The Dry Matter Digestibility Characteristics of Tropical Tree Legumes Using Menke *in vitro* Gas Production Technique

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ABSTRAK

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Sejumlah gas yang dihasilkan saat pakan diinkubasikan secara *in vitro* dalam cairan rumen berhubungan erat dengan kecernaan pakan. Suatu percobaan yang menggunakan teknik Menke produksi gas *in vitro* telah dilaksanakan untuk menentukan kecernaan bahan kering (BK) leguminosa pohon, yakni *leucaena*, *flemingia* dan *gliricidia*. Sampel daun diambil dari kebun. Institute of Animal Science, University of the Philippines Los Banos. Produksi gas diukur pada inkubasi 3, 6, 12, 48 dan 72 jam. Volume gas dihitung menurut persamaan yang dikutip dari TUAH; sedangkan persamaan MCDOLAND dirujuk untuk menentukan arah persamaan produksi gas. Pengaruh pencucian tidak diukur dan diasumsikan nol. Hasil pengamatan menunjukkan bahwa leucaena pada inkubasi 12 jam menghasilkan volume gas paling tinggi (1.217 ml/g BK). Volume gas dari substrat gliricidia dan flemingia menunjukkan hasil yang sama (1,60 ml/g BK *vs.* 1,60 ml/g BK). Kisaran potensial gas inkubasi 72 jam (nilai b) dan laju produksi gas (nilai c) ketiga substrat tidak menunjukkan perbedaan. Berdasarkan kurva logaritma gas yang diproduksi, leucaena cenderung menunjukkan peningkatan tertinggi yakni pada nilai b dan c. Perbedaan laju produksi gas pada setiap inkubasi mencerminkan kecernaan BK dari substrat di dalam rumen.

Kata kunci: Produksi gas *in vitro*, kecernaan bahan kering

ABSTRACT

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The amount of gas released when a feed was incubated *in vitro* with rumen fluid, was closely related to the digestibility of the feed. The experiment using Menke *in vitro* gas production was conducted to determine the dry matter digestibility of tropical tree legumes; *Leucaena leucocephala, Flemingia macrophyla,* and *Gliricida sepium*. The samples were collected from Institute of Animal Science farms of the University of the Philippines at Los Banos. The amount of gas produced were recorded at 3, 6, 12, 48 and 72 hours of incubation. To estimate the volume of gas, the equation of Tuah was adopted; while to describe the course of gas production, the equation of MCDONALD was used. The washing loss was not measured and assumed to be zero. Results showed that leucaena at 12 hours was the highest in the volume of gas produced (1.217 ml/g DM). There was a similarity of highly increase of the gas produced between gliricidia and flemingia at 24 hours of incubation i.e 1.60 ml/g DM and 1.60ml/g DM. The potential extent gas production at 72 hours (b value) and the gas production rate (c value) of three legumes were no significantly different. According to the logarithmic-curve of gas volume produced, leucaena tended to be the highest of increase in potential extent and the gas production rate. The different rate of gas production in related to incubation reflected the dry matter digestibility of the three legumes in the rumen.

Key words: *In vitro* gas production, dry matter digestibility

INTRODUCTION

Poor nutrition is one of the major constraint to livestock productivity in tropical regimes. This is because animals thrive predominantly on high-fibre feeds (straws, stovers and native pasture hay) which are deficient in nutrients (nitrogen, sulphur, minerals, phosphorus, etc) but essential for microbial fermentation. Consequently, the digestibility and intake of digestible nutrients are unavoidably low. These deficiencies can partly mitigated by supplementing roughage diets with feeds containing the deficient nutrients. Feeding practices develop in temperate countries are often inappropriate when applied to ruminant production systems in the tropics because temperate animals are fed straw as bulk in high density diets (OSUJI *et al*., 1992). Roughage diets and supplements may differ vastly in quality and therefore in the quantity eaten by animals. Previously digestibility and chemical composition were used to describe the nutritive value of fibrous feeds. This proved inadequate because these attributes give little indication of the

quantity of such feed an animal will eat and the quality of nutrients derived through digestion. An understanding of the factor which affect rumen digestibility of low-quality basal feeds and microbial protein production will assist scientists in designing more efficient diet.

Tropical tree legumes have the potential to produce large quantities of high protein leaf for animal feed. This is particularly important in areas where the majority of ruminants are currently fed forages and crop residues of low nutritive values (PRESTON and MURGUEITIO, 1992). Consequently, recent studies have examined the effect of supplementing these feed resources with leaves of tree and shrsub legumes, such as *Sesbania grandiflora* (L.) Poir, *Calliandra calothyrsus* Mewassn, *Gliricidia sepium* (Jacq.) Walp. and *Leucaena leucocephala* (Lam.) de Wit.

In experimental rubber plantations in Ghana, a flemingia mulch reduced the number of required weedings per year from six to two (ANONYMOUS, 1964). Temperatures at a soil depth of 10 cm were 7-8°C lower in a mulched plot (5000 kg DM per ha) than under bare soil. Soil moisture under a flemingia mulch has been shown to be significantly higher than under mulches of *Gliricidia sepium* and *Leucaena leucocephala.*

An alley farming trial in Nigeria compared the ability of fallows and mulches of flemingia, *Cassia siamea* and *Gliricidia sepium* to control weeds. The trees/shrsubs were not cut during a 2-year establishment period. In a 120-day test of the decomposition rate of foliage from the first cutbacks from these hedges, cassia lost 46% of its dry matter, flemingia 58%, and gliricidia 96% (YAMOAH *et al*., 1986a). For later pruning over two maize cropping seasons, gliricidia prunings decayed completely in a 120-day period, Acassia lost 85%, and flemingia 73%. However, Acassia showed the greatest potential for controlling weeds during both the 2-year fallow and the two maize crops, primarily because of the greater shade cast by its canopy during the establishment period.

The nylon bag technique described by ORSKOV *et al.* (1980) for the determination of the degradation of feedstuffs in the rumen at various incubation periods can be used to screen feeds at the initial stages of assessing their nutritive values. Applying the equation of MCDONALD (1981), $p= a + b (1- e^{-ct})$, to describe the course of degradation of the feeds, the constants, a, b, and c obtained can also be used to predict feed intake and growth rate (ORSKOV *et al.,* 1988). BLUMMEL and ORSKOV (1993) reported that the *in vitro* gas production technique developed by MENKE *et al.* (1979) could also be used to determine gas production at various incubation periods and these values could be used to describe the course of fermentation of the feeds, by applying the equation of MCDONALD (1981). These workers reported high positive correlation between the *in vitro* gas production and the dry matter digestibility values of the feeds at the various incubation periods (BLUMMEL and ORKSOV, 1993).

MATERIALS AND METHODS

The apparatus

Two hundred (200) mm long glass syringe (pisron pipette) calibrated to 100 ml, with capillary attachment. Silicon rubber tube about 45 cm long, plastic clip, analytical balance, water bath with grid for supporting syringes, suction bottle/erlenmeyer flask (2 liters), CO cylinder with regulator, glass syringe rack for storing the syringes, buchner funnel and flask, plastic bucket (4 liters) and cheese cloth, 10 ml automatic syringe (an aliquot dispenser), magnetic stirrer, thermometer.

Rumen fluid

Not more than 15 minutes before the trials starts, the sample of rumen fluids (about 1 liter) was collected in equal proportions from fistulated buffalo. The sample was than filtered through two layers of cheese cloth into a warm flask (kept in bucket of water at 37-38°C) and flush it with CO_2. The rumen fluid was taken before morning feeding.

Solution

Five (5) different solutions as media were prepared and mixed with the rumen liquor. The solutions were:

Solution A (13.2 g calcium chloride/$CaCl_2.2H_2O$); 10.0 g manganese chloride ($Mn\ Cl_2.4H_2O$); 1.0 g cobalt chloride ($Co\ Cl_2.6H_2O$): 8.0 g iron chloride ($Fe\ Cl_3.6H_2O$); made up to 100 ml with distilled water).

Solution B, Buffer solutions (39.0 g sodium hydrogen carbonate ($NaHCO_3$) or 35.0 g $NaHCO_3$ + 4.0 g ammonium hydrogen carbonate (NH_4HCO_3); made up to 1 liter with distilled water).

Solution C, Macro minerals (5.7 g disodium hydrogen phsophate/Na_2HPO_4; 5.2 g potassium dihydrogen phosphate/KH_2PO_4; 0.6 g magnesium sulphate/$MgSO_4.7$ H20 made to 1 liter distilled water).

Resazurin solution (100 mg resazurin made up to 100 ml distilled water)

Reducing solution (4 ml sodium hydroxide/1N NaOH; 625 mg sodium slphide/$Na_2S.9H_2O$, added to 95 ml distilled water).

***In-vitro* gas production procedures**

Samples of tree legumes as substrates were ground through a 1-mm sieve. Three replicates of each feed (each weighing about 200mg) were then put into 100 ml calibrated syringes together with a rumen fluid plus buffer solution (about 30ml) and incubated in a water bath maintained at 39°C. A Perspex lid with holes held the syringes upright in the water bath. The buffer solutions used have previously been described by MENKE *et al.* (1979). During any incubation time, there were also three blanks (rumen fluid + buffer solution) and three replicates of a hay standard. The rumen fluid was obtained from the same sheep (fed on the same diet) used for the nylon bag studies and the fluid was collected 1 hours after the morning feeding. The fluid was strained through two layers of cheese-cloth. The gas volume was recorded after 3,6,12, 24, 48, 72 and 96 hours of incubation. The initial volume of material in each syringe was also recorded before the commencement of the incubation of the samples.

The following equation was used to estimate the volume of gas produced at any incubation period (TUAH *et al*., 1996):

$$GPt = [(SVt - SVo) - (BVt - BVo) \times 0.200g]/ACW$$

where:

GPt = volume of gas produced at time "t"
SVt = syringe reading for the sample at time "t"
Svo = syringe reading for the sample at the beginning of incubation
Bvo = mean of three replicates of blank readings at the beginning of the incubation
BVt = mean of three replicates of blank readings at time t
ACW = actual weight of the sample incubated on dry matter basis

The equation of MCDONALD (1981) was used to describe the course of gas production. Washing losses were not measured and were assumed to be zero, because there was no gas was produced from unfermented feed. More often the following model was fitted to the data:

$$Y = b (1- e^{-ct})$$

where:

Y = the volume of gas produced with time (t)
b = the potential extent of gas production.
c = the gas production rate

The data of volume of gas produced was analyzed by using Completely Randomized Design (GOMEZ and GOMEZ, 1984).

RESULTS AND DISCUSSION

Data in Table 1 shows the volume of gas released at any incubation times. At the beginning of incubation (at 3 and 6 hours), flemingia produced the highest of volume (P<0.01) which were 0.57 ml/g DM and 0.77 ml/g DM; followed by gliricidia (0.35 ml/g DM and 0.45 ml/g DM) and leucaena (0.03 ml/g DM and 0.09 ml/g DM). This condition reflects that flemingia was more rapidly digested by rumen microbes than that of leucaena and *gliricidia* sp.

Table 1. *In vitro* gas production characteristic of tree legumes at sequencing time of incubation (ml/g DM)

Substrate	Time of incubation (hours)						
	3**	6**	12*	24^{ns}	36^{ns}	48^{ns}	72^{ns}
Leucaena 1	0.025	0.10	1.475	2.20	2.30	2.6	3.0
Leucaena 2	0.025	0.10	1.275	1.80	2.50	2.8	3.0
Leucaena 3	0.04	0.08	0.90	1.20	2.00	2.1	2.3
Average	0.03	0.093	1.217	1.73	2.267	2.50	2.767
Flemingia 1	0.70	0.80	1.30	2.30	2.40	2.8	2.9
Flemingia 2	0.40	0.60	0.60	0.80	1.60	2.1	2.4
Flemingia 3	0.60	0.90	1.00	1.70	2.00	2.6	2.9
Average	0.567	0.767	0.967	1.60	2.00	2.5	2.73
Gliricidia 1	0.40	0.60	0.60	2.10	1.60	2.5	3.0
Gliricidia 2	0.30	0.30	0.90	1.10	2.00	1.6	2.6
Gliricidia 3	0.4	0.4	1.2	1.6	2.0	2.0	2.4
Average	0.35	0.45	0.75	1.6	1.8	2.05	2.8

Ns : non significant; * : significant (P<0.05); ** : highly significant (P<0.01)

However after 6 hours those condition changed, especially at 12 hours incubation, there was a significant difference of gas volume produced where leucaena shows the highest (1.22 ml/g DM); followed by flemingia (0.97 ml/g DM) and gliricidia (0.75 ml/g DM). At 24 hours, flemingia and gliricidia showed similar result of the gas volume (1.61 ml/g DM and 1.60 ml/g DM, respectively). The different rate in the increase of gas volume produced of the substrates reflected the digestibility and the nutrient content. VAN SOEST and MCDOWELL (1987) discussed the interaction of the tannins in tree leaves with dietary protein and leucaena was more rapidly digested than gliricidia and flemingia due to protein and tannin contents. BEJO and SEVILLA (2001) divided gliricidia, leucaena and flemingia into trace, middle and high of tannin regarding to the criteria of condensed tannin. Several authors attributed of the low crude protein digestibility of the leaves due to presence of high tannins. In this experiment, it did not mean that flemingia has the highest of digestibility although it was the highest of the volume gas produced at 3 and 6 hours of incubation.

Table 2 shows the potential extent gas and the gas production rate of each substrate at 72 hours incubation. There was no significant difference on the potential extent gas although the potential extent gas of leucaena was the highest (13.83) followed by flemingia (13.67) and gliricidia (13.33). The same result was obtained on the gas production rate in which there was no significant difference of the substrates although flemingia produced the highest (0.0596) followed by leucaena (0.0509) and gliricidia (0.0496). TUAH *et al*. (1996) found that *in vitro* gas production of Maize leaves was 48.1 ml/200 mg DM at 72 hours incubation. Some reasons of the different results may be due to (1) the equipment used, i.e calibrated syringes, (2) nutritional content (CP, NDF), (3) possibility of anti nutritional effect on the microbial activity.

In general, the gas production rate (c value) of this experiment was higher than the report on straw (ORSKOV and RYLE, 1990). The values for husks and leaves were higher than that of straw in which these authors suggested as the minimum value to fill up energy requirements. Research on energy source of feeds showed that coffee pulp had digestibility values greater than those of untreated maize cobs at the 8, 16, 24 and 48 hours incubation periods. However, the *in vitro* gas production values exceeded those of untreated maize cob only at 3 and 6 hours incubation periods. Oil palm fruit bracts also had higher digestibility values than untreated maize cobs at 8, 16, and 24 hours incubation periods. (TUAH *et al*., 1996). The *in vitro* gas production values of oil palm fruit bracts were, however, greater than those of untreated maize cob at only 3 and 6 hours incubation periods. It was likely that the oil palm fruit bracts and coffee pulp may contain some anti-microbial factors which could affect the *in vitro* gas production values but did not affect the digestibility values. There was a build-up of these factors with time in the syringes while in the case of the *in sacco* dry matter digestibility technique, these factors were washed out from the bags into the rumen content and their effects would not be felt.

Figure 1, 2 and 3 show the logarithmic-curve of *in vitro* gas production characteristics of the substrate of tree legumes. In those curves were attached the trend-line of the gas produced.

Table 2. The potential extent gas at 72 hours incubation and the gas production rate of the substrate of tree legumes

Substrate	b value	c value
Leucaena 1	15.00	0.0564
Leucaena 2	15.00	0.0406
Leucaena 3	11.50	0.0557
Average	13.83	0.0509
Flemingia 1	14.50	0.0656
Flemingia 2	13.00	0.0305
Flemingia 3	14.50	0.0827
Average	13.67	0.0596
Gliricidia 1	15.00	0.0502
Gliricidia 2	13.00	0.0407
Gliricidia 3	12.00	0.0578
Average	13.33	0.0496

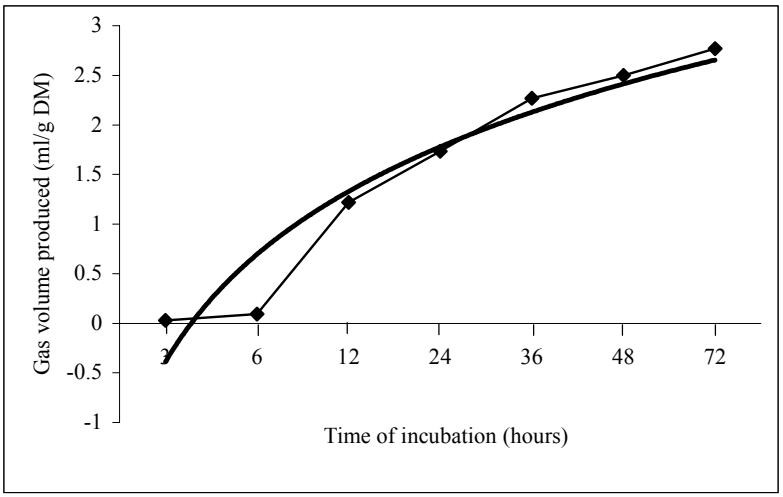

Figure 1. *In vitro* gas production characteristic of leucaena

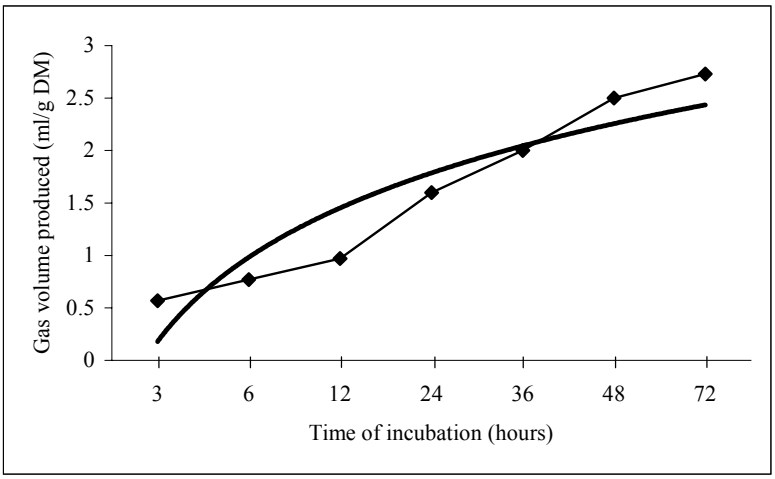

Figure 2. *In vitro* gas production characteristic of flemingia

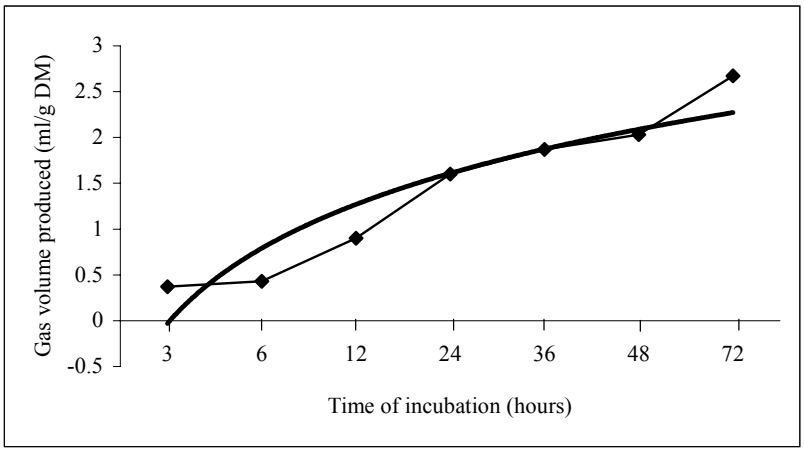

Figure 3. *In vitro* gas production characteristic of gliricidia

The gas volume released after incubation the sample at 3, 6, 12, 24, 36, 48 and 72 hours which are sharpened with logarithmic trend line reflect the value of how fast the substrate degraded in the rumen. LAI and PRESTON (1997) reported that gliricidia and leucaena at 96 hours incubation before washing, had *in vitro* gas production as of 33.1 ml/200 mg DM and 31.5 ml/200 mg DM respectively. Moreover, OSUJI *et al*. (1993) stated that the amount of gas released when a feed was incubated *in vitro* with rumen fluid are related to the digestibility of the feed. In this study it can be showed that leucaena was rapidly digested than flemingia and gliricidia although the trend of those curves are similar. TUAH *et al*. (1996) stated that the *in sacco* dry matter digestibility and *in vitro* gas production values at 24, 48, 72 and 96 hours incubation periods was highly significant ($P<0.01$) and positive. With this high crude protein content, either the *in sacco* dry matter digestibility or the *in vitro* gas production methods could be used to asses nutritive value, since there was a very good positive relationship between the two methods.

CONCLUSION

1. The *in vitro* dry mater digestibility of flemingia was the highest after 3 and 6 hours incubation.
2. Leucaena produced the highest was *in vitro* dry matter gas production after 12 hours incubation while glricidia was the lowest.
3. The potential extent gas at 72 hours (b value) and the gas production rate (c value) of three legumes were not significantly difference.

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