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Effect of Maturity and Fertilizer Application on *in vitro* Gas Production Characteristics of *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* Roth

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Abstract: An *In vitro* gas production study was conducted to determine the effect of age and soil treatments on fermentation characteristics of *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* Roth. The soil treatments were: T_1-not fertilized and T_2 received DAP fertilizer. Forage samples used were obtained from herbage materials harvested at 6, 8, 10, 12 and 14 weeks of growth. Dry samples (65°C for 24 h, 200 mg DM) of the harvested materials, were incubated in glass syringes with rumen fluid obtained from 2 Friesian steers fitted with permanent fistula. The gas volumes were recorded at 0, 4, 6, 12, 24, 36, 48, 72, 96 and 120 h and the data fitted to the model $P = a + b\,(1-e^{-ct})$ after correction with blanks using *in vitro* gas fit-curve program. Forage chemical analyses were done according to standard procedures. The resulting data was analyzed using SAS. Results showed that nutrient composition of this forage, varied with both age at harvest and treatment. The overall means of the fermentation fractions (b) and (a + b) in T_2 were higher than in T_1 ($p<0.01$) across the 3 forages. Strong correlation was observed between chemical composition and gas production. Pattern of gas production was strongly influenced by both treatment and age at harvest. It was therefore, concluded that, age at harvest, negatively affected rumen fermentation of these forages. Application of fertilizer improved *in vitro* gas production, suggesting enhanced fermentation.

Key words: *In vitro* gas production, forage, smallholder farms, feed quality

INTRODUCTION

In most tropical countries, tropical grasses, legumes and crop residues constitute the bulk of ruminant livestock diets. In Kenya, grasses such as *Pennisetum purpureum*, *Chloris gayana* and the natural species (such as *Themeda triandra*, *Cynodon dactylon*) are the sources of nourishment for ruminant livestock on smallholder farms during the wet season (Leng, 1989). During the dry season, crop residues assume a greater role as ruminant livestock feed resources on these farms (Lanyasunya *et al*., 2006c). These feed resources are characterized by low crude protein, high fibre content, low mineral content and low digestibility (Preston, 1995), which in turn adversely affect feed intake and therefore, performance of ruminant livestock. Supplementation of these feed resources with high protein legume forages has been advocated, as the most economically feasible option for improving their quality and therefore, animal performance on smallholder farms (Muinga *et al*., 1995). This cannot be done properly and in a cost effective manner, without sufficient understanding of the nutrient profiles of these feed resources and elucidation of those factors perceived to have considerable effect on their overall value as ruminant feeds (Close and Menke, 1986). It is this information gap that is presently being seen as the major draw back to efficient utilization of the many available feed resources on smallholder farms in Kenya (Lanyasunya *et al*., 2005). For instance, *Sorghum almum*, though known elsewhere for being a high biomass yielder with a potential for contributing to the alleviation of feed short on resource-poor farms (Kallah *et al*., 2000) is not well utilized in Kenya, primarily due to lack of information, essential for efficient utilization. The same was true for many potential tropical forages including *Commelina* sp. and *Vicia* sp. To enhance utilization of these forages, generation of technical information, is essential. It was on this premise that the current study was undertaken to characterize their *in vitro* gas production as influenced by age at harvest and fertilizer application.

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MATERIALS AND METHODS

This study was conducted at the department of animal science of Egerton University in Njoro, Kenya. Samples used were collected from *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* herbage grown under 2 soil treatments and harvested at 6, 8, 10, 12 and 14 weeks of growth. The soil treatments were T_1-No fertilizer (Control) and T_2-with fertilizer (50 g (~ 125 kg ha^{-1}) Di-ammonium phosphate), drilled with the seed during planting. Composite samples were collected at each harvest per plot for chemical analysis and dry matter determination (AOAC, 1990). *In vitro* gas production samples used in this trial were oven-dried at 65°C for 24 h and milled to pass 1.0 mm sieve (Menke *et al.*, 1979). The study was conducted following the procedure described by Menke *et al.* (1979). Sources of rumen liquor were 2 Friesian steers aged 47 months fitted with permanent rumen fistula and fed maize silage and supplemented with Lucerne hay at the ratio of 3:1. They also had free access to clean drinking water and mineral supplement. Rumen liquor was obtained by suction tubing through the fistula, strained through 2 layers of cheese-cloth into a warm clean thermos flask flushed with CO_2. Medium solution and rumen liquor were then mixed at the ratio of 2:1 (v/v mL) in a water bath maintained at 39±0.2°C. After weighing in duplicates, exactly 200 mg of the milled material (for each forage) was placed into each of the syringes (model Fortuna häberle labortehnik, Germany) using a carrier boat to ensure that no feed particles attach to the walls. The syringe pistons were lubricated with Vaseline (pure petroleum jelly) to ease their movement and also to prevent gas escape. The syringes were warmed at 40°C for 1 h prior to the injection of 30 mL rumen liquor-medium mixture into each one of them. This was immediately followed by insertion of the pistons, careful expulsion of the air in the syringe, closure of capillary tubing and incubation in a water bath maintained at 39±0.2°C. The syringes were then gently shaken every 30 min for the first 1 h and once every 2 h for the next 120 h of incubation. Readings of the gas volumes were made at 0h (V_0) and thereafter at 4, 6, 12, 24, 36, 48, 72, 96 and 120 h. The total gas values were corrected for the blanks (without feed sample) and the standards. The obtained data was stored in MS-Excel and analyzed using SAS (2002). The mean gas volume readings were fitted to the exponential equation: $p = a + b (1-e^{-ct})$ proposed by Menke *et al.* (1979) and gas production fermentation fractions were determine using *in vitro* gas fit-curve (Chen, 1997). Correlations of gas production with advancing plant maturity and chemical composition were also investigated.

RESULTS

From the results, wide variations were observed in nutrient concentration change patterns between the forages over the study period. Results showed that concentration of fibre components increased, whereas those of Ether Extract (EE) and Crude Protein (CP) decreased with advancing maturity of the forage. Under T_1, NDF concentration in *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* increased by 33.1, 23.9 and 17.9% respectively between 6 and 14 weeks. Under T_2, 32.3, 17.9 and 19.2% increases in NDF concentration, were observed in the 3 forages, respectively over the same period. On the other hand, CP concentration in *Sorghum almum* and *Commelina benghalensis* declined by 66.7 and 48% respectively from 156.3 and 175 g kg^{-1} DM at 6 weeks to 52.1 and 91.5 g kg^{-1} DM at 14 weeks. Crude protein content in *Vicia villosa* varied marginally over the same period. A sharp decrease in EE concentration in the 3 forages was also observed across the 2 treatments over the study period. Results showed that, EE concentration in *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* declined by about 73.4, 48.4 and 69.1%, respectively between 6 and 14 weeks. Table 1 compares the fermentation parameters of *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* over 120 h incubation period as influenced by both age at harvest and fertilizer application. The mean values for the fermentation parameters of the forages under study were variable. Strong effect of age at harvest and fertilizer application on the fermentation characteristics of these forages was evident. In T_1, potential gas production of *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* declined by 6.25, 19.32 and 15.13%, respectively from 40.99, 37.38 and 41.50 mL 200 mg^{-1} DM at 6 weeks to 38.43, 30.16 and 35.22 mL 200 mg^{-1} DM at 14 weeks. A similar trend was observed in T_2, with *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* recording a marginal decline of 1, 15.12 and 14.83%, respectively in the proportion of (a + b) fraction between 6 and 14 weeks. Within each treatment, *Sorghum almum* maintained significantly ($p<0.01$) higher mean values of both b and (a + b) fractions over the study period, compared to *Commelina benghalensis* and *Vicia villosa* (Table 1). Those recorded by *Vicia villosa* were inconsistent and fell between those recorded by *Sorghum almum* and *Commelina benghalensis* ($p<0.05$). Between treatments, T_2 recorded significantly higher ($p<0.01$) volumes of both b and (a + b) fractions than T_1 for both *Sorghum almum* and *Commelina benghalensis*. Between 6 and 14 weeks, the 'b' fraction in T_2 ranged between 20.46-33.74% in *Sorghum almum*, 6.05-25.18% in *Commelina benghalensis* and 1.54-8.17% in *Vicia villosa* higher than those recorded in T_1.

Table 1: *In vitro* fermentation characteristics of *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa* Roth as influenced by age at harvest and treatment

Age		Without fertilizer				With fertilizer			
(Wks)	Fract*.	*S. alm*	*C. beng*	*V. villo.*	SEM	*S. alm*	*C. beng*	*V. villo.*	SEM
	a	-3.71^{b}	-2.43^{a}	-4.02^{b}	0.32	-5.11^{b}	-3.65^{a}	-3.24^{a}	0.28
6	b	44.8^{b}	39.8^{a}	45.50^{b}	2.11	55.2^{c}	42.21^{a}	46.20^{b}	2.25
	a + b	40.99^{b}	37.38^{a}	41.50^{b}	2.08	50.08^{c}	38.55^{a}	42.96^{b}	2.14
	c	0.048^{b}	0.043^{a}	0.059^{c}	0.001	0.049^{a}	0.046^{a}	0.055^{b}	0.003
	a	1.65^{a}	-1.71^{b}	2.15^{c}	0.26	0.255^{a}	-0.79^{b}	3.60^{c}	0.31
8	b	37.61^{b}	37.10^{b}	36.30^{a}	2.21	50.3^{c}	42.41^{b}	37.20^{a}	2.42
	a + b	39.24^{b}	35.39^{a}	38.43^{b}	2.19	50.57^{b}	41.61^{a}	39.29^{a}	2.45
	c	0.058^{b}	0.045^{a}	0.054^{b}	0.002	0.039^{a}	0.042^{a}	0.053^{b}	0.003
	a	-2.49^{a}	-3.50^{b}	-2.71^{a}	0.19	-3.32^{c}	-3.41^{b}	-2.94^{a}	0.20
10	b	41.6^{c}	33.51^{a}	36.70^{b}	2.17	52.51^{c}	36.40^{a}	39.70^{b}	2.53
	a + b	39.11^{c}	30.01^{a}	34.0^{b}	2.13	49.18^{c}	33.01^{a}	36.76^{b}	2.07
	c	0.045^{a}	0.051^{b}	0.045^{a}	0.002	0.029^{a}	0.047^{c}	0.039^{b}	0.002
	a	-2.96^{b}	-1.78^{a}	-3.06^{b}	0.28	-0.59^{a}	1.34^{b}	-2.67^{b}	0.17
12	b	41.84^{c}	32.63^{a}	38.50^{b}	1.89	50.40^{c}	32.54^{a}	38.30^{b}	2.17
	a + b	38.87^{c}	30.81^{a}	35.44^{b}	2.03	49.82^{c}	33.87^{a}	35.62^{b}	2.23
	c	0.032^{a}	0.053^{b}	0.051^{b}	0.003	0.031^{a}	0.044^{b}	0.042^{b}	0.001
	a	-3.11^{c}	-2.97^{b}	-1.08^{a}	0.22	-2.95^{c}	-1.35^{b}	-1.16^{a}	0.11
14	b	41.52^{c}	33.15^{a}	36.30^{b}	2.21	52.70^{c}	34.06^{a}	37.75^{b}	2.23
	a + b	38.43^{c}	30.16^{a}	35.22^{b}	2.07	49.77^{c}	32.72^{a}	36.59^{a}	2.18
	c	0.047^{a}	0.047^{a}	0.081^{b}	0.08	0.029^{a}	0.045^{b}	0.046^{b}	0.002

Fract. -Fermentation fraction; *S. alm.* -*Sorghum almum; C. beng.* -*Commelina benghalensis; V. villo.* -*Vicia villosa*; * -Total number of samples incubated were 6 for each forage per treatment and harvest; sed-Standard error deviation; a-gas produced from the soluble fraction (mL 200 mg^{-1} DM); b-gas produced from the insoluble but fermentable fraction (mL 200 mg^{-1} DM); c-rate constant of gas production during incubation (h^{-1}); a + b – potential gas production (mL 200 mg^{-1} DM); Means with the same super script ($^{a, b, c}$) within the same row under the same sub-heading, are not significantly different ($p>0.05$)

Table 2: Relationship of *in vitro* gas with chemical nutrients and age of *S. almum*, *C. benghalensis* and *V. villosa* under two soil N regimes

	Correlation coefficients (*r*)			
	Without fertilizer		With fertilizer	
Forage	*r*	Pr > F	*r*	Pr > F
Sorghum almum				
Age at harvest (Weeks)	- 0.883	0.047	- 0.942	0.017
Crude Fibre (CF)	- 0.954	0.012	- 0.994	0.001
Neutral Detergent Fibre (NDF)	- 0.994	0.001	- 0.909	0.032
Ether Extract (EE)	+ 0.990	0.001	+ 0.990	0.022
Crude Protein (CP)	+ 0.905	0.035	+ 0.930	0.010
Commelina benghalensis				
Age at harvest (Weeks)	- 0.888	0.044	- 0.734	0.158
Crude Fibre (CF)	- 0.945	0.015	- 0.739	0.153
Neutral Detergent Fibre (NDF)	- 0.793	0.110	- 0.527	0.362
Ether Extract (EE)	+ 0.917	0.028	+ 0.990	0.214
Crude Protein (CP)	+ 0.943	0.016	+ 0.755	0.140
Vicia villosa				
Age at harvest (Weeks)	- 0.938	0.019	- 0.973	0.005
Crude Fibre (CF)	- 0.993	0.001	- 0.994	0.001
Neutral Detergent Fibre (NDF)	- 0.920	0.027	- 0.992	0.001
Ether Extract (EE)	+ 0.909	0.032	+ 0.890	0.034
Crude Protein (CP)	- 0.830	0.082	- 0.907	0.034

Similarly, the potential gas production fraction (a + b) also ranged between 12.01-29.51% in *Sorghum almum*, 3.13-17.58% in *Commelina benghalensis* and 0.53-8.12% in *Vicia villosa* higher than those recorded in T_1. There was however no evidence of effect of age at harvest and fertilizer application on gas production from the soluble fraction (a) and rate of gas production (*c*).

The relationship of *in vitro* gas production with chemical composition and age at harvest were also investigated (Table 2). The *r*-coefficients and *p*-values registered by the examined relationships varied widely between forages and treatments. Results showed that, across the 3 forages, gas production was strongly correlated with all the chemical components considered and age at harvest. Other than for *Commelina benghalensis* under T_2, most of the recorded correlations of *in vitro* gas with age at harvest and cell wall components were significant. In contrast however, the relationship of gas production with CP and EE in *Sorghum almum* and *Commelina benghalensis* were also strong, but positively related across the treatments with most of the correlations being significant. Though the correlations of gas with age and chemical composition of *Commelina beghalensis* in T_2 were strong, they however, did not result in significant relationships.

Figure 1 illustrates the effect of age at harvest and fertilizer application on the cumulative gas production patterns of *Sorghum almum* (Plate A), *Commelina benghalensis* (Plate B) and *Vicia villosa* (Plate C). Results showed that, forages harvested young (6 weeks) consistently exhibited higher gas production patterns than those harvested mature across the 3 forages and treatments. It was however, observed that, those forages, which received fertilizer during establishment comparatively, attained higher gas production peaks.

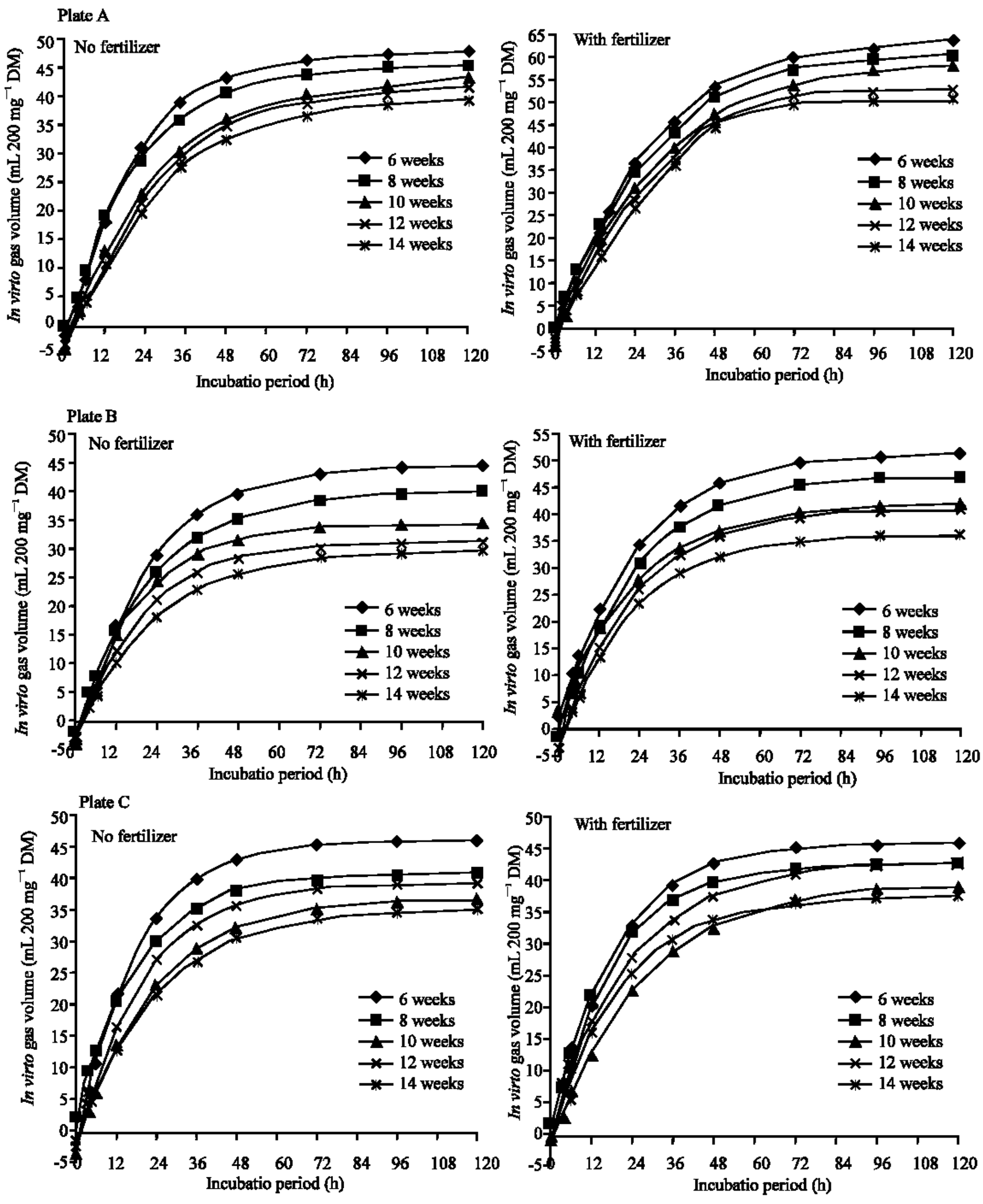

Fig. 1: Pattern of gas production as influenced by age at harvest and fertilizer application (Plate A: *Sorghum almum*; Plate B: *C. benghalensis* and Plate C: *Vicia villosa*)

DISCUSSION

The chemical nutrients of the 3 forages studied were comparable to those reported by Kallah *et al.* (2000) and Agyemang *et al.* (1998) in *Sorghum almum*, Gohl (1981) and Lanyasunya *et al.* (2006a) in *Vicia sativa* and Lanyasunya *et al.* (2006b) in *Commelina diffusa* and were found to vary with advancing plant maturity. This concurred with earlier research works by Leng (1992) and Kallah *et al.* (2000), who reported that chemical nutrients in most tropical forages vary with age of the plant. This study also showed that application of fertilizer significantly improved the overall CP concentration in the plant tissues. Similar effects of N-fertilizer on CP content in forages was also reported by Wouters (1985) in *Pennisetum purpureum* in Kenya, Olsen (1976) and Kallah *et al.* (2000) on *Sorghum almum* in Brazil and Nigeria, respectively and Snijders *et al.* (1992) in *Ipomoea batatus* in Kenya. Results also showed that, *in vitro* gas production equally varied between forages and with age at harvest and soil N regime. In general, however, the gas volumes recorded in the current study were found to compare well with the ranges reported by Abaş *et al.* (2005) for grass and vetch hay and those reported by Sadoval and Mendoza (2000) for many protein rich tropical shrubs. Graphical illustrations of the change pattern of the cumulative *in vitro* gas produced over the 120 h incubation period, showed that, all the charts followed the normal fermentation trends, as those reported by Menke and Steingass (1988) and Schofield and Pell (1995). From these charts, the effect of age at harvest on gas production was clearly evident across the 3 forages, with those in T_2 recording higher gas production peaks. The observed decrease in gas production was primarily attributed to the physiological changes associated with advancing plant maturity. The quantity of gas produced during fermentation, reflects the amount of substrate digested and therefore, microbial activity. Forage digestibility has been reported to depend largely on the actual digestibility of the cell wall matrix, which in turn depends on the extent of lignification (Van Soest, 1982; Akin, 1990). Lignification is caused by an array of closely interacting factors of which forage species, variety, stage of maturity at harvest, environment among others are the most critical (NRC, 2001; Shaver *et al.*, 2002). For instance, high tropical temperature increases lignification of the plant cell wall and promotes more rapid metabolic activity, which decreases the pool of metabolites in the cell (Akin, 1990), thereby impacting negatively on the value of the forage as a feed. Neutral Detergent Fibre (NDF), which includes hemicellulose and other insoluble components of plants' cell wall matrix cross-linked with lignin is particularly important in ruminant nutrition. It is reported that, as forage matures, they accumulate more NDF, which in turn decreases forage energy content and digestibility (Hoffman *et al.*, 2003). The observed changes in forage gas production over the study period, was partly attributed to these factors. Other research workers have also reported inability of microbes to function efficiently in rumen environments characterized by low N availability (Oba and Allen, 1999). The results of this study showed that the increase in CP concentration following the application of fertilizer further increased fermentation as evidenced by the observed higher gas production, suggesting that N availability was also an important factor determining the extent of fermentation.

CONCLUSION

Based on the results presented, it was concluded that age at harvest significantly influenced the digestibility of *Sorghum almum*, *Commelina benghalensis* and *Vicia villosa*. This places an emphasis on the need to identify the optimal stage to harvest these forages so as to maximize on both yield and quality. From the results, it was further concluded that application of N-fertilizer during establishment of *Sorghum almum* and *Commelina benghalensis* significantly improved their overall CP content and therefore fermentation characteristics. Though the N-fertilized *Vicia villosa*, recorded slightly higher gas production than the non-fertilized, it was concluded that, there would be no major advantage in applying N-fertilizer to boost its quality.

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