Comparison of Fermentation Kinetics (*In Vitro*) of Grass and Shrub Legume Leaves: The Pattern of Gas Production, Organic Matter Degradation, pH and NH_3 Production

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ABSTRAK

WIDIAWATI, Y. dan A. THALIB. 2006. Perbandingan pola fermentasi (*in vitro*) dari rumput dan daun pohon legum: Pola produksi gas, degradasi bahan organik, pH dan produksi NH_3. *JITV* 11 (4): 266-272.

Mikroba rumen mendegradasi karbohidrat dalam pakan menjadi bahan yang berguna seperti VFA, protein mikroba dan vitamin B, dan beberapa bahan yang tidak berguna seperti gas metan dan CO_2. Jumlah dan pola dari produk akhir fermentasi pakan oleh mikroba rumen tergantung kepada jenis pakan yang dikonsumsi. Pakan yang mengandung serat tinggi menghasilkan banyak VFA dan gas, sedangkan pakan mengandung protein tinggi menghasilkan banyak NH_3. Dua jenis pakan yaitu rumput dan tanaman legum memiliki struktur dan isi cell yang berbeda, sehingga diduga akan menghasilkan jumlah dan pola produk akhir fermentasi oleh mikroba rumen yang berbeda pula. Penelitian menggunakan metoda *in vitro* yang dikembangkan oleh Theodorou dan Brooks, untuk mengetahui produk akhir fermentasi dua jenis pakan yaitu rumput dan leguminosa oleh mikroba rumen. Hasil menunjukkan bahwa bahan organik dari tanaman legum lebih banyak yang didegradasi dalam waktu yang sama dibandingkan dengan rumput, jenis pakan ini juga menghasilkan lebih banyak NH_3. Berdasarkan jumlah bahan organik yang dicerna, maka rumput menghasilkan lebih banyak gas. Kedua jenis pakan tidak merubah pH di dalam rumen.

Kata Kunci: Rumput, Leguminosa, Produksi Gas dan Amonia, Kecernaan Bahan Organik

ABSTRACT

WIDIAWATI, Y. dan A. THALIB. 2006. Comparison of fermentation kinetics (*in vitro*) of grass and shrub legume leaves: The pattern of gas production, organic matter degradation, pH and NH_3 production. *JITV* 11 (4): 266-272.

Rumen microbes convert carbohydrate in a feed into useful product such as VFA, microbial protein and B-vitamin; and some waste products such as CH_4 and CO_2. The amount and pattern of each product depend on the type of feed consumed by the animal. High fiber diet produces high gas and VFA, while high protein diet produce high NH_3. Two types of feeds, grass and legume, have different cell structure and content, thus might have different pattern of rumen fermentation and product. *In vitro* method developed by Theodorou and Brooks was used to determine the pattern of rumen fermentation end product of two types of feeds, namely grass and legume. Result shows that legume has higher amount of OM degraded and produce higher amount of NH_3 compared to grass. On the basis of OM degraded, the grass yielded higher volume of gas than that of legume. The two types of feeds did not change the pH of rumen fluid.

Key Words: Grass, Shrub Legume, Gas And Ammonia Production, Organic Matter Digestibility

INTRODUCTION

The degradation of organic matter (OM) of the shrub legume will differ from that of other feeds such as grasses or crop residues because they have different cell structures. Grasses and other fibrous feeds, contain high cell wall but less cell content, while most of the shrub legumes would have larger cell content (MINSON, 1990). The cell-wall fraction of plants is degraded slowly in the rumen (POPPI and NORTON, 1995) and is more resistant to rumen microbial degradation when lignification of the plant occurs. Lignification takes places in most cell wall of grasses, as these mature. The cell wall of shrub legumes, on the other hand, does not undergo lignification to the extent that occurs in the cell wall of grasses (WILSON and HATFIELD, 1997).

Dietary nutrients made available to the ruminant animal depend to a large extent on microbial activities in its rumen. The microbes, in the process of fermentation, normally convert major fractions of carbohydrates and proteins in a feed to useful end-products, such as volatile fatty acids (VFA), microbial protein and B-vitamins. Associated with such a process also are some waste products, such as the gases methane (CH_4) and carbon dioxide (CO_2). Ammonia (NH_3) is released when proteins are degraded in the rumen. A certain proportion of this is used by the rumen microbes for microbial protein synthesis, while the balance may traverse the rumen wall into the bloodstream. Fermentation of a readily digestible protein in the rumen would result in increased NH_3 production.

Therefore the experiment was conducted in order to determine the pattern of end product of rumen microbial fermentation of two types of feeds, namely grass and shrub legume. The Elephant grass was used as representative of grass, because it is commonly used as fibre source feed for ruminant. While the shrub legume *Leucaena leucocephala* was used in the experiment because it is well known as high protein roughage and usually used as feed supplement by the farmers in Indonesia.

MATERIALS AND METHODS

Experimental design

Fermentation kinetics (*in vitro*) of two feeds, namely Leucaena and Elephant grass, as treatments that were replicated three times, was examined using a Completely Randomised Block Design (DANIEL, 1991). Each sample was incubated at the periods of 2, 6, 12, 18, 24, 36 or 48 h.

Feed samples

Fresh feeds were dried in a freeze-drier (Dynavac Freeze Drying Unit, UK) for about 4 days then were ground to pass through a 1 mm screen (Single Phase A. C. Industry Motor type JY2A-4, West Germany). Each ground feed sample was stored in a plastic bag at 4°C before being used in the experiment.

Microbial inoculum

Microbial inoculum was derived from rumen fluid taken from four rumen-fistulated sheep fed a mixed diet of Elephant grass and legume leaves (56:44) at 500 g dry matter (DM) $sheep^{-1}$ d^{-1}. The composition of the mixed diet was designed to provide a crude protein (CP) content of 13% on a DM basis. This level of CP is recommended for maintenance of tropical sheep (DJAJANEGARA *et al*., 1996). The ration was divided into two equal portions, one of which was offered at 0800 h and the other at 1600 h.

Incubation medium

The *in vitro* method developed by THEODOROU dan BROOKS (1990) was employed in the experiment. Materials used in the method included the feed samples as substrates, pooled rumen fluid as the microbial inoculum and a formulated solution as the incubation medium. The medium consisted of six reagents, namely micro-mineral (0.1%), buffer (22.2%), macro-mineral (22.2%), resazurine (0.2%), distilled water (51%) and a reducing agent (4.3%).

Sampling and laboratory analysis

Organic matter degradation

The feed organic matter (OM) degradation was determined by vacuum filtration through a pre-weighed crucible (Sintaglass, porosity, 70 mL capacity; Gallenkamp, Loughborough, UK). The crucibles containing feed residues were dried in an oven at 100 °C for 24 hours or to constant weight to determine DM. These then were ashed in a muffle furnance at 500 °C for 6 hours for OM determination by subtracting ash from dry matter. The amount of OM degraded was calculated using equation: OMDegraded = OM in feed sample - OM in residue in each incubation bottle.

Water-soluble, Water-insoluble but degradable OM in feed and the degradation rate of OM during the first 24 h and the last 24 h of incubation are shown in Figure 1.

The water-soluble fraction of OM *(a)* is the fraction of OM that disappeared during the overnight storage period of the culture bottle in the cold room at 4 °C. The water-insoluble but degradable OM *(b)* was the OM degraded in the inoculated culture bottle from time of inoculation to the end of incubation. The rates of degradation of feed OM during the first 24 h (0 - 24 h) was calculated by using equation: OM_{Deg24} = OM (mg) degraded during 0 - 24 h of incubation (d2) /period of incubation (24h) (d1).

The rates of degradation of feed OM during the last 24 h (24 - 48 h) of incubation were calculated by using equation: OM_{Deg48} = OM (mg) degraded during 24 - 48 h of incubation. (e2) /period of incubation (24h) (e1).

Gas production measurement

A detachable pressure transducer (Bailey & Mackey Ltd, Birmingham, UK) was used to monitor gas pressure in each inoculated culture bottle. The transducer had a range of 0 to 15 psi, with an accuracy of 0.1 $\pm$ 2% at 25°C. The pressure transducer was connected to the inlet of a disposable Luer-lock three-way tap (Robinet three-way tap, France). The first outlet of the three-way tap was connected to a disposable hypodermic needle (23 gauge x 1.5 inch) while the second outlet was connected to a disposable glass syringe of 60 mL capacity (Sterling Ltd., UK).

Gas volume produced from 1 g of feed sample degraded (*in vitro*) was calculated using equation:

$$GV = GVs - GVb$$

where GV is the volume of gas (mL) produced, GVs is the volume of gas (mL) produced from each bottle containing a feed sample, microbial inoculum and medium, GVb is the volume of gas (mL) produced from the Blank bottle containing microbial inoculum and medium.

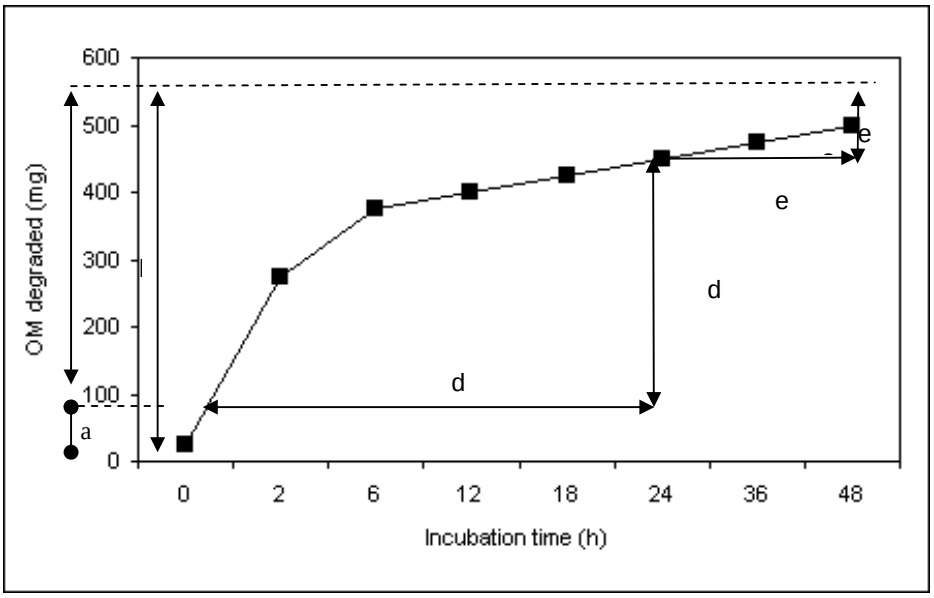

Figure 1. Curve of organic matter (OM) degraded in the inoculated bottle medium during 48 hours of incubation. *a* water-soluble fraction, *b* water-insoluble but degradable fraction and *c* water-soluble and water-insoluble but degradable fraction, *d1* the first 24 hours of incubation period, *d2* the amount of OM degraded during the first 24 hours of incubation period, *e1* the second 24 hours of incubation period, and e2 the amount of OM degraded during the second 24 hours of incubation period

pH and NH_3 production

The pH of the medium in each inoculated bottle was determined [using a CD 620 digital pH meter (Metler, UK)] immediately after the bottle was removed from the water bath.

Two mL aliquots of the medium from each bottle were transferred into 5 mL plastic storage vials for the determination of NH_3 concentration. The NH_3 concentration of the medium was determined by using the Conway technique developed by CONWAY and MALLEY (1942). This technique uses a glass Conway bowl, boric acid (2% g v^{-1}), sodium hydroxide (40% g v^{-1}), hydrochloric acid (HCl) (0.03 N), Conway indicator (bromo-cressol green: methyl red (1 : 2 wt/wt) in 95% ethanol solution) and Vaseline. The concentration of NH_3 was calculated by using the equation: NH_3 (mg/mL) = (mL HCl x N HCl x Mw NH_3) / Vol_{spl} (mL)

Statistical analysis

All raw data were tabulated using Microsoft® Excel 2000 for Windows 2000 (Microsoft Corporation, USA) and analysed using SPSS Version 7.0 for Windows 95 (SPSS Inc, USA). The data were analysed using one-way ANOVA (DANIEL, 1991) for Completely Randomised Block Design. Where significant effects from treatments were observed, differences among mean values were examined using Tukey's test (STEEL and TORRIE, 1980).

RESULTS AND DISCUSSION

Since the rumen fluid was taken from similar four sheep fed the same proportion of diet, which assumed to provide similar microbial population as the inoculum. It was assumed that any differences in fermentation characteristics in the cultured bottles would be due to differences in feeds sample examined in the experiment. The inoculum also was assumed to contain an adequate number and composition of rumen microbes to support optimal fermentation of the feed in a cultured bottle, because it was collected from rumen-fistulated sheep fed grass-legume mixture considerably providing enough protein (± 13%) and energy (8,5 MJ) recommended for maintenance of tropical sheep (DJAJANEGARA *et al.*, 1996). The inoculum also was collected at three hours after feeding when the rumen microbes were considered to be at maximal activity (CAMERO and FRANCO, 2001).

Nutrient content of feeds used in the experiment

The CP and neutral detergent fibre (NDF) contents of the feed samples used in the experiment were presented in Table 1.

Table 1. Crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) in Leucaena and Elephant grass fed to experimental animals

Nutrients	Treatments	
	Leucaena	Elephant grass
CP (%)	22	10
ADF (%)	28	49
NDF (%)	48	75

The legume leaves contained less cell-wall fraction, as indicated by lower ADF and NDF values compared to corresponding values from grass. The Elephant grass contained higher NDF and lower CP per unit of DM compared to that of Leucaena. This analysis was consistent to the report of MINSON (1990) that grass diet consists of large part of cell wall and less part of cell content, while legume diet consists of large part of cell content but less part of cell wall.

Organic matter degradation

Data on water-soluble, water-insoluble but degradable, total water-soluble plus water-insoluble but degradable fractions, and the rate of OM degradation in the inoculated bottles are presented in Table 2.

By using the OM degradation curve of a feed (Figure 1), it is possible to determine the water-soluble fraction of the feed (Table 2). The proportions of water-soluble and water-insoluble but degradable fractions were significantly affected by type of feed. The Elephant grass had lower content of water-soluble fraction compared that of Leucaena ($P<0.01$).

The water-soluble fraction of Leucaena (5.8%) was higher than that of the Elephant grass (4.5%). The higher water-soluble fractions of Leucaena would have contributed to the higher rate of OM degradation observed in this legume (2.1 mg h^{-1}) compared to the Elephant grass (1.9 mg h^{-1}). In addition, the structure differences in the cell wall of the legume and the grass would contribute significantly to the extent of the difference in OM degradation rate between the two types of feed.

These results was similar to the work of WILSON and HATFIELD (1997) who reported that cell wall of legume usually does not undergo lignifications as that occurs in the cell wall of grass. Thus the former diet is easier to be degraded by rumen microorganisms compared to the latter.

Gas production

Data on the total volume of gas produced and the rate at which the gas was produced from fermentation of the different feeds are presented in Table 3.

The total volume of gas produced in the culture bottles was significantly affected by the type of feed (Table 3). Total volume of gas produced was similar for Leucaena and Elephant grass. There were differences in the rates of gas production among the feeds during the first and second 24 h of incubation. In the first 24 h, Leucaena had higher rates of gas production than did Elephant grass ($P<0.01$). In the second 24 h of incubation, however, Elephant grass had higher rate of gas production ($P<0.05$) compared to that of Leucaena during the second 24 hours of incubation.

Table 2. Means* of water-soluble (*a*), water-insoluble but degradable (*b*) and *a* + *b* fractions at 24 h and 48 h of incubation and the degradation rate (0 - 24 h and 24 - 48 h of incubation) of organic matter (OM) from Leucaena and Elephant grass

Variable	Treatment			
	Leucaena	Elephant grass	SE	P
a fraction (mg)	26[a]	19[b]	2.9	0.005
b fraction (mg)				
24 h	375[a]	361[b]	22	0.000
48 h	426[a]	407[a]	156	0.000
a + *b* fraction (mg)				
24 h	401[a]	380[b]	29	0.001
48 h	452[a]	426[b]	20	0.000
OM degradation (mg h^{-1})				
0 - 24 h	16.7[a]	15[b]	0.95	0.000
24 - 48 h	2.1[a]	1.9[b]	0.10	0.003

Within rows, means with different superscripts differ significantly ($P<0.05$)

Table 3. Means* of total gas volume produced, and rate of gas production during fermentation (0 h - 24 h and 24 h - 48 h) of Leucaena and Elephant grass

Variables	Treatments			
	Leucaena	Elephant grass	± SE	P
Total gas volume produced (mL)	144[a]	143[a]	8.09	0.010
Gas production rate (mg h^{-1})				
0 - 24 h	4.7[a]	3.8[b]	0.30	0.001
24 - 48 h	1.3[b]	2.5[a]	0.37	0.035

* Within rows, means with different superscripts differ significantly (P<0.05)

The patterns of volume of gas production per unit of feed OM are presented in Figure 2. The volume of gas produced per unit of grass OM degraded in the culture bottles during incubation was above corresponding values from Leucaena (P<0.05).

When values for gas produced were expressed per unit of OM degraded, the Elephant grass produced 13%, more gas (P<0.02) than did Leucaena. This reflects the probable lower energetic efficiency associated with the conversion of the grass OM to absorbable nutrients.

The legume contained approximately 20 - 23% of protein in DM, which would have been fermented to produce NH_3 largely (BLUMMEL and BULLERDIECK, 1997) rather than the gases, CO_2 and CH_4. With its proportionally lower CP and higher carbohydrate (largely structural) compared with the legume (Table 1), Elephant grass, on fermentation, might be expected to produce proportionally higher acetate and consequently more CO_2 and CH_4. The production of acetate is accompanied by direct release of CO_2 (DOANE *et al*., 1997). Thus with the higher proportion of acetate produced from Elephant grass, it is suggested that a higher amount of CO_2 is released during fermentation of grass than of legume. Since the CO_2 contributes about 40% of total gas produced during fermentation (MCDONALD *et al*., 1995), the higher volume of gas per unit of OM degraded, observed for the Elephant grass might be explicable by the higher amount of CO_2 produced.

The higher rates of gas production from Leucaena compared Elephant grass during the 0 – 24 h of

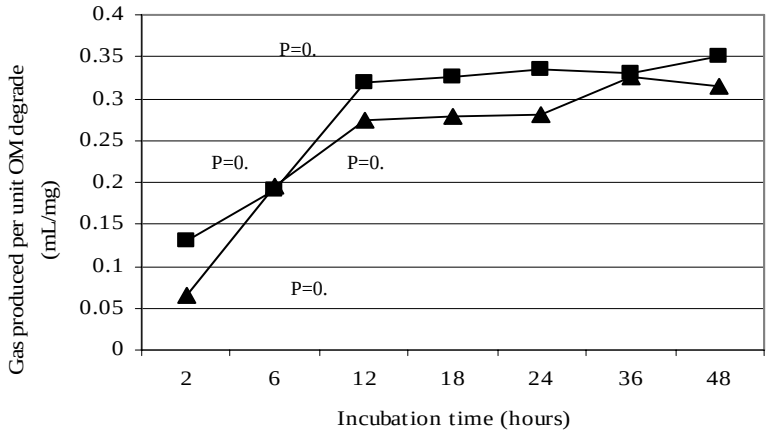

Figure 2. Means of volume of gas produced per unit of organic matter (OM) of Leucaena (■) and Elephant grass (▲) degraded in the culture bottle during the 48 hours of incubation

incubation period may reflect the higher proportions of water-soluble material in Leucaena (POPPI and NORTON, 1995). The Leucaena contained about 37% more water-soluble fraction than Elephant grass (Table 2).

pH and NH_3

Mean values of pH and NH_3 concentration of the medium in the culture bottles during the 48 h of incubation are presented in Table 4.

Table 4 Means* of pH and NH_3 concentration during fermentation of Leucaena and Elephant grass

Period	Treatment		
	Leucaena	Elephant grass	P
pH			
2 h	7.0	6.5	>0.05
6 h	6.8	6.8	>0.05
12 h	6.9	6.6	>0.05
18 h	6.8	6.9	>0.05
24 h	6.8	7.1	>0.05
36 h	6.7	6.7	>0.05
48 h	6.8	6.7	>0.05
NH_3 (mmol dl^{-1})			
2 h	1.45	1.30	>0.05
6 h	1.40	1.28	>0.05
12 h	1.55^a	1.28^b	0.049
18 h	1.45^a	1.27^b	0.001
24 h	1.65^a	1.50^b	0.010
36 h	1.75^a	1.40^b	0.049
48 h	2.10^a	1.35^b	0.008

- Within rows, means with different superscripts differ significantly ($P<0.05$).

The feed had no significant effect on the pH of the medium in the culture bottles during the 48 h incubation period. The pH values were relatively steady during the incubation period and ranged from 6.5 to 7.3 for the feeds examined (see Table 4). The result on the values of pH for elephant grass was similar to the work by THALIB (2002), which had the value of average pH 6.78 for Elephant grass during the *in vitro* experiment. While the pH value for Leucaena was similar to the work by NHAN (2000) which reported the value of pH ranging from 6.48 to 7.32 during the incubation period. The pH values of media in culture bottles were not significantly affected by the type of feed. Values in the rumen ranging from 6.5 to 7 are considered suitable (HUNGATE, 1966) for optimal activity and growth of the two major groups of bacteria, namely, the cellulolytic and proteolytic bacteria.

The dynamics of saliva secretion into, and absorption of VFA from the rumen were not represented in the closed *in vitro* system employed in the experiment. However, the buffer reagent used in the culture media appeared to be adequate for maintaining the pH values of the media within optimal limits for microbial activity during the 48 h period of incubation.

The NH_3 concentration value was affected significantly by the type of feed. Although values were similar for all the feeds at 2 h and 6 h of incubation, these started to differ significantly from 12 h onwards of incubation. Both feeds had similar values for NH_3 concentration during the 6 hours of incubation period. Ammonia concentration after 48 hours of incubation was 2,1mmol/100mL or about 350 mg L^{-1} for Leucaena. This value was similar with the value reported by NHAN (2000) for Leucaena namely 412 mg L^{-1}.

Overall, NH_3 concentrations for all the feeds were above the minimum values (50 mg L^{-1} or 0.3 mmole/100mL) required for rumen microbial activities (SATTER and SLYTER, 1974). The higher concentration of NH_3 resulting from fermentation of the legume was not unexpected as the fractions of water-soluble CP in the legume were greater than that in the Elephant grass.

The results of the experiments confirm the suggestions relating to digestibility and voluntary intake. The difference in OM degradability values between grass and legume largely explain the difference in nutritional values of the feeds. The important factor might be related to these is the differences in total VFA and NH_3 production from the grass and legume, respectively. The important implication from this observation would be, for example, on the use of Leucaena as a protein supplement in feeding with low quality roughage or grass. It seems that the production of VFA from such roughage in the rumen would not match the rate at which NH_3-N would be produced from Leucaena for optimal microbial activity. Thus, in a feeding situation similar to this, a delayed/staggered introduction of the Leucaena to feeding should be considered. These feeding strategy might be applied in both animals in the growing phase or for reproduction phase.

CONCLUSION

The amounts of OM degraded during fermentation were higher in the Leucaena, compared to the grass. In accordance with this, the total volumes of gas formed from the former group of feeds were higher than that of the latter feed. However, when expressed on the basis

of OM degraded, the grass yielded higher volume of gas. Rumen fermentation of legume leaves produced more NH_3 compared to that of grass. The feeds did not change the pH of rumen fluid.

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