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Comparison of Nutrient Composition and *In vitro* Digestion Characteristics of Four Forage Legumes from Two Agro-Ecological Zones of Rwanda

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Abstract: Ruminant production in sub-Saharan Africa countries including Rwanda is predominantly based on natural pastures as well as crop residues. The quantity and quality of these feed resources vary with season, herbage type and geographical location. A study aiming at comparison of nutritive values and *in vitro* digestibility characteristics of four forage legumes used by farmers in two different agro-ecological zones was conducted. Samples of forage legumes from High (HA) and Middle Altitudes (MA) of Rwanda were randomly collected. Samples were oven dried at 60°C for 48 h and Dry Matter (DM) and ash were calculated. Dried samples were proximately analysed for Crude Protein (CP), Phosphorus (P) and Calcium (Ca). *In vitro* fermentation medium consisted of 1:2 ruminal fluid: McDougall's buffer and the experiment was a completely randomised design. Each sample (0.2 g, DM basis) was put in a gas syringe in three replicates per forage species sample. Syringes containing samples with 30 mL of fermentation medium and 2 blanks were incubated in a water bath at 39°C for 72 h. Data for *in vitro* gas production: Metabolisable Energy (ME), Organic Matter Digestibility (OMD) and kinetic coefficients of fermentation were statistically analysed using PROC Nonlinear Model of SAS (9.3. Inc). Results showed that the nutritive values of four forage legumes from two agro-ecological zones (high and middle altitude) were highly significant difference ($p<0.01$). The CP of *Medicago sativa* (26.20%) and *Mucuna pruriens* (25.19%) from high altitude were higher than that of *Medicago sativa* (24.37%) and *Mucuna pruriens* (20.94%) from middle altitude while the CP of *Stylosanthes scabra* (15.73%) from middle altitude was higher than that of *Stylosanthes scabra* (12.76%) from high altitude. However, there was no significant difference ($p>0.05$) between *Desmodium intortum* from both sites (13.99 vs. 12.41%). Furthermore, there were no significant difference ($p>0.05$) of Ca and P content in forage legumes from high and middle altitudes. *In vitro* gas produced within 72 h showed that forage legumes from middle altitude produced more gas than those of high altitude. The OMD and ME of forage legume from middle altitude were higher than those of high altitude. However, the Digestible Crude Protein (DCP) of *M. pruriens* and *M. sativa* from high altitude were higher than that of *M. pruriens* and *M. sativa* from middle altitude. The evaluation of ecological environment of forage quality, especially potential degradable portion showed that the indicative of better quality forages from warmer environment was higher than those of cooler environment of Rwanda.

Key words: Middle altitude, high altitude, metabolisable energy, digestible crude protein, kinetic coefficients, Rwanda

INTRODUCTION

Growth of global population and the simultaneous increase in urbanisation and incomes has been linked to increasing consumption of livestock products, especially in sub-Sahara African countries (Stage *et al*., 2009). Livestock production in Rwanda faces many problems among which feed is a major constraint. Ruminant production in sub-Saharan Africa is predominantly based on natural pastures as well as crop residues. The quantity and quality of these feed resources vary with season, herbage type and geographical location. In efforts to meet animal's nutrient requirements, it is important to know the exact composition and feeding values of feeds in terms of protein, energy, fat, minerals and vitamins. Information on the nutritional qualities of a number of forage genotypes in Rwanda is available at limited parameters and a cursory review has depicted glaring knowledge gaps in digestion kinetics, Metabolisable Energy (ME) and protein quality attributes that are important indicators of intake as well as

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feed efficiency for maintenance and production. The nutritive value content of an animal feed is determined predominately by its digestibility (Preston, 1995). *In vivo* feed evaluation is good but not suitable for the rapid and routine feed evaluations. *In vitro* gas production methods are good alternative for measurement of the fermentation potential of a feed and it has good potentiality to predict *in situ* Dry Matter (DM) disappearance (Kamalak *et al*., 2004).

In many areas of East Africa, livestock especially cattle owners feed their animals with indigenous grass collected on roadside and/or marshland and these become scarce during dry season with low quality (Mupenzi *et al*., 2009). Improved forages are known for their rapid growth under favourable conditions leading to the production of good quantity and quality of feed. Improved fodder legumes such as *Medicago sativa*, *Desmodium intortum*, *Stylosanthes scabra* and *Mucuna pruriens* were found to be used by farmers in different areas of Rwanda including the High Altitude (HA) and Middle Altitude (MA) zones. Although, these forage legumes can provide a good quality of feed there is no support information to farmers across the named agro-ecological zones.

The aim of this study was to compare the nutritive values and *in vitro* gas production of four forage legumes used by farmers in the high and middle altitude zones of Rwanda. The objectives were to determine and compare nutritive values of four forage legumes from two agro-ecological zones of Rwanda to estimate and compare metabolisable energy and organic matter digestibility of four forage legumes according to agro-ecological zone and to determine and compare digestion kinetics of four forage legumes from two agro-ecological zones.

MATERIALS AND METHODS

Forage species: Four forage legumes namely; *Medicago sativa*, *Desmodium intortum*, *Mucuna pruriens* and *Stylosanthes scabra* were used as materials and each species was collected from two different agro-ecological zones (high and middle altitudes) of Rwanda. Four samples per forage legume were collected from Kinigi (high altitude: >1900 m a.s.l) and Rubona (middle altitude: 1750 m a.s.l) Research stations of the former Rwanda Agricultural Research Institute (ISAR: French acronym). These forage species were at the flowering stage of their third regrowth without fertiliser application.

Proximate analysis: Forage legume samples were air dried and then oven dried at 60°C for 48 h and Dry Matter (DM) and ash were calculated. Dried samples were ground to pass through 2 mm screen and were analysed for Crude Protein (CP), Calcium (Ca) and Phosphorus (P).

***In vitro* gas production:** Fermentation media consisted of micro-mineral two buffer solution B and C (Osuji *et al*., 1993) and ruminal fluid to provide the microbial inoculants. Ruminal fluid was obtained from fistulated steer and the material was delivered to the laboratory within 15 min in a vacuum flask. The ruminal content was macerated in fruit blender (Model No.: RM/161; Capacity 1.7 L with stainless steel blades). The macerates were squeezed from the fibrous mass into a plastic beaker (250 mL) through three layers of nylon cloth. During the squeezing the nylon cloth and contents were tightly fitted into the beaker to ensure minimum exposure to atmospheric oxygen.

Incubation and data recording: On the day of incubation buffer solution was constituted according to Osuji *et al*. (1993) and preheated at 39°C to reduce amounts of dissolved gases. Aliquots (20 mL) were dispensed into each gas syringes using veterinary drenching gun (Roux-Revolver®; Henke-Sass, Wolf GmbH). Any gas that was inadvertently introduced into the syringe was carefully expelled to avoid spillage of samples. Each sample (0.2 g, DM basis) was put in a gas syringe in three replicates per forage species and the experiment was a completely randomised design. Aliquot of rumen fluid (10 mL) was introduced into the same syringe to constitute a mixture rumen and buffer solution (1:2) (McDougall's buffer). Syringes containing samples with 30 mL of fermentation medium and 2 blanks were incubated in a water bath at 39°C for 72 h. The initial readings were recorded before the syringes were places into water bath set at 39°C. Readings of gas volumes were monitored at scheduled intervals >72 h of incubation.

Data analysis: Cumulative gas volumes were computed for each tube as the difference between the readings at time (t) and the initial reading adjusted for control readings at corresponding recording time. Data for *in vitro* gas production: Metabolisable Energy (ME), Organic Matter Digestibility (OMD) and kinetic coefficients of fermentation were statistically computed using PROC Non-Linear Model of SAS (9.3. Inc). The model was as follows (McDonald *et al*., 1981):

$$Y = a + b\left(i - e^{-c(t-tl)}\right) \quad (1)$$

Where:

Y = Dry matter disappearance at time
a = Intercept
b = Potentially degradable portion

c = Rate of degradation of b
tl = Time lag

$$OMD_H = 14.88 + 0.889V_{24} + 0.45CP + 0.0651Asah \quad (2)$$

Where:
OMD = Organic Matter Digestibility
Vg = Estimated gas volume at t = 24 h
CP = Crude Protein (% DM)

Energy values were estimated by using Eq. 3 (Menke *et al*., 1979):

$$ME(MJkg^{-1}\,DM) = 2.2 + 0.136V_{24} + 0.057CP + 0.0029CP^2 \quad (3)$$

Where:
ME = Metabolisable Energy
V_{24} = Estimated gas volume at t = 24 h
CP = Crude Protein (% DM)

Digestible Crude Protein (dCP) was estimated by using equations developed by Van Niekerk:

$$dCP(g\,kg^{-1}\,DM) = -32.6 + 0.94 \times CP(g\,kg^{-1}\,DM) \quad (4)$$

RESULTS AND DISCUSSION

The results for proximate analysis were calculated and presented in Table 1. Results showed that the nutritive values of four forage legumes from two agro-ecological zones (high and middle altitude) were highly significant difference ($p<0.01$). The CP of *Medicago sativa* (26.20%) and *Mucuna pruriens* (25.19%) from high altitude were higher than that of *Medicago sativa* (24.37%) and *Mucuna pruriens* (20.94%) from middle altitude while the CP of *Stylosanthes scabra* (15.73%) from middle altitude was higher than that of *Stylosanthes scabra* (12.76%) from high altitude. However, there was no significant difference ($p>0.05$) between *Desmodium intortum* from both sites (13.99 vs. 12.41%). Legumes used in the current study had a CP content of >12% DM. Njidda *et al.* (2009) reported that most tropical legumes species contain high CP and can be used to supplement poor quality roughages to increase productivity of ruminant livestock in tropical regions.

All selected legumes from middle altitude obtained higher ash than that of high altitude except *Desmodium intortum* from high altitude which produced higher ash than those of middle altitude.

Furthermore, there were no significant difference ($p>0.05$) of Ca and P content in forage legumes from high and middle altitudes. However, Ca and P of tested forage legumes were within the range reported by Njidda *et al.* (2009) for semi-arid browses. Generally, the minerals were within the range values and were likely adequate to meet the requirement for a dairy animal (Babayemi, 2006).

The results of metabolisable energy and digestible crude protein of selected forage legumes were calculated and shown in Table 2. Results showed that the metabolisable energy of forage of collected from middle altitude zone were higher than that of forage from high altitude. Nevertheless, digestible Crude Protein (dCP) of *Mucuna pruriens* (204.2 g kg^{-1} DM) and *Medicago sativa* (212.0 g kg^{-1} DM) from high altitude were higher than that of *Mucuna pruriens* (164.2 g kg^{-1} DM) and *Medicago sativa* (196.4 g kg^{-1} DM) of middle altitude while *Desmodium intortum* and *Stylosanthes scabra* collected from middle altitude were higher than those from high altitude (Table 2).

Considering the results of gas production within 24 h forage legumes from middle altitude produced higher gas volume than the forage legumes of the high altitude. The nutritive values of these forage species were similar to those reported by Whitbread *et al.* (2004) particularly found in stems and leaves of the tested forage legumes.

Evaluation of the Menke gas test of forages: The gas sproduction curve for the Menke gas was plotted to depict the behaviour of different forage under *in vitro* fermentation. The amount of gas produced by forage legumes collected from high altitude zone showed that *Stylosanthes scabra* was the top easily fermentable forages (Fig. 1a) during the 72 h of incubation. *Medicago sativa* and *Mucuna pruriens* constituted the medium cluster and did not differ significantly ($p>0.05$) from one another while *Desmodium intortum* was the least readily fermentable forage. All forage legumes showed a constant curve between the 67 and 72 h. This might be explained as rumen microbes were not active from this period. The gas production curve for forages from middle altitude (Fig. 1b) showed that *Medicago sativa* was the highest fermentable legume from the Ist 48 h it has been overtaken by *Stylosanthes scabra* which was the highest fermentable forages from the 48 h. At the 67th and 72nd h gas volumes became constant except *Stylosanthes scabra* which showed the increase of curve. *Mucuna pruriens* and *Desmodium intortum* were at the same fermentation level from the Ist 8 h but became significantly different ($p<0.05$) at 9th h.

Gas production comparison of forages collected in the two sites: *Stylosanthes scabra* and *Medicago sativa* were the highest forage legumes producing high gas volume in

Table 1: Nutritive values of four forage legumes according to agro-ecological zones

Sites	Forage species	DM (%)	CP (%)	Ash (%)	Ca	p-values
HA	*M. pruriens*	98.91	25.19	8.23	0.92	0.39
	D. intortum	96.48	12.41	10.04	0.76	0.39
	S. scabra	97.22	12.76	6.43	1.12	0.17
	M. sativa	96.02	26.20	10.18	1.19	0.35
MA	*M. pruriens*	89.19	20.94	9.37	1.91	0.33
	D. intortum	89.43	13.99	8.65	0.85	0.30
	S. scabra	90.99	15.73	8.93	1.72	0.18
	M. sativa	86.96	24.37	10.41	1.74	0.35

HA: High Altitude>1900 m; MA: Middle Altitude<1750 m

Table 2: Metabolisable energy and digestible crude protein of forage legumes calculated from gas produced within 24 h

Sites	Species	Mean gas vol_{24} (mL)	CP (kg^{-1})	CP (%)	ME (MJ kg^{-1} DM)	dCP (g kg^{-1} DM)
HA	*M. pruriens*	16.33	251.9	25.19	7.70	204.19
	D. intortum	14.33	124.1	12.41	5.30	84.050
	S. scabra	18.66	127.6	12.76	5.94	87.340
	M. sativa	19.33	260.2	26.02	8.28	211.99
MA	*M. pruriens*	24.00	209.4	20.94	7.93	164.24
	D. intortum	19.00	139.9	13.99	6.15	98.910
	S. scabra	21.66	157.3	15.73	6.76	115.26
	M. sativa	26.00	243.7	24.37	8.85	196.48

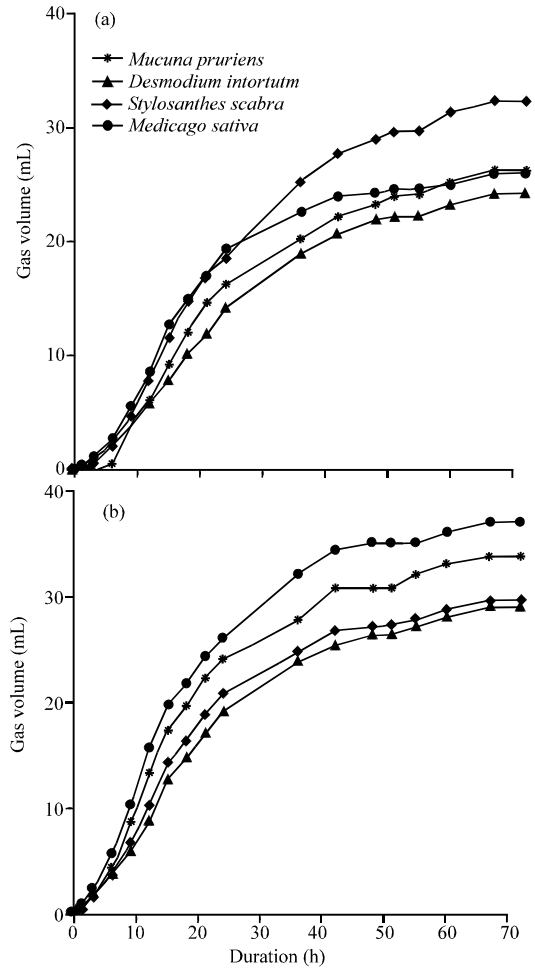

Fig. 1: *In vitro* gas production kinetics of selected forage species: a) High altitude zone; b) Mid altitude zone

Table 3: Analysis of variance for gas volume production

Source	df	SS	MS	Pr>F
Location	1	5596.38	5596.38	<0.0001
Species	3	7756.31	2585.43	<0.0001
Location *species	3	1502.21	500.74	<0.0001

DF: Degree of Freedom; SS: Sum of Square; MS: Mean Square; Pr: Probability; F: Fischer

high and middle altitude zones, respectively while the lowest was desmodium intortum in both sites. In general, forages from middle altitude produced more gas than those collected from high altitude zone (Fig. 1a, b). This difference might be due to the fact that forage from middle altitude produced more metabolisable energy which rumen microbes utilised to degrade feeds. This observation was in line with Pathak (2008) who stated that the increase of energy in the diet of ruminant animal will increase dry matter degradation in the rumen and De Boever *et al.* (2005) reported that the variation in gas production between the forages species can be attributed to compositional differences of forages, especially CP and fibre and may be other anti-nutritional components. These factors influence the amount of substrate OM that is fermented and the Short Chain Fatty Acids (SCFAs) produced upon fermentation.

Gas production coefficients: The results of Analysis of Variance (ANOVA) for kinetic coefficients extracted from gas production showed that there was significant difference ($p<0.01$) between forage legumes across sites and interaction site-forage species (Table 3). The gas production coefficients (Table 4) showed the levels of quickly (a), slowly degradable (b), rate of degradation (c), Potentially fermentable (PD), Effective Degradation (ED) substrates and Rate of Retention Time (RRT) for forage species.

Table 4: Gas production coefficients

		Mean±SE					
Sites	Species	a	b	c	PD	ED	RRT
HA	*D. intortum*	-1.29	31.27±1.28	0.026	29.98±2.01	13.34±8.38	37.83±1.26
	M. sativa	-1.79	29.60±8.53	0.044	27.80±8.11	15.80±5.74	22.75±8.97
	M. pruriens	-2.18	32.98±1.89	0.030	30.80±1.61	14.43±6.51	32.86±1.15
	S. scabra	-2.18	40.84±7.74	0.029	38.66±1.70	17.79±7.30	34.82±1.18
MA	*D. intortum*	-1.44	33.57±1.87	0.037	32.13±1.33	16.78±1.28	28.05±1.05
	M. sativa	-1.87	40.77±1.87	0.047	38.90±1.25	22.97±1.14	21.36±8.37
	M. pruriens	-2.07	37.30±2.53	0.046	35.22±1.88	20.47±9.56	21.80±6.69
	S. scabra	-2.01	39.82±1.83	0.035	37.81±1.81	19.54±9.68	28.25±2.10

SE: Standard Error; a: Intercept; b: Slowly degradable portion; c: Rate of degradation of b; PD: Potential Degradable; ED: Effective Degradable; RRT: Rate of Retention Time

Table 5: Analysis of variance for organic matter digestibility

Source of variation	df	SS	MS	Pr>F
Location	1	0.097	0.097	<0.0001
species	3	32.530	10.840	<0.0001
Location*species	3	3.180	1.060	<0.0001

DF: Degree of Freedom; SS: Sum of Square; MS: Mean Square; Pr: Probability; F: Fischer

Table 6: Kinetic coefficients for OMD from different sites

		Mean±SE				
Sites	Species	a	b	c	PD	ED
HA	*D. intortum*	15.27±0.34	27.90±1.75	0.027	43.18±2.06	28.32±0.66
	M. sativa	14.90±1.02	26.60±8.19	0.043	41.50±7.16	30.49±4.02
	M. pruriens	14.44±0.57	29.79±3.02	0.030	44.23±2.49	29.17±0.53
	S. scabra	14.41±0.58	36.36±2.72	0.029	50.79±2.20	32.05±0.20
MA	*D. intortum*	15.03±0.38	30.17±4.21	0.037	45.20±3.85	31.24±0.57
	M. sativa	14.76±0.29	36.28±1.82	0.047	51.05±1.57	36.87±0.53
	M. pruriens	14.52±0.36	33.51±3.10	0.046	48.03±2.89	34.56±0.88
	S. scabra	14.60±0.30	35.66±3.70	0.036	50.27±3.74	33.75±0.89

SE: Standard Error; a: Intercept; b: Slowly degradable portion; c: Rate of degradation of b; PD: Potential Degradable; ED: Effective Degradabl

It was found that *in vitro* gas production of selected forage legumes from middle altitude have higher gas production than those of high altitude. Considerin the degradable portion of forage legumes it was found that forage from middle altitude had more degradable portion except *S. scabra* of high altitude which obtained higher PD than that of middle altitude, however required more time to be degraded (34.82 h).

Organic Matter Digestibility (OMD): The results of ANOVA for kinetic coefficients extracted from organic matter digestibility showed that there was significant difference ($p<0.01$) between forage legumes across sites and interaction site-forage species (Table 5).

The evaluation of ecological environment of forage quality showed that the indicative of better quality forages from warmer environment was higher than that of cool environment. This is due to potential degradability and effective degradability of forage legumes across the two sites within 72 h (Table 6). The results showed that *D. intortum* from both high and middle altitude started their digestibility quickly before they were put in the incubation while others went slowly. *Stylosanthes scabra* from high altitude and *M. sativa* from middle altitude were more slowly degraded than others from the same site but the rate of degradation showed that *M. sativa* from both high and middle altitude have high rate than others from the same site *S. scabra* from high altitude and *M. sativa* from middle altitude have high potential than others from the same site finally the effective degradable showed that forages from middle altitude were higher than those from high altitude.

The relationship between organic matter digestibilities varies with type of feed. Nevertheless, it has been adopted as a rapid method feed evaluation (Sallam, 2005) with sufficient sensitivity to predict intake (Santos *et al*., 2010).

CONCLUSION

Literature is awash with information in nutrient compositions of tropical feeds. But little effort has been made to estimate energy values and degradation kinetics. The results from this study used rumen liquor to compare quality of forage legumes from middle and high altitudes of Rwanda. Basing on the results of laboratory analysis, there was significant variation in nutrient composition across the two agro-ecological zones due to the

adaptability of each forage species. In addition, the results of gas volume produced within 24 h showed that forage legumes from middle altitude produced more gas volume than those from high altitude. This might due to the higher temperature (22°C) found in middle altitude than high altitude (20°C) because the high temperature increases the DMD of the forage due to increase of energy in the forage. This study is a first attempt to compare nutritive values of forage species from different environments of Rwanda by using *in vitro* gas production. The evaluation of forage legumes from high altitude and middle altitude, especially potential degradable portion showed that the indicative of better quality feed is likely to be forage legumes from middle altitude zone.

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