. maximum* and *F. africana* grass species and their plant parts were analysed using the GLM procedure of SAS (2010), in a two-way factorial design within a completely randomised experimental design. The following linear statistical model was employed:

$$Y_{ijk} = \mu + S_i + P_j + (S \times P)_{ij} + E_{ijk}$$

Where, Y_{ijk}= response variable, μ = population mean, S_i = effect of grass species, P_j= effect of plant parts, $(S \times P)_{ij}$ = effect of interaction between grass species and plant parts, and E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P \leq 0.05$. Least squares means (LSMEANS)

Table 1. Chemical composition (g/kg DM, unless otherwise stated) of *F. africana*, *P. maximum*, *E. superba* and *T. triandra* grass species and plant parts

		Grass species				
		F. africana	***P. maximum***	***E. superba***	***T. triandra***	**SE**
DM (g/kg)	Whole plant	961.2	961.9	956.1	956.2	7.67
	Leaves	957.5	954.7	950.7	951.5	
	Seed heads	954.0	965.3	949.7	957.0	
	Stems	972.2	965.7	967.8	960.1	
OM	Whole plant	896.0^{AB}	882.9	899.3	888.6	11.20
	Leaves	855.6^{B}	854.9	881.6	857.8	
	Seed heads	905.5^{AB}	889.3	896.3	897.0	
	Stems	927.0^{A}	904.5	920.1	911.0	
CP	Whole plant	87.5^{AB}	86.1^{AB}	58.5^{AB}	54.7^{AB}	10.47
	Leaves	103.7^{A}	95.5^{A}	80.8^{A}	72.6^{A}	
	Seed heads	108.0^{abA}	124.6^{aA}	76.1^{abA}	68.6^{bAB}	
	Stems	50.9^{B}	38.3^{B}	18.5^{B}	22.9^{B}	
NDF	Whole plant	733.3^{AB}	686.4^{AB}	756.4	752.7^{AB}	22.59
	Leaves	659.7^{B}	675.1^{AB}	707.8	706.7^{B}	
	Seed heads	716.0^{abAB}	629.0^{bB}	789.2^{a}	717.6^{aAB}	
	Stems	824.1^{A}	755.0^{A}	772.0	833.9^{A}	
ADF	Whole plant	491.9	488.9	479.6	476.5	36.01
	Leaves	403.3	426.6	432.3	418.5	
	Seedheads	518.2	490.3	456.9	471.5	
	Stems	554.1	549.7	549.6	539.5	
ADL	Whole plant	112.5	130.2^{AB}	127.1^{B}	119.4	7.25
	Leaves	112.8	132.1^{AB}	98.9^{B}	108.3	
	Seed heads	116.4	105.4^{B}	113.7^{B}	109.5	
	Stems	108.4^{b}	153.0^{aA}	168.8^{aA}	140.3^{ab}	

[ab]In a row, different lowercase superscripts denote significant (P<0.05) differences between the species; [ABC]In a column, different uppercase superscripts denote significant (P<0.05) differences between the plant parts

DM: Dry matter; OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; SE: Standard error

were compared using the probability of difference option in the LSMEANS statement of SAS.

Results and Discussion

Chemical composition: The effect of grass species and their plant parts on chemical constituents was recorded (Table 1). Effective strategies to enhance the utilisation of grasses as a major source of feed for ruminants depend on the assessment of the chemical constituents that form the diets and also the interactions of the chemical components (Barnes *et al*., 2007). The chemical composition of *T. triandra*, *E. superba*, *P. maximum* and *F. africana* grass species and their plant parts was quantified in this study to define the relevance and importance of these species to ruminant nutrition. There was a significant difference between plant parts in terms of OM, CP, NDF and ADL content, whereas there was no species difference in terms of DM, OM and ADF. All the grass species had similar (P>0.05) DM (950 - 972 g/kg) and ADF (403 - 554 g/kg DM) contents. *F. Africana* had higher (P<0.05) OM content (927 g/kg DM) in the stem than in the leaves (857 g/kg DM), whereas seed heads of *P. maximum* had higher (P<0.05) CP content (125 g/kg DM) than *T. triandra* seed heads (69 g/kg DM). Leaves and seed heads of all the grass species had similar (P>0.05) CP content. The lowest CP content was observed in the stems of all grass species compared to other plant parts, which did not differ (P>0.05).

The CP content in *P. maximum* reported in this study was higher than the CP values reported by Tefera *et al.* (2009) as well as Keba *et al.* (2013). These differences could be attributed to different growth environments (Ravhuhali *et al*., 2018) and genotypes (Rajora *et al*., 2017). There was a species difference in terms of NDF content of seed heads (P<0.05). Seed heads from *E. superba* and *T. triandra* had higher (P<0.05) NDF content than those of *P. maximum.* Stems of *F. africana* and *T. triandra* had higher NDF content than the other leaf parts. Leaves and stems from *P. maximum* had comparable

NDF content, but the stems had higher (P<0.05) NDF content (755 g/kg DM) than the seed heads (629 g/kg DM). Stems of *T. triandra* had a higher (P<0.05) NDF content (833.9 g/kg DM) than the leaves (707 g/kg DM), indicating that NDF is rich in grass stems. These findings were in line with Buxton and Redfearn (1997), who reported that leaves contain less NDF content than other plant parts. In addition, Mahala *et al.* (2009) stated that as plant matures the NDF, ADF and lignin proportions increase while CP content decreases, emphasizing that an increase in these parameters is influenced by the maturity stage of the grass species. Johnson *et al.* (1967) stated that fibre content in *P. maximum* increased by 7.3% from 2½ weeks to 2½ months of age. All these variations in the nutritive values could be because of the differences in ecotype, assay method, age of grass species or the geographical zone (Ismail *et al*., 2014; Kirwa *et al*., 2015). Francisco *et al.* (2014) also emphasised that the chemical composition of grasses can be influenced by soil type/fertility, climate conditions and inherited traits of species lineage.

A significant species × plant parts interaction effect was observed for ADL content. Stems of *P. maximum* and *E. superba* had higher ADL content than *F. africana*, which were all comparable (P>0.05) to the ADL content of *T. triandra*. Plant parts from *F. africana* and *T. triandra* had the same ADL content. Stems from *P. maximum* had higher ADL (153 g/kg DM) than the seed heads (105.4 g/kg DM), which means the seed heads may require crushing before utilisation in order to improve its digestibility. For *E. superba*, the stems had higher ADL content (169 g/kg DM) than all the other plant parts, suggesting that the stem is less digestible than the other parts.

The grass species, plant parts and their interactions had a significant influence on macro-mineral concentration (Fig 1). Seed heads of *P. maximum* had the highest (P<0.05) phosphorus (0.0935 g/kg DM) compared to *F. africana*, *E. superba* and *T. triandra* which were comparable. Leaves of *T. triandra* (0.0409 g/kg DM) had the highest (P<0.05) phosphorus concentration compared to its other plant parts. In general, the mineral values from this study were lower than those reported by NRC (1996) for livestock requirements. Ayanda *et al*. (2016) also observed lower levels of minerals in grass species grown in semi-arid environments similar to those of the North-West province. Ganskopp and Bohnert (2003) reported an average of 1.42 g/kg DM in grass species. The lower phosphorus concentration in these grass species may be due to the maturity stage of the grasses. This could be explained from the findings of Horn (2017), who stated that as grasses mature, the phosphorus content in the grazing area declines significantly. *P. maximum* parts had the same (P>0.05) calcium concentration.

Leaves and stems of *E. superba* and *T. triandra* had higher (P<0.05) calcium than their seed heads. However, seed heads of *F. africana* had higher (P<0.05) calcium (1.495 g/kg DM) than the other grass species. All the grass parts had a calcium concentration below 3.2 g/kg necessary to meet animal requirements (NRC, 1996), especially when the animal depends on grass as a basal diet. Horn (2017) emphasised that the amount of Ca in the diet of cattle and sheep should be at least 1.5 times greater than the amount of P, but should not exceed 1.7 times. Leaves of *P. maximum* had the highest (P<0.05) magnesium concentration (0.1336 g/kg DM), whereas the stems had the lowest concentration (0.0601g/kg DM). *E. superba* and *T. triandra* had comparable (P>0.05) magnesium concentration in all their plant parts. *F. africana* stems and whole plant had the same concentration of Na as the leaves and seed heads of the same species. *P. maximum* seed heads had a higher (P<0.05) Na concentration (0.0388 g/kg DM) compared to its other plant parts. *P. maximum* leaves had comparable (P>0.05) Na concentration as the stems and whole plant. All the stems from the different grass species had comparable (P>0.05) Na concentrations (Fig 1).

The grass species, plant parts and their interactions had a significant effect on the micro-mineral concentration, except for Zn and Cu (Fig 2). The seed heads and stems of *F. africana* had the highest (P<0.05) Fe value than the leaves. Seed heads of *E. superba* had the highest (P<0.05) Fe (95.31 mg/kg DM) concentration compared to its other plant parts. *P. maximum* leaves had a lower (P<0.05) Fe (19.65 mg/kg DM) concentration compared to its all other plant parts. For all the grass species and their seed heads, the Fe concentration was comparable (Fig 2).

Plant parts of *F.africana*, *E. superb* and *T. triandra* had similar (P>0.05) Mn concentrations. The stems of *P.maximum* had the highest (P<0.05) Mn concentration (76.02 mg/kg DM), whereas the leaves and seed heads had the lowest (25.21 and 21.17 mg/kg DM, respectively). Magnesium and copper concentrations were also below the recommended levels stated by NRC (1996). According to NRC, 1.15 g/kg of Mg and 9.6 mg/kg of Cu

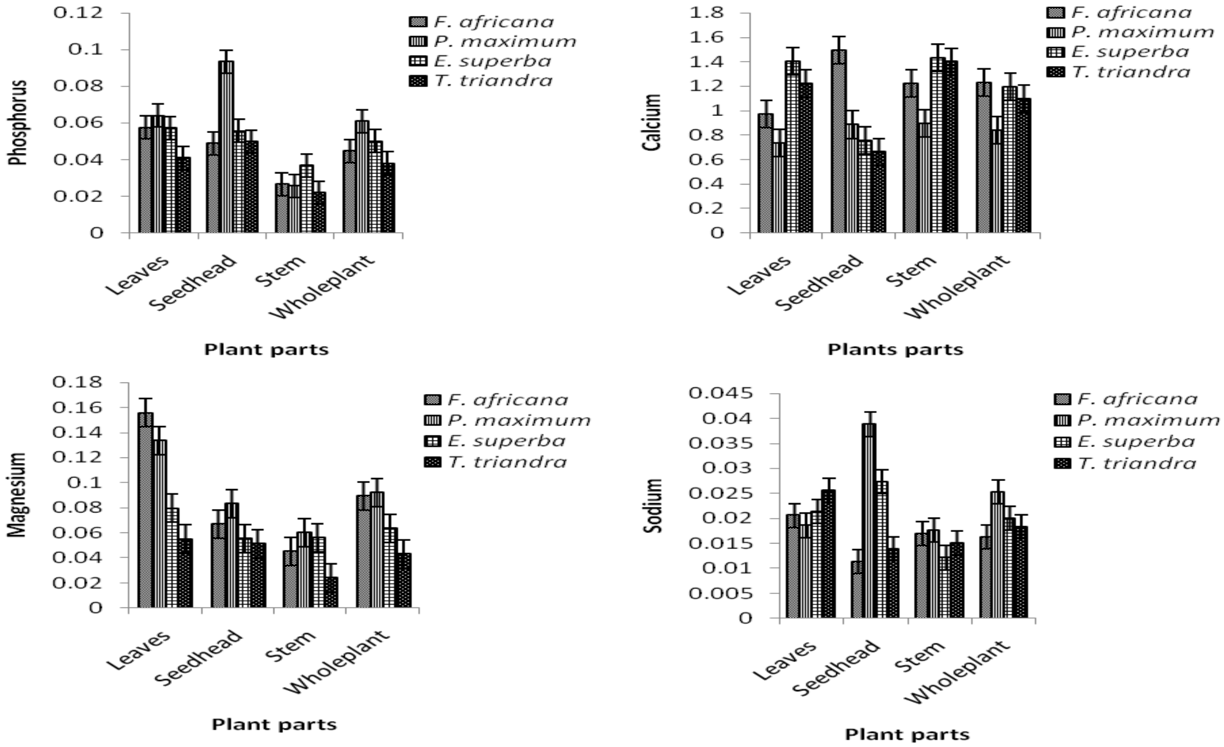

Fig 1. Macro-minerals concentration (g/kg DM) of *F. africana*, *P. maximum*, *E. superba* and *T. triandra* grass species and plant parts

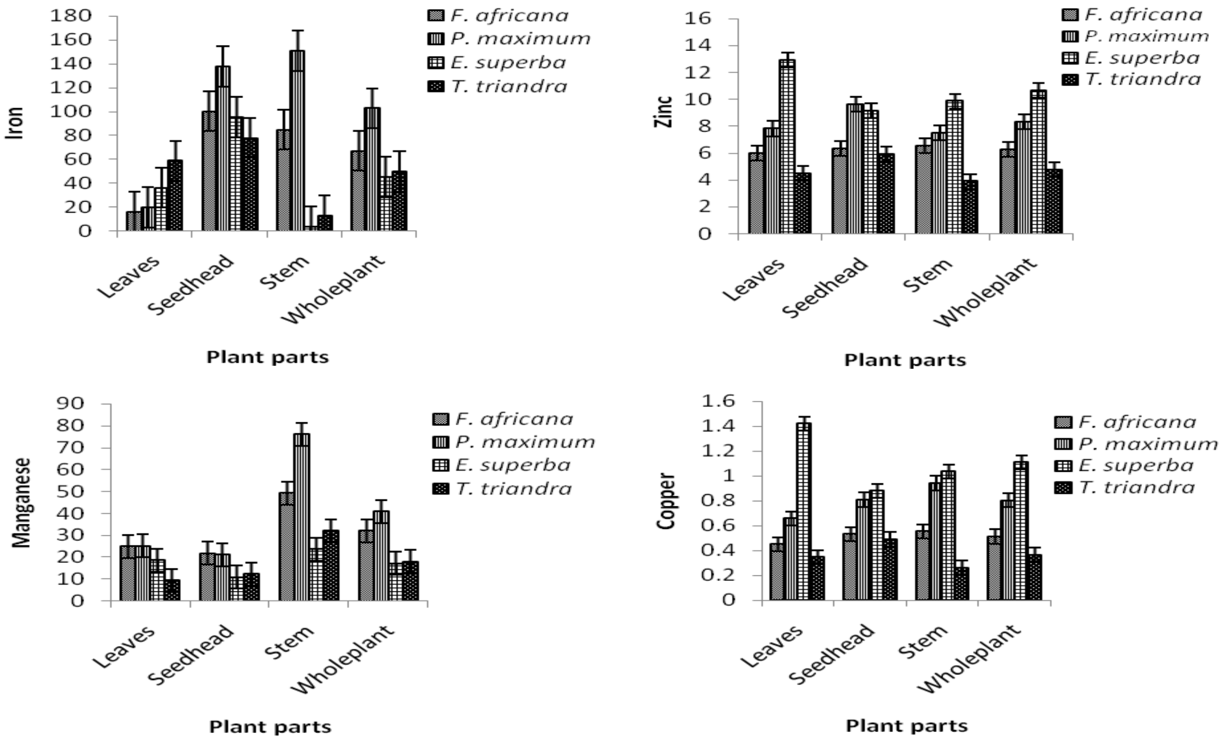

Fig 2. Micro-minerals concentration (mg/kg DM) of *F. africana*, *P. maximum*, *E. superba* and *T. triandra* grass species and plant parts

are sufficient to meet the requirements for livestock. A deficiency in Cu is known to cause ataxia, bleaching of hair, lameness, swelling of joints as well as some reproductive disorders in cattle (Maynard and Loosli, 1969; Ganskopp and Bohnert, 2003). Whereas a low concentration of Zn is always associated with its low mobility in neutral to alkaline soils (Mengel and Kirby, 1987), but contrary to this study, the soil pH from where the grass species were harvested was 4.5. This suggests that animals grazing in these rangeland need mineral supplementation to maintain growth, reproduction and production.

Prediction of dry matter intake: The prediction of the DMI through NDF concentration of the different grass species and their plant parts was made (Table 2). *F. africana* leaves had the highest DMI (8.2 g/kg), which was comparable to seed heads (P>0.05). *P. maximum* stems had the lowest DMI (7.2 g/kg) than the other parts. *E. superba* leaves had higher (P<0.05) DMI than the stems. *T. triandra* stems had the lowest DMI (6.5 g/kg) than the other parts, which were comparable (P>0.05).

Whole plants of *F. africana* and *P. maximum* had higher (P>0.05) DMI than *E. superb* (Table 2). Leaves from all the grass species had the same DMI (P>0.05). *P. maximum* stems had the highest DMI (7.2 g/kg), whereas *T. triandra* stems had lowest (6.5 g/kg). Seed heads of *P. maximum* had the highest DMI (8.6 g/kg), followed by *F. africana and T. triandra* (P>0.05) (7.5 g/kg) and the least was from *E. superba* (6.8 g/kg). These results indicated an inverse relationship between NDF content and DMI. In this current study, species and plant parts with high fibre had lower DMI. These findings could be attributed to Ismail *et al*. (2014), who stated that grasses with low fibre but high CP contents exhibit higher DMI.

Table 2. Predicted dry matter intake (DMI, g/kg) of grass species by animal unit (AU) based on neutral detergent fibre concentration

	Dry matter intake			
	Whole plant	**Leaves**	**Stems**	**Seed heads**
F. africana	7.4^{abB}	8.2^{aA}	6.6^{abC}	7.5^{bAB}
P. maximum	7.9^{aB}	8.0^{aAB}	7.2^{aC}	8.6^{aA}
E. superba	7.1^{cAB}	7.6^{aA}	7.0^{abAB}	6.8^{cB}
T. triandra	7.2^{bcA}	7.7^{aA}	6.5^{bB}	7.5^{bA}

^{abc}In a column, different lowercase superscripts denote significant (P<0.05) differences between the species; ^{ABC}In a row, different uppercase superscripts denote significant (P<0.05) differences between the plant parts; AU: Animal unit of 450 kg.

In vitro ruminal DMD of grass species: Effect of grass species and plant parts on the *in vitro* ruminal dry matter degradability (DMD) at different intervals 12, 24, 48 and 72 h (DMD12, DMD24, DMD48 and DMD72 g/kg) were also recorded (Table 3). Leaves of *P. maximum* had the

Table 3. *In vitro* ruminal dry matter degradability (g/kg) of grass species and grass plant parts

		Grass species				
		F. africana	***P. maximum***	***E. superba***	***T. triandra***	**SE**
DMD12	Whole plant	172.3	229.8	174.9^{AB}	133.6	20.7
	Leaves	202.4^{ab}	252.1^{a}	224.4^{abA}	143.5^{b}	
	Seedheads	182.2	208.1	102.7^{B}	120.6	
	Stems	132.2^{ab}	229.3^{a}	195.9^{abAB}	116.8^{b}	
DMD24	Whole plant	301.2	336.1	247.7^{AB}	274.9	31.0
	Leaves	341.7	370.1	365.1^{A}	283.0	
	Seedheads	339.3^{a}	324.0^{a}	109.1^{bB}	340.7^{a}	
	Stems	222.4	314.2	270.6^{AB}	220.9	
DMD48	Whole plant	362.7	427.3	333.8^{AB}	378.4	37.8
	Leaves	348.3	465.3	455.5^{A}	366.6	
	Seedheads	436.1^{a}	369.5^{ab}	179.0^{bB}	481.2^{a}	
	Stems	303.8	447.2	366.8^{AB}	287.6	
DMD72	Whole plant	463.3	544.6	406.3^{AB}	466.9	50.1
	Leaves	519.2	617.3	586.4^{A}	549.3	
	Seedheads	479.9^{a}	510.7^{a}	173.1^{bB}	439.2^{a}	
	Stems	390.9	505.8	459.4^{AB}	412.1	

^{ab}In a row, different lowercase superscripts denote significant (P<0.05) differences between the species; ^{AB}In a column, different uppercase superscripts denote significant (P<0.05) differences between the plant parts.

DMD12: dry matter degradability at 12 hours; DMD24: dry matter degradability at 24 hours; DMD48: dry matter degradability at 48 hours; DMD72: dry matter degradability at 72 hours; SE: Standard error at 12, 24, 48 and 72, respectively.

highest DMD12 value (252.1 g/kg DM) whereas *T. triandra* leaves had the lowest DMD12 value (143.5 g/kg DM). Leaves of *E. superba* had the highest (P<0.05) DMD12 (224.4 g/kg) and DMD24 (365.1 g/kg) values when compared to its other plant parts. The *E. superba* seed heads had the lowest DMD12 and DMD24 values (102.7 and 109.1 g/kg, respectively).

Seed heads from both *F. africana* and *T. triandra* had higher DMD48 values than *E. superba*. Leaves of *E. superba* had higher (P<0.05) DMD72 value (586.4 g/kg) than the seed heads (173.1 g/kg). The grass species leaves had higher DM degradability compared to all other plant parts (Table 3). Although the values from this study were lower than those reported by Huws *et al.* (2014), but they were in line with those reported by Masuda (1977), Chaves *et al.* (2006) and Huws *et al.* (2014). The authors reported that stem material had lowest degradability than leaves even during maturity stage. High lignin content especially on stems was a reason for lower degradability.

The high contents of lignin had a negative influence on degradability, which caused a decrease in the availability of nutrients. The structural carbohydrate such as lignin increased, while *in vitro* degradability of CP and DM were decreased (Bayble *et al*., 2007; Rambau *et al*., 2016). Huws *et al.* (2014) stressed that variation in DMD on plant parts might have been caused by different bacteria attachment to different plant parts material. Masuda (1977), Wilson and Mertens (1995) as well as Jung and Allen (1995) stated that weight proportion of the epidermis, the amount of cuticle and the degree of cross-linkage to other cell wall polymer within the plant, lignified parenchyma and the vascular bundles on the stems might be a reason for the lower degradation of stems.

Conclusion

It was concluded that the plant parts of all the grass species have a potential to complement each other as a protein source for ruminant animals. Furthermore, leaves of all the grass species had higher DMD values than other plant parts suggesting that livestock should be fed grasses with more foliage to enhance degradability in the rumen. The mineral status of the grasses were lower than NRC recommendations, thus there is a need for mineral supplementation for ruminants grazing in these growing conditions. Future research is recommended to determine the stage of growth where the nutritional value of these grasses is optimal for ruminants.

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