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Rumen Liquor from Slaughtered Cattle as a Source of Inoculum for *in vitro* Gas Production Technique in Forage Evaluation

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Abstract: The two experiments were conducted with the overall objective of adapting Holhiem Gas System (HGS) to working conditions in the East and Central Africa (ECA) Region. The first experiment employed rumen contents from abattoir as source of rumen fluids and inoculum for feed evaluation. The second trial determined fermentation kinetics and Metabolisable Energy (ME) contents of seven forage species/accessions under preliminary trials at the Rwanda Agricultural Board (RAB), Karama Research Station. Results showed that Organic Matter Digestibility (OMD) and ME contents derived from gas production technique (GME) were highly correlated with Tilley and Terry *In Vitro* Organic Matter Digestibility (IVOMD; $R^2 = 0.73$) and ME derived from IVOMD (TME) ($R^2 = 0.92$). The obtained values were used to estimate ME and kinetic coefficients of 17 improved forages available in Rwanda as a contribution to the on-going exercise of developing the ECA feed database and national forage germplasm development. Using cluster analysis 14 promising forage accessions were identified for next phase of evaluation in other agro-ecologies across the country.

Key words: East and Central Africa, feed database, rumen fluid, fermentation kinetics, metabolisable energy, organic matter digestibility

INTRODUCTION

Limited capacity to produce adequate quantities of quality feeds at the right time and their rational utilization are considered to be some of the reasons for low livestock productivity in developing countries. Rational use of feed resources requires information on nutrients contents of the feeds, their availability in the digestive tract and efficiency of metabolising the absorbed nutrients for maintenance and production. Considerable amount of work has been done to determine feed quality for ruminant livestock in developing countries. But comprehensive databases are lacking, especially in Sub-Saharan Africa (SSA) because the pieces of information are scattered and often incomplete. For example, in the feed database for SSA (CGIAR SLP, 2011), <25% of the entries have information on digestibility, Metabolisable Energy (ME). Information on digestion kinetics is not available. Yet these are important indicators of animal response and performance, especially when simulation models are used to derive best-bet options for rational feed production utilization (Tibayungwa *et al*., 2011). Using live animals to determine ME and digestion kinetics is prohibitively costly in terms of resources (animals, infrastructures, equipment and labour) for feeding, data collection and laboratory analysis of feeds, faeces and urine. *In vitro* systems where anaerobic fermentation is followed by removal of microbial biomass using enzymes (Tilley and Terry, 1963) or neutral detergent solution (Goering and van Soest, 1970) is also criticized for being laborious and unnecessarily costly for estimating ME and the kinetics of feed degradation. The problem is compounded if rumen fluid from fistulated animals is used because such animals are costly to acquire and maintain in poorly developed countries like those in SSA (Jones and Barnes, 1996). Therefore, forage germplasm evaluation systems in developing countries are often limited to agronomic data and nutrient contents as major criteria for selection. These problems can be overcome by adopting the gas production technique which has been extensively validated and used (Blummel and Orskov, 1993), as well as automated to reduce the tedium of data collection (Pellikaan *et al*., 2011). Adapting the use of rumen liquor from slaughtered animals as inoculum sources negates the cost of using surgically prepared animals and the need for compliance to animal welfare laws (Mohamed and Chaudhry, 2008). However, this will require the capacity for effectively ensuring that anaerobic conditions are maintained up to the time of incubation. Conventionally, this is done using continuous flux of CO_2 which is an

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additional cost that can discourage adoption of *in vitro* gas systems in developing countries. The objectives of this study, therefore is to validate the use of rumen liquor from slaughtered cattle as a source of inoculum for *in vitro* gas production and feed evaluation in the absence of CO_2 supply and justify the use of the protocol as a tool in forage germplasm evaluation and feed database development in developing countries.

MATERIALS AND METHODS

Study area: The study was conducted at the Karama Research Station of the Eastern Zone Division of the Rwanda Agriculture Board (RAB). The station is located at 30°25' East, 2°30' South at an altitude of 1400 m a.s.l (Munyemana, 2001). According to the Koppen classification, the climate of Bugesera is of the Aw type 3-4 with the average temperature of the coldest month being higher than 18°C and the duration of the dry season ranging from 3-4 months (Munyemana, 2001). The rainfall is frequently <1000 mm with an average of 950 mm year^{-1} while the daily temperatures generally vary between 15 and 28°C with an average of 21.5°C (Rwicaninyoni, 1987). The average relative sunshine for 18 years is 51.5%, the relative humidity of the air is approximately 73.5% and the mean velocity of the wind is of 3.68 km h^{-1}. The vegetation is predominantly dense formations of xerophilous thickets; consisting of a hundred species dominated by *Carissa* sp., *Haplocoelum* sp., *Olea* sp. and small lawns of graminaceous species (especially *Brachiaria* sp.) which grow between the thickets. These thickets characterize the local pastures (Munyemana, 2001). RAB uses the station for evaluated indigenous and introduced accessions of forages for low rainfall and drought tolerance.

Forage samples: Forage samples were taken from a germplasm collection of 27 species/accessions (Table 1). Samples from herbaceous species were standardization cuts at 120 days after planting. Fresh herbage from 1 m^2 quadrant was weighed for yield determination (not reported here). Samples from browse species were fresh twigs (leaves, petioles and succulent stems) from third cuts of the crop after 2 years of establishment. Subsamples were taken and air dried under shade for 7 days followed by oven drying at 60°C for 48 h. Air drying under shade was adopted as a convenient way of handling forage samples at locations that are distant from the laboratories and inadequate infrastructure (electricity and ovens) for drying samples immediately after harvests (Coppock and Reed, 1992). Dried subsamples were ground to pass 2 mm screen and stored for subsequent analysis.

Chemical analysis and *in vitro* digestibility: Proximate analysis of the samples was done at Sokoine University of Agriculture (SUA). Crude ash was determined as the weight of residual material after incinerating 1 g of sample at 550±20°C for 24 h expressed as a proportion (g kg^{-1} DM) of the original sample. Dry matter was estimated at 105°C. Crude protein was estimated as a factor of 6.25 of the nitrogen content in sample. The nitrogen content was determined using macro Kjeldahl Method by digesting 0.5 g of sample in concentrated sulphuric acid (12N H_2SO_4) in a heating block at 450°C for 1 h using selenium catalyst (AOAC, 2001). Ammonia from the digesta was recovered using alkaline solution (40% NaOH; w/v) followed by steam distillation into 25-30 mL of boric acid solution (10 g L^{-1}; w/v) using a fully automated system (Kjeldahl Analyzer; Pro Nitro, Model = Pro Nitro III). The ammonia concentration was determined by titration using a standard acid solution (0.1 M HCl). Crude fat was determined by petroleum ether extraction method as adapted in the SUA laboratory practice. The samples (3 g) were weighed into labelled thimbles. Extraction cups of known weight after drying at 105°C for 24 h were filled with 40-50 mL of petroleum ether and attached to the Soxhlet extractor. Extraction was initiated by immersing the thimble in boiling solvent for 15 min. This was followed draining the solvent in into extraction cup and refluxing the solvent through the thimble of 30-45 min. The solvent in the extraction cup was evaporated for about 20 min. The cup with the fatty residue was placed in a desiccators overnight and weighed next day. Crude fat content was computed as the difference between the weight of the cup containing the ether extract and the empty weight of the same cup, expressed as the proportion of the original dry weight of the sample. Forages were also analyzed at SUA for cell wall constituents (NDF and ADF) according to Goering and van Soest (1970). *In vitro* digestibility was determined according to Tilley and Terry (1963) technique.

Gas production and fermentation kinetics: Gas production tests were conducted at Rubona Research Station (Rwanda) in two sets of trials. The first set of trial examined the gas production characteristics in 7 out of 27 forage species that were analysed at SUA (Table 1): *Flemingia macrophylla*, *Desmodium distortum*, *Brachiaria* hybrid cultivar (cv.) Mulato II, *Desmanthus virgatus*, *Leucaena diversifolia* and *Clitoria ternatea* as proof of concept that fermentation kinetics could be reproduced using rumen liquor from slaughtered cattle in absence of CO_2 flux. Three sessions of incubation were conducted. In each session triplicates samples of each

Table 1: Chemical composition, *in vitro* dry matter and organic matter digestibility (g kg^{-1} DM) of forages under preliminary yield trial in Karama, Rwanda

			Feed components and digestibility values (g kg^{-1})						
Forage species	Common names	Accession No.	DM	CP	IVDMD	IVOMD	Ash	NDF	ADF
Cenchrus ciliaris	African foxtail grass	African foxtail grass	927.5	61.70	41.14	394.90	146.7	711.7	389.3
Pennisetum purpureum	Napier/Elephant grass	Napier/Elephant grass	926.1	48.80	462.00	454.40	106.6	738.1	386.2
Panicum coloratum	Bambatsi panic	-	934.1	59.10	439.20	413.70	90.8	709.3	358.7
Chloris gayana	Rhodes grass	-	931.0	48.10	415.70	399.00	103.3	763.0	409.4
Lablab purpureus	Dolichos lablab	-	939.7	186.80	554.50	541.40	102.3	498.1	300.6
Canavalia brasiliensis	Brazilian jackbean	CIAT 19038	929.9	159.40	556.70	532.50	128.0	480.3	272.9
Centrosema molle	Centro	CIAT 15160	948.8	139.00	438.70	409.40	109.2	614.0	348.0
Chamaecrista rotundifolia	Round leaf cassia	-	936.5	126.10	530.10	515.30	76.2	555.8	35844.0
Clitoria ternatea	Butterfly-pea	CIAT 20692	942.7	171.30	532.50	518.10	76.4	566.2	327.8
Cratylia argentea	**	CIAT 18516	928.0	179.40	439.80	421.50	146.0	558.3	299.3
Desmanthus virgatus	Dwarf koa	-	933.9	143.50	324.90	327.10	79.7	585.0	380.8
Desmodium distortum	**	-	922.5	136.60	528.30	50.17	113.9	457.8	295.5
Desmodium intortum	Greenleaf desmodium	-	937.9	153.20	442.20	416.70	97.0	594.8	381.7
Desmodium tortusum	**	ILRI 174	931.0	244.10	649.60	644.30	71.4	416.1	22611.0
Desmodium uncinatum	Silverleaf desmodium	-	925.4	190.10	357.30	336.30	114.4	728.4	307.7
Desmodium vetulinum	**	CIAT 33443	927.6	1494.00	513.30	492.20	80.6	478.4	264.2
Flemingia macrophylla	Apa apa	CIAT 20618	935.4	130.40	271.90	255.70	57.6	714.1	390.4
Gliricidia sepium	Gliricidia	ILRI 14504	935.9	191.80	535.20	505.20	127.6	413.4	258.2
Leucaena diversifolia	Diversifolia	-	938.3	227.00	35.85	398.10	76.4	478.4	264.2
Leucaena pallida	Guaja	ILRI 14203	930.6	170.70	370.70	374.20	54.6	624.1	356.4
Macroptilium atropurpureum	Siratro	-	926.6	187.90	465.50	464.30	110.5	511.8	278.9
Macrotyloma axillare	Acher axillaris	-	925.4	171.20	557.30	542.50	86.8	463.1	286.0
Medicago sativa	Alfalfa	-	931.5	15.06	530.00	499.40	95.0	516.8	329.8
Mucuna pruriens	Mucuna	-	926.9	197.80	498.70	495.80	71.1	588.3	328.1
Stylosanthes guianensis	Stylo	-	933.6	151.70	537.40	518.70	84.8	655.9	344.4
Stylosanthes scabra	Shrubby stylo	-	936.3	135.40	475.70	453.00	71.0	624.7	410.6

DM = Dry Matter; CP = Crude Protein; IVDMD = *In Vitro* Dry Matter Digestibility; IVOMD = *In Vitro* Organic Matter Digestibility; NDF = Neutral Detergent Fibre; ADF = Acidic Detergent Fibre; **Common name not found

specie/accession were incubated in rumen fluid from one animal. Thus, each specie/accession was incubated with rumen liquor from three animals. The second set of trials involved incubation of 17 other forages in triplicates in a sequence of three batches. In all cases, the feed samples each weighing 200 mg were incubated in airtight graduated gas syringes (100 mL) in a fermentation media that consisted of micro-mineral, two buffer solutions B and C and rumen liquor (Osuji *et al*., 1993). Rumen liquor was obtained from fresh rumen contents which were taken from slaughtered cattle immediately after evisceration and incision of the rumen. The material was delivered to the laboratory within 1 h in a warm vacuum flask. The rumen content was macerated in fruit blender purchased from local supermarket (Model No: RM/161; Capacity 1.7 L with stainless steel blades). The resultant slurry was squeezed from the fibrous mass into a spoutless plastic beaker (250 mL) through three layers of nylon cloth. In the absences of CO_2 flux, exposure to oxygen was minimised as follows: The vacuum flask was filled with boiled water and capped tightly until the time for collecting rumen contents at the slaughter house. The water was quickly replaced with rumen content immediately after the rumen was incised. The cap of the vacuum flask was screwed tightly, leaving no head space and avoiding leakage during the enter period of delivery to the laboratory. At the laboratory, the nylon cloth was spread loosely across the mouth of the beaker. The rumen contents were quickly emptied onto the loose nylon cloth, causing it to sag quickly into the beaker and in the process expelling air from the beaker by physical displacement. The contents were then squeezed, making sure that at least two thirds of the cloth was immersed in the fluid. Aliquots (20 mL) of mixed buffer solution 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. Then, aliquot of rumen liquor (10 mL) was introduced into the same syringe to constitute the incubation medium of the required ratio of buffer solution to rumen fluid (2:1 v/v). The two blank syringes contained incubation media without feed samples. All the syringes were incubated in water bath at 39°C for 48 h. When space was limited in the water-bath, some of the syringes were placed in oven (Model 600 memmert) set at the same temperature. Readings of gas volumes were taken at convenient time intervals with more frequency readings (1-2 h intervals) during the first 12-24 h of incubation. Thereafter the frequency was progressively longer (3-6 h intervals).

Data analysis: Cumulative gas volumes were computed for each tube as the difference between the readings at time (t_i) and the initial reading (t_0), adjusted for control

readings (blank) at corresponding recording times. The fermentation kinetic coefficients were computed from nonlinear regression model:

$$Y_{mg} = a_{mg} + b_{mg}\left(1 - e^{-ct}\right)$$

Where:

y_{mg} = Gas volume at time (t)
a_{mg} = Gas volume from rapidly degradable fraction of feed (intercept)
b_{mg} = Gas volume from slowly but degradable fraction of feed
c = Rate constant for fermentation (Orskov and McDonald 1979)

The model was run using NEWAY1. SAS model obtained from the International Livestock Research Institute (ILRI) which also estimated asymptotic gas production as proxy indicators for organic matter degradability. Organic Matter Degradability (OMD_{GP}) was derived from the gas fermentation kinetics using the equation:

$$OMD_{GP} = 14.88 + 0.889V_{24} + 0.45CP + 0.0651\,\text{Ash}\left(\text{Menke}\,et\,al.,1979\right)$$

Where:

OMD = Organic Matter Digestibility
V_{24} = Gas volume (mL) at 24 h
CP = Crude Protein (% DM)

Metabolisable energy (ME_{IVOMD}, MJ kg^{-1} DM) values were estimated from *In Vitro* Digestibility (IVOMD) from SUA laboratory results using equations:

$$ME_{IVOMD} = 0.81 \times 18.4\%\ \text{IVOMD}\left(\text{ARC},1980\right)$$

Metabolisable energy from *in vitro* gas kinetic (ME_{GP}, MJ kg^{-1} DM) was computed as:

$$ME_{GP} = 2.2 + 0.136V_g + 0.057CP + 0.0029CP^2$$
$$\left(\text{Menke}\,et\,al.,1979\right)$$

Statistical analysis: Differences in kinetic coefficients were examined Linear Model for complete randomized design:

$$Y_{ijk} = \mu + T_i + B_k\varepsilon_{ijk}$$

Where:

Y_{ij} = The jth volume reading of the ith forage sample of the kth batch of incubation
μ = The population mean
T_i = The mean for the ith forage
ε_{ij} = Random error

The relationships between OMD_{GP} and IVOMD and the relationship between ME_{IVOMD} and ME_{GP} were examined using linear regression analysis: y = a+bx, where y and x are dependent and independent variables, respectively. OMD_{GP} and ME_{GP} were the dependent variables (y) and IVOMD and ME_{IVOMD} were independent variables (x) in the respective equations. Cluster centres for fermentation kinetics (A-C) and for ME were generated using SPSS 16.0 in order to group the forages into feed groups to facilitate selection.

RESULTS AND DISCUSSION

Chemical composition: Laboratory analysis showed that DM of samples did not differ significantly because they were prepared according to standard procedures for analysis. As expected average CP contents were higher in legumes (158.2 g kg^{-1} DM) than in grasses (54.4 g kg^{-1} DM; $p<0.01$). The minimum and the maximum CP contents were registered in *Chloris gayana* and *Desmodium tortuosum*, respectively (Table 1). Among the grasses, *Cenchrus ciliaris* had the highest CP content. The values for *Chloris gayana* and *Pennisetum purpureum* were similar. Among the legumes, CP contents were lower than average in five species, especially *Chamaecrista rotundifolia* and *Flemingia macrophylla*. Mean fibre (NDF and ADF) contents were higher in grasses than in legumes except in *Flemingia macrophylla* and *Desmodium uncinatum* where the fibre contents were similar to the contents in grasses. IVOMD estimates were higher in legumes than in the grasses. NDF contents were negatively correlated with CP and IVOMD ($p<0.01$). The correlation between protein and IVOMD, though positive was not significant ($p>0.05$) (Table 2).

Fermentation kinetics of forages: The amount of gas produced during the 44 h of fermentation (Fig. 1) showed *Desmodium distortum*, *Brachiaria* hybrid (cv. Mulato II) and *Desmanthus virgatus* to be the top three easily fermentable forages by rumen microorganisms. *Leucaena*

Table 2: Pearson correlations of chemical composition and *In Vitro* Organic Matter Digestibility (IVOMD) of selected forage species

	Chemical compounts					
Samples	DM	CP	IVOMD	Ash	NDF	ADF
No. of entries	26	26	26	26		
DM	1					
CP	0.086	1				
IVOMD	-0.072	0.340	1			
Ash	-0.241	-0.205	0.036	1		
NDF	0.000	-0.698**	-0.657**	-0.012	1	
ADF	0.093	0.116	0.378	-0.244	-0.205	1

DM = Dry Matter; CP = Crude Protein; IVOMD = *In Vitro* Organic Matter Digestibility; ash = Ash; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; **High correlation

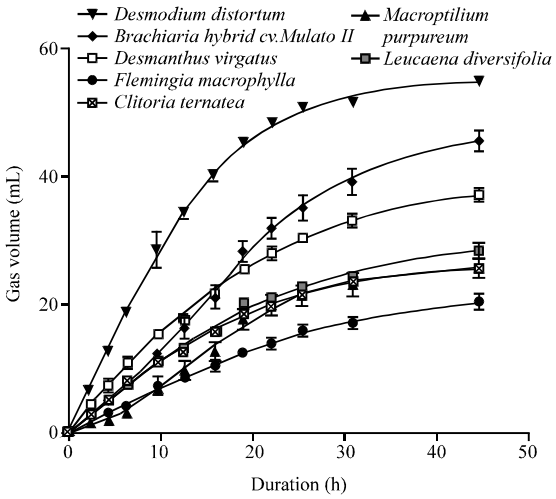

Fig. 1: *In vitro* gas production kinetics of selected forage species when incubated in rumen fluid

Table 3: Gas kinetic parameters ($y = A+B\ (1-e^{Ct})$) of tested forage species

Species	A	B	C	A+B
Desmodium distortum	4.4^{c}	526.0^{a}	0.0262^{c}	530.2^{a}
Clitoria ternatea	-6.9^{e}	171.0^{e}	0.0492^{b}	164.5^{c}
Macroptilium atropurpureum	-14.9^{b}	182.5^{c}	0.0409^{b}	167.5^{c}
Leucaena leucocephala	16.7^{d}	182.5^{c}	0.0402^{b}	165.5^{c}
Desmanthus virgatus	-18.6^{d}	308.0^{b}	0.0788^{a}	289.5^{b}
Brachiaria hybrid Mulato II	-13.9^{b}	353.5^{b}	0.0291^{c}	339.5^{b}
Flemingia macrophylla	-0.1^{a}	122.5^{d}	0.0495^{b}	147.4^{d}

A = Gas volume from rapidly degradable fraction of feed (intercept); B = Gas volume from slowly degradable fraction of feed; C = Rate constant for fermentation; Means in the column followed by the same superscript letter (a-d) are not significantly different ($p>0.05$)

leucocephala; *Clitoria ternatea* and *Macroptilium atropurpureum* constituted the medium cluster and did not differ significantly from one another ($p>0.05$). The fermentation kinetic coefficients associated with the cumulative gas production are shown in Table 3.

Negative values were recorded in all forages except *Flemingia macrophylla* but all the intercepts were not significantly different from zero ($p>0.05$). The segment of the curve associated with slowly degradable fractions (B) of the substrates was lowest in the *Flemingia macrophylla* and highest with *Desmodium distortum*. This coefficient was intermediate in the rest of the species in the experiment. The fermentation rate coefficient was highest in *Desmodium distortum*; least in *Flemingia macrophylla* and *Desmanthus virgatus* and intermediate in the rest of the species. The combined effects of slowly fermentable fractions and rates of fermentation were reflected in the potentially degradability (A+B). The OMD_{GP} was highly correlated with IVOMD ($R^2 = 0.7295$; $p<0.001$). The regression slope was approximately 15%, <1 (Fig. 2). Similarly ME_{GP} was highly correlated with ME_{IVOMD} ($R^2 = 0.9192$; $p<0.001$) but with the slope of

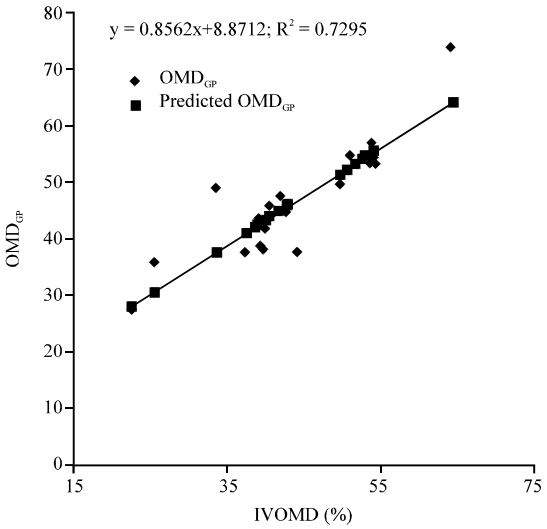

Fig. 2: Linear relationship between *In Vitro* Organic Matter Digestibility (%IVOMD) and organic matter digestibility estimates from Menke gas production (OMD_{GP})

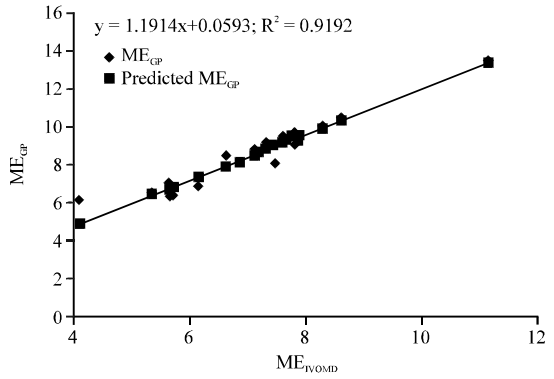

Fig. 3: Linear relationship between ME estimated from *in vitro* digestibility (ME_{IVOMD}) and ME by Menke gas systems (ME_{GP})

regression approximately 19% more than expected with perfect correlation of one (Fig. 3). Cluster analysis grouped the forages into 4 clusters (Table 4). The 1st cluster has the lowest ME contents. Fractional rates of fermentation (C) and the asymptotic gas volume (A+B) were also low. Category 2 had moderate ME content but very low fractional rates of fermentation. The potential fermentable fraction was very high. Only one fodder was placed into this category. The 3rd cluster had the highest ME content as well as moderately fractional rate of fermentation and potentially fermentable substrate. Cluster 4 had moderately high ME values; very high fractional rate of fermentation but low potentially fermentable substrates.

Table 4: Feed clusters of selected forages according to their *in-vitro* gas fermentation kinetics and metabolisable energy estimates

	Cluster centres					Fermentation kinetic parameters			
Cluster No.	A	B	C	ME	Forage species	A	B	C	ME
1	17.2	54.1	0.0194	5.6	*Cenchrus ciliaris*	15.8	59.4	0.02080	5.3
					Chloris gayana	16.0	58.7	0.01680	5.2
					Flemingia macrophylla	20.7	29.1	0.02760	5.6
					Pennisetum purpureum	15.6	35.9	0.06290	5.2
2	20.2	20.2	0.0028	6.8	*Stylosanthes scabra*	20.2	202.0	0.00285	6.8
3	22.9	65.8	0.0335	9.3	*Canavalia brasiliensis*	21.8	63.0	0.05650	7.6
					Desmodium tortuosum	24.0	68.6	0.01050	11.0
					Chamaecrista rotundifolia	22.0	37.6	0.05730	7.5
4	21.9	34.8	0.051	7.1	*Desmodium intortum*	21.9	32.5	0.05150	7.2
					Desmodium uncinatum	23.4	37.0	0.03900	7.5
					Desmodium velutinum	22.2	36.0	0.05040	7.1
					Gliricidia sepium	23.4	33.7	0.06940	8.3
					Lablab purpureus	23.1	34.9	0.06290	7.8
					Leucaena diversifolia	24.1	31.1	0.04120	7.2
					Leucaena pallida	20.6	46.9	0.01340	6.0
					Macrotyloma axillare	22.6	39.8	0.04770	7.8
					Panicum coloratum	16.2	51.3	0.02640	5.7

A = Gas volume from rapidly degradable fraction of feed (intercept); B = Gas volume from slowly degradable fraction of feed; C = Rate constant for fermentation; ME = Metabolisable Energy

In a conventional breeding program, clusters 1 and 2 are candidates for rejecting in preliminary yield trials because of low energy and rates of fermentation which can adversely affect intake. Cluster 3 and 4 can be considered for advanced yield trial because of indicatively high rates of fermentation; short retention time in the rumen and hence high intake. Despite differences among forages the contents of the chemical constituent were within ranges of values reported in tropical forages (Melaku, 2004). The differences most likely reflected differences associated with plant phenology and their effects on the composition and digestibility of organic components of the feed, especially protein and cell wall components. The CP contents in grasses were suboptimal for normal rumen function (Satter and Roffler, 1975). Pearson correlation confirmed that CP and NDF contents were negatively correlated (R^2 = 0.698, $p<0.01$). NDF contents were in turn negatively correlated with IVOMD (R^2 = -0.657; $p<0.01$). Nevertheless, NDF content accounted for only 43.1% of the differences in IVOMD in the forages. *Flemingia macrophylla* was least readily fermentable forage. This could be attributed to tannin contents in the *Flemingia foliage* that reduces degradability (Mui *et al*., 2001).

In addition to economic and logistical reasons, animal welfare reinforces that justification of using slaughter cattle as sources of rumen fluid in *in vitro* feed evaluation (Mohamed and Chaudhry, 2008). This trial went further to eliminate CO_2 which other researchers used. The gas production profile (Fig. 1) depicted a typical pattern that is obtained from gas syringes (Menke *et al*., 1979). The rates of gas production (mL g^{-1}) were within reported ranges (Huang *et al*., 2011). The fermentation kinetic coefficients associated with the cumulative gas production had negative values for the readily available component (A) in all forages except in *Flemingia macrophylla*. However, all the intercepts were not significantly different from zero ($p>0.05$) (Table 3). The negative intercepts can be misleading because they could reflect lag time associated with microbial attachment to the particulate material as indicated in the review by Craig *et al.* (1986). The intercepts can also reflect model misfits to the data generated (Castro *et al*., 2003).

The segment of the curve associated with slowly degradable fractions (B) of the substrates was lowest in the *Flemingia macrophylla* and highest with *Desmodium distortum*. Except for *Desmodium distortum*, the asymptotic gas volume was within the range of values reported from incubations using rumen liquor from fistulated animals (Rymer *et al*., 2005). The high asymptotic gas value of *Desmodium distortum* could be associated with microbial lyses during the advanced phases of fermentation hence the need to relate the incubation time with the partitioning factor.

The IVOMD accounted for 78% of the variations of OMD_{GP}. Other studies using the two-stage *in vitro* method obtained similar results (Castro *et al*., 2003). The slope of regression suggested that %IVOMD overestimated true digestibility by approximately 15% and this could arise from predictions based on soluble but unfermentable materials, such as soluble phenolics. The relationship between ME_{IVOMD} and ME_{GP} suggested that estimates of ME from gas production were higher than ME estimates from conventional *in vitro* digestibility measurements. This is contrary to results from other studies. Using 24 h incubation period Zhou *et al*. (2011) reported 12-56% lower ME estimates from gas production than ME derived from IVOMD of tropical forages of

similar characteristics to those used in this study. The inference is that less organic matter actually fermented was producing more metabolisable energy than the energy derived from the same amount of organic matter using conventional *in vitro* method of Tilley and Terry (1963). This phenomenon can be partially explained low efficiency in the *in vitro* system, resulting into more gas and less microbial biomass for the same amount of organic matter fermented (Blummel *et al*., 1997). Estimates of Partitioning Factor (PF) are lacking to confirm this inference. It can also reflect uncoupled fermentation that is associated with substrate exhaustion after extended periods of fermentation. Therefore, the durations of incubation are important to ensure that measurements are taken during the growth phase of microbial activity. Blummel *et al*. (2005) used the estimates of the half time ($t_{1/2}$) of asymptotic gas volume because the coefficient was highly correlated PF and efficiency of microbial production *in vivo*.

Metabolisable energy estimates in this study were also lower than published estimates in sub-Saharan Africa feed database. However in literature, it is possible to find ME estimates that fall within the range depicted in Table 3 for tropical forages (Foster and Bright, 1983). According to Rymer *et al*. (2005), getting similar results from gas production within one laboratory is more probable than getting the same results across different laboratories. But, *in vitro* gas production is likely to rank the feeds in the same order across different laboratories. Researchers would therefore, expect rumen fluids from different slaughter animals and days of slaughter but the ranking to be the same across days. The day to day discrepancies could be reduced by using reference samples of known ME contents and gas production values as gold standards in order to compare inter-laboratory results and readings from different days that slaughtered cattle provided rumen liquor. The gold standard requires feeding trials as next steps for improvement and scaling out the technique.

Researchers in sub-Saharan Africa rely predominantly on agronomic information and chemical composition in screening forage germplasm for productivity and nutritional values. Because of the relationship with intake (Blummel *et al*., 2005); gas production kinetics provides additional parameters that can enrich the selection criteria of forage germplasm. In this context, fermentation kinetics and estimates of ME suggests that cluster 3 are the most promising forages because they have the highest ME (9.3 MJ kg^{-1} DM), potential production rates (A+B: 440 mL g^{-1} of substrate) accompanied by a short half-life in the rumen ($t_{1/2}$ = 20.7 h). Cluster 2, rank second to cluster 3 because of they have the shortest half-life in the rumen ($t_{½}$ = 13.5 h) and ME (7.1 MJ kg^{-1}). The biological significance and ranking of the feeds would be improved if PF values were also estimated (Blummel *et al*., 2005).

CONCLUSION

From the gas production curve that was generated researchers conclude that rumen fluid from slaughtered cattle can be used for feed evaluation using *in vitro* gas production technique; even in absence of CO_2 delivery system. Since, this source of liquor is easily accessible, forage germplasm improvement programs in developing countries can adopt *in vitro* gas techniques in routine forage evaluation. Considerable variation in gas kinetic coefficient is likely to occur. But, the ranking of forages are likely to be similar when sources of rumen contents were different animals from different days of slaughter. Therefore, the technique can be used to identify a few promising species/accessions for advance yield and feeding trials using management numbers of animals. Gas kinetics per se is not adequate to make conclusive inference in the nutritional value of the feeds. Therefore, the evaluation protocol should include estimates of PF ratio in the forage evaluation protocol. There is also need to develop gold standards to facilitate cross-referencing results from different sources of inoculums and laboratories using *in vivo* calibrations.

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