Variety, harvest date after planting and plant fraction of Napier grass influence *in vitro* gas production

T Ansah, E L K Osafo* and H H Hansen**

Department of Animal Science, University for Development Studies, Tamale, Ghana
ansah.terry@uds.edu.gh
*** Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana**
**** Department of Large Animal Sciences, Copenhagen University, Denmark**

Abstract

An experiment was conducted to assess the relative superiority of digestibility of 4 varieties (V), 3 harvest dates (HD) and 2 plant fractions (PF) of Napier grass cultivated and harvested in the humid forest zone of Ghana. The varieties were; Local, 16798, 16786 and 16840. Except for the Local variety, all were improved varieties from ILCA (now ILRI). The HD was at 60, 90 and 120 days after planting and PF were leaf and stem fractions. Relative in-vitro gas production (IVGP) per gDM sample from in-vitro rumen fermentation was used as an indicator of superiority. The automated Ankom^{RF} system was used to measure the IVGP at 5 minute intervals over a period of 48 hours. Gas production at 12, 24 and 48 hours was evaluated using a 4×3×2 factorial randomized design.

No differences were found in ml gas production per gram DM incubated at 12 hours between stems, leaves, varieties or harvest dates. The stem fractions produced significantly more gas per gram dry matter than leaves at 24 hours (56.1 vs 64.0 ml; p=0.0376). No significant differences between varieties or harvest dates were found at 24 hours. At 48 hours differences were dependent on variety and harvest date. Total gas production for the stem fraction for all varieties was less at increasing harvest date. The leaf fraction of variety Local and 16798 followed the same trend with less gas produced at increasing harvest date. However, varieties 16786 and 16840 produced most gas from samples harvested at 90 days and 16786 increased proportionately more from 60 to 90 days than 16840 (58% vs. 7%). At 120 days, these two varieties produced only slightly less or almost the same amount of gas from samples harvested at 90 days. The leaf fraction of variety 16798 consistently produced the most gas across the harvest dates, but the same was not found for the stem fraction of this variety. The stem fraction of variety 16786, while producing 8% less gas than the best (16798), was the most stable gas producing variety when comparing samples harvested at 60 or 90 days and is therefore recommended for harvest at 90 days. If harvesting at 60 days, variety 16798 is recommended. If harvesting at 120 days variety local and 16840 will be the best option as gas production from stems at 120 days was relatively higher than the other varieties.

Key words: Ankom^{RF}, degradability, gas production system, leaf and stem

Introduction

Forages, and in particular grasses, represent the single most important feed resource for livestock in developing countries. The nutrient composition and digestibility of tropical grasses are influenced by species, variety, plant maturity at harvesting, soil fertility and climate (Minson 1990). Leaves have generally more crude protein and digestible carbohydrates (cell contents) than stems which have more structural carbohydrates.

The content of structural tissue components increase with plant maturity and this leads to an increase in the relative amount of structural carbohydrates (cellulose and hemicelluloses) and lignin as well as a decrease in relative cell content and protein concentration. Digestibility of forage in the rumen is related to the proportion and extent of lignification (Van Soest 1994).The overall digestibility of plants, as they mature, decreases and this is attributed to the amount and spatial arrangements of lignin within the plant. Increased synthesis of lignin reduces the digestibility of cellulose and hemicelluloses. Rapid accumulation of cell wall carbohydrate, at the latter stages of growth, causes a decrease in relative crude protein content and therefore affects digestibility (Kidunda et al 1990; Van Soest 1994; and Seyoum et al 1998).

Differences have been reported in the digestibility and nutrient composition of different fractions (leaf, stem and whole plant) of grass with plant maturity within the same species and varieties. Woodard and Prine (1991) and Williams and Hanna (1995) reported that the rate of decline in crude protein content is more rapid in stems than in leaves.

Gas is produced by microbial degradation of carbohydrates. The amount and composition of the gas produced is dependent on total dry matter and composition of the carbohydrates (Lopez et al 1998). Total gas production can therefore be used as an indication of digestibility (France et al 2000) and relative gas production can be used in comparative studies. It was against this background that a study was conducted to assess the relative superiority of digestibility of 4 varieties (V), 3 harvest dates (HD) and 2 plant fractions (PF) of Napier grass cultivated and harvested in the humid forest zone of Ghana.

Materials and methods

The experiment was conducted between March, 2008 and June, 2009 at the Department of Animal Science KNUST and the Department of Large Animal Sciences of the University of Copenhagen, Denmark.

Location and climate of area where the Napier grass was cultivated

The Department of Animal Science farm of the Kwame Nkrumah University for Science and Technology (KNUST) was the site used for the cultivation of the Napier grass. The area falls within the latitude 06^0 41N and longitude 01^0 33W and is at an altitude of 261.4meters above mid sea level (MSL). The site is within the humid semi-deciduous forest belt of Ghana and with a bimodal rainfall pattern.The rainfall during the experiment at the study area was 168.4±61mm and the temperature was 31.4±1.5 ^oC.

Source of planting material

The planting materials used for this study were made up of three varieties obtained from International livestock Center for Africa (ILCA) now International Livestock Research

Institute (ILRI) preserved at the herbarium of the KNUST and a local variety (Local) from Ghana. The four varieties were selected based on measures of best total stover yield and/or dry matter digestibility in the study conducted by Dzimale (2000). The leaf was separated from the stem by pulling the leaf blade down along the stem until it detached from the stem. The leaf as used in this report represents both leaf blade and leaf sheath. The chemical composition and biomass yield results of these plants have been reported elsewhere (Ansah et al 2010).

Sample preparation and in vitro gas technique

The Ankom^{RF} gas production technique was used for the incubation (Ankom 2008). The Napier grass samples harvested were chopped into lengths of 2cm and dried at 60^0C for 48 hours. Samples were ground using a laboratory mill (Wiley mill) to pass through 1mm sieve screen. Fifty grams of each sample was transported to the University of Copenhagen in Denmark for the *in vitro* gas studies.

Each experimental unit consisted of a 260 ml glass jar with attached module top. The module tops used contained the communication system. This is a radio frequency wireless system that monitors gas pressure, allows the release of gas produced during the fermentation and maintains a low positive pressure in the bottle headspace, thereby avoiding the entrance of air (Tagliapietra et al 2011). Gas accumulating in the headspace of bottles was automatically released when the pressure inside the units reached 6.9 kPa above ambient pressure. Pressure was measured every 5 minutes. Approximately 500mg of the milled Napier grass sample was weighed into 260ml glass jar and incubated at 39.5^0C overnight.

Experimental design

A 4×3×2 factorial in a completely randomized design was used for the gas production studies. The main factors were the varieties [4]; (Local, 16798, 16786 and 16840), harvesting days [3]; (60, 90 and 120 days) and plant fraction [2]; (leaf and stem). There were 5 runs of 48 hours of fermentation with 36 units in each. This resulted in between 5 and 8 fermentation values for each combination of factors.

Source of rumen mixture and procedure for gas measurement

Two rumen cannulated Jersey heifers were used for rumen fluid collection. They were fed low quality hay ad libitum with a minimal amount of concentrate per day. The concentrate (approx.1kg) was fed at least 4 hours before the rumen fluid was collected. Roughage and water were withheld two hours and 30minutes respectively before rumen fluid was taken. The fluid was collected into pre-heated thermos-flask. .

The buffer was prepared according to Menke and Steingass (1998), and buffer mixed with rumen fluid 2:1. A mixture of 60ml of this media was added to preheated units containing the grass samples. The glass jars were then closed and put into an incubator. Media and incubation preparation were done under anaerobic conditions by constantly flushing CO_2, at a temperature of about 39^0C-40^0C and a pH of about 6.8-7. The samples were incubated without disturbance for 48 hours.

Calculations and statistical analyses

The average cumulative pressure measured for each sample was calculated for consecutive 30 minutes intervals from the 5 minute measurements. Pressure was converted to ml of gas at standard temperature and pressure and gas produced per gram dry matter substrate incubated used was thereafter calculated.

Cumulative gas values at 12, 24 and 48 hours were selected for statistical analysis.

Statistical analyses were performed using the software R (R Development Core Team,2012).The following full model was tested for volume of gas produced at STP per ml substrate for results from 12, 24 and 48 hours. Reduced models were subsequently tested and only significant variables included.

$$\gamma_{xijk} = \mu + \delta + \alpha + \beta + (\delta\alpha_{xi}) + (\alpha\beta_{ij}) + (\delta\beta_{xj}) + (\delta\alpha\beta_{xij}) + \theta + \epsilon_{xijk}$$

Where γ is the ml gas produced per g DM substrate with plant fraction *x*, variety *i* and harvest date *j; μ* is the overall mean,δ is the plant fraction effect (fixed), α is the variety effect (fixed), β the effect of harvest date (fixed), $\alpha\delta$; $\delta\beta$; $\alpha\beta$; are the two way interaction between PF and V, PF and HD and V and HD respectively. $\delta\alpha\beta$ are the three way interaction between PF, HD and V, and θ QUOTE θ$^{\theta}$ the random effect of the run. The error terms and the random effect variable are assumed to have a normal distribution with mean zero and variances $\sigma_{eR}{}^2$ (residual error), $\sigma_{eV}{}^2$(V) $\sigma_{eHD}{}^2$(HD*)* and σeR^2(run). Model validation was carried out using visual inspection of residuals and Cook's distances. Significance is determined using a 0.05 level

Results

No significant differences or interactions were found in ml gas production per gram DM incubated at 12 hours between stems, leaves, varieties or harvest dates. The stem fractions produced significantly more gas per gram dry matter than leaves at 24 hours (74 vs. 66 ml; p=0.0418). No significant interactions or differences between varieties or harvest dates were found at 24 hours.

At 48 hours differences in leaves and stems varied depending on variety and harvest date. There was no difference between varieties for the stem fraction samples but significant differences were recorded for harvest date. The stem samples harvested at 60 and 90 were significantly greater than those harvested at 120 days (p=0.002), but not significantly different from each other. There was no difference between harvest dates for leaves but the varieties differed significantly. The leaves of variety 16798 differed significantly from the other varieties ($p<0.05$), while the other varieties didn't vary from each other (Table 1). Total gas production for the stem fraction for all varieties was less at increasing harvest date except, variety 16786 where an increase of 10% was recorded from 60 to 90 days (Table 2). The leaf fraction of two of the varieties (Local and 16798) followed the same trend with less gas produced at increasing harvest date (Table 2). However, varieties 16786 and 16840 produced most gas from samples harvested at 90 days (Table 2). The increase from 60 to 90 days was most pronounced for variety 16786 compared to 16840 (58% vs. 7%) (Table 3). At 120 days, these two varieties produced only slightly less or almost the same gas from samples harvested at 90 days. The leaf fraction of variety 16798 consistently produced the most gas across the harvest dates, but the same was not found for the stem fraction of this variety

(Table 3). The stem fraction of variety 16786, while producing 8% less gas than the best (16798), was the most stable gas producing variety when comparing samples harvested at 60 or 90 days (Tables1;3).

Table1: Gas production (ml/g DM) from fermentation of Napier grass at 48hours

		Stems	**Leaves**
Variety	Local	107	108^{b}
	16840	103	111^{b}
	16786	107	108^{b}
	16798	115	145^{a}
Harvest date	60	131^{a}	121
	90	110^{a}	122
	120	86^{b}	116

Means with different superscripts within the same column section are significantly different at ($p<0.05$)

Table 2: Ranking of variety and harvest date for each fraction at 48hrs

Variety/Harvest date	**Stem**			**Leaf**		
	60	**90**	**120**	**60**	**90**	**120**
Local	2 (125)	4 (88)	2 (82)	2 (107)	4 (98)	4 (89)
16840	4 (107)	3 (93)	1 (88)	3 (95)	3 (102)	3 (104)
16786	3 (112)	1 (123)	3 (79)	4 (72)	2 (114)	2 (105)
16798	1 (138)	2 (97)	4 (69)	1(154)	1 (125)	1 (117)

(Figures in parenthesis are volume (ml) of gas production per gram dry matter at 48hrs)

Table 3: Proportion of increase or decrease from early harvest to later harvest

Variety	**Stem**			**Leaf**		
	60-90	60-120	90-120	60-90	60-120	90-120
Local	30%	34%	7%	8%	17%	9%
16840	13%	18%	5%	*7%*	*10%*	1%
16786	*10%*	30%	38%	*58%*	*46%*	8%
16798	30%	50%	29%	19%	24%	6%

Percentages underlined indicates a decrease whilst those in italics indicate an increase

Discussion

Storage space for harvested forage is a challenge to most ruminant farmers in developing countries. This has resulted in farmers harvesting grass daily or at most weekly to feed small ruminants. This means that plant maturity at harvest increases thereby varying the nutrient composition and digestibility of the grass. The leaf to stem ratio will increase in the early stages of plant phenology while it will steadily decrease with increasing maturity after reaching a maximum. Therefore it is relevant to have knowledge of the digestibility of both leaves and stems in order to give farmers tools to find the best harvest date in a given situation. The in vitro gas production technique is a useful method for comparative studies of substrate degradation. The technique measures gas produced (mainly carbon dioxide and methane) as an indication of the extent to which feed is being digested (France et al 2000). Cumulative gas production is measured over a period of time and used as a basis to investigate the digestion kinetics of the substrate. In this study, gas accumulated at 12, 24 and 48 hours is reported. Menke and Steingass (1988) discuss the optimum duration of fermentation, and concluded that 24 hours is sufficient for maize, dried grass meal and easily fermentable feedstuffs, because the delayed fermentation in cellulose rich substrate is no longer detectable at this time. However, these authors also discuss that the incubation time chosen is less dependent on the time needed to achieve maximum gas production, but more on the expected rumen retention time. It could be expected that Napier grass stems would remain in the rumen for at least 48 hours and therefore this endpoint was chosen with intermediate points at 12 and 24 hours. The pattern of gas production from leaf and stem degradation at 24hrs conforms to the findings of Tolera and Sundstol (1999) who also found that stems produced more gas (at 24 hours) than leaf sheath and leaf blade of different varieties of maize but partly disagrees with the report of Tang et al (2008) who recorded no significant differences between cumulative gas production of maize leaf blades and stems from 26 to 72 hours. Tang et al (2008) found that the cumulative gas was consistently greater for the leaf sheath fraction compared to the stem. Poor crude protein degradability in the rumen can reduce the amount of ammonium nitrogen available for microbial cell synthesis and could be the cause of reduced microbial fermentation and hence low gas production. The higher gas production from the stem fraction, despite the high CP reported in the leaf, may therefore be an indication that the CP in the leaf fraction was poorly degraded at this time. Ansah et al. (2010) reported a higher CP for the leaf fraction of Napier grass (122.2g/kg) than stems (61.8g/kg) from the same plants cultivated and harvested for the in vitro gas study in Ghana. However, there was not an overall difference in the cumulative gas production of the stems vs. the leaves at 48 hours, suggesting an equal fermentative value of both if the rumen retention time is 48 hours. Gas production from stem and leaf was only significantly influenced by the harvest date and variety respectively at 48 hours. The stem fraction of the 60 and 90 days harvest produced more gas per g DM incubated than did the samples harvested at 120 days and this is attributed to the relatively high lignin content and low CP content of the stems at 120 days (Ansah et al 2010).

The leaf fraction of variety 16798 produced the most gas in relation to the other varieties at 48 hours and this is most likely due to the relatively low insoluble cell wall fraction reported for this variety (Ansah et al 2010). This relatively low level of insoluble fiber and lignin of this variety combined with a relatively high CP from the leaf fraction (Ansah et al 2010) could have also contributed to relatively high gas production.

The results indicate that leaf and stem fraction of the four varieties and three harvest days, could provide alternatively useful feed sources for livestock farmers. Overall, variety 16798 should be recommended if possible to harvest at 60 days and either use the entire harvest directly or store the harvest (table 2). When feeding the entire plant with no expectation of animal selectivity of leaf over stem, harvest at 60 or 90 days for any of the tested varieties should be recommended as harvest at 120 days gave significantly lower digestibility for the stems and numerically lower for leaves (table 1). Varieties local and 16840 is recommended if harvest at 60 and 90 days is not possible, as they only lost 5-7% of their original values and were not significantly different, but numerically greater than the other varieties when harvested at 120 days (Table 3).

Conclusions

- Variety 16786 is recommended for harvest at 90 days because of its relatively high gas production and stability from 60 to 90 days. If harvesting at 60 days is possible or necessary, variety 16798 is recommended due to its relative superiority of both leaf and stem at 60 days. If harvest at 60 and 90 days is not possible, then the varieties local and 16840 will be the best option as their gas production for stem at 120 days was relatively higher than the other varieties.

Acknowledgement

The authors will like to thank the Danish Development Research Network (DDRN) for funding the trip to Copenhagen University for the *in vitro* gas analysis and also thank the Department of Large Animal Science, Copenhagen University for providing funds for the chemicals used in the *in vitro* gas analysis.

References

AnkomRF Gas Production Operators Manual 2008 Ankom Technology 2052 O` Neil Road, Macedon, NY 14502. (RFS, RFS500N)

Ansah T, Osafo E L K and Hansen H H 2010 Herbage yield and chemical composition of four varieties of Napier (*Pennisetum purpureum)* grass harvested at three different days after planting. Agriculture and Biology Journal of North America 2010 1 (5): 923-929

Dzimale G J K, 2000 Herbage yield and Nutritive Value of ten Varieties of Elephant grass, *Pennisetum purpureum,* in Ashanti Region of Ghana. A thesis submitted to the school of postgraduate studies, Kwame Nkrumah University of Science and Technology, Kumasi, in partial fulfillment of the requirements for the MSc. (Animal science) Degree (unpublished).

France J, Dijkstra J Dhanoa M S and Bannink A 2000 Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro; derivation of models and other mathematical considerations. British Journal of Nutrition 83, 143-150.

Kidunda R S, Lowga A B and Mtengeti E J 1990 Utilization of research results on forage and agricultural by products as animal feed resources in Africa. PANESA/ARNAB (Pasture Network for Eastern and Southern Africa/African Research Network for Agriculture By-products), pp. 36 - 56. Proceedings of the first joint workshop held in Lilongwe, Malawi, 5-9 December, 1988, PANESA/ ARNAB, Addis Ababa, Ethiopia.

Lopez S, Carro M D Gonzalez J S and Ovejero F J 1998 Comparison of different in vitro and in situ methods to estimate the extent and rate of degradation of hays in the rumen. Animal Feed Science and Technology 73: 99-113

Menke K H and Steingass H 1988 Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid Animal Research and Development 28:7.

Minson D J 1990 Forage in ruminant nutrition Academic Press, San Diego, CA, 482 pp.

R Development Core Team 2012 R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://WWW.R-project.org/.

Seyoum B, Zinashi S Tadesse T T and Liyusew A 1998 Evaluation of Napier (*Pennisetum purpureum*) and pennisetum hybrids (*Pennisetum purpureum* x *Pennisetum typhoides*) in the Central Highlands of Ethiopia. Proceedings of the Fifth Conference of Ethiopian Society of Animal Production (ESAP), ESAP, 15- 17 May 1997, Addis Ababa, Ethiopia, pp. 194-202.

Tagliapietra F, Cattani M Hansen H H Hindrichsen I K Bailoni L and Schiavon S 2011 Metabolizable energy content of feeds based on 24 or 48 h *in situ* NDF digestibility and on *in vitro* 24 h gas production methods. Animal Feed Science and Technology170: 182–191.

Tang S X, Gan J Sheng L X Tan Z L Tayo G O Sun H Z Wang M and Ren G P 2008 Morphological fractions, chemical compositions and *in vitro* fermentation characteristics of maize stover of five genotypes. Animal *12* (2):1772-1779.

Tolera A and Sundstøl F 1999 Morphological fractions of maize stover harvested at different stages of grain maturity and nutritive value of different fractions of the stover. Animal feed Science and Technology. 81: 1-16.

Van Soest P J 1994 Nutritional Ecology of Ruminants, 2nd ed.Cornell University Press, 476 pp.

Williams M J and Hanna W W 1995 Performance and nutritive quality of dwarf and semi-dwarf grass genotypes in south-eastern USA Tropical Grasslands 29:122-127.

Woodard K R and Prine G M 1991 Forage yield and nutritive value of elephant grass as affected by harvest frequency and genotype. Agronomy Journal 83:541–546.

Received 14 January 2013; Accepted 3 April 2013; Published 1 May 2013

Go to top